AGAT-/- mice: a metabolic puzzle of energy deficiency and insulin sensitivity

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Introduction: Arginine: glycine amidinotransferase (AGAT, EC 2.1.4.1), catalyzes the first and rate-limiting step of creatine (Cr) biosynthesis. Its phosphorylated form (PCr) is the main reservoir of energy and is rapidly recruited to secure ATP needs in tissues with high or fluctuating energy demand, like brain and muscle. Scarce uptake of Cr from diet matched with deficient AGAT expression causes several disarrangements in brain and muscle [1, 2]. Interestingly, AGAT $^{-/-}$ mice maintained on a Cr free diet have reduced body weight despite a doubled food intake, compared to their wild-type controls (WT). We hypothesized that AGAT $^{-/-}$ have enhanced substrate catabolism, possibly due to improved insulin response. The role of Cr in metabolism of glucose and lipids which are critical substrates in the (pre-) diabetic state has not been studied. Therefore, we assessed whole body glucose disposal and insulin production. Moreover, we measured the hepatic triglyceride (HTG) pool and estimated its synthesis due to dietary fatty acids and newly synthesized triglycerides, i.e. *de novo Lipogenesis* (DNL) by ^{1}H and ^{2}H NMR spectroscopy analyses of liver tissue.

Materials and methods: AGAT$^{-/-}$ and WT mice were kept for 5 months in Cr free diet, *ad libitum*, until the complete depletion of Cr/PCr levels. *In vivo* ^{31}P MRS were acquired at 7T (SMIS, Surrey, UK). *I.p. Glucose tolerance test (IPGTT) and insulin production*: Glucose (1.5 g/Kg body weight) was dissolved in NaCl 0.9% (w/v) and injected intraperitoneally. Blood samples were withdrawn from tail vein and glucose was quantified before and 15, 30, 60, 90 and 120 minutes after the bolus injection. Insulin levels were determined at 0, 15, 60 and 120 minutes after the glucose bolus by ELISA (Millipore, USA). *Hepatic DNL.* Mice were injected with $^{2}H_2O$ to reach 3% enrichment of body water. Enrichment was maintained overnight with 3% $^{2}H_2O$ drinking water. HTG were extracted from frozen livers by Folch method, dried with N_2 (g) and dissolved in chloroform prior to the NMR analysis. NMR spectra were acquired at 11.7T (Brucker Instruments, Billerica, MA). Proton decoupling for ^{2}H-NMR spectroscopy was performed using a WALTZ-16 pulse sequence.^{2}H enrichment of the body water was determined by proton ^{2}H decoupled spectra with 32 transients, acquired at 25ºC without field frequency lock, with a 25º pulse, 2s acquisition and 8s as pulse delay [3]. ^{2}H enrichment in HTG pool was determined by the previous NMR protocol, though with a 90º pulse, 2s acquisition and 2s pulse delay. All spectra were processed with MestreNova (MestreLab research, Spain) PC program. Total HTG and DNL fraction were quantified by ^{1}H and ^{2}H-NMR against Pyrazine/$(^{2}H)_4$Pyrazine mixture as internal reference [4]. The fraction of HTG derived from DNL was estimated as the HTG ^{2}H-methyl/body water ^{2}H enrichment. Dietary contribution for HTG pool (%) is 100-DNL, assuming no other endogenous source of HTG.

