Tissue-specific effects of leptin administration on the abundance of mitochondrial proteins during neonatal development

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Abstract

Many tissues undergo a rapid transition after birth, accompanied by dramatic changes in mitochondrial protein function. In particular, uncoupling protein (UCP) abundance increases at birth in the lung and adipose tissue, to then gradually decline, an adaptation that is important in enabling normal tissue function. Leptin potentially mediates some of these changes and is known to promote the loss of UCP1 from brown fat but its effects on UCP2 and related mitochondrial proteins (i.e. voltage-dependent anion channel (VDAC) and cytochrome *c*) in other tissues are unknown. We therefore determined the effects of once-daily jugular venous administration of ovine recombinant leptin on mitochondrial protein abundance as determined by immunoblotting in tissues that do (i.e. the brain and pancreas) and do not (i.e. liver and skeletal muscle) express UCP2. Eight pairs of 1-day-old lambs

received either 100 µg leptin or vehicle daily for 6 days, before tissue sampling on day 7. Administration of leptin diminished UCP2 abundance in the pancreas, but not the brain. Leptin administration had no effect on the abundance of VDAC or cytochrome *c* in any tissue examined. In leptin-administered animals, but not controls, UCP2 abundance in the pancreas was positively correlated with VDAC and cytochrome *c* content, and UCP2 abundance in the brain with colonic temperature. In conclusion, leptin administration to neonatal lambs causes a tissue-specific loss of UCP2 from the pancreas. These effects may be important in the regulation of neonatal tissue development and potentially for optimising metabolic control mechanisms in later life.

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Introduction

Leptin is a 16 kDa polypeptide hormone which is principally synthesised and secreted by adipose tissue and acts to regulate energy homeostasis and a range of neuro-endocrine and reproductive functions in the adult (Schwartz *et al.* 1996, Friedman & Halaas 1998, Ahima & Flier 2000). However, its exact role in the neonate has yet to be fully determined. In the human fetus, plasma leptin concentrations increase with gestational age (Yuen *et al.* 1999, Cetin *et al.* 2000). In the newborn sheep, plasma leptin concentrations decline during the immediate 6 h after birth to then increase up to 7 days of age (Bispham *et al.* 2002). These temporal changes in leptin coincide with development of the hypothalamic–pituitary–adrenal axis with respect to the regulation of cortisol production

coincident with the rapid activation of uncoupling protein (UCP)-1, which is unique to brown adipose tissue (BAT) (Clarke *et al.* 1997a, Ricquier & Bouillaud 2000), and is followed by the gradual loss of UCP1 (Clarke *et al.* 1997b). In the neonatal sheep, leptin administration results in reduced UCP1 mRNA and protein abundance, in conjunction with maintained colonic temperature and plasma non-esterified fatty acid (NEFA) concentration, therefore not affecting thermogenic potential (Mostyn *et al.* 2002). The peak in UCP1 abundance at birth is accompanied by parallel increases in other mitochondrial proteins including the voltage-dependent anion channel (VDAC) located on the outer mitochondrial membrane, and cytochrome *c*, present within the inter-membrane space (Mostyn *et al.* 2003). VDAC regulates the supply of mitochondrial ADP and ATP into and out of the mitochondria. VDAC is also

a component of the mitochondrial permeability transition pore (MPTP), which is composed of a number of proteins which combine and make contact sites between the inner and outer mitochondrial membranes (Crompton 1999); there is evidence to suggest that the MPTP is utilised in some capacity during apoptosis in the release of cytochrome *c* from the mitochondria (Gottlieb 2000). There is potential for this event in all tissues, but the role of the MPTP has been most widely studied in organs most susceptible to ischaemic-reperfusion injury such as the heart (Crompton 1999). Cytochrome *c* is an essential component of the mitochondrial respiratory chain and is an mobile electron transporter, involved in the electron transfer from complex III to complex IV (Ludwig *et al.* 2001, Jiang & Wang 2004).

