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Supplemental Information

Hepatic Diacylglycerol-Associated Protein Kinase Cε Translocation Links Hepatic Steatosis to Hepatic Insulin Resistance in Humans

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Table S1. Hormones and Metabolites during Hyperinsulinemic-Euglycemic Clamp Studies. Related to Figures 1 and 2.

	No steatosis (n = 52)	Mild steatosis (n = 41)	Severe steatosis (n = 40)	p[a]
Basal (fasting)				
Insulin (pmol/l)	84 ± 5	131 ± 9**	145 ± 16***	< 0.001
Glucagon (ng/l)	94 ± 5	98 ± 6	81 ± 5	0.129
Cortisol (nmol/l)	228 ± 13	214 ± 14	213 ± 22	0.754
FA (mmol/l)	0.66 ± 0.03	0.65 ± 0.03	0.63 ± 0.03	0.702
Low-dose insulin infusion (step 1)				
Insulin (pmol/l)	301 ± 13	343 ± 18	326 ± 24	0.249
Glucagon (ng/l)	78 ± 4	80 ± 5	76 ± 4	0.858
Cortisol (nmol/l)	271 ± 16	252 ± 20	259 ± 15	0.562
FA (mmol/l)	0.11 ± 0.01	0.16 ± 0.01*	0.17 ± 0.01**	0.004
High-dose insulin infusion (step 2)				
Insulin (pmol/l)	765 ± 30	811 ± 47	777 ± 35	0.664
Glucagon (ng/l)	67 ± 4	70 ± 6	65 ± 4	0.778
Cortisol (nmol/l)	226 ± 16	218 ± 20	213 ± 15	0.842
FA (mmol/l)	0.03 ± 0.00	0.04 ± 0.00	0.05 ± 0.00*	0.009

Data are mean ± SEM. No, mild and severe hepatic steatosis were defined as IHTG < 5.56%, 5.56 – 15%, or > 15%, respectively. Fatty acids (FA), intrahepatic triglycerides (IHTG).

[a] Groups were compared by 1-way ANOVA with Bonferroni correction for multiple testing.

* $p < 0.05$ vs no steatosis. ** $p < 0.01$ vs no steatosis. *** $p < 0.001$ vs no steatosis.

