# Fibroblast Growth Factor-21 and its Relationship to Human Liver Fat Synthesis; Impact of a New Therapeutic Agent

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## ABSTRACT

FGF21 is a newly-discovered hormone linked to fat stored in the liver. The present human study tested a new drug to block liver fat synthesis to determine whether FGF21 would also be reduced with treatment.
Male subjects (n=12, age 34-56y, BMI 31.4 - 44.0 kg/m$^2$) were tested before (PRE) and after (POST) 10d of drug treatment (50 mg/d, n=6; 100 mg/d, n=4; 150 mg/d, n=2). Fat synthesis was quantitated using a stable-isotope labeled, oral-fructose tolerance test (OFTT). Blood was drawn to assess concentrations of lipids, hormones, plasma FGF21, and liver fat measured by MRI. FGF21 concentrations were stimulated after the OFTT. Peak plasma drug levels correlated with PRE-POST changes in both fasting (r=0.60, *P*=0.040) and peak FGF21 (r=0.75, *P*=0.005). After treatment at all drug dosages, liver fat synthesis was reduced 0-90% in the fasting state (*P*=0.003) and 14-85% at peak after the OFTT, (*P*=0.0004). The greater the drug effects on peak FGF21, the more liver fat synthesis was inhibited (r=0.64, *P*=0.024). Lastly, absolute changes in peak FGF21 were related to greater absolute reductions in liver fat measured by MRI (r=0.61, *P*=0.035). These data suggest that increases in FGF21 following sugar consumption may represent a healthy metabolic response and that stimulation of liver lipid synthesis restrains the natural increase in FGF21 following sugars. Future studies are needed to determine whether there is a direct or indirect relationship between FGF21 and fat synthesis, both of which are elevated in subjects with fatty liver disease.

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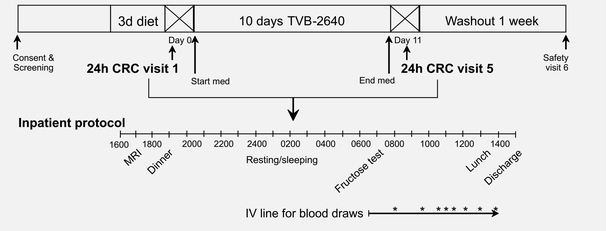
## BACKGROUND

Consumption of dietary sugars induces an increase in hepatic de novo lipogenesis (DNL), which promotes liver inflammation, ultimately leading to the development of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH). Similar to this, dietary sugars robustly increase serum FGF21 concentrations (Dushay JR. 2014, Maratos-Flier E. 2013, Soberg S. 2017, Soberg S. 2018, ). FGF21 is only secreted into circulation by the liver and elevated levels have been linked to humans with metabolic syndrome characteristics (Xinmei Z. 2008), however, its functions have not been clearly identified. In animal models, FGF21 has been shown to be a positive regulator of lipid synthesis (Fisher FM. 2016), improve glycemic control (Emanuelli B. 2014, Wang N 2018, Bernardo B. 2015), and exogenous treatment has improved the lipid profile (Talukdar S. 2016) and weight status (Talukdar S. 2016, Coskun K. 2008, Bernardo B. 2015). In high-fat, high-sugar diet-fed mice, pharmacologic inhibition of fatty acid synthase (FASN), a key enzyme in the DNL pathway, with a TVB-2640 analog prevents steatosis, inflammation, and fibrosis (3-V Biosciences 2017). In cancer patients, relatively high doses of the first-in-class FASN inhibitor, TVB-2640, reduced markers of DNL systemically (3VBio, FDA investigational drug application). The purpose of this study was to test the effect of TVB-2640 to reduce hepatic DNL in obese men with characteristics of the metabolic syndrome, to asses the relationship between FGF21 and TVB-2640, and to determine FGF21s role in lipid synthesis.

## METHODS

**Figure 1** is the study design. Male subjects with metabolic syndrome characteristics were consented and screened (n=12). All subjects were fed a 3-day, isocaloric diet, based on individual food records and energy requirements to maintain body weight, prior to drug treatment. A similar diet was continued during the 10-day drug treatment phase. Subjects completed a PRE and POST 24-hour, inpatient visit to measure, 1) hepatic DNL via $^{13}C_1$-acetate infusion and an oral sugars tolerance test (OSTT), followed by measurement of labeled palmitate in VLDL by GC/MS, 2) body composition via DEXA, 3) liver fat via MRI, MRS, and FibroScan™, and 4) FGF21 concentrations in fasting and following the OSTT via ELISA kit (BioVendor, #RD191108200R). The sugar content of the OSTT was based on the subject's weight with an average content of 109g fructose and 36g dextrose. The OSTT content was identical between PRE and POST. Throughout the 10 day treatment period, subjects had 3 safety visits to assess adverse, drug-related side effects. Six days following drug treatment cessation, subjects had a final safety visit. Statistical analysis was conducted using Statview and Excel.