UCP2 is present in a large number of tissues with its abundance being greatest in the lung, pancreas and skeletal muscle (Fleury *et al.* 1997). In the case of the lung it has recently been established in the sheep that UCP2 abundance peaks soon after birth and then rapidly decreases over the first month of life (Gnanalingham *et al.* 2005). This developmental pattern of expression is similar to that described for UCP1 in ovine BAT, suggesting UCP2 has a critical role in metabolic adaptation at birth. In this regard the high expression of UCP2 within the pancreas could be important in enabling the newborn to effectively adapt to the pronounced increase in plasma glucose concentration following the commencement of independent feeding (Phillips *et al.* 1978). The role of leptin in the abundance of UCP2, VDAC and cytochrome *c* proteins has not been previously determined. Administration of leptin to *ob/ob* mice, which do not produce leptin and are thus hypothermic, hyperphagic and obese, restores a normal body temperature, despite a 50% reduction in food intake (Pelleymounter *et al.* 1995). These changes in body temperature have been linked to increased abundance of UCP1 (Scarpace *et al.* 1997) and UCP2 (Gong *et al.* 1997) in BAT by some studies, but not by others (Memon *et al.* 2000). Moreover, these changes appear to be unique to rodents, as leptin treatment of large mammals, such as sheep and pigs, has been found to have a minor role in thermogenesis (Mostyn *et al.* 2002, Litten *et al.* 2004).

Leptin receptors are present in the β -cells of the pancreas, where UCP2 is also expressed (Kieffer *et al.* 1996, Chan *et al.* 1999). Leptin at physiological doses has been shown to inhibit both basal and glucose-stimulated insulin secretion *in vitro* (Emilsson *et al.* 1997, Roduit & Thorens 1997), and to lower circulating insulin levels *in vivo* in adult rodents (Sivitz *et al.* 1997, Bryson *et al.* 1999). Levels of rat pancreatic islet UCP2 mRNA are enhanced by recombinant adenoviral-induced leptin expression (Zhou *et al.* 1997) and UCP2 has also been genetically linked to type II diabetes (Fleury *et al.* 1997). In the brain, UCP2 is expressed discretely in neurons located in the subcortical regions that are involved in the central regulation of autonomic and metabolic processes including thermogenesis, and UCP2-producing neurons

have been found to be targets for peripheral hormones, including leptin (Horvath *et al.* 1999). In addition, i.c.v. leptin administration in rats causes depletion of adipocytes by apoptosis (Qian *et al.* 1998). Leptin has been proposed to increase glucose uptake in BAT and muscle and to reduce hepatic glycogen stores associated with increased glucose production (Nonogaki 2000). Liver UCP2 mRNA was increased by i.c.v. leptin administration in rats (Cusin *et al.* 1998), while skeletal muscle UCP2 mRNA was unaffected (Combatsiaris & Charron 1999). Interestingly, interleukin-1 β and tumour necrosis factor- α , which regulate leptin (Zumbach *et al.* 1997, Faggioni *et al.* 1998b), have been shown to upregulate UCP2 mRNA in liver, muscle and adipose tissue of adult mice (Faggioni *et al.* 1998a), although there is now debate whether UCP2 is present in the liver and skeletal muscle (Pecqueur *et al.* 2001). No study to date has investigated the effect of chronic leptin administration on the abundance of UCP2 in the pancreas, liver, skeletal muscle and cerebral cortex in the neonatal period in a precocial species, such as the sheep.

The aims of this study were thus to determine whether maintaining high plasma leptin concentrations during neonatal development promotes the loss of the mitochondrial proteins UCP2, VDAC and cytochrome *c* from either the pancreas, liver, skeletal muscle or brain (cerebral cortex) of neonatal sheep at 7 days postnatal age. This is the age when mitochondrial protein abundance is near maximal in many tissues including the lung and adipose tissue (Mostyn *et al.* 2003). In addition we determined whether any significant associations existed between these mitochondrial proteins and plasma leptin or NEFA concentrations and colonic temperature as measured through the study.

Materials and Methods

Full details of materials and methods have been previously published (Mostyn *et al.* 2002). In brief, eight pairs of weight-matched female triplet lambs were entered into the study. These were all conceived naturally and born normally at term to Bluefaced Leicester cross Swaledale ewes. Each lamb fed freely from their mother as they all remained with their mother throughout the study (including the one untreated triplet per ewe). At the end of the study the ewe kept the remaining triplet in order to avoid the need for the mother to prematurely cease lactating. Every lamb gained weight over the course of the study and there were no differences in growth rates between those treated with and without leptin. All operative procedures and experimental protocols had the required local ethical and Home Office approval as designated by the Animals (Scientific Procedures) Act of 1986. All pairs of lambs were selected on the basis of matched body weight ($\pm 10\%$) and a jugular vein catheter was inserted into each animal under local anaesthetic on day 1 of age (2% xylocaine) to enable

