Online-Only Appendix

TABLE 1. Blood glucose and plasma insulin levels during the insulin-stimulated (clamp) conditions and glucose turnover under basal (non-stimulated) conditions in wild-type and AMPKα2$^{-/-}$ mice

	Wild-type			AMPKα2$^{-/-}$		
	Chow	cHF	cHF+F	Chow	cHF	cHF+F
Blood glucose before clamp (mmol/l)	7.0 ± 0.8	8.0 ± 1.0	7.8 ± 0.8	8.5 ± 0.6	5.9 ± 0.1	7.4 ± 0.9
Steady-state blood glucose (mmol/l)	5.7 ± 0.5	5.7 ± 0.2	5.8 ± 0.2	5.8 ± 0.1	5.8 ± 0.7	5.9 ± 0.3
End-point plasma insulin (μU/ml)	30 ± 10	29 ± 7	24 ± 8	21 ± 7	25 ± 7	28 ± 9
Basal glucose turnover (μmol kg^{-1} min^{-1})	108 ± 11	86 ± 15	90 ± 15	118 ± 7	83 ± 23	104 ± 17

The data are presented as means ± SE (n = 5-8). Wild-type and AMPKα2$^{-/-}$ mice were fed either a Chow diet or corn oil-based high-fat diets without (cHF) or with 15% of the lipids in the form of n-3 LC-PUFA concentrate (cHF+F) for 9 weeks.

TABLE 2. Triglycerides, NEFA and cholesterol levels in plasma of wild-type and AMPKα2$^{-/-}$ mice under different metabolic conditions

	Wild-type			AMPKα2$^{-/-}$		
	Chow	cHF	cHF+F	Chow	cHF	cHF+F
Triglycerides (mmol/l)						
Fasted	0.46 ± 0.03	0.49 ± 0.03	0.46 ± 0.04	0.57 ± 0.05	0.44 ± 0.02	0.46 ± 0.02
Fed	1.17 ± 0.08	1.24 ± 0.11	0.62 ± 0.07*†	1.04 ± 0.06	1.22 ± 0.08	0.73 ± 0.07*†
Clamp	0.44 ± 0.05	0.64 ± 0.09	0.41 ± 0.04†	0.38 ± 0.06	0.55 ± 0.05	0.53 ± 0.08
NEFA (mmol/l)						
Fasted	1.16 ± 0.07	0.84 ± 0.08*	0.78 ± 0.05*	1.34 ± 0.05‡	0.89 ± 0.06*	0.87 ± 0.05*
Fed	0.90 ± 0.05	0.94 ± 0.05	0.59 ± 0.05*†	0.88 ± 0.04	0.99 ± 0.06	0.68 ± 0.04*†
Clamp	0.25 ± 0.02	0.39 ± 0.05	0.27 ± 0.03†	0.21 ± 0.03	0.41 ± 0.11	0.37 ± 0.07
Cholesterol (mmol/l)						
Fasted	1.77 ± 0.07	2.81 ± 0.22	2.00 ± 0.15†	1.78 ± 0.04	2.67 ± 0.12	2.02 ± 0.08†
Fed	2.25 ± 0.08	4.12 ± 0.25*	3.10 ± 0.20*†	2.10 ± 0.06	3.94 ± 0.18*	2.75 ± 0.14*†
Clamp	2.26 ± 0.13	2.98 ± 0.22	2.51 ± 0.15	1.83 ± 0.09	2.99 ± 0.24	2.63 ± 0.22

The data are presented as means ± SE (for fasted and fed mice, n = 13-15; for clamp, n = 8-14). Wild-type and AMPKα2$^{-/-}$ mice were fed either a Chow diet or corn oil-based high-fat diets without (cHF) or with 15% of the lipids in the form of n-3 LC-PUFA concentrate (cHF+F) for 9 weeks. Plasma parameters were assessed at 8 weeks (fasted values) or 9 weeks (fed and clamp values), respectively. *$P < 0.05$ vs. genotype Chow; †$P < 0.05$ vs. genotype cHF; ‡$P < 0.05$ vs. wild-type on respective diet.

