Measurement of ectopic fat deposition in genetically modified mice fed a high fat diet

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Abstract

In-vivo MRI and localized ^{1}H-MRS were used to monitor ectopic fat storage induced by high-fat feeding in estrogen-related receptor α (ERRα) deficient mice. After 2 months on the diet, intramyocellular lipids (IMCL) in the *tibialis anterior* muscle of wild-type mice increased by ~3-fold while virtually no change was detected in ERRα knockout mice. In contrast to the expected phenotype, ERRα knockout mice also displayed a ~5-fold increase in intrahepatic lipid contents and levels of visceral adiposity similar to wild-type mice, suggesting normal lipid absorption.

Introduction

Localized proton (^{1}H) MRS has proven to be a sensitive and precise tool for the quantification of gross tissue fat content and has been used for studying type II diabetes-related changes in muscle and liver lipid metabolism *in vivo*, both in humans and rats. As main outcomes of these studies, high intramyocellular lipid (IMCL) and intrahepatic lipid (IHL) levels were found to predict insulin resistance. It is critical that such readouts can also be measured in mice, especially that an affluence of knockout ("loss of function") and transgenic ("gain of function") animals is now widely used for pharmacological studies. The estrogen-related receptor α (ERRα) interacts with the peroxisome proliferator-activated receptor (PPAR) coactivator 1-α (PGC-1α) to control mitochondrial biogenesis and hence may play a key role in the control of energy expenditure (1). Unexpectedly, initial characterization of ERRα deficient "knockout" (ERRα KO) mice displayed reduced fat mass and resistance to high-fat diet induced obesity (2). In view of this, the present study aimed at further characterizing ERRα KO mice by repeatedly measuring ectopic fat storage – i.e. abdominal adiposity, IHL and IMCL contents – over a two-month high-fat diet period.

Methods

Experiments were carried out on 3 to 6-month old male mice (wild-type WT: 27.4±1.2g, n=7; knockout KO: 26.3±2.2g, n=6). Following baseline measurements on day 0, mice were fed a high-fat (HF) diet (58% fat calories) for 9 to 10 weeks. All NMR data were obtained under 2% isoflurane anesthesia, using a Bruker Biospec 7T/30cm instrument equipped with a 12-cm i.d. gradient insert. MR sessions occurred on days 0, 7, 14, 21, 28, 35 and 67. Whole-body fat distribution, liver volume and IHL contents were measured by using a 35 mm ^{1}H birdcage resonator while IMCL contents were determined by a using a 72-mm birdcage resonator for transmission and a 2-cm surface coil for reception. For fat distribution, contiguous transversal 1mm-thick slices were obtained using a turbo-spin echo sequence with 31 echoes/excitation and 128 phase encoding steps (273μm^2 in-plane resolution), optimized for short TE (6.95ms) and short TR (220ms) to allow for signal suppression from tissues other than fat. 2D image series were imported into IDL software for pixel counting-based determination of fat volumes. A signal threshold was used to exclude non-fat tissues in each slice. Fat distribution was determined by manually outlining the visceral (Visc) as opposed to the subcutaneous (SC) fat along the well-defined parietal peritoneum line. For liver volume measurements, 35 contiguous 2-mm thick FISP images (flip angle 45º, matrix size 128x128, TE/TR 1.63/3.27ms, 16 averages) were acquired. For IHL measurements, localized ^{1}H-MR spectroscopy in the presence of localized ^{1}H PRESS spectra (TE 20ms, TR 2s, SW 10kHz, 128 averages) were obtained from a 2x2x2mm^3 voxel placed typically in a distal region of the left lobe away from major vascular structures and distinct hyperintense fatty deposits. Respiratory gating was used to minimize motion artifacts. Spectral analysis was performed by integrating peak areas after line fit of the water (i.e. peak at 4.7ppm) and fat (i.e. $(CH_2)_n$ methylene peak at 1.3ppm) resonances using the Nuts-PPC software package (AcornNMR, Inc., Fremont, CA). Absolute amounts of IHL were assessed after correction for T1 and T2 relaxation as well as the liver volume as described previously (3). For IMCL measurements, proton spectra were obtained from the *tibialis anterior* (*TA*) muscle of the left leg, using also a PRESS sequence (1x1x1mm^3 voxel, TR/TE=1.5s/20ms, 1024 averages) with water CHESS suppression. Scout images were acquired to carefully position the volume of interest. Peak areas for total creatine (tCr: 3.0ppm, internal reference), EMCL (1.5ppm) and IMCL (1.3ppm) were quantified using a line fitting procedure similar to before. Data are presented as means±SE.

Results

Both mouse strains responded in a similar fashion to the 2-month high-fat diet in terms of food intake (~2.5g/day) and body weight gain (~4.5g/month). However, data obtained from fat distribution analysis showed that while on day 0 the relative contribution of visceral fat to total fat was higher in WT than in KO (48% vs. 34%) mice, this difference faded away after 3 weeks on the HF diet (~36% for both groups). Upon the HF diet, IHL levels increased similarly between both groups, from ~0.03mmol/ml liver on day 0 to ~0.15mmol/ml liver on day 68. In contrast, IMCL levels were markedly increased in WT mice only while no change or even a slight decrease was measured in KO mice (fig.1).

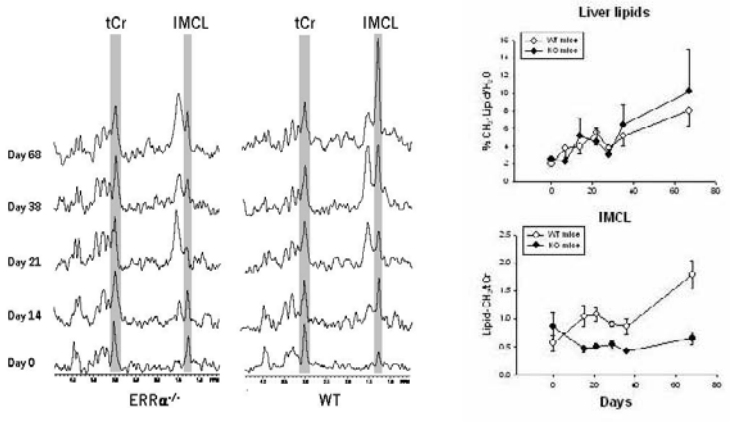

Fig 1. Time-course changes in IHL and IMCl over the 2-month high-fat diet

Discussion

These preliminary results show that ERRα KO mice have decreased IMCL levels with no change found in IHL. Published results in young animals (4) found that lipid absorption by the intestines in knockout mice was decreased; however, this does not seem to play a prevalent role in mature animals. In conclusion, a robust measurement of IMCL, IHL and fat distribution is possible in the anesthetized mouse. Such readouts offer complementary information which may prove useful when studying lipid related disorders in specific mouse models. The determination of IMCL levels may be considered as a useful endpoint in experiments looking at muscle insulin resistance.

References

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