Available online at www.sciencedirect.com
ScienceDirect
Domestic Animal Endocrinology 35 (2008) 130–141
DOMESTIC ANIMAL ENDOCRINOLOGY
www.journals.elsevierhealth.com/periodicals/dae

Uncoupling protein expression in skeletal muscle and adipose tissue in response to *in vivo* porcine somatotropin treatment☆

T.G. Ramsay *, A.D. Mitchell, M.P. Richards

Growth Biology Laboratory, USDA-ARS, Beltsville, MD 20705, USA

Received 1 December 2006; received in revised form 8 November 2007; accepted 5 December 2007

Abstract

These experiments examined the potential roles of somatropin (pST) and IGF-I in the regulation of uncoupling protein (UCP)2 and UCP3 and their regulatory proteins peroxisome proliferator activated receptor (PPAR) α, γ and δ using *in vivo* pST treatment of swine and *in vitro* supplementation of pST or IGF-I to adipose slices. Six, 90 kg barrows were treated with recombinant pST (10 mg) for 2 week while another six pigs were injected with buffer. Total RNA from outer subcutaneous adipose (OSQ) and middle subcutaneous adipose (MSQ) tissues, leaf fat, liver and longissimus (LM) was amplified by reverse transcription-PCR with quantification of transcripts by capillary electrophoresis with laser-induced fluorescence detection. UCP2 mRNA abundance increased in liver ($P<0.001$) and all three adipose tissues by pST treatment ($P<0.05$). Administration of pST increased UCP3 mRNA abundance by 42% in LM ($P<0.01$). PPARα mRNA abundance increased with pST treatment by 29% in liver ($P<0.05$), while decreasing 25% in LM ($P<0.05$). PPARγ mRNA abundance decreased 32% ($P<0.01$) while PPARδ increased 48% in LM ($P<0.01$) with pST administration. *In vitro*, pST reduced UCP2 mRNA abundance in OSQ and MSQ tissue slices ($P<0.05$). UCP3 mRNA abundance decreased in OSQ ($P<0.05$) but increased in MSQ ($P<0.05$) with pST. In contrast, IGF-I increased UCP2 and UCP3 mRNA abundance in both MSQ and OSQ slices ($P<0.05$). These experiments suggest pST, IGF-I and metabolic adaptations to pST contribute to regulating UCP2 and UCP3.
Published by Elsevier Inc.

Keywords: Uncoupling protein; PPAR; Somatotropin; IGF-I; Swine

1. Introduction

The uncoupling proteins (UCPs) have been grouped into a family of proteins based upon their *in vitro* capacity to uncouple mitochondrial respiration [1]. Uncoupling protein 1 was the first of these proteins identified and shown to contribute to non-shivering thermogenesis [2], but is not expressed in swine due to the absence of brown adipose tissue [3]. Other member of this family, UCP2 and UCP3, share 55% and 57% amino acid identity with UCP1 and 73% with each other [4,5]. However, UCP2 and UCP3 demonstrate much weaker uncoupling activity [6] and through a number of studies have been shown to have primary functions independent of a significant role in uncoupling activity and heat production [7,9,10]. Uncoupling protein 2 has been associated with metabolism of free radicals and mild uncoupling activity [5,7]. Using UCP3 knockout mice, UCP3 has been associated with changes in mitochondrial energy production that suggests some minor uncoupling activity specific to skeletal muscle [8] while the majority of studies have

☆ Mention of a trade name, vendor, proprietary product or specific equipment is not a guarantee or a warranty by the U.S. Department of Agriculture and does not imply an approval to the exclusion of other products or vendors that also may be suitable.

* Corresponding author at: BARC-East, Building 200, Room 207, Beltsville, MD 20705, USA. Tel.: +1 301 504 5958; fax: +1 301 504 8623.
E-mail address: timothy.ramsay@ars.usda.gov (T.G. Ramsay).

0739-7240/$ – see front matter. Published by Elsevier Inc.
doi:10.1016/j.domaniend.2007.12.001

indicated a more specific role for UCP3 with regulation of fatty acid metabolism and ATP-dependent processes [9,10]. Damon et al. [11] and Spurlock et al. [12] have reported that both UCP2 and UCP3 mRNA are detected in porcine skeletal muscle and adipose tissue.

