Eindrapportage ‘Metabolic effects of ketohexokinase inhibition on individuals with non-alcoholic fatty liver disease’ NL80131.068.22 / METC22-006

Name of Sponsor/Company: Institute of Nutrition and Translation Research in Metabolism (NUTRIM), Maastricht University	**Individual Study Table Referring to Part of the Dossier** N.A.	
Name of Finished Product: PF-06835919		
Name of Active Ingredient: PF-06835919		

Title of Study:
Metabolic effects of ketohexokinase inhibition on individuals with non-alcoholic fatty liver disease

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Publication (reference):
Results are not yet published

Studied period (years): June 2022-November 2023	**Phase of development:** Phase IIa

Objectives:

Primary Objective:

To assess the effect of ketohexokinase inhibition (KHKi) on hepatic insulin sensitivity in overweight/obese individuals with non-alcohol fatty liver disease.

Secondary Objective: To assess the effect of KHKi on fat distribution, adipose tissue insulin sensitivity, and fat oxidation in overweight/obese individuals with non-alcoholic fatty liver disease.

Explorative Objectives:

To explain the incomplete urinary fructose excretion upon KHKi treatment:

- To assess in vivo ketohexokinase (KHK) activity upon KHKi in overweight/obese individuals with non-alcoholic fatty liver disease.
- To assess the contribution of alternative metabolic pathways upon KHKi.
- To assess the involvement of gut microbiota upon KHKi in overweight/obese individuals with non-alcoholic fatty liver disease.

Methodology:

In this double-blind placebo-controlled cross-over trial fifteen males and females were treated with PF-06835919 (300 mg/daily) and placebo for 6 weeks, divided by a 5-week wash-out period. At baseline, habitual daily fructose was calculated, and supplemented to 60 g/day with sugar sweetened beverages (SSB) during the treatment periods, to ensure a sufficient effect size. The participants started consuming these SSB's already one week before the start of the medication during the run-in period. A 2-step hyperinsulinemic-euglycemic clamp (10 vs. 40 mU/m^2/min) with D-[6,6-$^2H_2$]-glucose tracer infusion was performed to assess hepatic and peripheral insulin sensitivity. Indirect calorimetry (ventilated hood and respiration chamber) was used to determine substrate oxidation rates during the clamp and during the night. Intrahepatic lipid (IHL) content and composition was quantified with ^{1}H-magnetic resonance spectroscopy (MRS). *In vivo* fructose metabolism was measured with ^{31}P-MRS and visceral and subcutaneous adipose tissue volume was measured with MRI. 24-hour urine and faecal samples were collected, body composition was assessed (BodPod) and a muscle biopsy was taken. All measurements were done at day 41 and 41 and were identical for both periods.

Number of patients (planned and analysed):

Planned: 17 (incl. drop-out rate)

Analyzed: 15

Diagnosis and main criteria for inclusion:

Inclusion criteria:

- Participants are able to provide signed and dated written informed consent prior to any study specific procedures
- Men and (postmenopausal) woman
- Aged ≥ 40 and ≤ 75 years
- Body mass index (BMI) 27 – 38 kg/m2
- Hepatic steatosis (i.e. IHL ≥ 5.56%)
- Stable dietary habits (no weight loss or gain > 3 kg in the past 3 months)
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Exclusion criteria:

- Type 2 diabetes
- Patients with congestive heart failure and and/or severe renal and or liver insufficiency
- Uncontrolled hypertension
- Any contra-indication for MRI scanning

- Alcohol consumption of >3 servings per day for man and >2 servings per day for woman
- Smoking
- Unstable body weight (weight gain or loss > 3kg in the last 3 months)
- Engagement in structured exercise activities > 2 hours a week
- Previous enrolment in a clinical study with an investigational product during the last 3 months or as judged by the investigator which would possibly hamper our study results
- Use of drugs that inhibit organic anion transporting polypeptide (OATP) transporters (e.g. rifampicin, gemfibrozil, cyclosporine, erythromcyin and clarithromycin)
- Subjects who do not want to be informed about unexpected medical findings

Test product, dose and mode of administration, batch number:
PF-06835919 (100 mg/tablet), 300 mg (3 tablets) per day, once a day in the morning, oral tablets 22-BU-00333

Duration of treatment:
6-weeks

Reference therapy, dose and mode of administration, batch number:
Placebo, 100 mg/tablet, 3 tablets daily, once in the morning, oral, 22-BU-00333

Criteria for evaluation:
Compliance was checked by counting the returned tablets and urinary fructose excretion.

