Differential fat distribution in UCP3 over-expressing transgenic mice.

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Introduction

Uncoupling proteins (UCP family) allow protons extruded from mitochondria by the electron transport chain, during the process of oxidative phosphorylation, to re-enter without the synthesis of ATP from ADP. The main role of such uncouplers (UCP1) is fat hydrolysis and thermogenesis and hence is associated with brown adipose tissue mitochondria [1]. More recently another uncoupling protein, UCP3 has been associated with similar modes of action as UCP1, however the mitochondrial location and heat producing activity of UCP3 in mammalian cells still needs to be demonstrated.

Unlike UCP1, which is found predominantly in brown adipose tissue, UCP3 resides in skeletal muscle. Clapham *et al* [2] have recently created transgenic mice which specifically over-express human UCP3 protein within muscle. These mice were hyperphagic but weighed less than there wild-type littermates. The animals exhibit lower fasting plasma glucose and insulin levels and increased glucose clearance rate following an oral glucose load. This provided direct evidence that skeletal muscle UCP3 may influence metabolic rate and glucose homeostasis in the whole animal. It also suggested a role in fat metabolism implying derangements in deposition due to increased fat hydrolysis.

An area of great debate is that the site of fat deposition may be indicative of future clinical manifestations such as coronary heart disease, diabetes and obesity. These transgenic mice could have implications for such diseases and hence, two questions needed to be answered. Firstly, how much fat is deposited as subcutaneous, visceral and intermuscular in control and high UCP3 expressing mice? Secondly, if UCP3 is primarily associated with muscle, should fat deposition within this tissue be compromised the most.

Methods

Animals: Mice were housed and maintained in accordance with procedures outlined in the Home Office Animals (Scientific Procedures) Act 1986, UK., and allowed free access to a standard chow diet and water. Two groups (wild type controls & high UCP3 expressors) of age matched animals (n=6 per group) were scanned at ages 6,8,10,12,14, and 18 weeks. Animals were anaesthetised using 4-5% isoflurane and maintained using 1.5% with 60%:40% O2:N2 at a rate of 500ml - 1L/min through a face mask. Mice were placed within the bore of a 18.3 cm 7T Oxford magnet and physiologically monitored using ECG and respiratory motion.

A pre-requisite for this study was to differentiate between subcutaneous, visceral and intermuscular fat. For this purpose a 2D spin-echo sequence was employed with and without fat suppression. This allowed us to produce high quality transverse images from the hindquarters through to the base of the kidneys.

MRI: Images were obtained on a Bruker AMX300 interfaced to the magnet. A multislice 2D spin echo sequence was used with a field of view of 4 x 4 cm and a matrix size of 128 x 128. Experimental time was 23 minutes using a single echo of 12.6ms, a TR of approximately 5.3 s, 2 averages and a spectral width of 100kHz. A total of 32 slices were acquired with a slice thickness of 1.5 mm. Following acquisition of the slices a chemical shift selective fat suppressed image was acquired. Processing of the images was aided by subtraction of the fat suppressed and non-fat suppressed images. This allowed fat associated with the subcutaneous, visceral and intermuscular areas to be delineated. Absolute quantitation of the fat depots over a confined area of the mouse (ie from the testis to the base of the right kidney) was performed. Absolute volumes (cm3) are represented as means ± SEM; statistical significance was calculated using the Students´ t test.

Results

Figure 1 depicts the different fat deposition characteristics between control wild type and high expressing UCP3 mice. All three sites showed a significant divergence between the two groups as the animals grew older, with the UCP3 animals consistently laying down less adipose tissue. Interestingly, week 12, which coincides approximately with sexual maturity, produced an acceleration in fat deposition within each group. Despite UCP3 remaining a muscle residing protein general fat distribution was affected. With respect to final fat concentrations, the UCP3 mice had 34 ($p<0.02$), 42 ($P<0.01$) and 40% ($P<0.01$) lower fat deposits in the subcutaneous, visceral and intermuscular spaces, respectively compared to their wild type counterparts. Interestingly, there was no significant increase in intermuscular fat at 18 weeks compared to week 6, however the wild type exhibited a 130% increase ($P<0.001$). In both the subcutaneous and visceral regions, significant increases of 180 - 220% were noted over the 12 week study for both UCP3 expressing and wild type mice. Standardisation of all values for individual weight and/or body volume produced equivalent results.

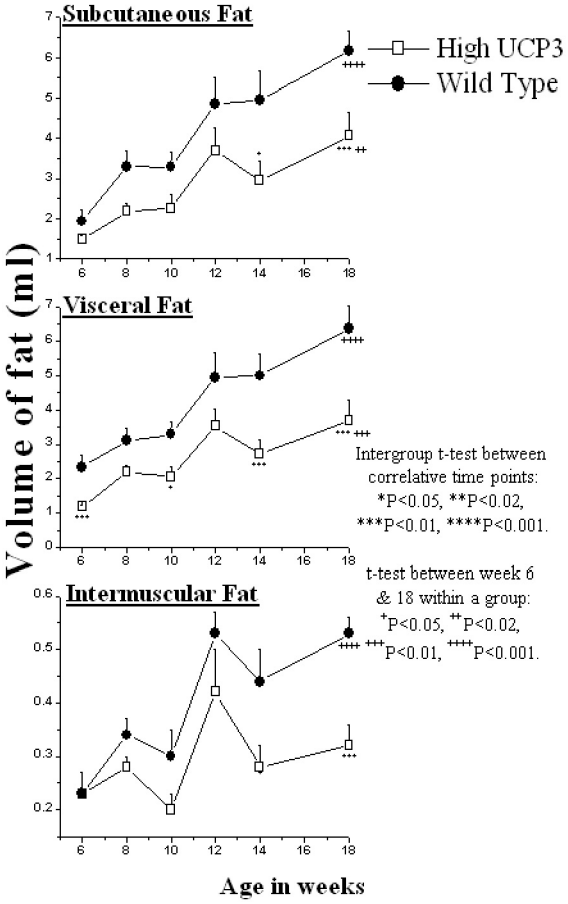

Figure 1

Discussion

It is clear from the results that over-expression of muscle UCP3 prevents the same fat deposition characterised by control animals. Intermuscular changes in the UCP3 mice were not significantly different between week 6 and 18, unlike the control, indicating the increased role that UCP3 plays within muscle tissue. However, both visceral and subcutaneous fat were still significantly lower when compared directly to the controls at each time point. Muscle metabolism plays a pivotal role in whole body homeostasis and thereby UCP3, although confined to this tissue, exerts a much wider ranging influence on systemic energy metabolism.

References

1. Boss, O. *et al.* FEBS Lett. **408**, 39-42 (1999).
2. Clapham J. *et al.* Nature **406**, 415-418 (2000).