

Supporting Information

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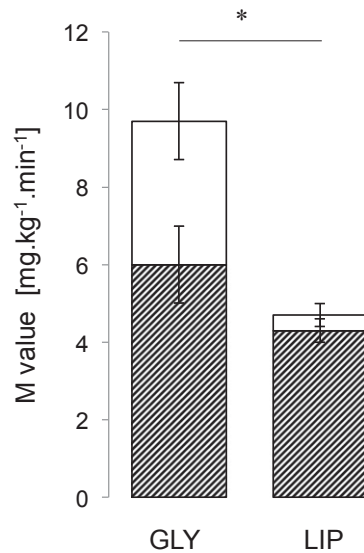


Fig. S1. Whole-body glucose disposal in lean healthy young humans after 4 h glycerol (GLY) or lipid (LIP) infusion ($n = 23$), glucose oxidation (striped), and lipid oxidation (plane). M value was reduced during lipid infusion ($P < 10 \cdot 10^{-7}$). Data are given as means \pm SEM.

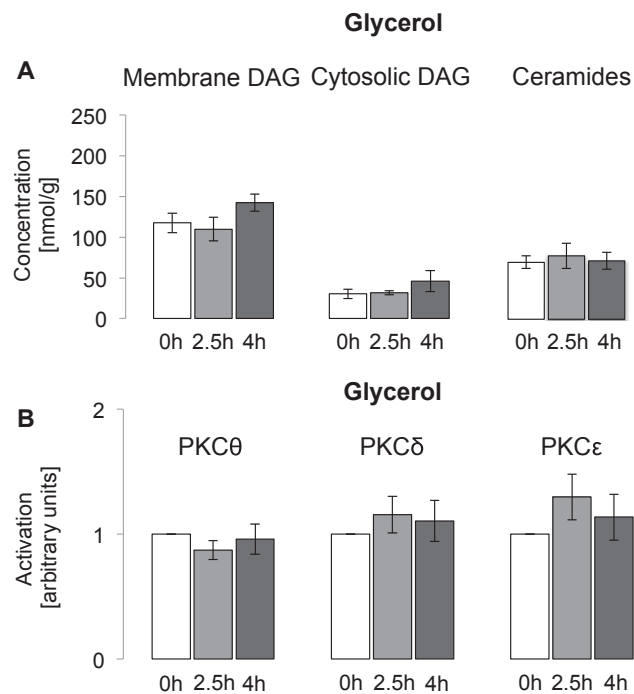


Fig. S2. (A) Myocellular diacylglycerol (DAG) concentrations in the membrane and cytosolic fraction and ceramide concentrations during glycerol infusion in young lean healthy controls (CON) ($n = 10$). Myocellular ceramides did not change during lipid infusion. (B) Activation of myocellular PKC θ , PKC δ , and PKC ϵ during glycerol infusion in young lean healthy controls ($n = 10-14$). Data are given as means \pm SEM.

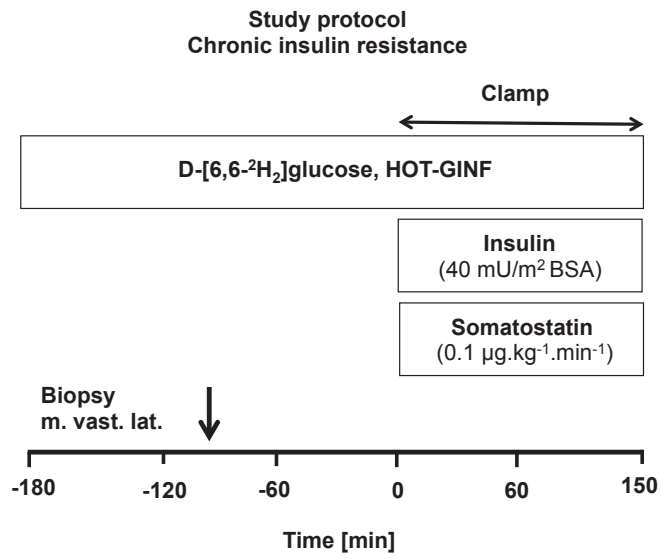


Fig. S7. Study protocol for common insulin resistance.

