JOURNAL OF CARDIOVASCULAR MAGNETIC RESONANCE®
Vol. 6, No. 1, pp. 355–358, 2004

Poster Abstracts: Experimental MRS

472. Evaluation of Intramyocellular Lipids Related to Insulin Resistance and Metabolic Syndrome I\in Lipoatrophic Diabetes by Proton Magnetic Resonance Spectroscopy

Fabio Berezowsky Rocha, MD,[1] Marcello Zapparoli, MD,[2] Marcia Lima de Oliveira Mugnaini, MD,[2] Marly Maria Uellendahl, MD,[2] Miguel Rosario, MD,[2] Fátima Cristina Pedroti, MD,[1] Luiz Francisco Rodrigues de Ávila, MD, PhD,[2] Roberto Kalil-Filho, MD, PhD,[1] Bernardo Léo Wajchenberg, MD, PhD,[1] Cláudio Campi de Castro, MD, PhD,[1] José Rodrigues Parga, MD, PhD,[2] Carlos Eduardo Rochitte, MD, PhD.[2]
[1]*Cardiovascular Magnetic Resonance Laboratory, Heart Institute–InCor–University of São Paulo Medical School, São Paulo, Brazil,* [2]*Cardiovascular Magnetic Resonance Laboratory, Heart Institute–InCor–University of São Paulo Medical School, Sao Paulo, Brazil.*

Introduction: Recently, proton magnetic resonance spectroscopy (MRS) has been shown to differentiate lipids from extra cellular and intracellular space in leg muscles. The increase in intra cellular or intramyocellular lipid (IMCL) in the soleum muscle has been associated to peripheral insulin resistance, metabolic syndrome and diabetes mellitus. It is proposed that long chain fatty acids like acilCoa could be storaged in the cell cytoplasm when inducted by hormonal and metabolic factors leading to fatty acids excess over the oxidative process. Ultimately, this intracellular fat accumulation will cause insulin resistance. H^1 MRS with specific technique show two lipid peaks described as the extramyocellular lipid (EMCL) and IMCL and located at 1.6 ppm and 1.4 ppm, respectively. The IMCL/creatine ration has been shown to be higher in patients with insulin resistance (9.5 ± 0.9) than in those without it in normals (3.0 ± 0.5). However, several technical questions still remain over this method.

Purpose: To test H^1 MRS technique in the analysis of the lipids (extra and intra cellular) in lipoatrophic diabetes and normal subjects, analyzing the soleum muscle.

Methods: We studied three patients with this rare disease, characterized by an extreme decrease in extra cellular lipids within the muscles and two normal subjects. MRS studies were performed in a Signa LX/CVi 1.5T–GEMS, Milwaukee, Wisconsin with an extremity volumetric coil positioned at the center of the soleum muscle of right leg. MRS utilized a stimulated echo acquisition mode (STEAM) sequence to localize and acquire magnetic resonance signal from a voxel of 8 cm3 within the soleum muscle, excluding vascular and fascia structures. Parameters were TR = 2000 ms, TE = 12 ms and TM = 13,7 ms. The H^1 MRS analysis was done with specific software *SAGE®*, reconstructing the spectrum by apodization, zero filling and auto phasing. The values were set in reference to the water peak of 4.7 ppm. Peak, range and integral of the EMCL, IMCL, creatine, water and total lipids were measured. EMCL/Creatine and IMCL/Creatine ratios were calculated.

Results: Analyzed, the lipoatrophic spectroscopy results are shown in the Table 1.

Table 1.

Patient	EMCL/ creatine	IMCL/ creatine	EMCL peak	IMCL peak
A	1.339927121	2.122436231	1.47	1.29
B	0	7,106310924	Null	1,27
C	0	11.98607427	Null	1.31

DOI: 10.1081/JCMR-120028319
1097-6647 (Print); 1532-429X (Online)

Table 2.

Patient	EMCL/ creatine	IMCL/ creatine	EMCL peak	IMCL peak
A	5.825905725	5.378475456	1.44	1.27
B	6.490519789	5.74628121	1.49	1.28

Normal group is shown in Table 2.

