Supplementary materials for

Impaired Ketogenesis and Increased Acetyl-CoA Oxidation Promote Hyperglycemia in Human Fatty Liver

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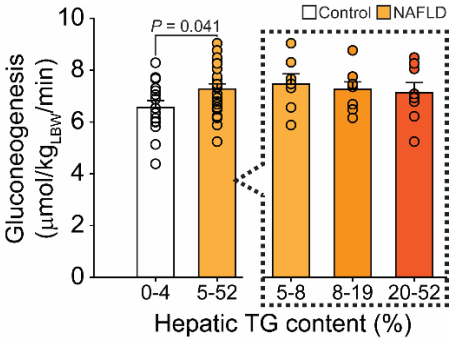

Figure S1. Gluconeogenesis. Rates of hepatic gluconeogenesis among control (N = 15) and NAFLD subjects (N = 23), broken out by tertiles of liver fat among the NAFLD population. *Note:* Glucose isotopic data was unavailable for two control subjects. Significance was determined using the two-tailed Student's *t*-test for unpaired data and two-way repeated measures ANOVA.

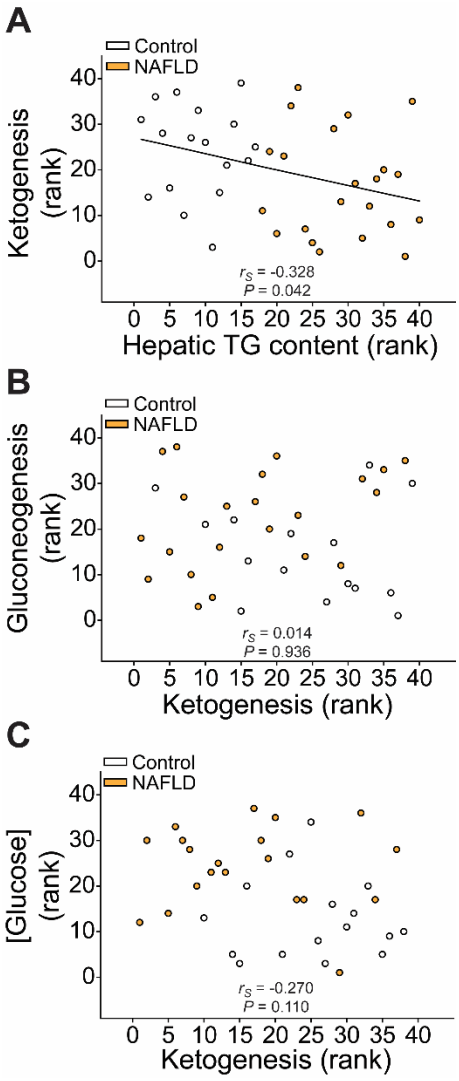

Figure S2. Correlations related to ketogenesis. (**A**) Ketogenesis was inversely correlated with hepatic TG content. Despite a prior report, there was no significant relationship between (**B**) gluconeogenesis or (**C**) plasma glucose concentrations and ketogenesis in the study population. *Note:* Ketone isotopic data was unavailable for two NAFLD subjects (N = 38). Strength and significance of correlations were determined by Spearman's rank order and scatter plots were therefore presented as ranked data. Local polynomial regression was used to fit a smooth curve between significantly correlated variables.