TABLE 3. The basal rates of lipid metabolism in isolated hepatocytes of wild-type and AMPKα2$^{-/-}$ mice

	Wild-type			AMPKα2$^{-/-}$		
	Chow	cHF	cHF+F	Chow	cHF	cHF+F
Fatty acid oxidation (pmol/h/mg protein)	13 ± 2	8 ± 1*	9 ± 1*	15 ± 2	12 ± 1‡	6 ± 1*†
Lipogenesis (pmol/h/mg protein)	108 ± 9	74 ± 24	43 ± 10*†	79 ± 10	51 ± 6	34 ± 6*

The data are presented as means ± SE (n = 3 analyzed in triplets). Wild-type and AMPKα2$^{-/-}$ mice were fed either a Chow diet or corn oil-based high-fat diets without (cHF) or with 15% of the lipids in the form of n-3 LC-PUFA concentrate (cHF+F) for 9 weeks. *P < 0.05 vs. genotype Chow; †P < 0.05 vs. genotype cHF; ‡P < 0.05 vs. wild-type on respective diet.

TABLE 4. The content and composition of hepatic lipids in wild-type and AMPKα2$^{-/-}$ mice killed in ad libitum fed state

	Wild-type			AMPKα2$^{-/-}$		
	Chow	cHF	cHF+F	Chow	cHF	cHF+F
Total ceramide (nmol/g)	121.45 ± 9.33	119.71 ± 6.43	125.89 ± 14.66	123.34 ± 4.84	117.91 ± 7.53	123.82 ± 6.42
Triglyceride species						
Total fatty acids (μmol/g)	38.10 ± 3.18	116.55 ± 14.51*	80.35 ± 8.80†	50.31 ± 4.60	128.04 ± 18.59*	76.80 ± 6.63†
Myristic (14:0) (μmol/g)	0.38 ± 0.05	1.25 ± 0.22*	0.63 ± 0.08†	0.44 ± 0.04	1.27 ± 0.25*	0.59 ± 0.06†
Palmitic (16:0) (μmol/g)	11.12 ± 0.94	30.39 ± 4.10*	20.27 ± 2.88†	14.44 ± 1.06	35.25 ± 6.10*	17.17 ± 1.86†
Palmitoleic (16:1n7) (μmol/g)	2.82 ± 0.35	1.54 ± 0.46*	0.54 ± 0.10*	3.01 ± 0.37	1.35 ± 0.41*	0.81 ± 0.41*
Stearic (18:0) (μmol/g)	0.85 ± 0.09	1.90 ± 0.15*	1.60 ± 0.08*	0.98 ± 0.08	2.52 ± 0.31*‡	1.83 ± 0.14*†
Oleic (18:1n9) (μmol/g)	13.23 ± 1.22	27.05 ± 3.33	15.08 ± 1.72*†	16.68 ± 1.18	30.81 ± 5.84	15.52 ± 1.60*†
Linoleic (18:2n6) (μmol/g)	7.68 ± 0.61	47.52 ± 5.98*	25.98 ± 2.67*†	12.16 ± 2.60	49.22 ± 5.78*	25.81 ± 2.43*†
Arachidic (20:0) (μmol/g)	0.08 ± 0.01	0.23 ± 0.04*	0.18 ± 0.01*	0.12 ± 0.02	0.28 ± 0.05*	0.19 ± 0.02†
α-Linolenic (C18:3n3) (μmol/g)	0.43 ± 0.03	1.42 ± 0.24*	0.81 ± 0.09†	0.56 ± 0.06	1.52 ± 0.28*	0.79 ± 0.07†
Behenic (22:0) (nmol/g)	71.89 ± 5.67	89.37 ± 11.63	31.13 ± 2.65*†	81.32 ± 3.67	98.81 ± 15.97	43.53 ± 7.20*†
Arachidonic (20:4n6) (μmol/g)	0.56 ± 0.04	3.15 ± 0.58*	0.41 ± 0.04†	0.84 ± 0.12	3.72 ± 0.79*	0.47 ± 0.06†
Lignoceric (24:0) (nmol/g)	7.30 ± 0.80	10.28 ± 1.07	9.93 ± 1.04	8.14 ± 0.70	14.66 ± 3.10*	11.19 ± 0.89
Eicosapentaenoic (20:5n3) (μmol/g)	0.13 ± 0.01	0.25 ± 0.04	1.91 ± 0.21*†	0.13 ± 0.01	0.23 ± 0.05	1.33 ± 0.13*†‡
Nervonic (24:1n9) (nmol/g)	7.43 ± 1.01	8.23 ± 0.58	10.01 ± 0.89	6.94 ± 0.28	10.04 ± 1.30*	12.30 ± 1.17*
Docosahexaenoic (22:6n3) (μmol/g)	0.73 ± 0.06	1.74 ± 0.29	12.90 ± 1.08*†	0.84 ± 0.05	1.75 ± 0.31	12.21 ± 1.21*†
Diacylglycerol species						
Total fatty acids (μmol/g)	2.57 ± 0.34	2.51 ± 0.29	1.85 ± 0.18†	1.76 ± 0.11‡	2.47 ± 0.25*	2.66 ± 0.40*‡
Myristic (14:0) (μmol/g)	0.08 ± 0.01	0.08 ± 0.01	0.06 ± 0.01	0.06 ± 0.00	0.06 ± 0.01	0.06 ± 0.01