The absolut values of EMCL are reduced in lipoatrofic diabetic patients compared with normal one's. The absolut values of IMCL are increased in lipoatrophic diabetic patients compared with the normals. The peaks of EMCL and IMCL mantained the normal intervals in both groups. The results obtained in the spectral anaylisis showed higher differences between the IMCL/CREATINE and EMCL/CREATINE ratios in lipoatrophic diabetic patients compared to normal subjects (Figures 1 and 2).

Conclusions: MRS demonstrated ability to differentiate and quantify EMCL and IMCL values. Moreover, in lipoatrophic diabetic patients with known severe EMCL decrease, this MRS technique provided reliable results, demonstrating not only the EMCL decrease but also detecting precisely the IMCL increase.

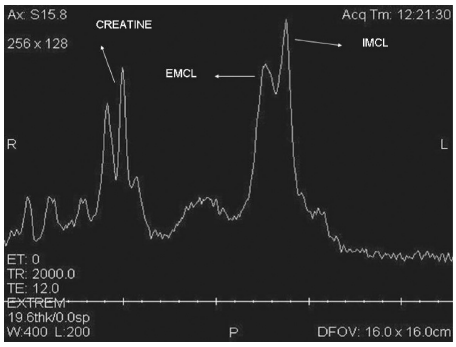

Figure 1.

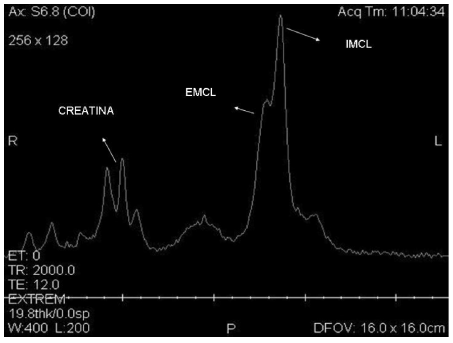

Figure 2.

473. Effects of Blocking the NHE and the NBC on Ischemic Na_i^+ Overload in Rat Hearts

Michiel Ten Hove, PhD,[1] Marcel G. J. Nederhoff,[1] Cees J. A. Van Echteld, PhD.[2] [1]*Interuniversity Cardiology Institute of the Netherlands, Utrecht, Netherlands,* [2]*Cardiology, Heart Lung Center Utrecht, University Medical Center Utrecht, Utrecht, Netherlands.*

Introduction: Blocking the Na^+/H^+ exchanger (NHE) during ischemia has been shown to reduce Na^+ overload during ischemia and to improve post-ischemic contractile recovery in experimental situations. However, the results from a clinical trial (GUARDIAN) were disappointing. The effect of ischemic blockade of the Na^+/HCO_3^- co-transporter (NBC) as well as blockade of both, the NHE and the NBC, on ischemic Na_i^+ overload is unknown. Combined blockade of both pH_i regulating transporters might provide more cardioprotection than NHE blockade alone.

Methods: Isolated, Langendorff perfused rat hearts were subjected to 30 minutes of global ischemia and 30 minutes of reperfusion. Cariporide (3 μM) or bicarbonate free HEPES buffer was used to block the NHE, the NBC or both, respectively. $[Na^+]_i$ and pH_i were measured using simultaneous ^{23}Na and ^{31}P MRS, respectively, on a three channel BrukerAvance NMR spectrometer equipped with a 9.4 T magnet. $TmDOTP^{5-}$ was used as a shift reagent to discriminate between Na_i^+ and Na_e^+.

Results: Reduction of ischemic Na_i^+ overload (Figure 1) by NHE blockade was 43%, by NBC blockade 21% and by combined NHE and NBC blockade 52%. End-ischemic pH_i (Figure 2) was 6.09±0.06 in bicarbonate perfused, untreated hearts, 5.85±0.02 when the NHE was blocked, 5.81±0.05