Participants were asked to maintain their habitual diet and regular physical activity pattern. Three days before the test days, participants were instructed to refrain from strenuous activities and to continue the medication with the last dose on the morning of day 42. Body weight was monitored during the treatment period. In week 1 and week 5 of the treatment, a telephonic meeting was planned, to check compliance, general well being and side-effects. During week 3 of the treatment period an intermediate evaluation visit was planned, during which several safety measurements were performed, and compliance and side-effects were checked. Body composition was determined to check for diet induced weight changes and changes in physical activity levels were checked by a questionaire after the intervention.

Statistical methods:
Results are presented as mean ± SEM when normally distributed and median (95% CI) when not-normally distributed using Shapiro-Wilk normality test. The intervention effect was analyzed using the paired Wilcoxon signed-rank test. Statistics were performed using SPSS 24.0 and GraphPad Prism, and a two-sided $p<0.05$ was considered statistically significant.

Summary - Conclusions

Compliance:

Tablet counting and analysis of urinary fructose excretion after 6-weeks of KHKi compared to placebo indicated excellent compliance. Participants remained weight stable (difference: 0.37 kg; 95% CI: -0.12, 0.83; p=0.12) between both treatment arms and body composition did not also not change (fat % difference: 0.45 %; 95% CI: -0.3, 1.2; p=0.28). Urinary fructose excretion increased 100 fold compared to placebo. The rise in phosphomonoesters after a 60 g oral fructose load, observed after placebo treatment was completely abolished after PF-06835919 treatment, indicating effective suppression of KHK-mediated fructose metabolism (difference iAUC placebo vs pf-06835919 p<0.001).

Safety Results:

No adverse events were reported based on the blood and urine safety analyses. Reported (side-)effects during PF-06835919 were all mild and transient, and included flatulence/bloating (n=3), change in bowel movement (n=3), flu-like symptoms (n=1), abdominal pain (n=1) and muscle cramps (n=1), which were also partly reported during placebo treatment. No serious adverse events were reported related to the treatment with PF-06835919

Efficacy

Intrahepatic lipid content was lower after PF-06835919 versus placebo (absolute difference: -2.5%; 95% CI: -3.3, -1.8; p<0.001) and, the percentage of saturated fatty acids (SFA) of IHL also decreased (absolute difference: -2.9%; 95% CI: 1.3, 4.5; p=0.002). Despite the beneficial effect on IHL, hepatic insulin sensitivity was not statistically significantly changed after KHK inhibitor treatment compared to placebo, although ten out of fifteen participants showed an increased EGP suppression (difference: 6.0%; 95% CI: -3.4, 15.4; p=0.156). Adipose tissue insulin sensitivity was higher after PF-06835919 treatment compared to placebo (difference: 7.3%; 95% CI: 2.0, 12.0; p=0.02). Additionally, peripheral insulin sensitivity was significantly higher (p=0.002) after 6 weeks of PF-06835919 (mean: 21.4 μmol/kg/min) as compared to placebo (mean: 17.4 μmol/kg/min). These results show that six weeks of KHK inhibition suppresses intrahepatic fructose metabolism, and reduces IHL content and the SFA fraction of liver fat. These changes were accompanied by improvements in peripheral and adipose tissue insulin sensitivity, although the change in hepatic insulin sensitivity did not reach statistical significance. These findings suggest a the detrimental role for KHK-dependent fructose metabolism per se on metabolic health.

Date of report

04/11/24