Hormonal regulation of these genes has been demonstrated *in vitro*, with somatotropin (GH) reducing uncoupling protein UCP2 and UCP3 mRNA abundance in adipose tissues slices from finishing pigs [13]. However the potential role of GH in the regulation of the uncoupling proteins in other major metabolic tissues of the pig has not been examined. Somatotropin is a potent inducer of lipid metabolism [14] as well as affecting free radical metabolism [15]. Since the uncoupling proteins are believed to have roles in these processes, GH may alter UCP expression in peripheral tissues *in vivo* through an indirect mechanism.

For the *in vivo* situation, the interaction of hormones in the endocrine milieu is important for overall metabolic regulation. Perhaps due to the variety of endocrine interactions *in vivo*, the potential role of GH in the regulation of the uncoupling proteins within adipose tissue is unclear [16–18]. Any and all *in vivo* studies with GH can be confounded by the known impact of GH on insulin-like growth factor I (IGF-I) secretion and the subsequent actions of IGF-I on peripheral tissues. The present study was designed to determine if the porcine uncoupling proteins are regulated *in vivo* by somatotropin, a modifier of energy metabolism and secondly to determine if IGF-I has a role in regulation of the uncoupling proteins in swine. The overall metabolic effects of GH on peripheral metabolism would suggest that UCP expression would be increased in peripheral tissues with GH treatment through its actions on IGF-I secretion and fatty acid metabolism. Furthermore, peroxisome proliferator activated receptor (PPAR) α, δ and γ mRNA abundance were analyzed because of their suggested role in the regulation of UCP gene expression [19–21] in an attempt to identify a mechanism for any changes in UCP mRNA abundance.

2. Materials and methods

Twelve crossbred barrows (Yorkshire × Landrace), weighing 70 kg, were individually penned in environmentally controlled housing. Animals were individually fed a basal diet containing 18% CP, 1.2% lysine, and 3.5 Mcal of DE/kg *ad libitum* as previously reported [22].

At 90 kg, six randomly selected pigs were treated with daily injections of sterile recombinant pST (10 mg; Southern Cross Biotech, Toorak, Victoria, Australia) in sodium bicarbonate buffer (pH 9.4). This dosage was based upon previous studies in pigs of this size [23,24]. The other six pigs served as controls and were injected with sterile bicarbonate buffer alone. Injections (1.0 mL) were performed into the extensor neck muscles between 08:00 and 08:30. Feed was presented at 09:00. With initiation of pST treatment, the feed was offered at 85% of calculated *ad libitum* intake [25], based upon BW and adjusted every 3 d. Animals were maintained on treatment for 2 week. A blood sample was obtained from each pig on d 14 of treatment at 15:00, 6 h after feed presentation. Animals were euthanized on d 15 at 08:00.

Various tissues were acquired following euthanasia by electrical stunning and exsanguination according to procedures approved by the Institutional Animal Use and Care Committee. Dorsal subcutaneous adipose tissue samples were collected from between the second and fourth thoracic vertebrae and subsequently outer subcutaneous adipose (OSQ) and middle subcutaneous adipose (MSQ) layers were separated at the connective tissue fascia separating the layers according to Anderson et al. [26], diced and frozen in liquid nitrogen. In addition, samples of liver, leaf (perirenal) fat and longissimus (LM) muscle were collected, diced and frozen in liquid nitrogen. Outer and middle subcutaneous adipose tissues were separated for analysis because of their known differences in metabolic activity, while leaf fat has a different metabolic profile from either subcutaneous adipose tissue [26]. The LM was selected as it represents a large, economically important muscle that responds to pST [27].

2.1. Hormone and metabolite analysis

Blood samples were centrifuged at 600 × *g* and serum samples were collected and stored at −70 °C for later analyses of hormones and metabolites. Concentrations of T_3 and cortisol were determined by homologous RIA using commercial kits (Diagnostics Products Co., Los Angeles, CA). Intraassay CV was 4.9% for T_3 and 3.4% for cortisol. Interassay CV was 6.6% for T_3 and 5.2% for cortisol. Serum insulin was measured using a homologus RIA kit with human standards (Linco Research Inc., St. Charles, MO). Dilutions of both the quality control solution and pig serum pool dilutions were parallel to the standard curve. Recovery of known amounts of unlabeled insulin yielded an average recovery of 97.6% of the added amount. Intraassay CV for insulin was 7.0%, while the interassay CV was 9.4%. Serum IGF-I was acid–ethanol extracted to remove binding proteins and then assayed using a heterologous immunoradiometric kit (Diagnostics Systems Laboratory Inc., Webster, TX) which was previously validated for swine [28]. Intrassay CV for IGF-I was 6.1% while the interassay CV was