Palmitic (16:0) (μmol/g)	1.02 ± 0.13	0.95 ± 0.13	0.70 ± 0.06	0.69 ± 0.05	0.90 ± 0.09	1.12 ± 0.22‡
Palmitoleic (16:1n7) (μmol/g)	0.09 ± 0.01	0.03 ± 0.00*	0.01 ± 0.00*	0.06 ± 0.01‡	0.02 ± 0.00*	0.02 ± 0.01*
Stearic (18:0) (μmol/g)	0.70 ± 0.18	0.54 ± 0.10	0.48 ± 0.11	0.31 ± 0.02	0.40 ± 0.04	0.65 ± 0.16
Oleic (18:1n9) (μmol/g)	0.28 ± 0.02	0.25 ± 0.03	0.13 ± 0.01*†	0.24 ± 0.02	0.25 ± 0.04	0.19 ± 0.03
Linoleic (18:2n6) (μmol/g)	0.23 ± 0.02	0.49 ± 0.07*	0.27 ± 0.03†	0.24 ± 0.02	0.57 ± 0.07*	0.36 ± 0.05†
Arachidic (20:0) (nmol/g)	15.23 ± 4.54	11.84 ± 1.95	11.87 ± 2.85	7.67 ± 0.56	9.47 ± 0.62	10.94 ± 0.92
α-Linolenic (18:3n3) (nmol/g)	12.61 ± 1.52	12.60 ± 1.88	6.55 ± 0.50*†	7.64 ± 0.64‡	10.73 ± 1.52	13.23 ± 2.62‡
Arachidonic (20:4n6) (nmol/g)	72.11 ± 3.89	83.54 ± 5.32	30.86 ± 4.35*†	65.43 ± 4.89	100.95 ± 16.46*	37.75 ± 4.28*†
Eicosapentaenoic (20:5n3) (nmol/g)	10.43 ± 0.75	8.59 ± 0.89	19.97 ± 1.62*†	8.58 ± 0.58	8.37 ± 8.84	20.29 ± 1.27*†
Nervonic (24:1n9) (nmol/g)	0.80 ± 0.10	1.36 ± 0.32	1.18 ± 0.12	0.83 ± 0.06	1.38 ± 0.37	1.38 ± 0.21
Docosahexaenoic (22:6n3) (μmol/g)	0.05 ± 0.00	0.06 ± 0.01	0.13 ± 0.01*†	0.06 ± 0.01	0.14 ± 0.05*‡	0.17 ± 0.02*
Phospholipid species						
Total fatty acids (μmol/g)	67.96 ± 1.61	65.83 ± 1.09	70.75 ± 1.39†	68.20 ± 0.97	67.62 ± 1.40	74.45 ± 2.44*†
Myristic (14:0) (μmol/g)	0.14 ± 0.01	0.22 ± 0.08	0.16 ± 0.01	0.26 ± 0.08	0.15 ± 0.02	0.15 ± 0.01
Palmitic (16:0) (μmol/g)	18.38 ± 0.46	13.80 ± 0.24*	17.38 ± 0.21†	18.16 ± 0.30	14.18 ± 0.34*	18.20 ± 0.87†
Palmitoleic (16:1n7) (μmol/g)	1.40 ± 0.12	0.17 ± 0.01*	0.25 ± 0.11*	1.36 ± 0.06	0.17 ± 0.01*	0.33 ± 0.19*
Stearic (18:0) (μmol/g)	11.21 ± 0.36	14.39 ± 0.28*	13.53 ± 0.47*	11.56 ± 0.25	14.81 ± 0.33*	14.30 ± 0.36*
Oleic (18:1n9) (μmol/g)	5.23 ± 0.21	3.34 ± 0.14*	3.80 ± 0.21*	5.02 ± 0.14	3.42 ± 0.14*	4.11 ± 0.36*†
Linoleic (18:2n6) (μmol/g)	12.37 ± 0.29	14.27 ± 0.39*	14.89 ± 0.42*	12.00 ± 0.21	14.38 ± 0.45*	15.91 ± 0.41*†
Arachidic (20:0) (μmol/g)	0.10 ± 0.01	0.11 ± 0.01	0.15 ± 0.01*†	0.10 ± 0.00	0.11 ± 0.01	0.15 ± 0.01*†
α-Linolenic (C18:3n3) (μmol/g)	0.07 ± 0.01	0.04 ± 0.00*	0.05 ± 0.00	0.07 ± 0.01	0.05 ± 0.00*	0.06 ± 0.01
Behenic (22:0) (μmol/g)	0.13 ± 0.01	0.03 ± 0.01*	0.04 ± 0.01*	0.13 ± 0.01	0.02 ± 0.00*	0.04 ± 0.02*
Arachidonic (20:4n6) (μmol/g)	11.83 ± 0.66	13.70 ± 0.30*	5.60 ± 0.47*†	12.85 ± 0.18	13.75 ± 0.79*	6.62 ± 0.80†
Lignoceric (24:0) (nmol/g)	9.90 ± 1.00	15.69 ± 6.14	11.32 ± 0.70	13.82 ± 3.82	10.46 ± 0.72	10.29 ± 0.83
Eicosapentaenoic (20:5n3) (μmol/g)	0.44 ± 0.08	0.08 ± 0.01*	2.01 ± 0.17*†	0.27 ± 0.01	0.16 ± 0.10	1.78 ± 0.19*†
Docosahexaenoic (22:6n3) (μmol/g)	6.64 ± 0.39	5.64 ± 0.18	12.88 ± 0.65*†	6.42 ± 0.13	6.40 ± 0.53	12.80 ± 0.75*†

