In vivo Assessment of Ca^{2+}-related Glucose Homeostasis in Skeletal Muscle using Manganese-enhanced Magnetic Resonance Imaging

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Introduction

In healthy subjects, blood glucose levels are maintained within a narrow range by the regulation of ß-cell insulin secretion, peripheral glucose uptake and hepatic glucose production. Calcium ions (Ca^{2+}) signaling plays a critical role in secretion of insulin from pancreatic ß-cells. Insulin, in turn, stimulates the translocation of vesicles containing glucose transporter-4 (GLUT-4) to the plasma membrane in myocytes. Skeletal muscle is the major site of glucose disposal *in vivo*, being responsible for 75% of insulin-dependent glucose uptake. Release of GLUT-4 to the plasma membrane is similar to the exocytotic release of insulin and neurotransmitters, and also mediated by Ca^{2+}. The liver is also a major site of glucose metabolism, where hepatic glucose production is inhibited by insulin and induced by glucagons: both hormones inducing Ca^{2+} influx in hepatocytes *in vitro*. Mn^{2+} is a positive T_1 contrast agent and as a surrogate of Ca^{2+}, has been used by *in vivo* manganese-enhanced MRI (MEMRI) techniques to monitor Ca^{2+}-related cellular activities in neurons, myocardiocytes and pancreatic ß-cells (1, 2). Here, we explore the application of MEMRI for assessing Ca^{2+}-related processes in skeletal muscle glucose homeostasis.

Materials and Methods

All MRI studies were performed on a 4.7T MRI scanner. C57BL/6 mice (n = 12) were fasted overnight and imaged under terminal inhalation anesthesia. 3D magnetization-prepared rapid gradient echo (MP-RAGE) MRI was performed prior to, during and following i.v. $MnCl_2$ infusion (2 mM, 0.1 ml, 0.2 ml/h) with parameters: TR = 10 ms, TE = 2 ms, TD = 2 s, TI = 740 ms, Flip angle = 10°, FOV = 100 × 50 × 32 mm, matrix = 256 × 128 × 64, 1 scan with a temporal resolution of 4 min. For the glucose challenge, a bolus i.p. injection of 2 g/kg glucose was administered at 16 min after the start of $MnCl_2$ infusion (n = 6/group): (control animals received saline). The SI was measured from ROIs in the gastrosplenic pancreas, liver and thigh, and MEMRI data presented as: % SI change = $(SI_t - SI_0) / SI_0 \times 100$ %. (SI_0 is the average SI of the three MRI datasets acquired prior to $MnCl_2$ infusion and SI_t is the SI at time t.)

Results and Conclusions

Figure 1 shows the MEMRI SI profile in skeletal muscle, pancreas and liver with and without glucose stimulation (n = 6/group). In skeletal muscle, Mn^{2+} uptake was insignificant in the absence of glucose stimulation (Figure 1A). Following glucose stimulation, the SI increased rapidly, reaching a maximum (20%) at 36 min post-infusion and remaining so for the duration of the study ($P < 0.01$, GEE; Figure 1A). In the pancreas, the SI rose following $MnCl_2$ administration reaching peak enhancement 6 min after the end of infusion and remained so for a further 60 min (Figure 1B). The pancreas MEMRI SI profile of mice receiving glucose was significantly different from controls ($P < 0.05$, GEE; Figure 1B). In the liver, the SI rose following $MnCl_2$ administration reaching peak enhancement 6 min after the end of infusion and then gradually declined (Figure 1C). No difference in SI was observed in the liver with and without glucose stimulation ($P > 0.05$, GEE; Figure 1C).

Discussions

MEMRI revealed significantly greater SI enhancement in skeletal muscle and pancreas and following glucose challenge. The greater SI enhancement, suggesting induction of Ca^{2+} influx, is consistent with the known stimulation of Ca^{2+} influx by glucose in these organs. The MEMRI profile was essentially unchanged by glucose administration in the liver, possibly as both insulin and glucagon can facilitate Ca^{2+} influx into hepatocytes, leading to an influx of Ca^{2+} (and Mn^{2+}) in both the fasted and fed states.

Conclusions

MEMRI may be capable of non-invasively assessing glucose homeostasis in skeletal muscle and pancreas.

References: 1.Antkowiak PF, et al. *Am J Physiol Endocrinol Metab* 2000. 2. Silva AC, et al. *NMR Biomed* 2004.

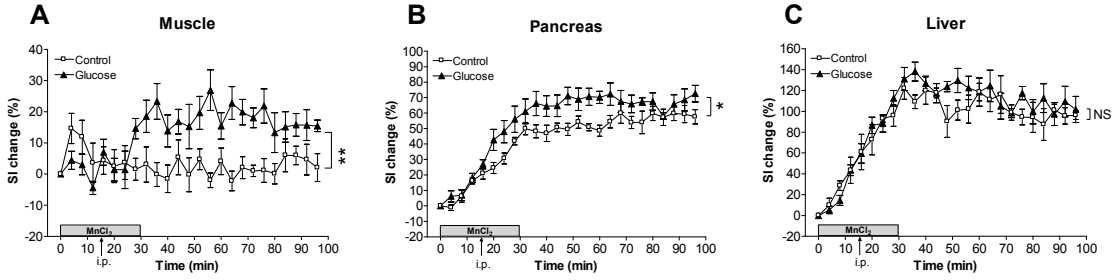

Figure 1 The percentage SI change in A) muscle, B) pancreas and C) liver, acquired by MP-RAGE MRI following infusion of $MnCl_2$ and i.p. bolus injection of glucose (2 g/kg) or water (control). The grey bar indicates the start and duration of the i.v. $MnCl_2$ infusion. The arrow indicates the timing of the bolus i.p. injection of glucose or water at 16 min; n = 6/group; Data is presented as mean ± sem; Data analyzed by GEE; * = $p < 0.05$, ** = $p < 0.01$, NS = not significant.