In vivo [1]H NMR spectroscopic study of muscle lipids in hyperlipidemic and diabetic Watanabe rabbits

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Introduction

Diabetes and hyperlipidemias are becoming a major global health challenge with escalating costs [1]. It is currently thought that the pathogenesis of type II diabetes, for instance, involves reduced mitochondrial activity in skeletal muscle leading to IMCL deposits [2]. ^{1}H NMR spectroscopy *in vivo* has become a valuable tool for studying skeletal muscle insulin resistance. In skeletal muscle, lipids are stored in two compartments, as extramyocellular lipids (EMCL) in macroscopic plates along fasciae, and intramyocellular lipids (IMCL) in cytoplasm droplets within muscle cells. Due to compartmentation differences, the lipid resonances from EMCL and IMCL can be seen as separate peaks in the MR spectrum [3]. EMCL is metabolically relatively inert, whereas IMCL can be mobilized and utilized rather easily [4]. In the present study, we have characterized the ^{1}H NMR metabolite and lipid levels in skeletal muscle of control and diabetic Watanabe hereditable hyperlipidemic (WHHL) rabbits to assess the role of hereditary hyperlipidemia in combination with diabetes.

Methods

Watanabe hereditable hyperlipidemic (WHHL) were used to study lipids in skeletal muscle. The animals are characterized by inborn LDL-receptor deficiency and chronic dyslipidemia, similarly to humans [5]. The weights of all rabbits were 2.5-3.9 kg. Diabetes was induced by Alloxan (Sigma Aldrich) at least two months prior to the study. Blood glucose levels in diabetic rabbits were between 16.1-34.0 mmol/l. The ^{1}H NMR spectra were acquired from *biceps femoris* muscles of control (n=4) and diabetic (n=5) animals. For measurements, the major axis of the muscle was always aligned parallel to the magnetic field to prevent differences in orientation-dependent effects between animals. MRS was performed in a 4.7 T magnet, equipped with actively shielded field gradients interfaced to a Varian ^{UNITY}*INOVA* console. A quadrature surface coil was used as both receiver and transmitter. Gradient-echo images were acquired to place the voxel to the muscle. For metabolite analysis, the LASER method incorporating a water-suppression scheme was used (TR=6s, TE=34ms, NS=128), non-water-suppressed spectra were obtained similarly (NS=8) to provide reference. Metabolite concentrations were calculated based on water content, not corrected for relaxation differences. Data-analysis was performed in the time domain using jMRUI software (http://carbon.uab.es/mrui/). Student's t-test was used to check statistical significance.

Results

Typical spectra are shown in Fig. 1. The IMCL methylene levels in diabetic animals were significantly increased when compared to controls (71±12 vs. 32 ± 9 mmol/kg w.w., see Fig. 2). EMCL also appeared to be higher in diabetic animals (764 ± 237 mmol/kg vs. 222 ± 57 mmol/kg w.w.), however there was large variation between animals, and the difference was not significant (P = 0.10). Trimethylammonium (TMA), total creatine (tCr) levels were not significantly different between normal and diabetic animals (Fig. 2).

Conclusions

Our results show modest accumulation of IMCL in control WHHL rabbits, indicating decreased insulin sensitivity already in the control animals. In humans, it has been shown that muscle insulin resistance appears to be associated only with hypertriglyceridemia [6], and not combined dyslipidemia, as in the case with WHHL rabbits. However, in the diabetic WHHL rabbits, significant accumulation of intramyocellular lipids could be detected, consistent with increased skeletal muscle insulin resistance. The results show that NMR spectroscopy of muscle has the power of detecting IMCL accumulation in diabetic animals even against the background of hyperlipidemia and co-existing insulin resistance. This has important implications for clinical studies, where diabetic patients often present with combinations of diabetes and dyslipidemias in conjunction with diabetes and obesity.

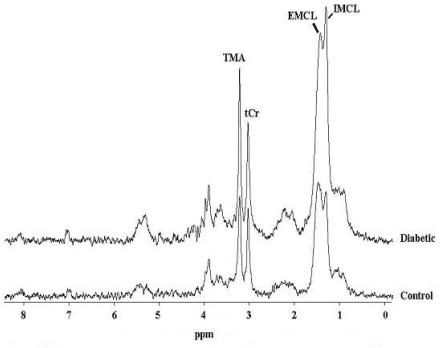

Figure 1. [1]H MR spectra from *biceps femoris* of WHHL rabbit. Trimethylammonium (TMA), total creatine (tCr), extramyocellular lipids (EMCL) and intramyocellular lipids (IMCL) are shown

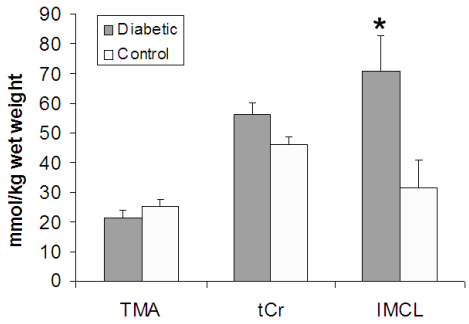

Figure 2. Metabolite and lipid concentrations in *biceps femoris* of WHHL rabbit. Values are means ± SEM. * P<0.05, control vs. diabetic

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