Loss of OCT1 function lowered liver fat content in humans: Sex dependent effects and potential molecular mechanisms

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Background

NAFLD

The term non-alcoholic fatty liver disease (NAFLD) describes steatosis of hepatocytes without excessive alcohol consumption. Newest research establishes it as the cause of a range of diseases. Specific treatment is lacking until this day.[1]

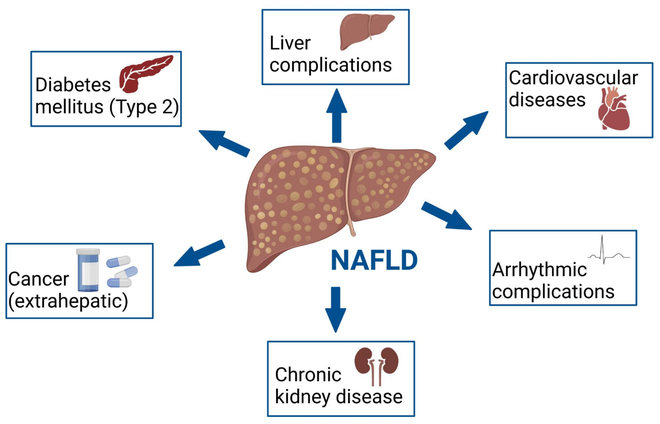

Figure 1. Diseases caused by NAFLD. (Created with BioRender)

OCT1

Organic cation transporter 1 (OCT1) is a polyspecific hepatic cation transporter. It transports commonly used drugs (e.g. metformin, sumatriptan and tramadol) as well as thiamine (vit. B1).[2] OCT1 is genetically highly variable with common alleles leading to a loss of OCT1 function.[3]

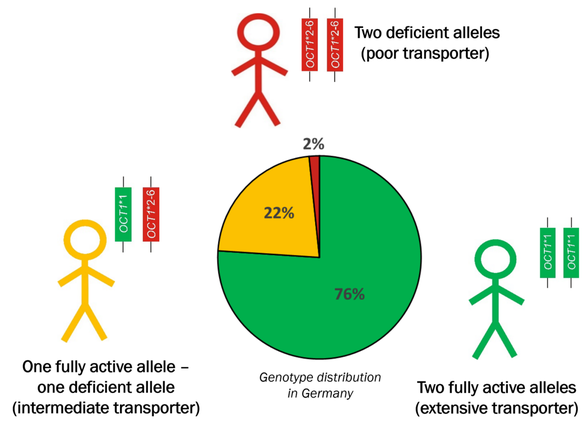

Figure 2. Distribution of OCT1 genotypes in German population.

OCT1 and NAFLD

OCT1 has been found to influence liver fat content in mice. Oct1 knock-out mice showed lower liver weight that was caused by a lower content of triglycerides in the liver.[4]

Aims

The aim of this study was to transfer findings in mice to humans by determining whether genetically determined loss of OCT1 function influenced liver fat content in humans. Further research focuses on revealing the molecular mechanisms that are influenced by OCT1.

Methods

We took advantage of the existing data of the Study of Health in Pomerania (SHIP). 2512 datasets of whole-body MRI were used, allowing us to quantify liver fat content. The OCT1 genotype was imputed using genome-wide SNP array data. We applied non-parametric tests and regression analysis to determine the influence of genetically determined OCT1 deficiency on liver fat content.

For analysis of molecular mechanisms, we used Human embryonic kidney (HEK293) cells overexpressing human OCT1.

Transferring findings in mice to men

Forty percent of the population had mild to severe hepatosteatosis with sex specific differences

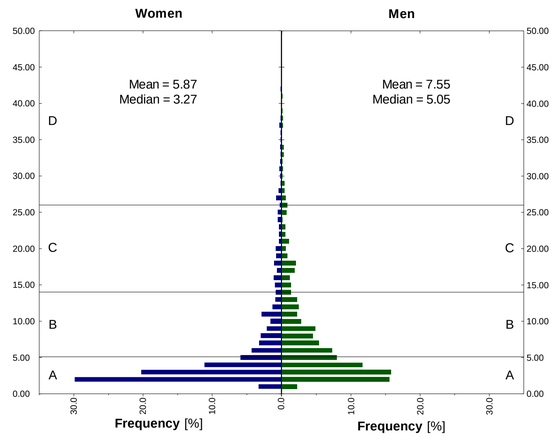

Figure 3. Histogram of liver fat content in study population by sex.

