Cardiac Lipid Accumulation and Hypertrophy in a Murine Model of Non-alcoholic Fatty Liver

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Purpose: Diabetic cardiomyopathy occurs frequently in patients with type 2 diabetes and it is well known that non-alcoholic fatty liver (NAFL) and insulin resistance are associated with increased cardiovascular risk. In this context, the increased lipid availability in NAFL may lead to enhanced lipid deposition and oxidative stress in cardiomyocytes followed by functional impairment. Thus, the present study was aimed at investigating how NAFL and insulin resistance relate to all-over body lipid homeostasis and cardiac function.

Methods: For this purpose, we used transgenic mice with adipose tissue-specific overexpression of the sterol regulatory-element binding protein-1c (tg-SREBP-1c$^+$) which develop NAFL secondarily due to ectopic lipid deposition. Adult female C57Bl/6 wild-type (WT) and tg-SREBP-1c$^+$ mice at the age of 8 months were fed a standard chow diet and received tap water ad libitum. ^{1}H MRS and MRI was performed at a vertical 9.4 T Bruker Avance^{III} Wide Bore NMR spectrometer using a microimaging unit (Micro2.5) equipped with an actively shielded 40-mm gradient set (1 T/m maximum gradient strength, 110 µs rise time at 100% gradient switching). A 30-mm saw resonator was used for assessment of all-over body fat by ^{1}H MRI and hepatic fat by ^{1}H MRS (respiratory-triggered PRESS, 2×2×2 mm^3 voxel located in the right liver lobe, outer volume suppression (OVS)). For determination of abdominal fat composition, a 1×1×1 mm^3 voxel was placed in almost fat-only tissue. Quantification of intra- and extramyocellular lipids in tibialis anterior (TA) muscle was carried out using a 10-mm saddle coil. Localized ^{1}H MR spectra from TA muscle were acquired from a 1.3×1.3×3 mm^3 voxel using PRESS with eddy current compensation, VAPOR water supression, and OVS (TR, 1000 ms; TE, 20 ms, averages, 1024). Finally, cardiac function and morphology was determined from ECG- and respiratory-gated cine-FLASH movies using a 25-mm saw resonator. For quantification of cardiac lipids, a 1×2×3 mm^3 voxel was placed in the septum as shown below (Fig. 1b; ECG- and respiratory-gated PRESS; CHESS water suppression, OVS, TR 1000 ms, TE 9.1 ms, averages 1024). Hyperinsulinemic-euglycemic clamps were used to assess insulin sensitivity, and reactive oxygen species production from isolated heart mitochondria was determined by the Amplex Red method.

Results and Discussion: Analysis of abdominal fat by ^{1}H MRI and localized ^{1}H MRS revealed a pronounced lipodystrophy in tg-SREBP-1c$^+$ mice. Both visceral and subcutaneous fat contents were strongly reduced by 60 and 90%, respectively, while ^{1}H MRS showed an almost three-fold increase in liver fat in the mutant as compared to WT (n=9 each, p<0.001). Furthermore, tg-SREBP-1c$^+$ mice exhibited a profound shift in body triglyceride composition in that levels of physiological important polyunsaturated fatty acids dramatically decreased (by 50%, p<0.01) accompanied by an increase in monounsaturated fatty acids by approximately 50%. To determine ectopic lipid deposition in the skeletal muscle, we analyzed intra- and extramyocellular lipid (IMCL + EMCL) contents by localized ^{1}H MRS from the tibialis anterior. Again transgenes displayed strongly increased lipid levels in that EMCL were increased twice (p<0.05) whereas IMCL were even tripled as compared to WT controls (p<0.001). Concomitantly to the alterations in fat distribution and composition, we observed a decrease in whole body insulin sensitivity by 70% in the mutants (p<0.05). In the next step, we analysed the consequences of these metabolic deteriorations on cardiac function. As shown in Fig. 1a, tg-SREBP-1c$^+$ mice were characterized by substantially increased wall thicknesses in both dia- and systole associated with a rise in myocardial mass by 40% (p<0.05). On the other hand, this was not accompanied by any functional impairment since global ejection fraction and also local wall thickening was unchanged. However, localized ^{1}H MRS revealed that transgenic hearts clearly showed an increased lipid content as compared to WT controls (Fig. 1c, bottom and top, respectively). Quantification of the spectra demonstrated an almost 100% increase in lipids for tg-SREBP-1c$^+$ hearts (Fig. 1d; n=9, p<0.01). Of note, subsequently we also found a doubled release of reactive oxygen species from isolated mitochondria in these hearts (p<0.05).

Conclusions: Insulin resistance in a mouse model of NAFL associates with pronounced lipodystrophy and left ventricular hypertrophy. Although heart function remains still intact in this setting, long-term increased cardiac lipids and oxidative stress are likely to render the heart vulnerable for ischemic intolerance and impaired myocardial function with age.

Figure 1: **(a)** Cardiac hypertrophy in tg-SREBP-1c$^+$ mice without compromized cardiac function. **(b)** Representative longitudinal cardiac ^{1}H MRIs in end-diastole of WT and tg-SREBP-1c$^+$ mice indicating the location of the voxel (blue rectangle) used for ^{1}H MRS of cardiac lipids. **(c)** Characteristic cardiac ^{1}H MR spectra from the septum of WT (top) and tg-SREBP-1c$^+$ mice (bottom). Abbreviations: Cho, choline; Cr, creatine; Tau, taurine; Δ-1 and Δ-1$_p$, protons bound next to mono- and polyunsaturated carbons; α, β, ω, protons bound to α, β, and ω carbons of the fatty acid chain, α+γ$_g$, protons bound to α+γ carbons of the glycerol backbone. **(d)** Quantification of cardiac lipids related to the total non-suppressed water signal.