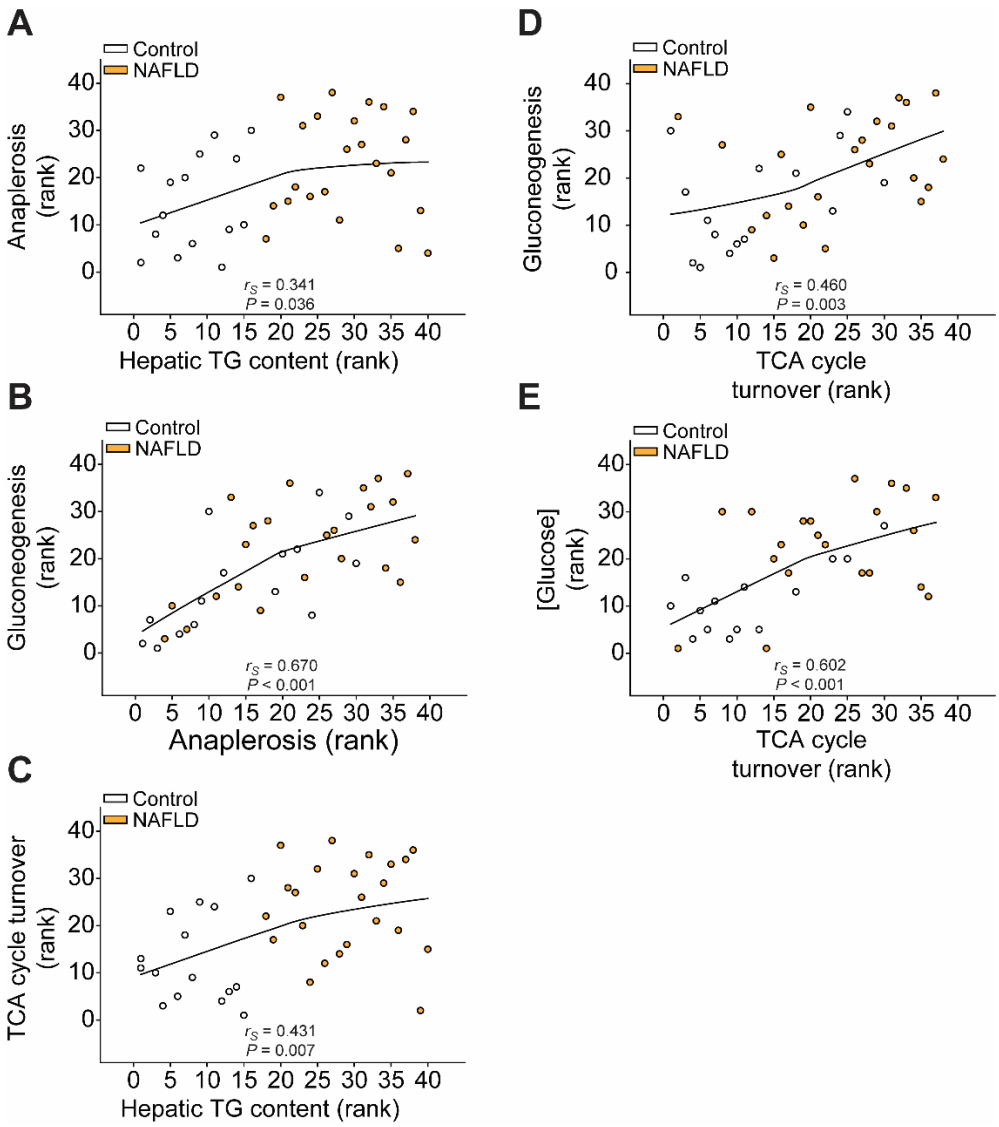

Figure S3. Correlations related to pathways centered on the TCA cycle. Anaplerosis, of which pyruvate carboxylase flux is a major component, was positively correlated with (**A**) hepatic TG content and (**B**) gluconeogenesis. The oxidation of hepatic acetyl-CoA in the TCA cycle was positively correlated with (**C**) hepatic TG content, (**D**) gluconeogenesis, and (**E**) plasma glucose concentrations (N = 38). *Note:* Glucose isotopic data was unavailable for two control subjects. Strength and significance of correlations were determined by Spearman's rank order and scatter plots were therefore presented as ranked data. Local polynomial regression was used to fit a smooth curve between significantly correlated variables.

