The *PNPLA3* I148M variant increases intrahepatic lipolysis and beta oxidation and decreases *de novo* lipogenesis and hepatic mitochondrial function in humans

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INTRODUCTION

The *PNPLA3* I148M variant at rs738409 is the strongest genetic risk factor of NAFLD/NASH [1] but the underlying pathophysiology remains unclear.

NASH is associated with hepatic mitochondrial dysfunction [2].

Mitochondria are key organelles in the catabolism (*i.e.* beta oxidation, ketogenesis and tricarboxylic acid (TCA) cycle oxidation) and anabolism (*i.e. de novo* lipogenesis, DNL) of fatty acids [3].

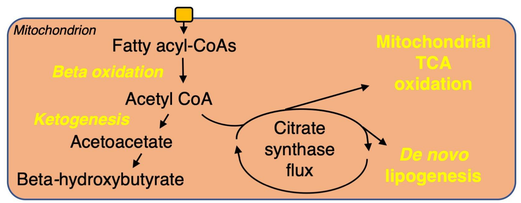

Figure 1. *Pathways of fatty acid metabolism in hepatic mitochondria.*

AIM

To study the effect of the *PNPLA3* I148M on intrahepatic metabolism *in vivo* in humans under multiple physiological conditions by combining a recall-by-genotype approach with state-of-the-art stable isotope techniques.

We hypothesize that the PNPLA3 I148M will cause hepatic mitochondrial dysfunction.

METHODS

93 healthy participants; 37 homozygous carriers (*148MM*) and 56 non-carriers (*Control*) of the *PNPLA3* I148M variant.

Hepatic DNL was assessed after an overnight fast using D_2O [4].

Hepatic fate of exogenous fatty acids (FA) was determined using a mixed meal enriched in ^{13}C-labeled FA [5].

Hepatic mitochondrial citrate synthase flux was assessed before and after a 6-day ketogenic diet by Positional Isotopomer NMR Tracer Analysis (PINTA) by infusing [3-^{13}C]-lactate and [$^2H_7$]-glucose [6].

Intrahepatic triglyceride content was assessed by ^{1}H magnetic resonance spectroscopy [4 5 6].

Hepatic mitochondrial redox state was assessed by the ratio of plasma [BOHB] and [acetoacetate] [4 6].

RESULTS

Overnight fast study

	Control	148MM
Participants (n)	36	19
Age (years)	50.6 ± 1.0	52.5 ± 1.8
Sex (women/men)	28/8	16/3
Body mass index (kg/m^2)	28.2 ± 0.8	28.5 ± 1.4
Intrahepatic triglycerides (%)	3.2 ± 0.4	10.1 ± 2.3 **

Increased ketogenesis from endogenous fatty acids in 148MM

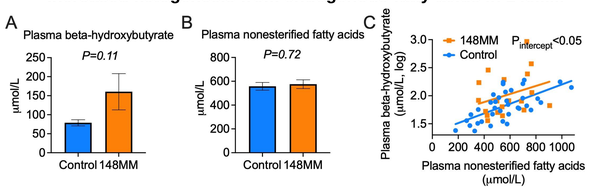

Figure 2. *Plasma (**A**) beta-hydroxybutyrate and (**B**) nonesterified fatty acids and (**C**) a linear regression between the two variables in the groups.*

Decreased *de novo* lipogenesis in 148MM

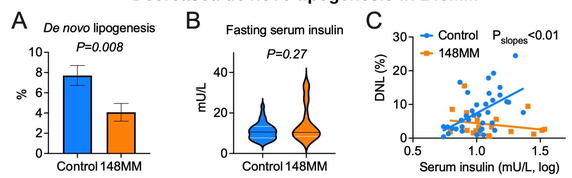

Figure 3. *(**A**) Hepatic DNL, (**B**) fasting serum insulin and (**C**) a linear regression between the two variables in the groups.*

Mixed meal study

	Control	148MM
Participants (n)	14	12
Age (years)	52.4 ± 1.8	53.1 ± 2.2
Sex (women/men)	11/3	10/2
Body mass index (kg/m^2)	31.8 ± 1.5	31.8 ± 2.0
Fasting serum insulin (mU/L)	6.2 (3.6 – 8.7)	6.0 (4.5 – 11.2)
Intrahepatic triglycerides (%)	1.9 (1.0 – 6.6)	6.3 (4.8 – 12.7) *

