Available online at www.sciencedirect.com
SCIENCE DIRECT®
Domestic Animal Endocrinology 28 (2005) 351–366
DOMESTIC ANIMAL ENDOCRINOLOGY
www.journals.elsevierhealth.com/periodicals/dae

Regulation of uncoupling proteins 2 and 3 in porcine adipose tissue☆

T.G. Ramsay*, R.W. Rosebrough

Growth Biology Laboratory, USDA-ARS, BARC-East, Bldg. 200, Rm. 207, Beltsville, MD 20705, USA

Received 18 July 2004; accepted 22 December 2004

Abstract

This study was performed to determine whether or not uncoupling protein 2 (UCP2) and UCP3 expression in porcine subcutaneous adipose tissue are hormonally regulated in vitro and whether their expression is correlated with changes in metabolic activity. Tissue slices (approximately 100 mg) were placed in 12-well plates containing 1 mL of DMEM/F12 with 25 mM Hepes, 0.5% BSA, pH 7.4. Triplicate slices were incubated with basal medium or hormone supplemented media at 37 °C with 95% air/5% CO_2. Parallel cultures were maintained for either 2 or 24 h to evaluate metabolic viability of the tissue. Slices were transferred to test tubes containing 1 mL of DMEM/F12 with 25 mM Hepes, 3% BSA, 5.5 mM glucose, 1 μCi ^{14}C-U-glucose/mL and incubated for an additional 2 h at 37 °C. Glucose metabolism in 2-h incubations did not differ from 24-h (chronic) incubations, indicating viability was maintained ($P > 0.05$). Expression of UCP2 and UCP3 was assessed in slices following 24 h of incubation with various combinations of hormones by semi-quantitative RT-PCR. Expression of UCP2 was induced by leptin (100 ng/mL; $P < 0.05$). Growth hormone (100 ng/mL) inhibited UCP2 expression ($P < 0.05$). Expression of UCP3 was inhibited by growth hormone (100 ng/mL; $P < 0.05$), triiodothyronine (10 nM; $P < 0.05$) or leptin (100 ng/mL; $P < 0.05$). Changes in UCP expression could not be associated with overall changes in glucose metabolism by adipose tissue slices in chronic culture. Published by Elsevier Inc.

Keywords: Uncoupling protein 2; Uncoupling protein 3; Adipose tissue; Swine

☆ 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 on approval to the exclusion of other products or vendors that also may be suitable.

* Corresponding author. Tel.: +1 301 504 5958; fax: +1 301 504 8623.
E-mail address: tramsay@anri.barc.usda.gov (T.G. Ramsay).

0739-7240/$ – see front matter. Published by Elsevier Inc.
doi:10.1016/j.domaniend.2004.12.003

1. Introduction

The presence of an uncoupling protein was first detected in brown adipose tissue of hibernating animals and believed accountable for non-shivering thermogenesis [1]. This protein, uncoupling protein 1 (UCP1), was demonstrated to mediate ATPase independent proton leakage at the inner mitochondrial membrane [2]. UCP1 dissipates the transmembrane electrochemical potential by transporting protons from the intermembrane space back toward the matrix of the mitochondria, promoting a proton leakage and generating energy. The consequence of UCP1 activity is heat production through uncoupling ATP formation from cellular respiration. However, proton leakage exceeds all that can be accounted for by UCP1 activity, implicating additional proteins and leading to the discovery of additional uncoupling proteins.

UCP2 and UCP3 share 55% and 57% amino acid identity with UCP1 and 73% with each other [3,4]. UCP2 mRNA has been shown to be expressed in a range of tissues, including adipose tissue and skeletal muscle [3]. The actual UCP2 protein has been confirmed to be present in adipose tissue by Western analysis, but not present in skeletal muscle [5]. Feeding a high fat diet in rodents produces an increase in the expression of UCP2 in adipose tissue [3,6]. This response by UCP2 has been proposed to result in an increase in the formation of reducing equivalents necessary for lipogenesis [7].

Uncoupling protein 3 is expressed primarily in skeletal muscle in rodents, with limited expression in adipose tissue [8]. Using UCP3 knockout mice, UCP3 has been associated with changes in mitochondrial energy production that suggests uncoupling activity specific to skeletal muscle [9]. The combined results of the research into the potential functions of UCP2 and UCP3 may suggest a function for them in tissue specific energy metabolism/expenditure.

The porcine UCP2 and UCP3 sequences have been cloned [10]. The pig UCP2 and UCP3 sequences are 89% and 88% homologous, respectively, to the human homologues. Comparison of the pig UCP2 and UCP3 sequences reveals them to be 76% identical. Damon et al. [11] have reported that both UCP2 and UCP3 are expressed in porcine skeletal muscle and adipose tissue. However, the regulatory mechanisms for UCP2 or UCP3 expression have not been examined in swine adipose tissue. The present study was performed to determine whether or not UCP2 and UCP3 expression in porcine adipose tissue is hormonally regulated.

To accomplish this goal requires an in vitro system for chronic incubation ($\geq$24 h) of porcine adipose tissue. Previous studies have been unable to produce a chronic incubation system wherein the metabolic rate has not declined with time [12,13]. Therefore, the second objective of this experiment was to develop a chronic incubation system for adipose tissue slices and to determine if changes in tissue metabolism are related to changes in UCP2 or UCP3 expression.

2. Material and methods

An acute regulation of UCP2 or UCP3 expression has not been demonstrated in adipose tissue. These proteins appear to respond primarily to more chronic stimuli. Therefore, a