Results & Discussion: After five months on a Cr free diet the muscles of AGAT-/- were completely devoid of Cr and PCr (Fig. 1). AGAT$^{-/-}$ mice had lower body weight (19.3±2.2 g *vs* 33.9±5.6 g, p<0.0001), and lower blood glucose levels after glucose load, (889±59 *vs* 1396±89 area under the curve, p<0.0001) compared to WT mice **(**Fig.2 A). The normal pancreatic response to the glucose load is an increase in insulin production, but in AGAT $^{-/-}$ mice this production was significantly lower than in WT mice, (34.0±3.7 *vs* 65.5±5.9, area under the curve, p<0.01) consistent with AGAT$^{-/-}$ mice being more insulin sensitive (Fig.2 B). The ^{1}H spectra of the hepatic lipid extracts showed that AGAT$^{-/-}$ mice had a lower HTG pool compared the respective WT controls (106±19 µmol/gww *vs* 195±20 µmol/gww, p=0.03). The Cr depletion in AGAT$^{-/-}$ mice doubled DNL contribution to the HTG pool, compared to WT mice (16.9±2.7 % *vs* 7.1±1.2 %, p=0.002, Fig.3 A and B). Hence, dietary fatty acids contributed to HTG pool with 83.1±2.7 % *vs* 92.9±1.2 %, in AGAT $^{-/-}$ and WT mice, respectively.

Conclusion: The data demonstrate that AGAT $^{-/-}$ have an insulin sensitive phenotype, and an increased DNL contribution to a reduced HTG pool. Dietary fatty acids contribute less to HTG, suggesting a re-direction of dietary fatty acids to high energy demanding tissues as muscle. Thus Cr/PCr seems to play a role in insulin sensitivity and related substrate metabolism, which calls for further investigation into the exact mechanism of action.

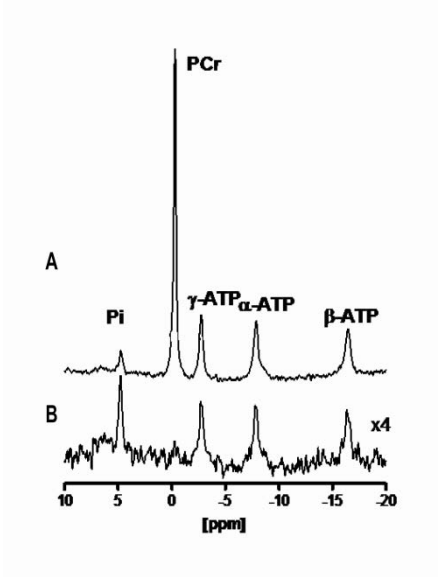

Figure 1 ^{31}P of muscle of WT (A) and AGAT$^{-/-}$ (B) after five months of creatine free diet.

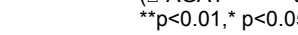

Figure 2 Plasma glucose levels (A) and insulin response (B) during an ipGTT. (□-AGAT $^{-/-}$ and ■-WT) ***p<0.001, **p<0.01,* p<0.05.

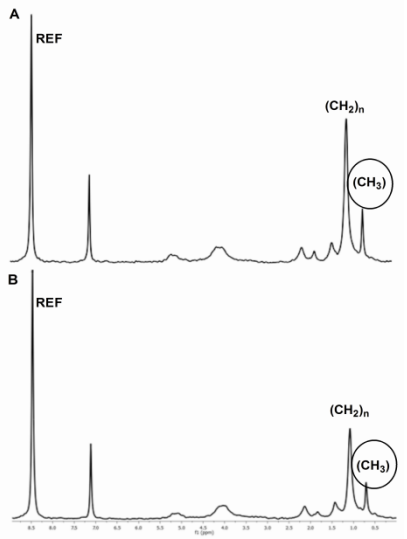

Figure 3 ^{2}H-NMR spectra of HTG of AGAT$^{-/-}$ (A) and WT (B). Fraction of HTG derived from DNL was determined by ^{2}H-enrichment of $CH_3/^{2}H$ enrichment in body water. REF-Pyrazine $(^{2}H)_4$ reference.

References [1] Item CM *et al*., Am J Hum Gen (69) 2001. [2] Nabuurs CIHC *et al*., abstract s1437and 2676 ISMRM meeting, 2009. [3] Jones JG *et al*., Magn Res Med (45) 2001. [4] Delgado TC *et al*., NMR Biomed (22) 2009.

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