OCT1 deficiency influenced liver fat content with a bordering significance

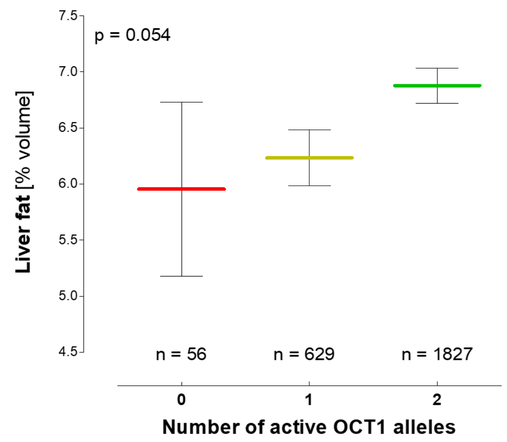

Figure 4. Liver fat content by number of active OCT1 alleles. (mean ± SEM | n.s.: p>0.05 | Jonckheere-Terpstra-test to account for trends between OCT1 genotypes)

Loss of OCT1 function lowered liver fat content in women

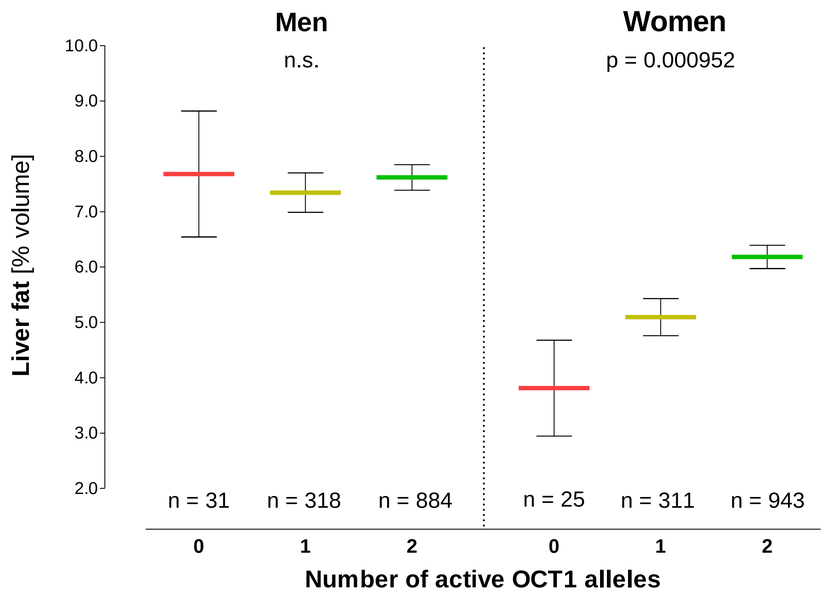

Figure 5. Liver fat content by number of active OCT1 alleles, stratified by gender. (mean ± SEM | n.s.: p>0.05 | Jonckheere-Terpstra-test to account for trends between OCT1 genotypes)

Linear model of predictors of liver fat content

Transformation of liver fat content by natural logarithm

Parameter	b	SE b	p
Constant	-1.401	0.110	<0.001
Age	0.009	0.001	<0.001
BMI	0.093	0.003	<0.001
Alcohol consumption	0.005	0.001	<0.001
Sex	-0.174	0.028	<0.001
Number of active OCT1 alleles	0.070	0.026	0.006

R^2 = 0.363 for the model with all known risk factors
ΔR^2 = 0.002 for model after addition of OCT1 (p = 0.006)
p < 0.001 for both models

Revealing molecular mechanisms

OCT1 expressing cells had a more energetic, mitochondrial based metabolic phenotype under glucose admission