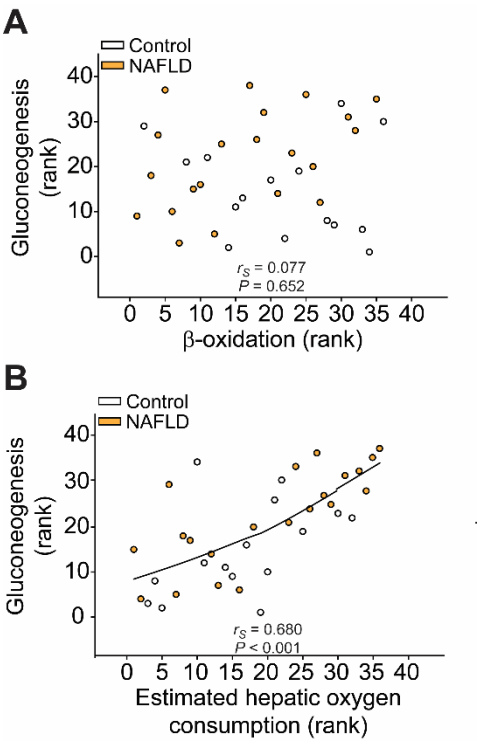

Figure S4. Correlations related to combined isotopic data. (**A**) There was no relationship between β-oxidation (acetyl-CoA production) and gluconeogenesis. (**B**) Hepatic oxygen consumption was highly correlated with gluconeogenesis (N = 36). *Note:* Combined isotopic data was unavailable for two control and two NAFLD subjects. Strength and significance of correlations were determined by Spearman's rank order and scatter plots were therefore presented as ranked data. Local polynomial regression was used to fit a smooth curve between significantly correlated variables.

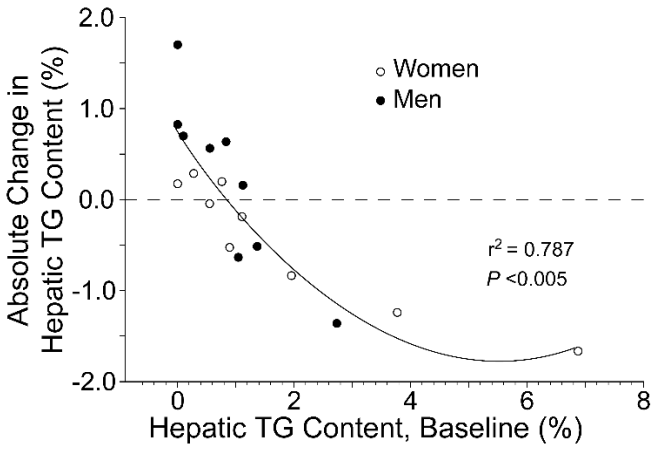

Figure S5. Change in hepatic TG content in humans after a 48-hour fast. Unpublished data from a prior human study examining metabolic changes that occur during a 48-hour fast (8 men, 9 women) (1). Hepatic TG content was measured by ^{1}H-MRS at baseline and after an observed 48-hour fast. Individuals with liver fat content above ≈0.8% experienced a decrease in hepatic TG content after fasting, consistent with mobilization by lipolytic release. Those subjects who began the fasting period with a hepatic TG content of less than ≈0.8% experienced either no change or an increase in liver fat after a 48-hour fast. Data analyzed by regression analysis.

1. Browning JD, Baxter J, Satapati S, and Burgess SC. The effect of short-term fasting on liver and skeletal muscle lipid, glucose, and energy metabolism in healthy women and men. *J Lipid Res.* 2012;53(3):577-86.