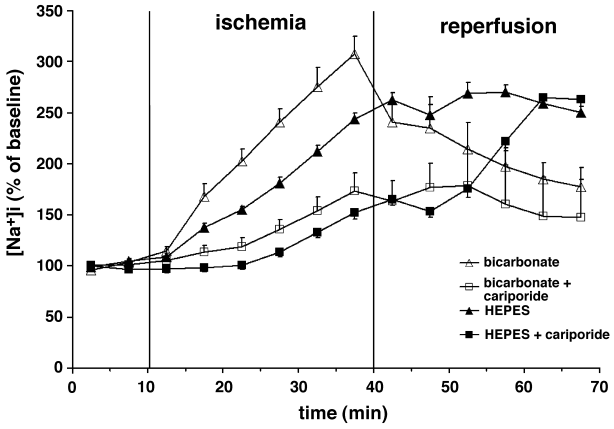

Figure 1. $[Na^+]_i$ during ischemia and reperfusion.

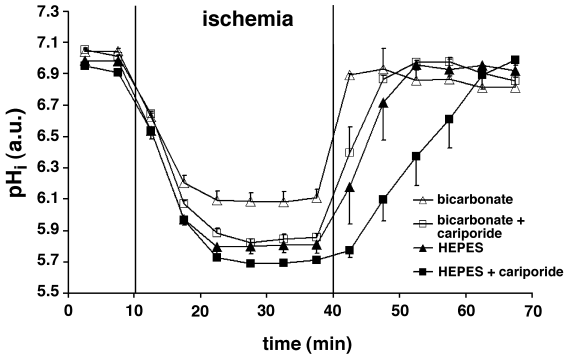

Figure 2. pH$_i$ during ischemia and reperfusion.

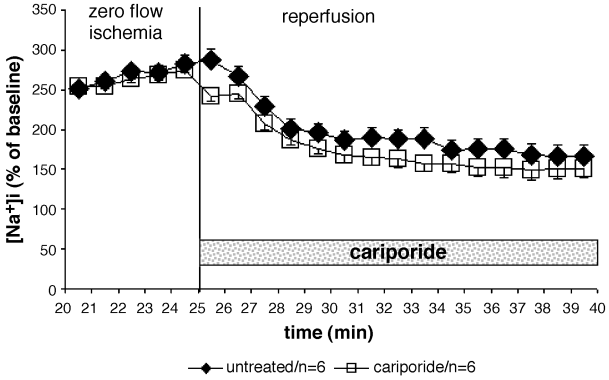

Figure 1. [Na$^+$]$_i$ in rat hearts with or without post-ischemic NHE blockade after 25 min. zero flow ischemia.

when the NBC was blocked and 5.70±0.01 when both the NHE and the NBC were blocked. NHE blockade improved post-ischemic systolic and diastolic contractile recovery, NBC blockade and combined blockade did not. Combined blockade of the NHE and the NBC conserved H_i^+ load during reperfusion and lead to massive Na^+ influx when blockades were raised. Omission of bicarbonate under conditions of NHE blockade severely impaired coronary flow.

Conclusion: Without blockade, both the NHE and the NBC mediate acid equivalent efflux in exchange for Na^+ influx during ischemia, the NHE much more than the NBC. Blockade of either one does not affect the other one. The lack of contractile recovery in hearts after blockade of both, the NHE and the NBC can be explained by the impaired coronary flow. Combined blockade of the NHE and the NBC is therefore potentially dangerous.

474. Why Post-Ischemic NHE Blockade Offers Only Limited Protection

Michiel Ten Hove, PhD,[1] Cees J. A. Van Echteld, PhD.[2] [1]*Interuniversity Cardiology Institute of the Netherlands, Utrecht, Netherlands,* [2]*University Medical Center Utrecht, Utrecht, Netherlands.*

Introduction: Ischemic blockade of the Na^+/H^+ exchanger (NHE) has been found to be cardioprotective in many studies. However, reports on the efficacy of specific NHE blockers when administrated only during reperfusion are inconsistent. Differences in the severity of ischemia and in drug delivery may explain these inconsistencies. Little is known about the primary goal of post-ischemic NHE blockade, i.e. reduction of Na_i^+ overload.

