Use of MRI for Longitudinal In Vivo Phenotyping of Obese Mouse Models Following a Dietary Intervention

A. Bidar[1], K. Ploj[2], C. Lelliott[2], K. Nelander[3], L. Storlien[2], and P. Hockings[1]
[1]DECS Imaging, AstraZeneca R&D, Mölndal, Sweden, [2]CVGI, Bioscience, AstraZeneca R&D, Sweden, [3]DECS Discovery Statistics, AstraZeneca R&D, Sweden

Introduction
In preclinical drug discovery, experimental rodent models of obesity/diabetes are used for the investigation of metabolic disorders. Repeated in vivo measurements of adipose tissue depots and intraorgan fat can provide longitudinal data with greatly reduced usage of experimental animals. The aim of the present study was threefold: (i) to validate in vivo MRI/S determinations of brown adipose tissue (BAT) at the scapular region, total, intra-abdominal and subcutaneous white adipose tissues (TAT, IAT, SAT) as well as intrahepatocellular lipids (IHCL) against ex vivo measurement; (ii) to address the "Reduce" aspect of the 3R's (Reduce, Refine, Replace) mandate, by presenting a statistical power analysis to study the benefits in performing a longitudinal study when each individual is used as its own control, compared to a cross-sectional study when a different cohort is measured at each time; and (iii) to characterize the phenotypic and metabolic switch of the "cafeteria diet" mouse model [1] during a dietary intervention.

Method
Groups of female C57Bl/6J mice (n = 6/group) were fed a regular chow (R3) or high fat and sugar (HFD) diet for 16 weeks and compared to a group fed HFD for 12 weeks and then switched to R3 for a further 4 weeks. MRI determinations were made at 9 and 15 weeks with autopsy performed at 16 weeks, see Fig 1. MRI/S was performed on a 4.7T Bruker scanner. The protocol consisted of: (i) acquisition of two sets of respiratory gated high resolution 3D FISP scans covering the upper and lower extremity of the animal with TR/TE/α: 4.2ms/2.1ms/45°, field of view: 50x50x50 mm and matrix size: 256x192x192, and (ii) acquisition of a localized PRESS ^{1}H spectra obtained from a 3x3x3 mm^3 voxel in the liver for IHCL measurement, TR: 3 s, TE: 6.8 ms, SW: 4006 Hz, 64 averages, and 2048 data points. Areas of water peak (Aw) at 4.7 ppm and lipid methylene (CH2) peak (Af) at 1.3 ppm were measured and IHCL expressed in percent as: IHCL = 100 x Af / (Aw+Af). 3D images were evaluated using a semi-automatic in-house procedure for body fat segmentation. At each time point the mass of each adipose compartment was measured. Post-mortem adipose tissues were harvested and weighed. Liver was weighed and hepatic lipid measured by an enzymatic/colorimetric method. Results are expressed as mean ± SEM. Inter-group comparisons were analyzed using a ANOVA test. Correlations between measured ex vivo and in vivo measurements were assessed by linear regression. Pearson's correlation coefficient (r) was determined. The level of significance of the p-value was set at 5%.

Results
There were very strong correlations between the 15 week in vivo MRI data and 16 week ex vivo data for intrabdominal fat (r=0.99), intrascapular brown fat (r=0.93) and intrahepatic fat (IHCL; r=0.97; all p<0.0001). The result of the power calculations showed that for intrabdominal and intrahepatic fat variables, group sizes and total animals needed can be reduced by using a non-invasive method such as MRI where each animal acts as its own control (Table 1). IHCL was plotted against intra-abdominal fat as % of BW (%IAT) for all in vivo measurement points (Fig. 2). IHCL did not change when %IAT ranged from 0-5% but showed a linear rise with a shallow slope in the range of 5 to 15% and a slope that was almost vertical at %IAT above 15 (corresponding to a body weight above 45 g). A similar pattern was seen when IHCL was plotted against body weight.