vehicle or leptin administration and blood sampling. The ovine leptin was produced recombinantly (Gertler *et al.* 1998). Lambs were entered into the study on day 1 of life and injected daily for 6 days at ~0930 h with either 100 µg leptin or vehicle (100 µl sterile water). Body weight was measured daily and blood samples were taken on each study day before treatment. Colonic temperature was measured daily at ~0900 h using a digital thermometer (VWR International, Leics, UK). On day 7, blood samples were taken at between 1100 and 1200 h from each lamb that was then humanely killed by i.v. administration of 100 mg/kg pentobarbital sodium (Euthatal: RMB Animal Health, UK). All major organs were rapidly removed, placed in liquid nitrogen and stored at -70°C until subsequent laboratory analysis.

Laboratory analyses

Protein detection Mitochondria were prepared from 1 g frozen head of pancreas, liver, skeletal muscle (i.e. quadriceps) and cerebral cortex area of the brain, and protein content of each preparation determined by the Lowry *et al.* (1951) method. Western blotting was utilised to measure the abundance of each protein. Following electroblotting of the polyacrylamide gel onto a nitrocellulose membrane, Ponceau red staining was used to visually confirm that similar amounts of protein had been transferred before subjecting the membranes to immunodetection (Mostyn *et al.* 2003). Abundance of cytochrome *c* was determined on 10 mg mitochondrial protein using an antibody (SC7159; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) at a dilution of 1 in 1000. VDAC abundance was determined using an antibody raised in rabbits to ovine VDAC, purified from the kidney of a newborn lamb as described by Mostyn *et al.* (2003), and was used at a dilution of 1 in 2000. Abundance of UCP2 was determined using the same antibody as described by Pecqueur *et al.* (2001), at a dilution of 1 in 10 000, which was raised against human UCP2. UCP2 was detected in the pancreas and cerebral cortex at 7 days of postnatal life, but not in skeletal muscle or liver, where the protein detected was not at the correct molecular mass, and hence was not UCP2 (Pecqueur *et al.* 2001, Mostyn *et al.* 2003). Densitometric analysis was performed using AIDA (Aida version 2.0; raytest Isotopenmeßgeräte GmbH, Straubenhardt, Germany) on each membrane following image detection using a Fujifilm LAS-1000 cooled CCD camera (Fuji Photo Film Co., Ltd, Tokyo, Japan) and all values are expressed in densitometric units. Specificity of detection was confirmed using non-immune rabbit serum. A range (10–68 kDa) of molecular mass markers was included on all gels. All gels were run in duplicate and a reference sample (an appropriate ovine mitochondrial sample) was included on each to allow comparison between gels.

GDP binding The thermogenic activity of mitochondrial protein prepared from skeletal muscle as described above

was assessed from the *in vitro* activity of the mitochondrial conductance pathway using GDP at a concentration of 2 mM, with non-specific binding measured using a 200 mM concentration of GDP using the same methods described by Symonds *et al.* (1992). In addition, mitochondrial protein prepared from perirenal adipose tissue from a 1-day-old sheep acted as the positive control on this assay and all measurements were made in triplicate.

Plasma leptin, glucose and NEFA concentrations

Plasma concentrations of glucose and NEFA were measured enzymatically (Clarke *et al.* 1994). Plasma leptin concentration was determined using a validated double-antibody RIA as described by Delavaud *et al.* (2000). Plasma concentrations of leptin were assayed in duplicate 200 µl samples using a rabbit anti-ovine leptin primary antibody, iodinated ovine leptin and sheep anti-rabbit secondary antibody. The limit of leptin detection was 0.1 ng/ml and the intra- and inter-assay coefficients of variation were 4.2 and 9.1% ($n=5$) respectively.

Statistical analyses

All data are presented as means $\pm$ S.E.M. Tests of normality as determined by the Kolmogorov–Smirnov test revealed that the data were non-parametric. Statistically significant ($P<0.05$) differences between values obtained from vehicle-controls and leptin-treated groups were determined by the Mann–Whitney *U* test and correlations within individual groups by Spearman's rank order test (SPSS v11.0; SPSS, Inc.).