The data are the means ± SE (n = 8-14). Wild-type and AMPKα2$^{-/-}$ mice were fed either a Chow diet or corn oil-based high-fat diets without (cHF) or with 15% of the lipids in the form of n-3 LC-PUFA concentrate (cHF+F) for 9 weeks and killed in ad libitum fed state. *P < 0.05 vs. genotype Chow; †P < 0.05 vs. genotype cHF; ‡P < 0.05 vs. wild-type on respective diet.

The intake of n-3 LC-PUFA resulted in a marked increase in EPA and DHA concentration in all lipid fractions, independently of the genotype and with relatively small changes in the diacylglycerol fraction. Except for EPA and DHA (and a relatively low content of arachidonic acid and high content of nervonic acid), levels of individual fatty acids in the triglyceride fraction reflected the total tissue content of triglycerides. The fatty acid profile of the phospholipid fraction was only marginally affected by either diet or genotype. In diacylglycerol fraction, the content of several fatty acids (i.e., palmitic, palmitoleic, oleic, and α-linolenic acid) was differentially affected by the cHF+F diet, depending on the genotype and explaining the effect of the cHF+F diet on total hepatic content of diacylglycerols (see Fig. 5A of the main text). α-linolenic acid was by far the most differentially affected fatty acid, with a ~1.9-fold decrease in response to cHF+F diet in wild-type mice, and a ~1.2-increase produced by the same diet in AMPKα2$^{-/-}$ mice. This fatty acid also contributed to the lower content of liver diacylglycerols in AMPKα2$^{-/-}$ compared to wild-type mice fed the Chow diet.