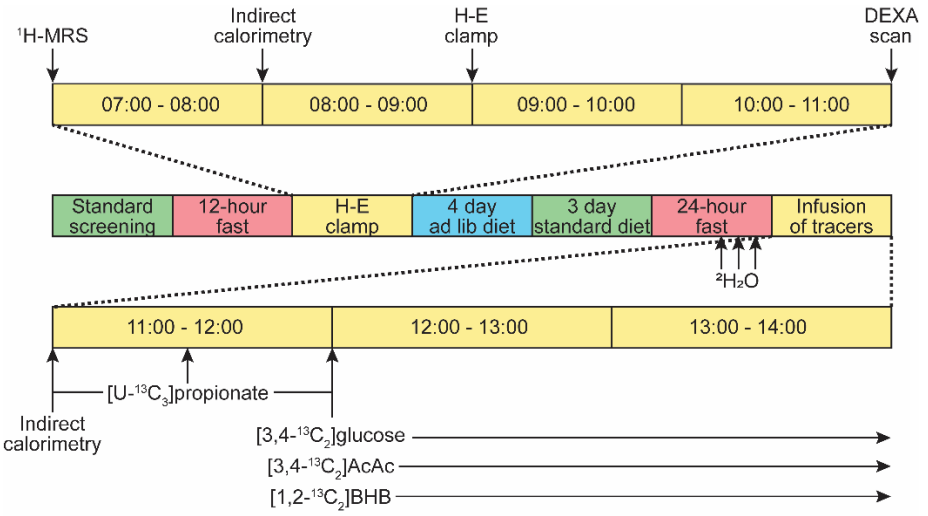

Figure S6. Study schema. After screening, recruited subjects presented at 07:00 after a 12-hour fast. Between 07:00 and 09:00, ^{1}H-MRS was performed to determine hepatic TG content followed by indirect calorimetry to assess respiratory quotient. At 10:00 a hyperinsulinemic-euglycemic (H-E) clamp was initiated for 120 minutes. Subjects then underwent dual-energy x-ray absorptiometry (DEXA) to assess body fat distribution and lean mass. Subjects then maintained an ad lib diet for four days, keeping a record of food intake to allow individual daily caloric intake to be determined. Subjects were then placed on a three-day standard meal plan with a daily caloric intake reflective of their ad lib diet. The final standard meal occurred at 12:00 the day prior to isotopic studies, marking the beginning of a 24-hour fast. Subjects presented at 08:00 the following day to undergo indirect calorimetry followed by the isotope studies. Between 22:00 the day prior and 12:00, subjects received two tracers orally: divided doses of 70% [^{2}H]water at 22:00, 02:00, and 06:00 and [U-$^{13}C_3$]propionate at 11:00, 11:30, and 12:00. The infusion began with a bolus of infusate at 12:00 (24-hr after last meal) followed by a 2-hour infusion of [3,4-$^{13}C_2$]glucose, [3,4-$^{13}C_2$]acetoacetate, and [1,2-$^{13}C_2$]β-hydroxybutyrate. At the end of the infusion period, a 50 cc blood draw was performed.

Table S1. Characteristics of matched subjects at enrollment.

	Group		
	Control (N=12)	**NAFLD** (N=12)	*P*-value
Age (yr)	40 ± 4	46 ± 4	0.297
Sex (F:M)	7:5	7:5	1.000
Ethnicity/Race (n)			
Non-Hispanic White	5	5	1.000
Non-Hispanic Black	5	2	0.375
Hispanic	2	3	1.000
Asian	0	1	1.000
American Indian	0	1	1.000
Body Mass Index (kg/m^2)	27 ± 1	29 ± 1	0.125
Body Fat (%)	32 ± 3	35 ± 2	0.404
Glucose (mg/dl)	87 ± 1	103 ± 6	0.027
Hemoglobin A1c (%)	5.4 ± 0.1	5.9 ± 0.3	0.109
M value (mg/kg/min)	7.2 ± 0.6	3.8 ± 0.3	<0.001
Total cholesterol (mg/dl)	174 ± 11	220 ± 16	0.031
HDL-c (mg/dl)	58 ± 4	50 ± 4	0.182
LDL-c (mg/dl)	98 ± 13	132 ± 14	0.089
Triglycerides (mg/dl)	87 ± 16	189 ± 25	0.003
AST (U/l)	24 ± 4	36 ± 8	0.175
ALT (U/l)	24 ± 5	51 ± 14	0.088
Albumin (mg/dl)	4.5 ± 0.1	4.6 ± 0.1	0.535
Platelet Count (x 1000)	227 ± 17	238 ± 14	0.598
Hepatic TG Content (%)	1 ± 0	18 ± 4	0.002

Note: Values are mean with SE. Data analyzed by unpaired *t*-test and Fisher Exact test. *Abbreviations:* ALT, alanine aminotransferase; AST, aspartate aminotransferase; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; TG, triglyceride. Conversions: triglycerides, HDL-c, and LDL-c (mg/dl) x 0.02586 = mmol/l; glucose (mg/dl) x 0.05551 = mmol/l.