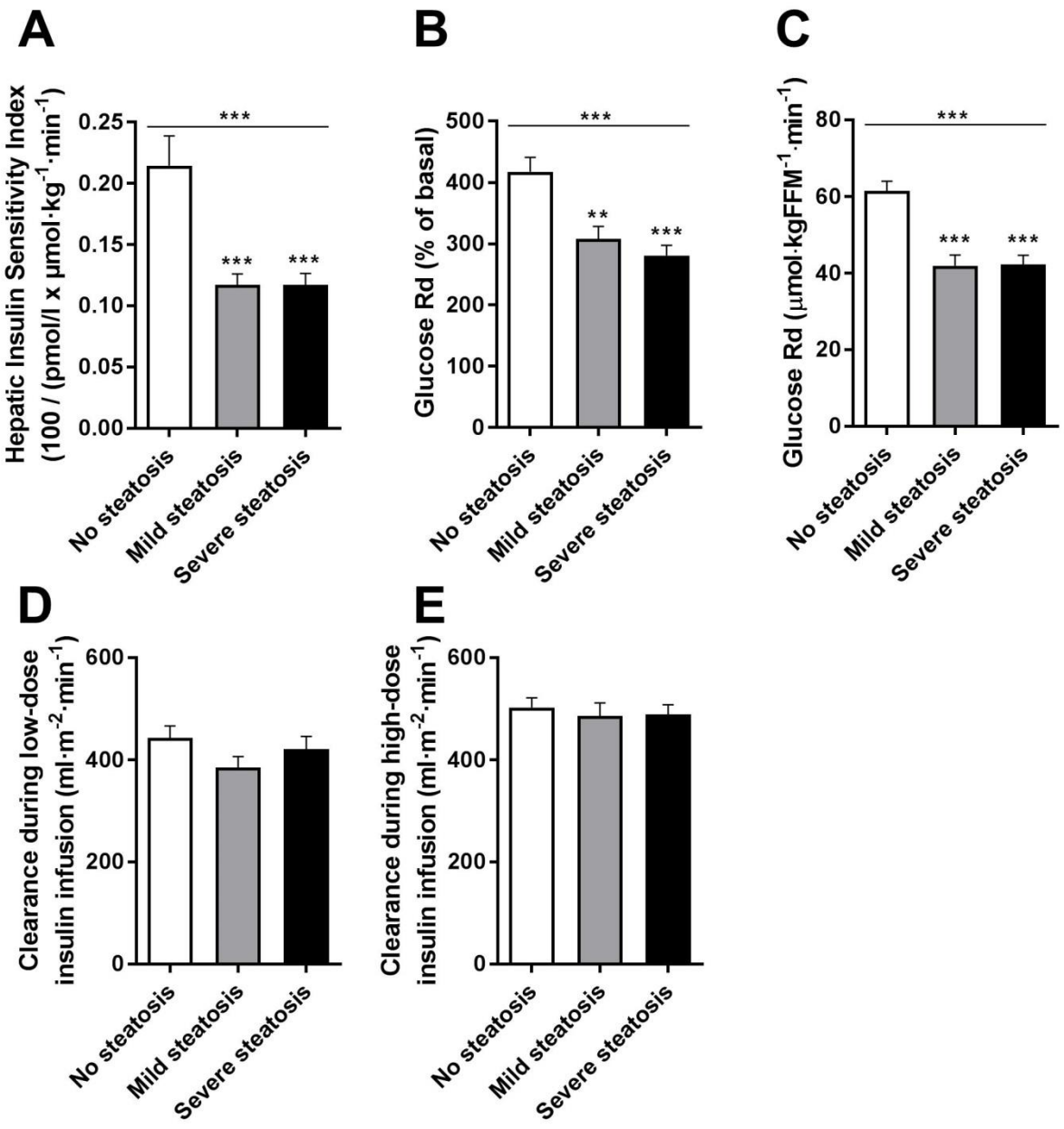

Figure S1. Additional Metabolic Flux Data. Related to Figure 1.

(A) The Hepatic Insulin Sensitivity Index was reduced in subjects with hepatic steatosis, but did not differ between subjects with mild or severe steatosis. The index was calculated as 100 / (fasting plasma insulin x basal endogenous glucose production).

(B) Expressing peripheral insulin sensitivity as percentage increase relative to basal glucose uptake yielded similar results.

(C) Expressing peripheral insulin sensitivity relative to lean body mass yielded similar results.

(D) Insulin clearance during low-dose insulin infusion (step 1 of the clamp) did not differ between subjects with varying extent of steatosis. Insulin clearance was calculated as insulin infusion rate / plasma insulin concentration during the clamp.

(E) Insulin clearance during high-dose insulin infusion (step 2) did not differ between subjects with varying extent of steatosis.

Data are mean ± SEM (n = 133). Rate of disappearance (Rd), fat-free mass (FFM).