TABLE 5. The content and composition of hepatic diacylglycerols in wild-type and AMPKα2$^{-/-}$ mice subjected to hyperinsulinemic-euglycemic clamp

	Wild-type			AMPKα2$^{-/-}$		
	Chow	cHF	cHF+F	Chow	cHF	cHF+F
Diacylglycerol species						
Total fatty acids (μmol/g)	1.90 ± 0.21	2.43 ± 0.32	2.57 ± 0.32	1.99 ± 0.12	2.72 ± 0.35	2.43 ± 0.48
Myristic (14:0) (μmol/g)	0.06 ± 0.01	0.05 ± 0.01	0.07 ± 0.01	0.05 ± 0.00	0.06 ± 0.01	0.06 ± 0.01
Palmitic (16:0) (μmol/g)	0.58 ± 0.07	0.67 ± 0.09	0.85 ± 0.17	0.62 ± 0.03	0.73 ± 0.08	0.75 ± 0.15
Palmitoleic (16:1n7) (μmol/g)	0.03 ± 0.00	0.01 ± 0.00*	0.01 ± 0.00*	0.03 ± 0.00	0.01 ± 0.00*	0.01 ± 0.00*
Stearic (18:0) (μmol/g)	0.53 ± 0.18	0.41 ± 0.05	0.68 ± 0.19	0.42 ± 0.05	0.48 ± 0.10	0.68 ± 0.26
Oleic (18:1n9) (μmol/g)	0.24 ± 0.04	0.29 ± 0.05	0.22 ± 0.04	0.31 ± 0.05	0.33 ± 0.05	0.21 ± 0.04
Linoleic (18:2n6) (μmol/g)	0.29 ± 0.05	0.81 ± 0.15*	0.49 ± 0.09	0.35 ± 0.04	0.91 ± 0.14*	0.47 ± 0.08*†
Arachidic (20:0) (nmol/g)	13.16 ± 4.54	9.63 ± 0.72	14.52 ± 3.47	9.63 ± 1.08	11.94 ± 2.51	16.70 ± 6.43
α-Linolenic (18:3n3) (nmol/g)	17.76 ± 5.65	13.88 ± 3.53	13.70 ± 4.46	11.91 ± 1.68	13.03 ± 2.17	11.09 ± 2.80
Arachidonic (20:4n6) (nmol/g)	83.84 ± 14.03	68.67 ± 2.07	33.82 ± 3.17*†	106.37 ± 11.29	80.26 ± 4.40*	39.60 ± 4.58*†
Lignoceric (24:0) (nmol/g)	1.29 ± 0.21	1.09 ± 0.12	1.56 ± 0.23	1.21 ± 0.08	1.02 ± 0.13	1.08 ± 0.21
Eicosapentaenoic (20:5n3) (nmol/g)	5.73 ± 0.45	5.10 ± 0.75	11.96 ± 0.99*†	6.26 ± 0.82	5.40 ± 0.58	12.48 ± 1.12*†
Nervonic (24:1n9) (nmol/g)	11.63 ± 2.56	9.83 ± 2.32	11.61 ± 2.24	10.87 ± 2.25	12.37 ± 2.85	11.64 ± 1.82
Docosahexaenoic (22:6n3) (μmol/g)	0.05 ± 0.01	0.07 ± 0.01	0.16 ± 0.03*†	0.07 ± 0.01	0.08 ± 0.01	0.16 ± 0.04*

The data are the means ± SE (n = 5-8). Wild-type and AMPKα2$^{-/-}$ mice were fed either a Chow diet or corn oil-based high-fat diets without (cHF) or with 15% of the lipids in the form of n-3 LC-PUFA concentrate (cHF+F) for 9 weeks. Liver tissue was collected immediately after the completion of hyperinsulinemic-euglycemic clamp. *$P < 0.05$ vs. genotype Chow; †$P < 0.05$ vs. genotype cHF; ‡$P < 0.05$ vs. wild-type on respective diet.

