ECTOPIC EXPRESSION OF WNT10B DECREASES ADIPOSITY AND IMPROVES GLUCOSE HOMEOSTASIS IN OBESE RATS

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Introduction: Wnt10b is a secreted glycoprotein is that activates Wnt/β-catenin signaling to regulate diverse developmental processes and adult tissue homeostasis. Activation of Wnt10b signaling in adipose tissue inhibits development of adipocytes, and transgenic mice are resistant to diet-induced and genetic obesity, and have improved insulin sensitivity and glucose homeostasis. The purpose of this study was to deliver Wnt10b cDNA into female rats via a viral vector and investigate the long-term metabolic effects of Wnt10b expression during diet-induced obesity (DIO). MRI and *in vivo* magnetic resonance spectroscopy (MRS) methods were used to determine whether increased expression of Wnt10b by gene transfer results in a decrease in both global and local muscular fat deposits in DIO rats.

Experimental: A recombinant adeno-associated virus (rAAV1-Wnt10b) was generated and administered in a single intramuscularly injection (10^{12} viral particles). Global body composition was determined using **MRI** (4.7-T; Bruker Avance) using a custom built quadrature transmit/receive birdcage coil with 11.4 cm inner diameter and 14 cm length. Respiratory-gated 3D images were acquired from Wnt10b treated, DIO, and diet-restistant (DR) rats using a fast spin echo imaging (FSE) sequence. Data were processed using 3D-Doctor (Able Software Corp). Muscle levels of total creatine (TCr), intramyocellular (IMCL), and extramyocellular (EMCL) lipids were measured in both the tibilias anterior (TA) and soleus muscles of Wnt treated, DIO, and DR rats using localized MRS (PRESS) at 11.1-T (Bruker Avance). All MRS measurements were made on a single rat hindlimb, using a custom-made quadrature transmit receive birdcage coil with 3.8 cm inner diameter and single tuned to 470.5 MHz. High-resolution, T_2 weighted multiplanar FSE images were acquired for the precise localization of a volume of interest (VOI) within the TA or soleus muscles. Magnetic field homogeneity (<50 Hz) was optimized over the VOI and water suppression was optimized using chemical shift-selective (CHESS) water suppression. TCR, IMCL, and EMCL levels were either determined using Xwin-nmr (Bruker BioSpin) or with jMRUI (http:/mrui.uab.es/mrui) using the AMARES algorithm. Metabolite chemical shifts were assigned based on a chemical shift of 3.05 ppm for TCr, and 1.3, 1.5 ppm for IMCL and EMCL, respectively. In addition, TCr content was also determined based on the ratio of TCR peak are to the total proton density based on an unsuppressed PRESS spectrum from the same volume. Extensive *in vitro* phantom work and *in vivo* imaging of VOIs confirmed the accuracy of the VOI placement.

Results and Discussion: rAAV1-Wnt10b was found to inhibited adipogenesis in 3T3-L1 preadipocytes. Rats on a high-fat diet and infected with rAAV1-Wnt10b showed a sustained reduction in body weight compared to controls, and expression of Wnt10b was accompanied by a reduction in hyperinsulinemia and triglycerides plasma levels. MRI and MRS revealed that the ectopic rAAV expression of Wnt10b resulted in a decrease in both global and local muscular fat deposits in DIO rats. In addition, decreased levels of IMCL in skeletal muscles measured by volume localized proton MRS correlated with improved glucose tolerance.

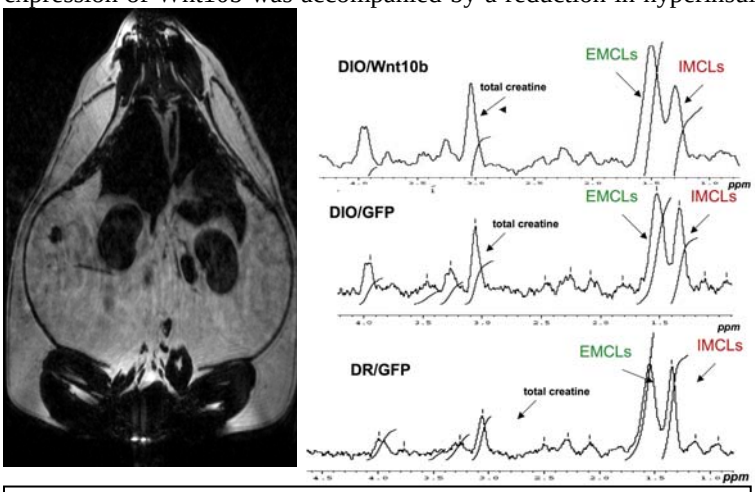

Figure 1: (**Left**) Single Slice from a 3D respiratory gated MRI of an DIO rat. (**Right**) Representative water suppressed spectra from DIO/Wnt10b, DIO/GFP, and DR/GFP muscle

Conclusions: The study demonstrates a potential therapeutic effect of ectopically expressed Wnt10b on adiposity and insulin sensitivity and the ability of MR techniques to follow changes in adiposity and glucose homeostasis *in vivo*. In addition the high magnetic field and large free bore size permits of the 11.1T allows for the acquisition of well-resolved *in vivo* ^{1}H spectra from small VOIs in large rodent muscles.

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