In vivo Measurement of Intramyocellular Lipids in Pre-Diabetic and Diabetic Female Zucker Diabetic Fatty Rats Using Magnetic Resonance Spectroscopy

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Introduction

Insulin resistance (IR) plays an important role in the pathogenesis of type II diabetes and metabolic syndrome. Although the precise mechanisms causing IR remain unclear, there is increasing evidence that impaired lipid metabolism is associated with the development of IR. One of the hallmarks of IR found in both human and animal studies is increased accumulation of intramyocellular lipids (IMCL) in skeletal muscle [1,2]. IMCL concentration is strongly related to IR, and is affected by many factors such as age, diet, exercise, and gender [2-4]. Among rodent models of IR/type II diabetes, male and female Zucker diabetic fatty (ZDF) rats represent two distinct metabolic phenotypes. Unlike male ZDF rats that spontaneously become diabetic with age, female ZDF rats, which are insulin resistant, remain normal-glycemic, unless fed with a specialized diabetogenic high fat diet (HFD). Several studies have documented IMCL levels in male ZDF rats [3,4], but limited IMCL data are available in female ZDF rats. In this study we characterized the time-course IMCL content in tibialis anterior (TA) in relation to the progression of diabetes and the effects of two anti-diabetic agents, rosiglitazone and metformin, on IMCL content in pre-diabetic and diabetic female ZDF rats.

Methods

Animal Protocols. A cohort of 6-week-old female ZDF rats and age matched female ZDF lean littermates were used. Protocol I: female ZDF rats were fed either with HFD (GMI C13004) or normal chow diet (ND) (PMI 5008). ZDF lean rats, fed with ND were used as controls. Post-prandial blood glucose (BG) was assessed every week via Precision PCx glucometers. Rats that had become diabetic after 4 weeks on HFD were treated with either rosiglitazone (10 mg/kg), or metformin (500 mg/kg) for two weeks. Time-course IMCL levels were measured (N=4-8/group) at 0, 0.5, 2, 4, and 6 weeks following the commencement of HFD. Protocol II: Rats fed with ND were divided into the following groups (N=6–8 /group): ZDF lean, ZDF vehicle, ZDF rosiglitazone (10 mg/kg), or ZDF metformin (500 mg/kg). Treatment was started at 6 weeks of age and continued for two weeks. IMCL was evaluated before and 2 weeks after the treatment in all animals. Both drugs were dissolved in 0.2% HPMC and water, and administered orally once a day.

IMCL by Proton MR Spectroscopy. All experiments were conducted on a Bruker 7T magnet using a surface coil. Localized proton spectra were acquired from a 2x2x2 mm^3 volume within the TA using a PRESS sequence (TR=2s, TE = 15 ms, NT=256). Water signal acquired using the same sequence without water suppression was used as the internal reference for relative IMCL concentration (IMCL/Water).

Results and Discussion

Female ZDF rats under normal diet were insulin resistant, obese, but remained normal-glycemic (BG: 110.7 –120.4 mg/dl). However they consistently developed diabetes within 4 weeks when fed with C13004 HFD. The average post-prandial BG reached 332.5±31.2 mg/dl by 4 weeks and further increased to 365.8±18.4 mg/dl after 6 weeks on the HFD. Figure 1 illustrates the time-course IMCL content in female ZDF rats that were either on ND or on HFD. IMCL levels in TA in ZDF rats were 3.1 times that of the lean littermates at 6 weeks of age. When fed with HFD, IMCL levels steadily increased with time up to 4 weeks, while IMCL content in ZDF rats on ND slightly decreased with age. No significant age-dependent changes in IMCL levels were observed in the ZDF lean littermates. Both rosiglitazone and metformin, as expected, significantly reduced post-prandial glucose levels from 365.8 mg/dl to 158.1 and 145.9 mg/dl, respectively, after 2 weeks of treatment. Meanwhile the IMCL accumulation in TA was also significantly decreased by both agents. We postulate that both rosiglitazone and metformin may have a direct effect on fatty acid oxidation in skeletal muscle via indirect activation of AMP-activated protein kinase, or in the case of rosiglitazone it may also work by shunting free fatty acids away from non-adipose tissues. Interestingly, when treatment was applied to pre-diabetic ZDF rats at age of 6 weeks for two weeks, only rosiglitazone significantly lowered IMCL in TA, whereas metformin had no effect (Figure 2). This may indicate that meformin's ability to lower IMCL is influenced by diet or disease state. Our current data provide important background information pertaining to the ZDF female rat model for future pharmacological studies in evaluating potential new anti-diabetic drugs. The differential drug-induced modulation of IMCL in pre-diabetic and diabetic rats may offer useful insights for understanding the mechanisms of actions of these drugs.

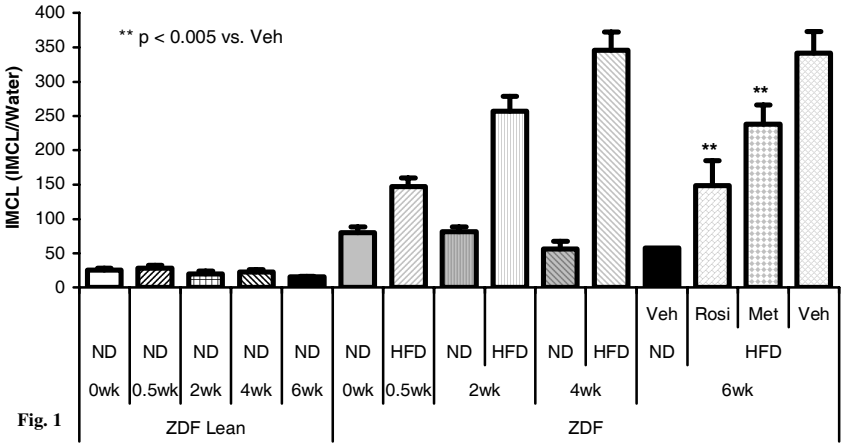

Fig. 1

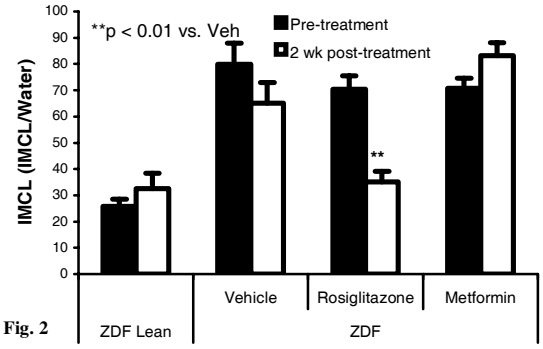

Fig. 2

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