RESEARCH DESIGN AND METHODS

Animals and treatments. Whole-body AMPKα2$^{-/-}$ mice and wild-type littermate controls generated on a hybrid C57BL/6 and 129/Sv genetic background (1) and backcrossed to C57BL/6J mice for nine generations were used. At four weeks of age, mice were weaned onto a standard laboratory Chow (lipid content ~3.4% wt/wt; extruded R/M-H diet; Ssniff Spezialdiäten, Soest, Germany) and maintained at 22°C on a 12 h light–dark cycle (light on from 6 a.m.) with free access to food and water.

Two separate experiments were performed, in which four-month-old wild-type and AMPKα2$^{-/-}$ mice (n = 13-15), caged in groups of 3-4 mice, were fed the following diets for nine weeks: (i) Chow; (ii) a corn oil-based high-fat diet (cHF; lipid content ~35.2% wt/wt); or (iii) a cHF diet supplemented with n-3 LC-PUFA concentrate (46% DHA, 14% EPA; product EPAX 1050 TG; EPAX, a.s., Lysaker, Norway) replacing 15% of dietary lipids (cHF+F). The macronutrient and fatty acid composition of all diets has been described before (2). Fresh rations of food were distributed every two days, while food consumption and body weights were recorded once a week. Mice from the first experiment in ad libitum fed state were first anesthetized by diethylether, exsanguinated through the cervical incision, then sacrificed by cervical dislocation (between 8 a.m. and 10 a.m.), and EDTA-plasma, liver, quadriceps muscles, epididymal and subcutaneous (dorsolumbar) adipose tissues were collected for various analyses (see below). When indicated, plasma samples were also collected after eight weeks from mice fasted for 15 hours (between 8 a.m. and 11 p.m.). In order to assess insulin sensitivity, mice from the second experiment were studied under hyperinsulinemic-euglycemic conditions.

Real-time RT-PCR analysis. Total RNA was isolated from liver samples (stored in RNA*later* Solution; Ambion, Austin, TX) homogenized in TRIzol Reagent (Invitrogen, Carlsbad, CA). A single-strand cDNA was synthesized from 0.5 µg of total RNA and gene expression was assessed by real-time PCR, using the LightCycler® 480 Instrument and the kit LightCycler® 480 SYBR Green I (Roche Diagnostics, Mannheim, Germany). Transcript levels were normalized according to the expression levels of eukaryotic elongation factor 1a (ELF-1a). The following oligonucleotide primers, designed by Lasergene software (DNAStar, Madison, WI), were used:
SCD1:
sense, 5'-ACTGGGGCTGCTAATCTCTGGGTGTA,
antisense, 3'- GGCTTTATCTCTGGGGTGGGTTTGTTA;
SREBP-1c:
sense, 5'- TACCCGTCCGTGTCCCCCTTTTC,
antisense, 3'- TGCGCTTCTCACCACGGCTCTG;
ELF-1a:
sense 5'- TGACAGCAAAAACGACCCACCAAT,
antisense, 3'- GGGCCATCTTCCAGCTTCTTACCA.

Activity of α1 and α2 AMPK isoforms. Livers were collected by freeze-clamping. AMPK was immunoprecipitated from tissue extracts and the activity was assayed using a peptide substrate (3). AMPKα1 or AMPKα2 isoforms were immunoprecipitated using specific antibodies (gift of Dr. D. Grahame Hardie), which were bound to Protein G Sepharose (Amersham Pharmacia Biotech, Little Chalfont, UK). Immunoprecipitates were mixed with AMP (1 mmol/l), [γ-

^{32}P]ATP (1 mmol/l) and AMARA peptide (1 mmol/l; Vidia, Prague, Czech Republic) dissolved in HEPES-Brij buffer. Reaction proceeded for 30 min at 30°C and it was stopped by dropping the filter spotted with a reaction mixture into 1% phosphoric acid solution. The activity of 1 U/mg protein corresponds to 1 nmol ^{32}P-AMARA peptide/mg protein per min.

