LACK OF EXTRACELLULAR ADENOSINE FORMATION PROMOTES LIPOLYSIS, INTROMYOCELLULAR LIPID DEPOSITION, AND PERIPHERAL INSULIN RESISTANCE

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Introduction: The purine nucleoside adenosine is a ubiquitous breakdown product of ATP and is involved in many physiological and pathophysiological events [1]. The final step of extracellular adenosine formation is catalyzed by the 5'-ectonucleotidase CD73. Adenosine is well known to inhibit lipolysis mediated by adenosine A1-receptors [2]. However, it is yet unknown, whether this effect is functionally relevant under *in vivo* conditions and whether adenosine formed extracellularly by CD73 is involved. We therefore aimed to characterize the metabolic consequences of impaired extracellular adenosine formation by using CD73-deficient ($CD73^{-/-}$) mice using ^{1}H MRI and $^{1}H/^{13}C$ MRS for analysis of all-over body fat content and composition as well as hepatic and myocellular lipid distribution.

Methods: Adult male C57/Bl6 wildtype (WT) and $CD73^{-/-}$ mice at the age of 6-8 months were fed a standard chow diet and received tap water *ad libitum*. $^{1}H/^{13}C$ MR was performed at a vertical Bruker Avance III Wide Bore NMR spectrometer at 400.1 MHz for ^{1}H and 100.6 MHz for ^{13}C using a Bruker Microimaging unit (Micro 2.5) 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 of hepatic fat by ^{1}H MRS (respiratory-triggered PRESS, 3×3×3 mm^3 voxel located in the right liver lobe, outer volume suppression (OVS)). Abdominal fat was associated to visceral and subcutaneous regions by analysis of anatomical images with and without fat suppression. For determination of fat composition, non-volume selective proton-decoupled ^{13}C MR spectra were recorded over the entire abdominal region using a 12×8 mm transmit/receive ^{13}C surface coil. Quantification of intra- and extramyocellular lipids (IMCL + EMCL) 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 (5.07 µl) voxel placed as shown in Fig. 1A using a PRESS sequence with eddy current compensation and VAPOR water supression as well as outer volume suppression to reduce EMCL signal contamination (TR, 1000 ms; TE, 20 ms; spectal bandwidth, 5000 Hz; data points, 2048; acquisition time, 409.60 ms; averages, 2048).

Results: Analysis of fat-selective MR images from WT and $CD73^{-/-}$ animals showed that the mutant is characterized by a distinctly altered fat pattern in particular within the subcutaneous areas. Quantification of the MRI data over the entire abdominal area (expressed in arbitrary units as ^{1}H MR integral per body volume analysed) demonstrated that the visceral fat content was almost unchanged, but the subcutaneous fat content was significantly reduced in $CD73^{-/-}$ mice. The classification of the subcutaneous fat in deep and superficial areas revealed that this was predominantly caused by a decrease in the superficial fat fraction (WT: 2.6±0.9; $CD73^{-/-}$: 1.4±0.5 (n=10); Fig. 1C right) and only to a minor extent by alterations in the deep subcutaneous fat. In the same experimental setting natural abundance ^{13}C MR spectra were acquired for the parallel analysis of the abdominal fat composition. However, no differences were found between the groups for the content of saturated, mono- and polyunsaturated fatty acids as well as the average fatty acid chain length. Similarly, localized ^{1}H MR spectra of the liver showed no significant differences in intrahepatic lipid accumulation and composition between WT and the mutant. To further characterize the consequences of CD73 deficiency on the lipid distribution we analyzed intra- and extramyocellular (IMCL + EMCL) lipid contents. For this purpose localized ^{1}H MR spectra were acquired from the tibialis anterior (TA) muscle (Fig. 1A). The sampling volume for the two representative spectra displayed in Fig. 1B was 5.07 µl for both WT and $CD73^{-/-}$ mice. As can be seen, spectra from $CD73^{-/-}$ TA muscle (top) clearly show an increased IMCL content as compared to WT controls (bottom). Quantification of the spectra revealed an almost 50% increase in IMCL levels for the transgenic group, whereas EMCL levels were not significantly altered (Fig. 1C left). Analysis of blood parameters unveiled significant increases in blood glucose (WT: 111±14 vs. 146±24 mg/dl ($CD73^{-/-}$)) and serum insulin levels (WT: 1.20±1.15 vs. 7.06±5.51 µg/l ($CD73^{-/-}$)) accompanied by increased serum free fatty acids (WT: 203±65 vs.: 354±141 µM ($CD73^{-/-}$)) and triglycerides (WT: 4.2±4.9 vs. 15.4±12.2 mg/dl ($CD73^{-/-}$)) in the mutant. Taken together, these results indicate an enhanced lipolysis when extracellular formation of adenosine is impaired resulting in an accumulation of lipids in muscle cells. Increased insulin and glucose levels provide additional evidence for an insulin resistance.

Conclusions: Disrupted adenosine formation due to lack of CD73 leads to an enhanced lipolysis accompanied by accumulation of intramyocellular lipids in skeletal muscle and increased insulin levels. These findings suggest that (i) CD73-derived adenosine is an important modulator of lipolysis and (ii) impaired extracellular adenosine release might be critically involved in development of insulin resistance and associated diseases like diabetes mellitus and the metabolic syndrome.

References: [1] Shryock JC et al, Am J Cardiol. 79: 2-10 (1997) [2] Fredholm BB et al, Adv Pharmacol. 61: 77-94 (2011).

Figure 1: Analysis of myocellular lipids levels and subcutaneous fat content in WT and $CD73^{-/-}$ mice, respectively. (A) Voxel localization for ^{1}H MRS voxel of tibialis anterior (TA) muscle. (B) Localized ^{1}H MR spectra of TA muscle and (C) quantification of myocellular lipids and subcutaneous fat in WT and $CD73^{-/-}$ mice. Abbreviations: Cho, choline; Cr, creatine; Glx, glutamine + glutamate; H_2O, residual non-suppressed water; IMCL/EMCL intra/extramyocellular lipids; Tau, taurine.