** $p < 0.01$ vs no steatosis. *** $p < 0.001$ vs no steatosis.

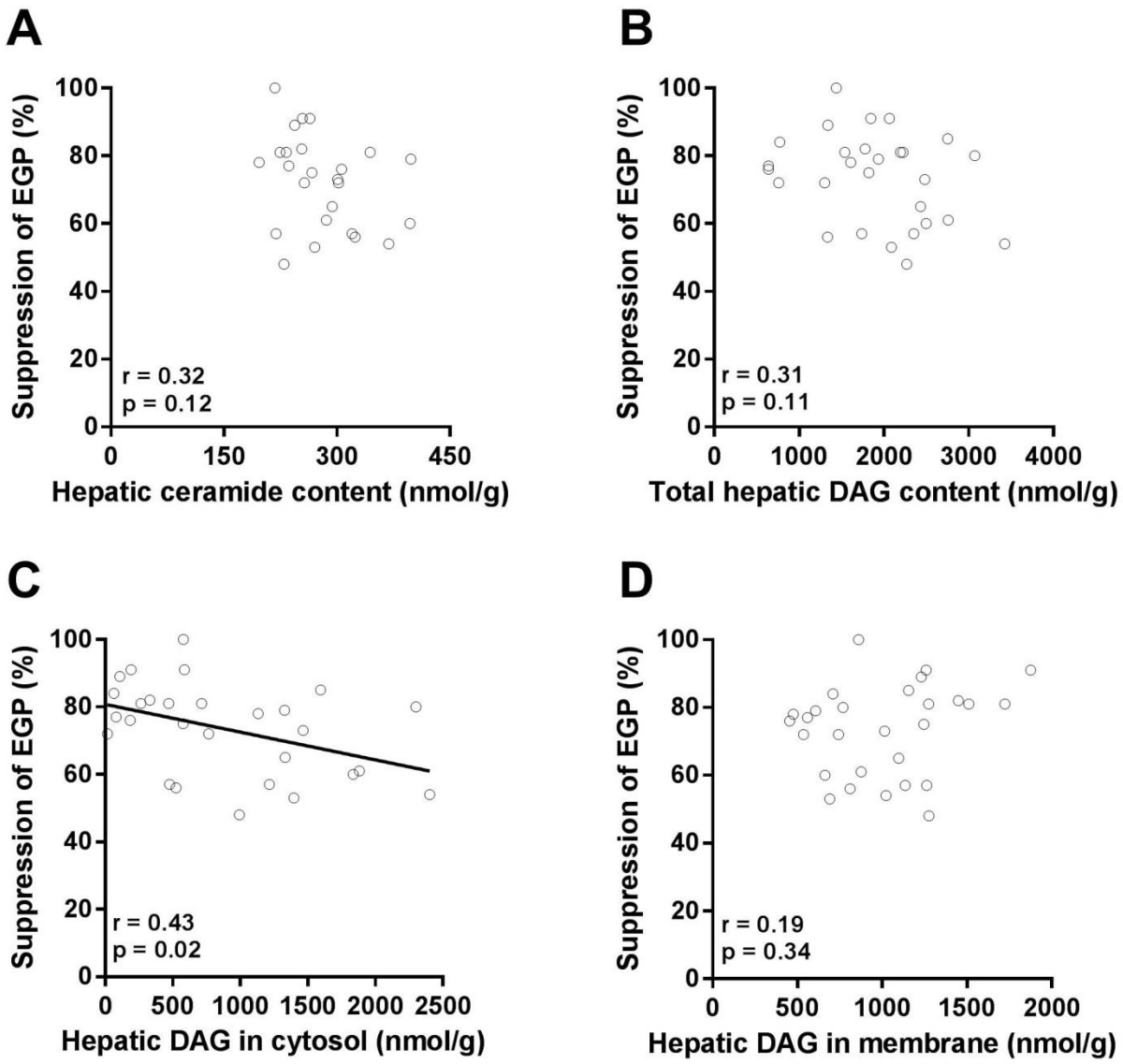

Figure S2. Hepatic DAG Accumulation in the Cytosolic Fraction, but Not Total DAG Content, Membrane DAG Content, or Ceramide Content, Predicted Hepatic Insulin Sensitivity in Obese Subjects. Related to Figure 3.

(A) Hepatic ceramide content and insulin suppression of EGP.

(B) Total hepatic DAG content and insulin suppression of EGP.

(C) Hepatic DAG content in the cytosolic fraction and insulin suppression of EGP.

(D) Hepatic DAG content in the membrane fraction and insulin suppression of EGP.

Lines were fitted by least squares method (n = 25-29). Diacylglycerol (DAG), endogenous glucose production (EGP).

Table S2. Correlations Between Individual Hepatic DAG Species and Insulin Sensitivity of EGP Suppression (n = 29). Related to Figure 3.

	Cytosolic fraction			Membrane fraction		
Species	r[a]	p	Abundance [mean ± SEM (nmol/g)]	r[a]	p	Abundance [mean ± SEM (nmol/g)]
C18:1-C16:0	**-0.44**	**0.019**	**222.6 ± 33.9**	0.01	0.948	215.2 ± 14.6
C20:4-C20:5	-0.12	0.551	17.1 ± 2.3	**0.41**	**0.028**	**84.7 ± 6.9**
C16:0-C20:4	-0.36	0.061	0.7 ± 0.1	0.39	0.041	1.6 ± 0.1
C18:0-C20:4	-0.27	0.172	0.6 ± 0.1	0.41	0.032	2.0 ± 0.1
C18:0-C18:0	-0.47	0.012	15.2 ± 2.1	-0.12	0.559	15.3 ± 0.9
C18:2-C18:0	-0.33	0.083	7.9 ± 1.2	0.34	0.078	12.9 ± 0.8
C18:1-C18:0	-0.42	0.027	27.6 ± 4.2	0.06	0.760	32.8 ± 2.4
C16:0-C16:0	**-0.47**	**0.013**	**73.5 ± 12.0**	-0.21	0.296	71.0 ± 4.5
C18:0-C16:0	-0.45	0.015	23.5 ± 3.5	-0.10	0.630	20.6 ± 1.3
C18:1-C18:1	**-0.41**	**0.030**	**326.4 ± 51.2**	0.17	0.377	377.4 ± 34.2
C18:1-C18:2	-0.33	0.091	59.6 ± 9.5	0.34	0.076	65.8 ± 6.8
C18:2-C18:2	-0.23	0.233	9.8 ± 1.6	0.38	0.044	10.4 ± 1.1
C16:0-C18:2	-0.33	0.085	79.0 ± 12.4	0.29	0.130	100.9 ± 5.9

Highly abundant species that were also related to hepatic insulin sensitivity are highlighted. Diacylglycerol (DAG), endogenous glucose production (EGP).

[a] Correlations were evaluated by Pearson's correlation coefficient.

Table S3. Correlations Between Individual Hepatic Ceramide Species and Insulin Sensitivity of EGP Suppression (n = 25). Related to Figure 3.