The analysis of blood samples from hyperinsulinemic-euglycemic clamps. To assess D-[3-^{3}H]glucose, $^{3}H_2O$ and total glucose concentrations, blood samples were firstly deproteinized by precipitation with a $ZnSO_4/Ba(OH)_2$, followed by centrifugation (4). The first aliquot of the supernatant was evaporated to dryness to determine the radioactivity corresponding to D-[3-^{3}H]glucose. The second aliquot was used to determine the radioactivity of both D-[3-^{3}H]glucose and $^{3}H_2O$. Plasma $^{3}H_2O$ radioactivity was then calculated as the difference between radioactivity in the second and the first aliquot. In the third aliquot of the supernatant, the total glucose concentration was assessed by the glucose oxidase method (Glukosa God 1500, PLIVA-Lachema, Czech Republic). The D-[3-^{3}H]glucose specific activity was calculated by dividing the D-[3-^{3}H]glucose enrichment of plasma (dpm/ml) by the total plasma glucose concentration (mg/ml). Time points, in which the steady-state D-[3-^{3}H]glucose specific activity varied more than ± 15 %, were not taken into account (4). The rates of insulin-stimulated glucose turnover (GTO) were determined as the ratio of the [3-^{3}H]glucose infusion rate (dpm/min) to the specific activity of plasma glucose (5). The rate of glycogen synthesis in quadriceps muscle was quantified by measuring the incorporation of D-[3-^{3}H]glucose into glycogen and calculated by dividing the radioactivity of D-[3-^{3}H]glucosyl units in glycogen (dpm/kg) by the mean specific activity of D-[3-^{3}H]glucose in plasma during the last hour of the clamp.

The rate of lipogenesis in isolated hepatocytes. De novo lipogenesis was assesed using ^{14}C-Acetate (1uCi/ml) (PerkinElmer, USA). Cells were incubated in 2 ml of a medium (M199, Gibco) containing ^{14}C acetate with or without 25 mM glucose and 100 nM insulin (Actrapid MC, Novoindustri, Denmark). After 2 hr of incubation at 37 °C (5% CO_2 and 95% O_2) cells were washed by PBS and harvested into 600 ul KOH (shaking at 70°C). ^{14}C incorporation into saponifiable FA was estimated (6) and expressed as pmol of acetate converted to fatty acid/mg protein per hour. Identical incubations in parallel wells without radioactivity were performed to determine protein concentrations.

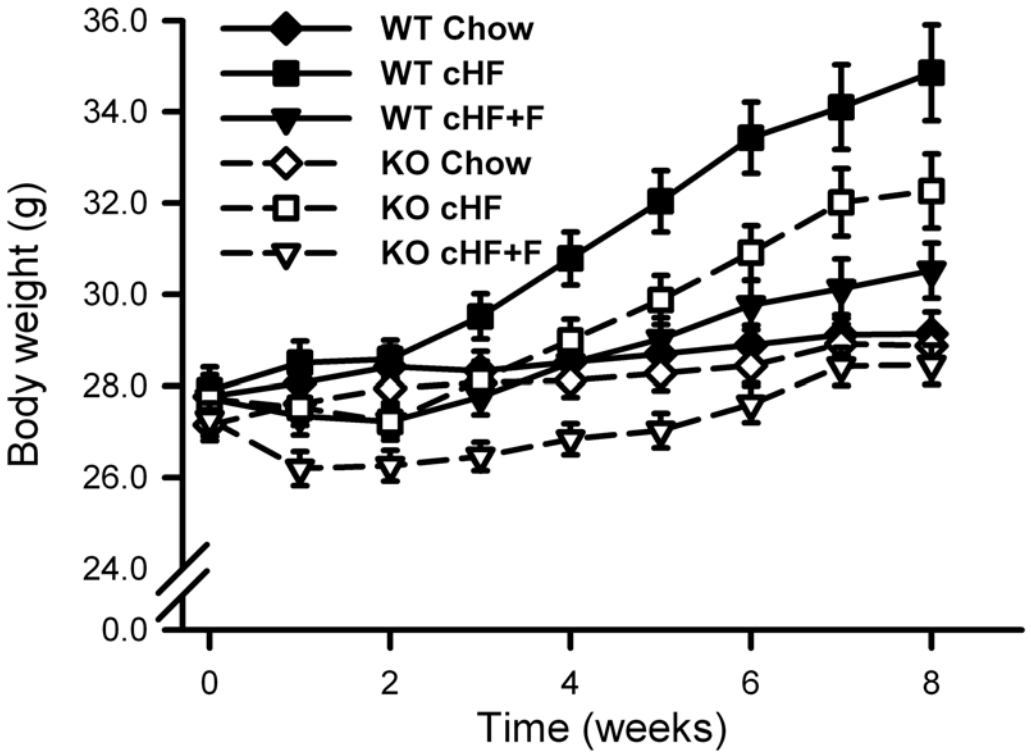
The rate of fatty acid oxidation in isolated hepatocytes. Palmitate oxidation was assesed using [1-^{14}C] palmitate (PerkinElmer, USA). Cells were incubated for 45 min at 37°C in 800 ul of a medium (M199) supplemented with 1% BSA, 50 μM cold palmitate and [1-^{14}C] palmitate (0,38 μCi/reaction) with or without 1 mM AICAR. The reactions were terminated by aspiration of the media, cells were washed by PBS and then incubated in 800 ul of 5 % perchloric acid for 15 min at room temperature. Palmitate oxidation was determined by measuring production of ^{14}C-labeled acid-soluble metabolites (ASM), a measure of tricarboxylic acid cycle intermediates and acetyl esters. The ASM were assessed in supernatants of the acid precipitate. Identical incubations in parallel wells without radioactivity were conducted to determine protein concentrations.

