Fat composition assessment by 1H and 13C spectroscopy in mice

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Introduction

For many years, the body fat composition has been studied by means of 13C spectra of adipose tissue extracts using high resolution MR (Fan, Clifford et al. 1994; Zancanaro, Nano et al. 1994; Falch, Storseth et al. 2007) and other techniques like gas chromatography (Sillerud, Han et al. 1986; Fernandez-Real, Broch et al. 2003). However, these methods require a large sample and a long acquisition time, and cannot be performed in vivo. One approach to in vivo measurement was introduced by Künnecke (Künnecke, Verry et al. 2004) using non anesthetized animals housed in polyethylene containers. We propose a straightforward method to quantitatively analyze fatty acid composition in wild type and genetically obese diabetic mice over several months.

Material and methods

A total of twenty-four mice (wild type, n=12 and genetically obese, n=12) divided into three groups of 5, 9, 18 months of age (eight animals per group) were studied in a Bruker Biospec 70/20 scanner using a 1H/13C surface coil. The coil was positioned over epididymal fat in the abdomen of the mouse. Animals were anesthetized with Sevofluorane (1%) and monitored. After a global shimming ('mapshim' protocol), we acquired 1H spectra (TR 1000ms, NEX 1, 2048 points, bandwidth 10KHz) and proton-decoupled 13C spectra (TR 4000ms, NEX 192, 2048 points, bandwidth 15KHz, 85 µsec bipolar pulse, decoupling with waltz-16 irradiation throughout the acquisition). 13C spectrum acquisition took about 12 minutes and 1H spectrum acquisition took about 2 seconds. We measured the areas of lipid and water resonances on the 1H spectra. Quantification of carbon resonances was based on Künnecke´s work. The spectrum is dominated by glycerides, which leads to six distinct groups of resonances: S1: carboxylic (172 ppm); S2: olefinic (130 ppm); S3: polyolefinic (128ppm); S4 and S4´ correspond to C2 and C1, C3 of glycerol backbone (69 and 62ppm); S5 methylene (30 ppm) and S6: methyl group (14 ppm). The fractional contr bution of saturated (FAs), monounsaturated (FAm) and doubly (poly) unsaturated (FAd) fatty acids to the total pool of fatty acids chain in glycerides was calculated with the following formulas: $FAs= (S_1-S_2/2)/S_1$; $FAd=S_3/2*S_1$; $FAm=(S_2-S_3)/2*S_1$.

Results

Figure 1 illustrates differences in 1H spectra between wild type and genetically obese mice aged nine months. Proportion of lipids to water in wild type mice is about 10-20% while in obese mice the lipid peak has practically the same area as the water peak. Figure 2 shows typical 13C spectra for both types of animals. Figure 3 shows plots of the fractional contributions of FAs, FAd and FAm against lipid/water content at different ages. The FAs increases when the mice are 9 months old and slightly decreases at eighteen months old. On the contrary, FAd values decreases at 9 months old. Only at the age of nine months, we find a linear relationship between lipid content and fatty acid saturation.

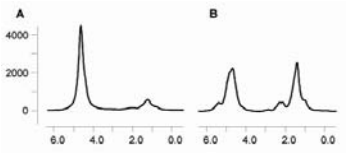

Figure 1 1H spectra from wild type (A) and genetically obese mice (B)

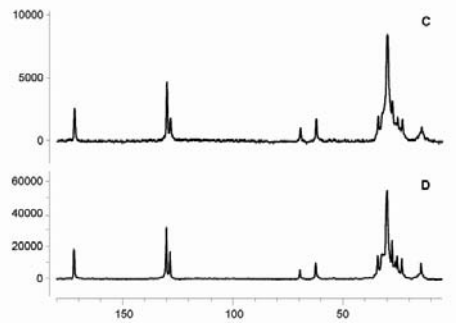

Figure 2 13C spectra from wild type (C) and genetically obese mice (D)

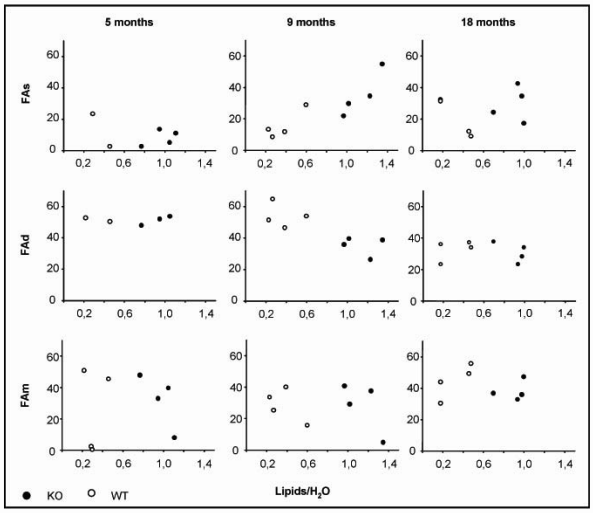

Figure 3 Fatty acid fractional contr bution plotted against lipid/water content for animals of 5, 9 and 18 months of age. Black dots= genetically obese mice, White dots=wild

Conclusions

This work proposes a straightforward method to study the body composition and fractional contribution of fatty acids by means of magnetic resonance spectroscopy in vivo. It has been tested by comparing results for epididymal fat in wild type and obese mice. We have found clear differences between wild and obese animals. This approach has proven to be valuable to non-invasively characterize lipid composition, and it is suitable for investigations in obesity-related disorders, such as insulin resistance and type-2 diabetes.

References

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