Species	r[a]	p	Abundance [mean ± SEM (nmol/g)]
C16:0	-0.27	0.187	58.8 ± 2.1
C18:0	-0.24	0.254	16.6 ± 0.6
C20:0	-0.28	0.180	43.8 ± 6.6
C22:0	-0.27	0.190	65.1 ± 2.4
C24:1	-0.16	0.447	40.9 ± 1.2
C24:0	-0.06	0.779	52.4 ± 2.1

Endogenous glucose production (EGP).

[a] Correlations were evaluated by Pearson's correlation coefficient.

Table S4. Characteristics of Subjects with Normal Hepatic Insulin Sensitivity or with Hepatic Insulin Resistance, Who Had Liver Biopsies Taken during Bariatric Surgery. Related to Figure 3.

	Sensitive (n = 10)	Resistant (n = 8)	p[a]
Female sex	6 (60)	3 (38)	0.343
Age (years)	51 ± 2	45 ± 4	0.135
Body mass index (kg/m^2)	41 ± 1	47 ± 3	0.055
Body fat content (%)	45 ± 1	47 ± 3	0.603
Fasting glucose (mmol/l)	4.8 ± 0.1	5.5 ± 0.3	0.013
Fasting insulin (pmol/l)	115 ± 20	183 ± 20	0.029
Basal EGP ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)	6.9 ± 0.3	7.2 ± 0.3	0.518
Insulin suppression of EGP (%)	87 ± 2	56 ± 1	< 0.001
IHTG content (%)	9.2 ± 3.0	14.2 ± 2.6	0.209

Data are count (%) or mean ± SEM. Hepatic insulin sensitivity and resistance were defined as insulin suppression of EGP > 80% and < 65%, respectively. Endogenous glucose production (EGP), intrahepatic triglyceride (IHTG).

[a] Groups were compared by 2-tailed independent samples t test.

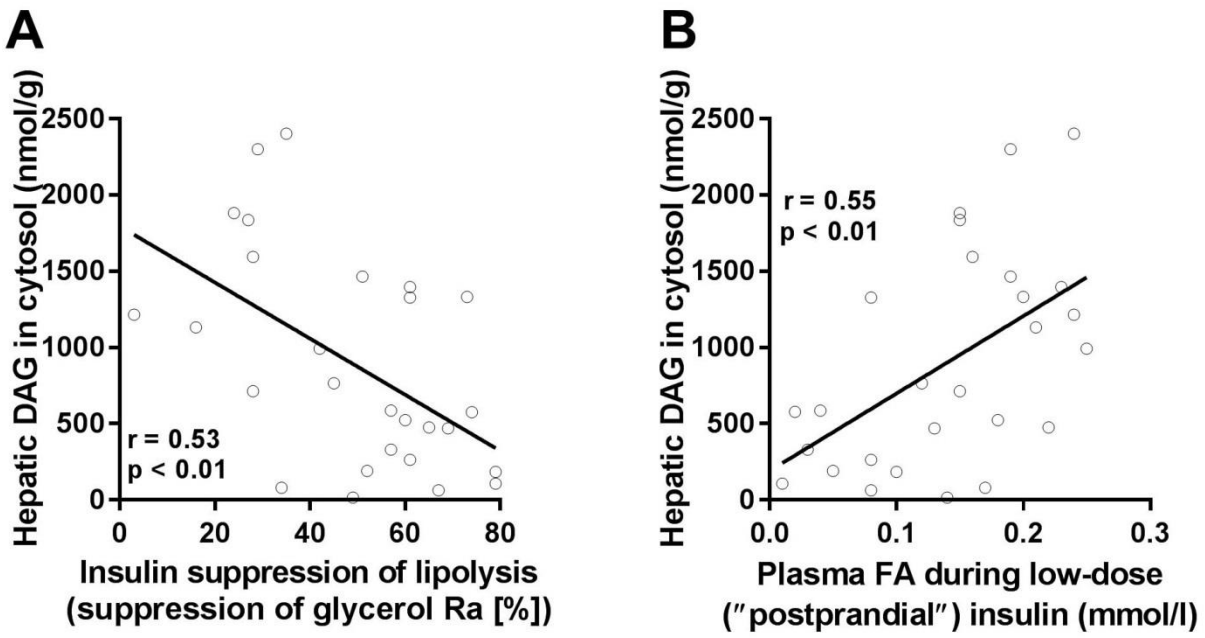

Figure S3. Release of FA from Lipolysis during Hyperinsulinemic Conditions Predicts Hepatic DAG Accumulation. Related to Figure 3.

(A) The rate of lipolysis during hyperinsulinemic-euglycemic clamps was assessed by tracer-dilution of [1,1,2,3,3-$^{2}H_5$]glycerol.

(B) Plasma FA concentrations during hyperinsulinemia.

Data were fitted by least squares method (n = 28). Diacylglycerol (DAG), fatty acids (FA).