**Figure 1. Study design**




## RESULTS

**Figure 2. Body weight and biochemical markers**

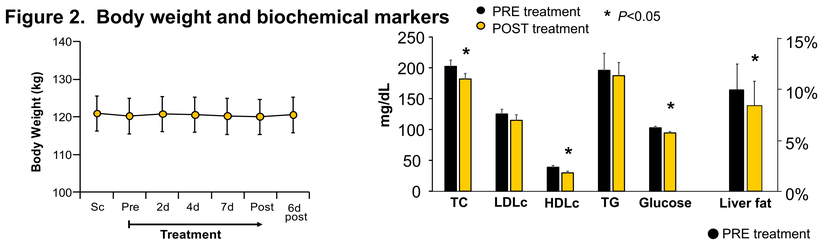



**Figure 3. Fractional DNL in VLDL-TG PRE and POST drug**

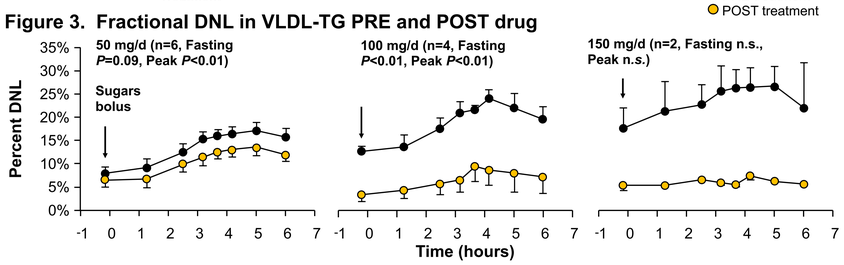



**Figure 4. FGF21 concentrations PRE and POST drug: Effect of sugar consumption**

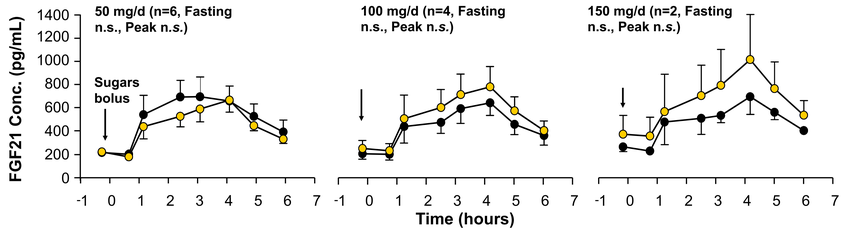



**Figure 5. Change in FGF21 and peak drug concentrations**

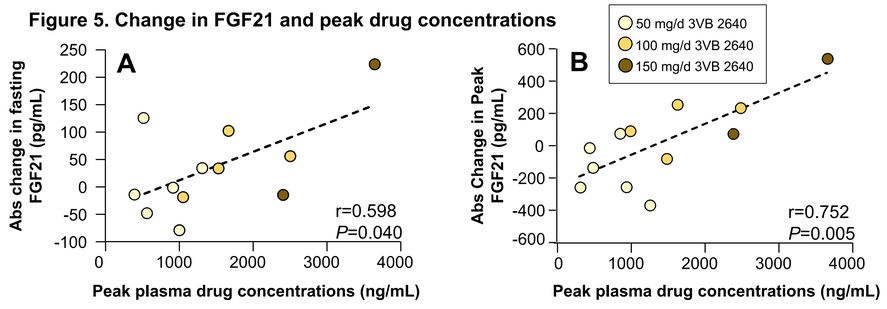



**Figure 6. Change in peak FGF21 concentrations and change in hepatic DNL and liver fat**

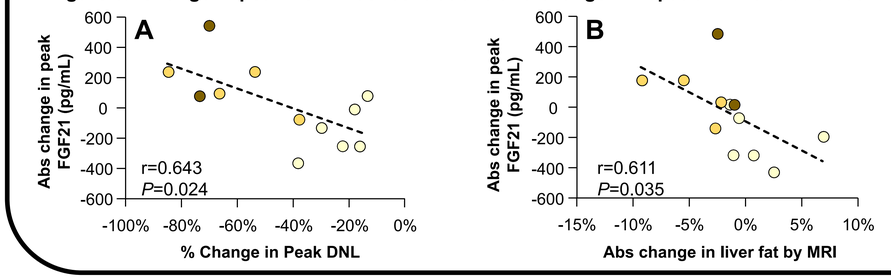



**Figure 7. Theoretical relationships between glycolytic flux and FGF21**

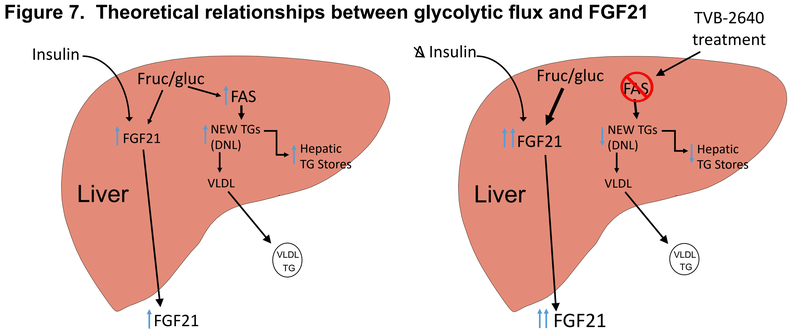
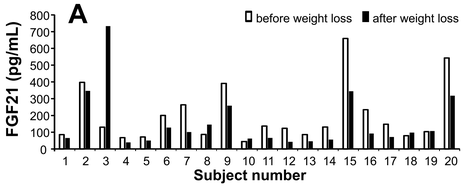
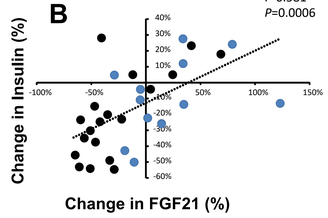



**Figure 8. Low-CHO diet study; 4-wks, CHO restriction of 600 kcal/d**

**Table 1. Metabolic changes**

| n=20, mean ±SEM | Baseline | 4 weeks | *P*-value |
|---|---|---|---|
| Weight (kg) | 102 ± 4 | 96 ± 3 | < 0.001 |
| Glucose (mg/dL) | 97 ± 2 | 91 ± 3 | 0.057 |
| Insulin (μU/mL) | 12.1 ± 1.1 | 8.9 ± 0.6 | 0.003 |
| FGF21 (pg/mL) | 203 ± 40 | 128 ± 24 | 0.002 |




## SUMMARY and CONCLUSIONS

- Subjects remained weight stable throughout the treatment (**Figure 2**) and cholesterol levels and liver fat were significantly reduced.
- **Figure 3.** Across all doses, fasting liver fat synthesis (de novo lipogenesis, DNL) was reduced from 0% to 90% (*P*=0.003), and peak DNL was reduced from 14-85% (*P*=0.0004).
