Pilot investigation of intramyocellular and abdominal lipid contents by 2D MR Spectroscopy and MRI in patients with Type2 Diabetes and Impaired Fasting Glucose

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Introduction: Insulin resistance (IR) and cardiovascular disease (CVD) have been linked by inflammation (the "common soil" hypothesis). Attempts to construct CVD risk prediction models utilizing inflammatory biomarkers and vascular imaging have remained imperfect. Work in the last decade has emphasized the importance of abdominal visceral fat deposits and intramyocellular lipid (IMCL) in metabolic syndrome and IR development. MRI enables localization, and quantification of lipid deposits and localized 2D MR Spectroscopy (MRS) approaches have been implemented recently to overcome limitations of inadequate spectral dispersion in localized 1D MRS (1). Use of MRI fat localization techniques and myocellular lipid estimation using MRS, if correlated with biomarkers of CVD, would allow us to understand the roots of development of IR and CVD risk. Our aim was to assess the relative relationship between visceral fat and IMCL deposits, and (a) hs-CRP: a well validated biomarker of inflammation; (b) Carotid intimal thickness (IMT) by ultrasonography (US): the gold standard for assessment of subclinical atherosclerosis; (c) HOMA IR (homeostasis model of insulin resistance).

Methods: We recruited 10 subjects (age 18-65 years), with impaired fasting glucose/early diabetes, all with glucose levels <140 mg/dL (Table 1). Anthropometry, venous blood testing and US of carotid arteries was performed. MRI was done by the standard T1-weighted spin-echo imaging with respiratory compensation and calf muscle spectroscopy using 2D L-COSY sequence (1) on a 3T MRI scanner. Abdominal fat distribution was determined by a Java based fuzzy c-means (FCM) clustering fat segmentation program running on a PC (2). MRS data was processed using Felix-2000 (Felix NMR Inc., San Diego, CA) and expressed as ratios of the volume under each peak to the diagonal (_d) creatine peak volume. Pearson correlation coefficients were calculated.

Results and Discussion: A representative 2D L-COSY spectrum is shown in Fig.1 [Peaks (F2, F1): Car_8: Carnosine (8, 8), Car_7: Carnosine (7, 7), Unsat_d: Unsaturated fatty acids (5.4, 5.4), TGFR: Triglyceryl backbone protons (4.3, 5.4), Ch_d: Choline (3.2, 3.2), Cr_d: Creatine (3, 3), MethylFat /FAT: Methyl/polymethylene protons of saturated/unsaturated fat (0.9, 0.9) /(1.4, 1.4), EMCL2 (5.5, 2.2) and IMCL2 (5.2, 2.0) arise from spin-spin coupling between olefinic and allylic methylene protons and EMCL1 (5.5, 2.9) and IMCL1 (5.2,2.7) arise from indirect spin-spin coupling between respective olefinic and diallylic methylene protons of extra-myocellular lipids (EMCL) and IMCL (3)]. The results are summarized in Tables 2 and 3 where IMCL and EMCL represent the respective averages of IMCL and EMCL 1 and 2 below the diagonal. Both IMCL/Cr and EMCL/Cr positively correlated with the IMT and thus development of atherosclerosis whereas only IMCL/Cr correlated with the level of IR. hs-CRP did not show a significant correlation with any other parameter. Additionally, the EMCL unsaturation index (U.I.) showed a positive correlation with HbA1C (r=0.783, p=0.007) and negative correlation with HDL (r=-0.806, p=0.005) whereas IMCL U.I. showed a positive correlation with HbA1C (r=0.919, p=0.027) and IR (r=0.926, p=0.023) but only in the obese subjects (n=5). Strong correlations of the Ch/Cr ratio with other parameters might be a reflection of changes in membranal composition or phospholipid metabolism in these patients. However, our data is limited by the small number of subjects.