Table S1. Myocellular DAG species in short-term and common insulin resistance

Study group and condition	C18:1, C16:0		C20:4, C20:5		C16:0, C20:4		C18:0, C20:4		C18:0, C18:0		C18:2, C18:0		C18:1, C18:0								
	Basal	4 h	Basal	4 h	Basal	4 h	Basal	4 h	Basal	4 h	Basal	4 h	Basal	4 h							
CON																					
Glycerol																					
Cytosol, nmol/g																					
Mean	2.67	3.21	3.71	1.04	0.85	1.06	0.31	0.29	0.41	0.35	0.31	0.43	3.76	3.73	5.81	0.77	0.81	1.04	2.98	3.00	4.41
SD	0.99	0.74	3.04	0.33	0.19	0.55	0.17	0.05	0.36	0.17	0.05	0.34	2.72	1.28	6.59	0.37	0.17	0.85	2.13	0.86	4.38
Membrane, nmol/g																					
Mean	11.64	12.82	15.56	11.49	8.77	13.98	0.48	0.46	0.69	1.73	1.45	2.24	3.78	4.24	6.11	2.62	2.72	3.42	4.38	4.74	6.32
SD	3.18	4.30	4.28	5.34	1.78	2.42	0.20	0.11	0.31	0.64	0.19	0.23	1.83	0.93	3.09	0.58	0.62	0.81	1.70	1.26	2.19
Lipid																					
Cytosol, nmol/g																					
Mean	3.15	8.24	3.96	1.44	1.59	1.64	0.07	0.11	0.11	0.12	0.14	0.12	5.17	5.92	4.24	0.37	0.74	0.45	1.32	3.47	1.48
SD	1.27	5.59	1.90	0.74	1.12	1.14	0.04	0.04	0.06	0.06	0.06	0.04	4.29	5.18	2.17	0.18	0.27	0.32	1.39	3.78	1.16
Membrane, nmol/g																					
Mean	14.48	27.64	19.70	15.74	20.07	17.78	0.18	0.35	0.29	0.78	1.16	0.94	5.55	6.98	6.66	1.75	3.34	2.60	3.48	6.37	4.91
SD	6.46	18.19	17.63	7.48	12.86	7.65	0.08	0.11	0.17	0.38	0.31	0.49	1.83	3.37	2.02	0.78	1.05	1.27	1.41	2.68	3.77
OBE																					
Cytosol, nmol/g																					
Mean	7.21			1.15			0.12			0.21			8.18			0.70			5.76		
SD	1.38			0.44			0.04			0.05			5.61			0.28			3.76		
Membrane, nmol/g																					
Mean	15.66			9.01			0.24			1.12			8.86			2.12			6.85		
SD	3.51			3.84			0.07			0.41			4.57			0.80			2.71		
T2D																					
Cytosol, nmol/g																					
Mean	9.35			1.51			0.15			0.29			11.41			1.04			7.40		
SD	7.13			0.67			0.07			0.16			14.01			0.65			6.06		
Membrane, nmol/g																					
Mean	20.68			11.78			0.30			1.67			10.41			3.28			9.65		
SD	8.15			5.15			0.10			0.90			7.98			1.56			6.84		