- The oral sugars tolerance test (OSTT) acutely stimulated an increase in FGF21 concentrations but no significance was found for the drug's PRE-POST FGF21 concentrations (**Figure 4,** Fasting *P*=0.17, Peak *P*=0.80). At greater drug concentrations, the OSTT appeared to increase FGF21 concentrations, however, no significance was found in OSTT time-averaged FGF21 concentrations, likely due to small sample size (**Figure 4**).
- At lower drug doses, both fasting and peak FGF21 remained relatively consistent with slight decreases, while as drug doses increased, both fasting and peak FGF21 concentrations increased (**Figure 5A,** *P*=0.040 and **5B,** *P*=0.005).
- The greater the peak FGF21 concentrations the greater the reductions in DNL(**Figure 6A,** *P*=0.02). Additionally, increases in peak FGF21 were associated with reductions in liver fat (**Figure 6B,** *P*=0.03).
- As shown in **Figure 7**, the data can be interpreted as follows. Insulin is known to stimulate FGF21 production as does glycolytic flux. Drug treatment may result in diversion of carbons away from DNL, potentially increasing glycogenic and gluconeogenic fluxes.
- This theory was tested by measurement of FGF21 in a separate setting of dramatic reductions of glycolytic flux and increases in gluconoogenesis through dietary carbohydrate restriction and weight loss.
- Twenty men and women undergoing carbohydrate restriction-induced weight loss (**Table 1**) exhibited significant reductions in fasting insulin, glucose, and FGF21 (**Figure 8A**). Data from both studies were combined and show a strong relationship between changes in insulin and FGF21 (**Figure 8B**).

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