Results

Daily jugular venous injection of leptin resulted in a persistent increase in plasma leptin throughout the course of the study (Fig. 1). Administration of leptin

Figure 1 Effect of chronic daily (6 days) administration of leptin (100 µg/day) on the plasma concentration of leptin as measured <24 h after i.v. leptin injection in neonatal sheep. Values are means $\pm$ S.E.M. ($n=8$ per group). * $P<0.05$, mean value significantly different from the vehicle-treated group.

Figure 2 Effect of chronic (6 days) administration of leptin (100 µg/day) on the abundance of UCP2 in the pancreas and cerebral cortex of neonatal sheep sampled at 7 days postnatal age. Examples of protein expression are given. Values are means ± S.E.M. ($n=8$ per group). * $P<0.05$, mean value significantly different from the vehicle-treated group.

resulted in a significant decrease ($P<0.01$) in UCP2 abundance in the pancreas at 7 days of postnatal age, but not in the cerebral cortex area of the brain (Fig. 2). In contrast, VDAC and cytochrome *c* protein abundance were unaffected by leptin in all tissues studied (Table 1). GDP binding in skeletal muscle was unaffected by leptin (vehicle, 7.8 ± 0.6 pmol/mg mitochondrial protein; leptin, 8.4 ± 0.6 ($n=8$ per group)) and was markedly lower than in BAT (vehicle, 44.6 ± 4.9 pmol/mg mitochondrial protein). Chronic leptin administration did not affect total body or tissue weights compared with controls (Table 2) or plasma glucose concentration throughout the study (e.g. day 4 vehicle, 5.21 ± 0.20 mM; leptin, 5.5 ± 0.16).

A number of significant tissue-specific associations were observed between plasma leptin and NEFA concentrations and the physiological and molecular indices measured following leptin administration as outlined in Table 3. In the pancreas, UCP2 was positively associated with VDAC

and cytochrome *c* abundance following leptin administration, but negatively associated with VDAC in the vehicle group and mean plasma NEFA concentration over 7 days of leptin administration. VDAC abundance in the pancreas was also positively associated with plasma leptin on day 7 following leptin administration. In the liver, VDAC abundance was positively associated with plasma leptin on day 7 and with cytochrome *c* content, whereas in skeletal muscle, plasma leptin on day 7 was positively associated with VDAC and cytochrome *c* abundance following leptin administration, and negatively associated with cytochrome *c* in the vehicle group. These associations observed following leptin administration were observed at the time at which plasma leptin was similar between groups (day 7 vehicle, 2.0 ± 0.3 ng/ml; leptin, 2.2 ± 0.3) and is not unexpected as on the final day of the study all animals were sampled more than 24 h after the previous leptin injection. After leptin administration, mean colonic temperature over 7 days was positively associated with

Table 1 Abundance of the mitochondrial proteins VDAC and cytochrome *c* in the pancreas, liver, skeletal muscle and cerebral cortex, as measured in neonatal sheep subjected to i.v. leptin (100 µg/day) administration or vehicle for 6 days. Values in arbitrary units are means ± S.E.M., $n=8$ per group

Tissue	VDAC		Cytochrome <i>c</i>	
	Vehicle	Leptin	Vehicle	Leptin
Pancreas	191.9 ± 14.2	176.2 ± 25.8	140.3 ± 9.7	141.7 ± 10.6
Liver	258.2 ± 34.8	240.5 ± 30.9	82.7 ± 5.6	80.2 ± 5.8
Skeletal muscle	80.7 ± 11.7	81.1 ± 10.0	109.5 ± 12.7	103.1 ± 11.3
Cerebral cortex	104.8 ± 13.0	98.6 ± 14.3	98.1 ± 8.2	101.8 ± 7.3

Table 2 Mean body and organ weights as measured in 7-day-old neonatal sheep subjected to i.v. leptin (100 µg/day) administration or vehicle for 6 days. Values are means $\pm$ S.E.M., $n=8$ per group

	Vehicle	Leptin
Body weight (kg)	5.9 $\pm$ 0.4	5.6 $\pm$ 0.4
Liver (g)	156.9 $\pm$ 9.9	165.8 $\pm$ 12.4
Brain (g)	46.8 $\pm$ 1.1	48.5 $\pm$ 1.4

GDP binding in skeletal muscle, and UCP2 abundance in the cerebral cortex.