Figure 1: A 2D L-COSY spectrum recorded from the soleus muscle of a 66 year old diabetic subject with TR/TE =2s/30ms, NEX=8 for each t1, 50 t1 increments and voxel size of 27ml.
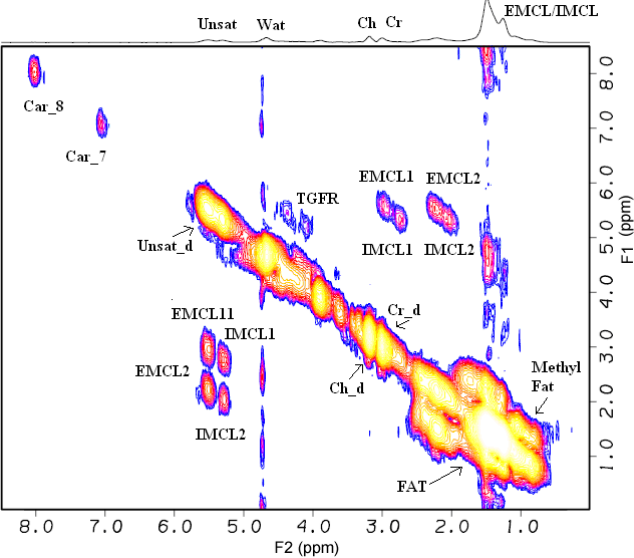

Table 1. Materials and Methods

EXCLUSION CRITERIA	VENOUS BLOOD CHEMISTRY
- HbA1C% >6.5% or <5.1% - Definite diabetic micro- and macro-angiopathy (by medical records) - Diabetes treated with any drugs - Severe renal disease - Severe hepatic disease - Malignancy - Chronic inflammatory disease - Contraindications to MRI	- HbA1C% - Fasting glucose and insulin - Liver function - Renal function - hs-CRP - LDL, HDL, triglycerides, total cholesterol

Table 2. Sample characteristics (Mean ± SD)

Age (years)	53.3 ±12.9
Hb A1C (%)	6.03 ± 0.47
HOMA IR	3.74 ± 1.85
hs-CRP (mg/dL)	3.25 ± 3.48
Visceral fat (cm3)	136.03 ± 59.6
Subcut. fat (cm^3)	204.13 ± 74.04
Height (cm)	168.64 ± 10.5
Weight (lb)	200.79 ± 48.9
Waist (cm)	114.55 ± 15.25
BMI	30.78 ± 5.39

Table 3: Pearson's correlation coefficients [r (p value)]

	IMCL/Cr	EMCL/Cr	HOMA IR	Ch/Cr	Subcut. Fat	Visceral Fat
IMCL/Cr		0.679 (0.031)	0.633 (0.049)	-0.75(0.013)	NS	NS
EMCL/Cr	0.679 (0.031)		NS	-0.73(0.017)	NS	NS
HOMA IR	0.633 (0.049)	NS		-0.701(0.024)	NS	NS
IMT (cm)	0.879 (0.002)	0.668 (0.049)	0.792 (0.006)	-0.93(0.000)	NS	NS
hs-CRP(mg/dL)	NS	NS	NS	NS	NS	NS
Unsat_d/Cr	0.93 (0.007)	0.97 (0.001)	NS	-0.709(0.022)	NS	NS
TGFR/Cr	NS	0.852 (0.02)	NS	NS	0.842(0.02)	NS

Conclusion: Our pilot findings suggest that in the early diabetes, intramyocellular fat is closely related to the extent of atherosclerosis and IR whereas inflammation and visceral fat are not closely related to fat deposition, atherosclerosis or IR. We infer that inflammation plays a minor role in initial development of metabolic derangements related to IR and atherosclerosis, and intramyocellular lipids are more significantly associated with IR and atherosclerosis than extramyocellular and visceral fat in early diabetes.

References: 1.Thomas MA, Yue K, Binesh N et al. Magn Res Med. 2001; 46:58-67. 2. Singh J, Rathore RKS, Gupta RK et al. Proc. Intl. Soc. Mag. Reson. Med 2007; 15: 3825. 3. Velan SS, Durst C, Lemieux SK et al. JMRI. 2007; 25:192–199.