Role of central adiposity on IMCL accumulation and glucose intolerance in a mouse model of insulin resistance

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Abstract

This study examined whether visceral fat as measured by MRI can act as a modulating factor of intramyocellular lipids (IMCL) as measured by localized ^{1}H-MRS at 9.4T in a mouse model of insulin resistance. The diabetogenic treatment with dexamethasone resulted in a 47% increase of IMCL in the *tibialis anterior* (*TA*) while visceral fat contents were also found 77% greater than in saline-treated mice. These results support the hypothesis that an enlarged visceral fat mass contributes to the development of insulin resistance through an increase in IMCL.

Introduction

In a previous study on *fa/fa* Zucker rats (1), we have shown that following a high-fat diet the increase in intramyocellular lipids (IMCL), a strong determinant of insulin resistance (2), was positively correlated not only to glucose intolerance but also to an accumulation of visceral fat, another predisposing factor for the development of type 2 diabetes (3). Yet the question remained as to whether visceral adiposity could modulate IMCL accumulation, for example through a mass effect mechanism involving circulating free-fatty acids. In the present study, in order to increase the window of our observations, insulin-resistant mice were treated with dexamethasone, a diabetogenic agent. Our assumption was that excess glucocorticoid increases the basal lipolytic rate in adipocytes. Possible interactions between regional adiposity and IMCL deposition were measured by MRI and localized ^{1}H-MR spectroscopy, respectively. To our knowledge, this is the first time that *in vivo* measurements of IMCL in the mouse are reported, thus opening a new range of applications in diabetes research.

Methods

Experiments were carried out on 4-week old male mice (27±1 g, n=26). In order to increase IMCL to NMR-detectable levels, all mice were fed prior to the dexamethasone challenge with a high-fat diet (54% fat calories) for 9 to 10 weeks. At 14-week of age, in combination with the same high-fat diet, a subgroup of 13 mice was given three x 11 mg/kg i. p. doses of dexamethasone (dexa) over the course of 5 days (d0, d2 and d4), while a second subgroup (n=13) was dosed with saline. All NMR data were obtained on a Bruker 9.4T micro-imaging instrument. Prior to and after the dexa-treatment, both whole-body insulin resistance and IMCL levels were assessed *in vivo* from an oral glucose tolerance test (OGTT at days -7 and 3 under 5-hour fasting conditions) and using localized ^{1}H-MR spectroscopy (days -5 to -3 and 4), respectively. The incremental area under the curve (AUC) from 0 to 120 min after an oral glucose load (1 g/kg) was calculated for plasma glucose concentrations to provide an index of insulin sensitivity. Proton spectra were obtained under anesthesia with 2% isoflurane from the *tibialis* muscle of the left leg, using a home-made 1.3-cm diameter coil and a PRESS sequence (3 mm^3 voxel, TR/TE=2s/25ms, 512 averages) with water CHESS suppression. Scout images were acquired to carefully position the volume of interest. Peak areas for total creatine (tCr: 3.0 ppm), EMCL (1.5 ppm) and IMCL (1.3 ppm) were quantified using a line fitting procedure. The creatine signal served as an internal reference for IMCL and EMCL quantification. In addition, abdominal fat distribution (i.e., visceral fat vs. subcutaneous fat depots in the pelvis-to-diaphragm region) was measured in sacrificed animals at day 5. To this end, contiguous transversal 1.0 mm-thick slices were obtained using a 35 mm ^{1}H birdcage resonator and a turbo-spin echo sequence with 32 echoes/excitation and 128 phase encoding steps (270 µm^2 in-plane resolution), optimized for short TE (25ms) and short TR (2s) to allow for signal suppression from other tissues 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. Data are presented as means±SEM.

Results

Both a coil of Helmotz type and an animal holder were built purposely to carry out *in vivo* ^{1}H-MRS measurements in mice at a high field. With this setup, well resolved ^{1}H spectra were obtained from the *TA* muscle of ~30 g mice, within less than 20 min, with a signal-to-noise ratio>32 as calculated from the creatine peak. After a 2-month period on a high-fat diet and prior to the dexa study (day -4), the IMCL/tCr ratio was similar for both groups studied (Dexa: 2.13±0.39 vs Saline: 2.74±0.51, p>0.05). Of note, these values are within close range of those observed in a preliminary study which in addition described a ~1.8-fold increase in IMCL content following the exact same 2-month diet. While inducing a slight hyperglycemia (e.g. plasma fasting glucose increased from 272±8 mg/ml to 300±10 mg/ml, p=0.1), the dexa treatment further impaired whole-body glucose tolerance by ~20% (p<0.05), as assessed from glucose excursion data (ΔAUC_{dexa}: +24% vs. ΔAUC_{saline}: -12%, p<0.02). Along with a greater body weight gain (dexa: +1.1 ± 0.6g vs. saline: -0.3 ± 0.5 g, p<0.03), visceral fat contents of dexa-treated animals were also 77% higher than in the saline group on day 5. Finally, a marked increase in IMCL/tCr by ~65% ($\Delta IMCL/tCr_{dexa}$ +1.27±0.57 vs $\Delta IMCL/tCr_{saline}$ -0.67 ±0.76, p=0.06) was measured in response to the diabetogenic treatment. On day 5, IMCL/tCr values appeared to be strongly related to the amount of visceral adiposity (r=0.69, *P*<0.01, Fig.1).

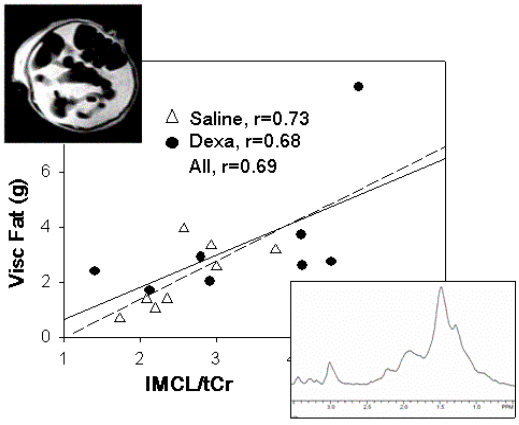

Fig.1 – Correlation between visceral fat content and IMCL changes

Discussion

This study was designed based on the assumption that visceral fat being more responsive to stress than subcutaneous fat, glucocorticoid stimulation might exacerbate the shift of lipids from the portal vein to ectopic regions such as the skeletal muscle. In agreement with this hypothesis, greater amounts of IMCL were detected in response to the diabetogenic treatment with dexamethasone, along with an impairment of glucose tolerance and fat deposition preferentially in the visceral region. This illustrates how visceral fat may impact muscle glucose uptake through IMCL changes; although a causative association still needs to be demonstrated. These results support the fact that IMCL may be used as an organ-specific and metabolic biomarker of peripheral insulin resistance.

References

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