Discussion

Leptin and UCP2 in the neonatal pancreas

In this study, we have shown for the first time that chronic leptin administration decreases UCP2 abundance in the neonatal pancreas. Importantly, this response was observed in the absence of any effect of leptin on food intake or behaviour (Mostyn *et al.* 2002). Our findings are therefore in agreement with the only report of UCP2 expression in human islets, demonstrating a 75% reduction in UCP2 mRNA expression following leptin administration *in vitro* (Brown *et al.* 2002). In contrast to our findings, Zhou *et al.* (1997) demonstrated that levels of rat pancreatic islet UCP2 mRNA and enzymes of fatty acid oxidation were enhanced by recombinant adenoviral-induced leptin expression. In the pancreas, the decreased UCP2 abundance with chronic leptin administration may improve insulin secretion and ameliorate the effects of type II diabetes, as reported in UCP2-deficient mice (Zhang *et al.* 2001). It is proposed that an absence of UCP2 leads to increased coupling in the β -cell mitochondria, causing higher ATP levels, inhibiting β -cell potassium-ATP channels, depolarising the cell, leading to calcium influx through voltage-gated calcium channels, with this

increased calcium leading to augmented insulin secretion (Zhang *et al.* 2001, Nedergaard & Cannon 2003).

The functional relevance of the modest decrease in pancreatic UCP2 content following chronic leptin administration on β -cell function remains to be determined. It could act to modulate insulin secretion and aid normoglycaemia, thereby explaining why leptin administration had no adverse effect on plasma glucose in the present study. Acute and chronic leptin treatment lowers circulating insulin levels in rodents *in vivo* (Sivitz *et al.* 1997, Bryson *et al.* 1999), although this has not been confirmed in human islets *in vitro* (Brown *et al.* 2002). Exposure of rat islets to elevated plasma NEFA concentration, which has long-term (inhibitory) and short-term (stimulatory) effects on glucose-stimulated insulin secretion, did increase UCP2 abundance (Lameloise *et al.* 2001). This contrasts with our negative association between mean plasma NEFA and pancreatic UCP2 following leptin administration, suggesting a decrease in UCP2 may allow the pancreatic β -cells to resist the detrimental effects of high NEFA exposure (Dubois *et al.* 2004, Joseph *et al.* 2004). Increased NEFA metabolism leads to an increase in energy flux through the electron transport chain, which can lead to enhanced production of reactive oxygen species in β -cells (Carlsson *et al.* 1999, Barbu *et al.* 2002). UCP2 may be involved in this interaction by limiting the NEFA-stimulated increase in reactive oxygen species production by dissipating the excess energy by activating proton transport mechanisms at the matrix side of the mitochondrial inner membrane (Echtay *et al.* 2002). The positive association between UCP2 and VDAC and cytochrome *c* proteins may be important in regulating the ATP/ADP ratio in β -cells, which also controls insulin secretion (Sweet *et al.* 2004). The recent localisation of VDAC in the plasma membrane (Bahamonde & Valverde 2003), its possible role in fluid secretion (Buettner *et al.* 2000) and its presence in the human pancreas (Huizing *et al.* 1998), suggest an additional role for VDAC in insulin secretion by β -cells – a role possibly enhanced by leptin, in view of the

Table 3 Significant relationships in the pancreas, liver, skeletal muscle and cerebral cortex between fetal plasma leptin and physiological and molecular indices measured in sheep neonates subjected to i.v. leptin (100 µg/day) or vehicle for 6 days ($n=8$ per group)

	Group	x axis	y axis	R ²	P value	Relationship
Tissue						
Pancreas	Vehicle	VDAC	UCP2 protein	0.37	0.034	–
Pancreas	Leptin	VDAC	UCP2 protein	0.74	0.015	+
Pancreas	Leptin	Cytochrome <i>c</i>	UCP2 protein	0.75	0.036	+
Pancreas	Leptin	Mean plasma NEFA over 7 days	UCP2 protein	0.42	0.047	–
Pancreas	Leptin	Plasma leptin on day 7	VDAC	0.39	0.014	+
Liver	Leptin	Plasma leptin on day 7	VDAC	0.45	0.036	+
Liver	Leptin	Cytochrome <i>c</i>	VDAC	0.34	0.046	+
Muscle	Leptin	Plasma leptin on day 7	VDAC	0.70	0.019	+
Muscle	Vehicle	Plasma leptin on day 7	Cytochrome <i>c</i>	0.67	0.032	–
Muscle	Leptin	Plasma leptin on day 7	Cytochrome <i>c</i>	0.96	<0.0001	+
Muscle	Leptin	Mean colonic temperature over 7 days	GDP binding	0.51	0.042	+
Brain	Leptin	Mean colonic temperature over 7 days	UCP2 protein	0.38	0.048	+

positive association between VDAC and plasma leptin on day 7 in the leptin group alone. Overall, these effects of leptin administration may be important in maintaining and protecting β -cell function. In this regard, leptin-deficient *ob/ob* mice have β -cell dysfunction (Lee & Romsos 2003).