Methods: Isolated rat hearts were subjected to either 25 minutes of zero flow ischemia or 60 minutes of low flow (0.2 ml/min.) ischemia. Hearts were reperfused with or without the selective NHE blocker cariporide added to the perfusate. In the low flow group cariporide was administrated 5 minutes prior to reperfusion. [Na$^+$]$_i$ and pH$_i$ were measured with simultaneous ^{23}Na and ^{31}P MRS, respectively, on a 3 channel Bruker Avance NMR spectrometer equipped with a 9.4 T magnet. TmDOTP^{5-} was used as shift reagent to discriminate Na_i^+ and Na_e^+.

Results: Administration of cariporide after zero flow ischemia caused the [Na$^+$]$_i$ to decrease during the first minute of reperfusion, followed by a partial and transient rise during the second minute (Figure 1). Untreated hearts showed a very small rise in [Na$^+$]$_i$

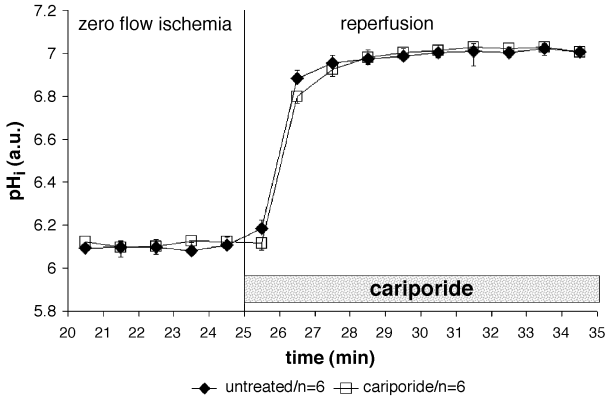

Figure 2. pH$_i$ in rat hearts with or without post-ischemic NHE blockade after 25 min. zero flow ischemia.

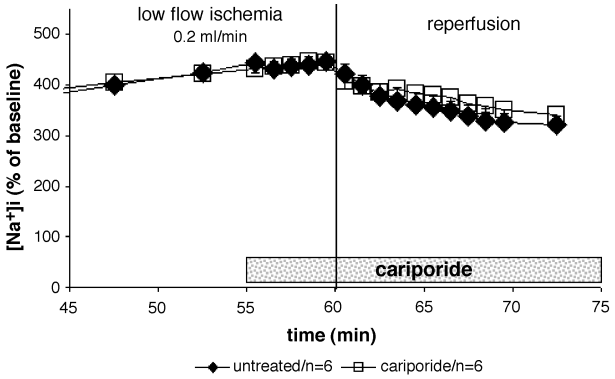

Figure 3. $[Na^+]_i$ in rat hearts with or without post-ischemic NHE blockade after 60 min. low flow ischemia.

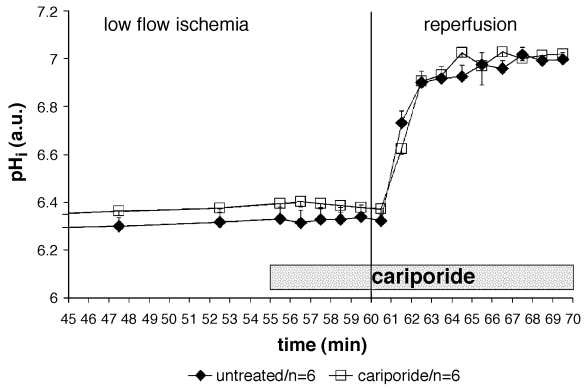

Figure 4. pH_i in rat hearts with or without post-ischemic NHE blockade after 60 min. low flow ischemia.

during the first minute. The pH_i recovered slightly slower in cariporide treated hearts than in untreated hearts (Figure 2). No effect of cariporide on the rate pressure product could be detected since the rate pressure product recovered fully in untreated hearts. The end-diastolic pressure, however, was increased during reperfusion in both groups, indicating that cariporide was not cardioprotective. After low flow ischemia the $[Na^+]_i$ (Figure 3) and the pH_i (Figure 4) recovered similarly in both groups. Recovery of the rate pressure product was poorly in both groups.

Conclusion: Post-ischemic NHE blockade only has a small and transient effect on $[Na^+]_i$ and pH_i after zero flow ischemia and no effect at all after low flow ischemia, which explains the lack of cardioprotectivity under these experimental conditions.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016