Increased ketogenesis from exogenous fatty acids in 148MM

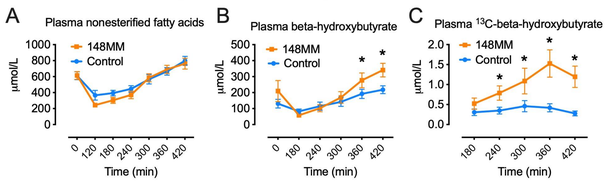

Figure 4. *Plasma (**A**) nonesterified fatty acids, (**B**) beta-hydroxybutyrate and (**C**) ^{13}C-beta-hydroxybutyrate concentrations in the groups after a mixed meal. * P<0.05 by t test.*

RESULTS

Ketogenic diet study

	Control	148MM
Participants (n)	6	6
Age (years)	56.2 ± 3.5	60.7 ± 1.6
Sex (women/men)	4/2	6/0
Body mass index (kg/m^2)	31.1 ± 2.3	32.6 ± 2.7
Fasting serum insulin (mU/L)	9.7 (9.5 – 10.7)	8.1 (7.0 – 11.5)

Increased intrahepatic lipolysis during a ketogenic diet in 148MM

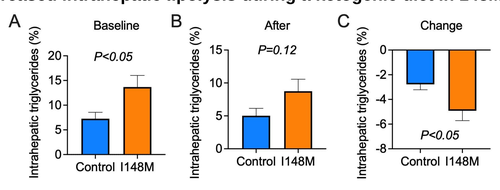

Figure 5. *Intrahepatic triglycerides (**A**) at baseline and (**B**) after a 6-day ketogenic diet and (**C**) change in intrahepatic triglycerides in the groups.*

Increased ketogenesis during a ketogenic diet in 148MM

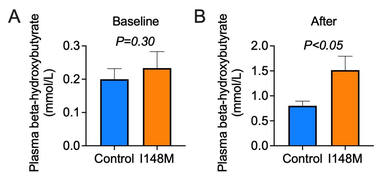

Figure 6. *Plasma beta-hydroxybutyrate concentrations (**A**) at baseline and (**B**) after a 6-day ketogenic diet in the groups.*

Decreased hepatic mitochondrial citrate synthase flux in 148MM

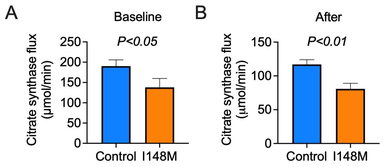

Figure 7. *Hepatic mitochondrial citrate synthase flux as assessed by PINTA (**A**) at baseline and (**B**) after a 6-day ketogenic diet.*

Increased mitochondrial stress markers in 148MM

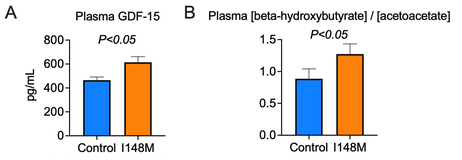

Figure 8. *Plasma (**A**) GDF-15 and (**B**) hepatic mitochondrial redox state, as determined by plasma beta-hydroxybutyrate-to-acetoacetate ratio, in the groups.*

CONCLUSIONS

Homozygous *PNPLA3* I148M carriers have alterations in both **intrahepatic anabolic/catabolic processes** and **mitochondrial function** as reflected by:

1) *increased rates of intrahepatic lipolysis*,

2) *decreased rates of DNL* and

3) *increased hepatic mitochondrial beta oxidation/ketogenesis*.

These changes were associated with an *increased mitochondrial redox state* and *a decreased hepatic mitochondrial citrate synthase flux*.

These results provide new insights in the mechanisms by which the *PNPLA3* I148M variant promotes liver disease.

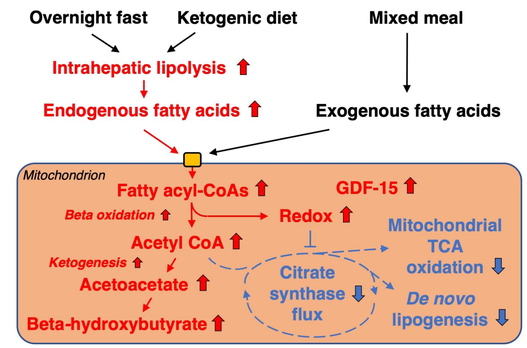

Figure 9. *Working model of the intrahepatic effects of the PNPLA3 I148M.*

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