Table S2. Myocellular DAG species in short-term and common insulin resistance

	C16:0, C16:0			C18:0, C16:0			C18:1, C18:1			C18:1, C18:2			C18:2, C18:2			C16:0, C18:2		
	Basal	2.5 h	4 h	Basal	2.5 h	4 h	Basal	2.5 h	4 h	Basal	2.5 h	4 h	Basal	2.5 h	4 h	Basal	2.5 h	4 h
CON																		
Glycerol																		
Cytosol, nmol/g																		
Mean	2.01	2.04	2.55	3.18	3.37	4.32	4.71	5.13	5.86	3.39	3.73	4.13	1.61	1.73	1.97	3.55	3.54	4.30
SD	1.09	0.48	2.03	1.91	0.80	3.86	1.53	0.85	3.63	1.09	0.64	2.48	0.67	0.48	1.67	1.27	0.52	2.91
Membrane, nmol/g																		
Mean	4.47	4.68	7.37	4.94	5.32	7.59	25.63	25.03	30.43	15.76	15.95	18.54	9.03	9.20	10.29	14.49	14.41	18.11
SD	1.34	1.13	2.87	2.00	1.15	2.57	6.30	7.23	9.76	3.66	5.57	6.83	2.26	2.09	2.05	3.09	3.42	4.30
Lipid																		
Cytosol, nmol/g																		
Mean	2.15	3.42	2.36	3.51	4.87	2.93	6.64	16.30	8.79	3.00	6.45	4.26	1.18	3.03	3.15	1.92	3.99	2.91
SD	0.70	1.99	0.66	2.43	5.03	1.29	3.55	12.95	6.21	1.26	2.66	2.23	0.53	0.51	1.81	0.75	1.77	0.91
Membrane, nmol/g																		
Mean	5.39	7.91	6.60	4.75	6.24	6.10	36.15	79.51	48.21	16.69	32.24	22.40	4.80	13.02	10.33	9.64	19.66	13.72
SD	1.78	4.02	3.77	1.32	2.35	2.65	21.37	61.92	42.50	9.62	13.91	13.40	2.84	4.33	5.32	4.35	6.60	6.96
OBE																		
Cytosol, nmol/g																		
Mean	3.11			6.66			9.97			7.08			3.33			4.86		
SD	0.97			3.88			4.85			4.50			1.97			1.84		
Membrane, nmol/g																		
Mean	6.04			7.93			28.45			17.32			10.13			16.38		
SD	1.01			2.57			11.20			7.78			5.34			5.64		
T2D																		
Cytosol, nmol/g																		
Mean	4.14			8.45			11.28			8.51			3.51			5.44		
SD	2.94			6.63			8.15			5.35			2.56			2.91		
Membrane, nmol/g																		
Mean	7.40			9.77			42.18			26.67			11.90			21.56		
SD	3.63			6.54			17.14			11.25			4.82			5.25		

Table S3. Myocellular DAG species correlate with PKC θ activation

DAG species	Cytosolic fraction		Membrane fraction	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
C16:0, C20:4	0.521	<0.005	0.447	<0.05
C18:0, C20:4	0.461	<0.01	0.362	<0.05
C18:2, C18:0	0.581	<0.005	0.428	<0.05
C18:1, C18:2	0.552	<0.005	0.417	<0.05
C18:2, C18:2	0.591	<0.005	0.532	<0.005
C16:0, C18:2	0.570	<0.005	0.529	<0.005

Pearson correlation coefficients (*r*) between myocellular DAG species and PKC θ activation across lean healthy humans (*n* = 11), young insulin-resistant obese humans (*n* = 10) and elderly obese humans with T2D (*n* = 10).

Table S4. Anthropometric data of study participants

	CON	OBE	T2D
<i>n</i> (male/female)	36 (24/12)	10 (5/5)	10 (5/5)
Age, y	28 ± 1	29 ± 2	59 ± 3*
Body mass index, kg/m ²	22.4 ± 0.4 [†]	41.4 ± 1.9	35.6 ± 1.2
Fasting glucose, mg/dL	86 ± 3	77 ± 3	131 ± 17*
Fasting free FA, mmol/L	0.52 ± 0.13	0.50 ± 0.18	0.76 ± 0.15
Fasting triglycerides, mg/dL	106 ± 13	137 ± 24	188 ± 20*
Hemoglobin A _{1c} , mmol/mol	33 ± 0	34 ± 1	54 ± 5*

Data are given as means ± SEM. FA, fatty acids.

**P* < 0.05 vs. lean healthy controls (CON) and insulin resistant OBE.

[†]*P* < 0.05 vs. insulin resistant obese and patients with T2D.