Leptin and the neonatal brain, skeletal muscle and liver

UCP2 was detected in the cerebral cortex of the neonatal sheep, although unaffected by leptin administration. In the rodent and primate brain, UCP2 is expressed predominantly in neuronal populations of subcortical regions that are involved in the central regulation of autonomic, endocrine and metabolic processes (Horvath *et al.* 1999, Diano *et al.* 2000). There is also a positive correlation between these areas and a high local brain temperature (Horvath *et al.* 1999). While the effects of chronic leptin administration on UCP2 abundance in the cerebral cortex have not been previously determined, leptin receptors have been co-localised to similar subcortical regions, through which leptin regulates appetite, energy balance and sympathetic nervous system activity (Della-Fera *et al.* 2001). Recently, circulating leptin has been proposed to mediate lipopolysaccharide-induced anorexia and fever in the rat, probably through a hypothalamic interleukin-1 β -dependent mechanism (Sachot *et al.* 2004). The stimulation of UCP2 transcription by the pyrogenic cytokines tumour necrosis factor- α and interleukin-1 β , which are also known to increase serum leptin levels *in vivo* (Zumbach *et al.* 1997, Faggioni *et al.* 1998b), suggests a similar role for UCP2 lipopolysaccharide-induced fever (Faggioni *et al.* 1998a). The positive association between UCP2 in the cerebral cortex and the mean colonic temperature over 7 days in the leptin group, suggests that such a mechanism, possibly involving interleukin-1 β , may be involved in the maintenance of colonic temperature despite the loss of UCP1 in neonatal BAT following chronic leptin administration in sheep (Mostyn *et al.* 2002).

In this present study, UCP2 was not detected in the neonatal liver or skeletal muscle, in agreement with Pecqueur *et al.* (2001), but in contrast to rodent studies that have only confirmed UCP2 mRNA abundance and its potential regulation by leptin in the liver and skeletal muscle (Cusin *et al.* 1998, Combatsiaris & Charron 1999, Ricquier & Bouillaud 2000) although the specificity of the antibodies used in these studies remains to be established. However, while UCP2 mRNA is widely expressed in a variety of tissues (Ricquier & Bouillaud 2000), the protein expression is limited to a few organs, due to translational regulation of the UCP2 mRNA by an upstream open reading frame located in exon two of the UCP2 gene which strongly inhibits the expression of the protein (Pecqueur *et al.* 2001). Moreover in the present study, the effects of leptin administration appear to be specific to the inner mitochondrial protein UCP2, since VDAC and

cytochrome *c* proteins, although present in all the tissues examined, were unaffected by leptin administration, which is in agreement with previous findings in neonatal sheep BAT following chronic leptin administration (Mostyn *et al.* 2002). Interestingly the positive associations between plasma leptin and VDAC and cytochrome *c* proteins in the pancreas, liver and skeletal muscle following leptin administration were only observed when plasma leptin had returned to basal concentration in these previously leptin-treated animals. It is therefore possible that although leptin had no direct effect on the abundance of these mitochondrial proteins, previous exposure to high leptin could potentially maximise either VDAC or cytochrome *c* abundance in that individual. This may explain why, for example, the abundance of cytochrome *c* was negatively correlated with basal leptin in controls but positively correlated in those animals given leptin. Ultimately this adaptation may imply augmented energy production within these organs following leptin administration (Lehninger *et al.* 1993, Crompton 1999, Gottlieb 2000). Indeed, the positive association between GDP binding and mean colonic temperature over 7 days in the skeletal muscle following leptin administration, suggests a possible role in thermogenesis; however, this would appear to be minor in comparison with neonatal BAT, which has markedly higher GDP binding activity, a reflection of the much greater potential thermogenic capacity.

In conclusion, we have shown for the first time that leptin administration to the neonate has tissue-specific effects on the abundance of UCP2. These effects may be important in the regulation of neonatal tissue development and potentially for optimising metabolic control mechanisms in later life.

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