Discussion
The results demonstrate that MRI can be used to follow whole body adiposity and intrahepatic lipid content longitudinally with excellent precision compared to ex vivo measurements. The power analysis illustrates the importance of MRI for serially and non-invasively monitoring the phenotypic changes after diet or an intervention when each animal is used as its own control. This allows for a substantial reduction in animal usage in agreement to the 3R's mandate. Obese mice undergoing a diet switch showed an improvement in body adiposity and intrahepatic fat content showing that these phenotypes are reversible upon adequate intervention. The results also demonstrate for the first time in this animal model the effect of increasing adiposity on liver fat accumulation with clear break points defining low and extremely high lipid accumulation trajectories. A simple explanation for this sudden steep increase in IHCL level may be related to the limitation in triglyceride deposition in adipose tissue storage depots, which may divert triglycerides for accumulation in other tissues such as the liver [2]. These results also provide baseline data set for comparing different animal models of obesity and diabetes and for evaluating the effect of pharmacological interventions in these mice.

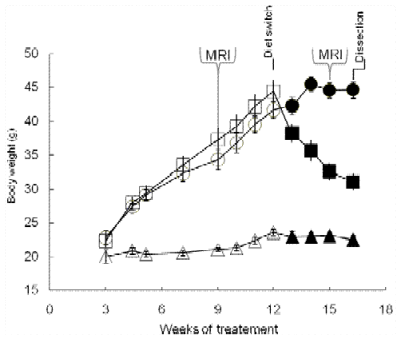

Fig. 1: Treatment and imaging schedule.

Magnitude of Treatment Effect to be detected, Difference (%)	IAT base value= 2.88g		IHCL base value= 14.45%	
	a) N 2 obs/animal	b) N 1 obs/animal	a) N 2 obs/animal	b) N 1 obs/animal
10	384	3484	2660	6028
20	96	872	666	1508
40	24	220	168	380

Table 1: Sample size calculations for IAT and IHCL for the two different experimental conditions (power 80%): (a) for the case where the same animal is measured twice (2 obs/animal) and (b) when a different cohort is measured at each time point (1 obs/animal). Here, N represents the total number of animal needed per experiment for two groups and two time point.

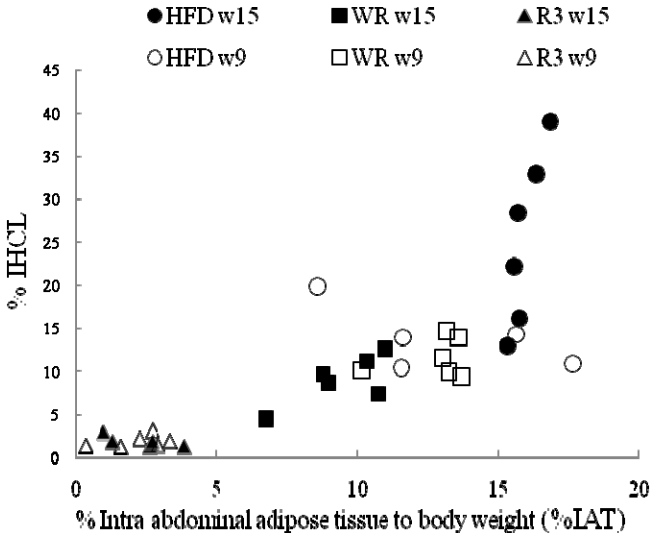

Fig. 2: Relationship between IHCL and IAT expressed as a % of body weight for mice fed a high fat diet and standard R3 chow at week 9 (HFD: ○, R3: Δ, WR: □) and at week 15 (HFD: ●, R3:▲, WR: ■). A clear cut off point is observed when % IAT reached 15 %.

References: [1] Li *et al.* Metabolism Clinical and Experimental 2008; 57: 1704–1710. [2] Garg A *et al.* J Clin Endocrinol Metab 2002; 87(7): 3019-3022