Quantification of intramyocellular lipids (IMCL) in mouse models of insulin resistance

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Introduction - Intramyocellular lipid (IMCL) content has been proposed as a biomarker for insulin resistance, a severe condition usually preceding overt type II diabetes [1,2]. To date, ^{1}H-MRS is the only modality to non-invasively assess IMCL levels *in vivo* [3]. It has been successfully applied to investigate IMCL in various species including man, rats and recently also mice [1-4]. Numerous studies have demonstrated tight correlation between insulin resistance and IMCL in human patients and various disease models in rats [1,2]. In pharmaceutical research, however, mice are often the preferred species for drug discovery. The aims of the present study were (i) to optimise ^{1}H-MRS in mouse hind leg such as to provide a high-throughput modality that allows absolute quantification of IMCL, (ii) to evaluate baseline IMCL in different mouse strains proposed as potentially useful animal models with polygenic predisposition and diet induced insulin resistance, and (iii) to demonstrate modulation of IMCL levels upon pharmacological intervention with an insulin sensitizer.

Methods - 102 Male mice of four different strains were studied. RCS10 (NONcNONZO10) and AKR/J mice were fed a high-fat feed (10% fat, 7w and 20% fat, 14w, respectively) to an age of 19 weeks. CD1 mice also received high-fat diet (34% fat, 7w) to an age of 10 weeks and an injection of streptozotocin (90mg/kg) to compromise pancreatic function. C57Bl/6 were kept on a very high-fat diet (60% fat, 21w) to an age of 29 weeks. An additional group of C57Bl/6 mice was fed a chow diet as a control. A subgroup of the CD1 mice underwent a 2-week treatment with the insulin sensitiser pioglitazone (30mg/kg/d, food admix). MR measurements were conducted on a Bruker Biospec 4.7T/40cm equipped with a V-shaped transmit/receive butterfly coil. Animals were anaesthetised with 2% isoflurane in O_2/N_2O. Axial scout images of the hind leg were obtained with a multi-slice FLASH sequence (TE/TR=9/150ms). Localised *in vivo* ^{1}H-MRS of the red gastrocnemius muscle was performed with a PRESS sequence (TE/TR=16/1100ms, (1mm)^3 volume of interest, CHESS water suppression, and 256 averages). A reference spectrum was acquired under identical conditions, however without water suppression and only 8 averages. Spectroscopic data were analysed in the time domain with the jMRUI software. IMCL levels were calculated relative to water and, after calibration with biochemically determined triglyceride contents, expressed on an absolute scale in mg/g tissue (mean ± SEM)

Results and Discussion - Workflow and data acquisition have been optimised in order to enable IMCL assessments in large-scale pharmacological studies. A sustained throughput of four mice per hour was achieved by reducing the time required for animal preparation with a mould that allows consistent positioning of the animals' legs, and by minimising data acquisition to the limit of reliable quantification. In contrast to previous work, IMCL were quantified on an absolute scale in mg/g to facilitate inter-strain comparison. For this purpose, relative IMCL levels were compared to analogous measurements of hepatic lipids, which were subsequently analysed with biochemical methods.

Figure 1 depicts the summary of quantitative IMCL assessments in red gastrocnemius muscle of four mouse strains potentially suitable as animal models of insulin resistance. As expected, IMCL levels in chow diet-fed C57Bl/6 mice were found to be low (0.35±0.03 mg/g (n=30)) and served as a reference. C57Bl/6, RCS10 and AKR/J mice on diabetogenic high-fat diet showed only slightly increased IMCL levels (0.45±0.06 mg/g (n=30), 0.66±0.25 mg/g (n=8), 0.74±0.25 mg/g (n=8)) over the controls. On the other hand, high fat-fed CD1 mice with streptozotocin-compromised pancreatic function had strongly increased IMCL levels reaching 3.19±0.52 mg/g (n=12). This high level of IMCL allowed its responsiveness to pharmacological intervention to be tested. Drug treatment with the insulin sensitiser pioglitazone indeed reduced the IMCL levels in these mice to 1.64±0.37 mg/g (n=14) after two weeks of treatment.

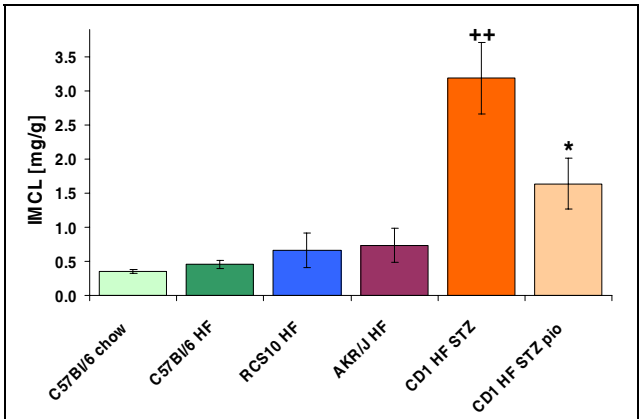

Fig. 1: *IMCL in red gastrocnemius of different mouse strains. Chow: chow diet; HF: high fat diet; STZ: streptozotocin; pio: pioglitazone treatment; ++: different from C57BL/6 chow (p<0.01); * different from CD1 HF STZ (p<0.05).*

Conclusions - The present data demonstrate that rapid IMCL assessment in mice by means of *in vivo* ^{1}H-MRS is readily feasible and suitable for pharmacological screening studies. Among the four tested mouse models of peripheral insulin resistance, streptozotocin treated CD1 mice on high fat diet had highly elevated IMCL levels that reliably allowed the beneficial effect of pharmacological intervention with an insulin sensitiser to be confirmed.

References

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