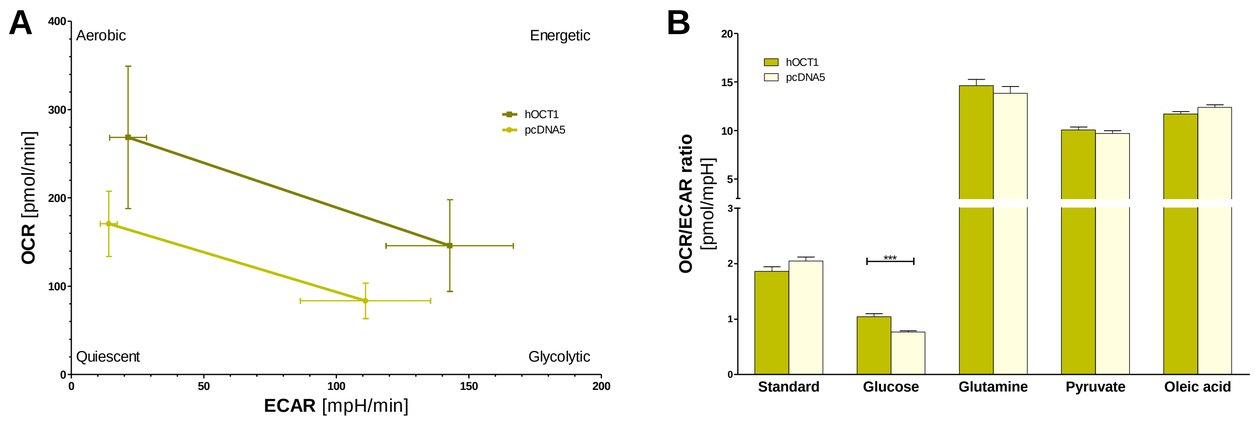

Figure 6. Metabolic phenotype of OCT1 over-expressing HEK293 cells and HEK293 cells expressing only the control vector (pcDNA5). We used *Agilent Seahorse* technology to assess oxygen consumption rate (OCR) and extracellular acidification (ECAR). **A –** OCR by ECAR in medium without any energy sources (left) and after admission of glucose (right). **B –** OCR to ECAR ratio in medium with different supplements as only energy source or standard medium containing all energy sources.
(mean ± SEM | *** p<0.0001 | n=2-3 | unpaired t-Test)

OCT1 expressing cells showed a shift in the metabolism of branched amino acids by a more efficient degradation of valine

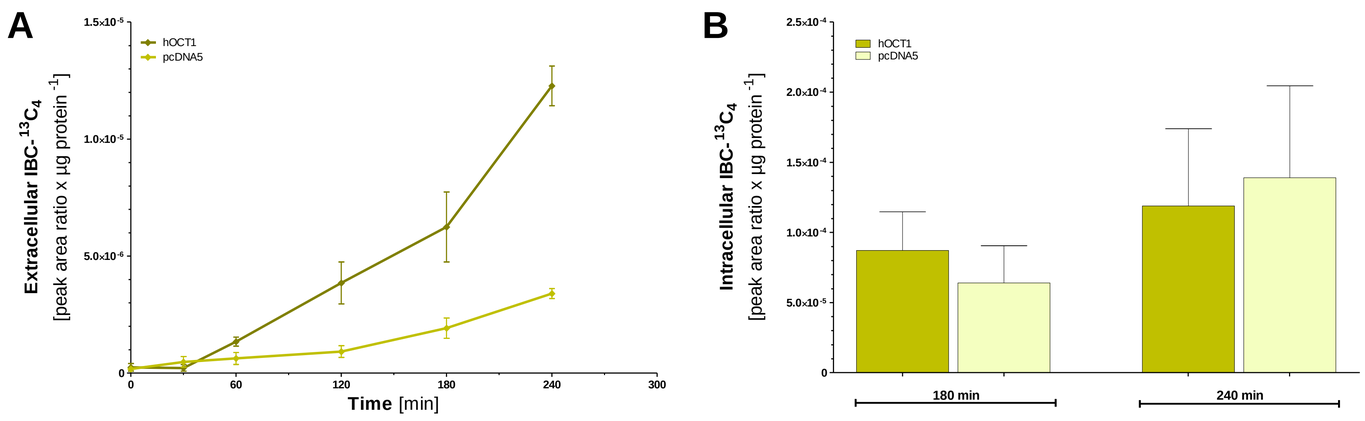

Figure 7. Accumulation of Isobutryl-Carnitine-$^{13}C_4$ (IBC-$^{13}C_4$) in OCT1 over-expressing HEK cells and HEK cells expressing only the control vector (pcDNA5). IBC-$^{13}C_4$ was formed from incubation with valine-$^{13}C_5$. **A –** Extracellular accumulation of IBC-$^{13}C_4$ over time. **B –** Intracellular accumulation of IBC-$^{13}C_4$ after 180 min and 240 min.
(mean ± SEM | n=3)

Conclusion

- Genetically determined OCT1 deficiency was **associated with lower fat content**
- This association **was sex dependent: strong in women and absent in men.**
- OCT1 expressing cells had a more **mitochondrial based metabolism.**
- **OCT1 influenced the degradation of valine.**

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