Total content and fatty acid composition of phospholipid, diacylglycerol, triglyceride, and ceramide fractions. Liver samples were pulverized in an aluminum mortar precooled in liquid nitrogen. The powder was transferred to a tube containing ice-cold methanol and 0.01%

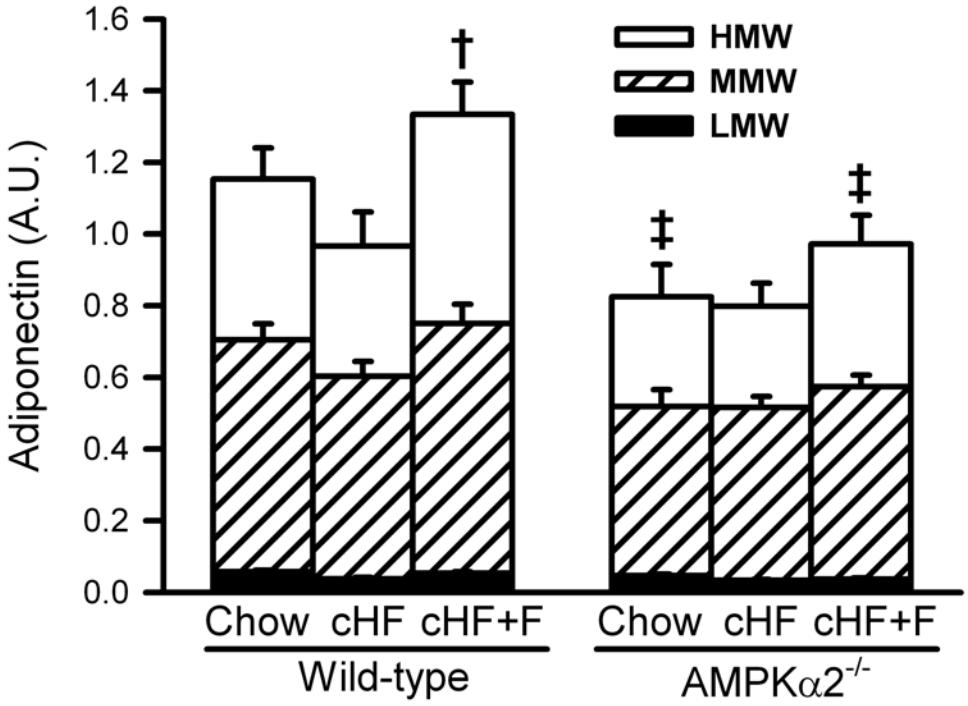
butylated hydroxytoluene (Sigma) as an antioxidant. Internal standards (1,2-diheptadecanoin, triheptadecanoin and 1,2-diheptadecanoyl-sn-glycero-3-phosphatidylcholine, Larodan Fine Chemicals, Sweden) were then added to the samples and lipids were extracted by the method of Folch. The fractions of total phospholipids, triglycerides and diacylglycerols were separated by thin-layer chromatography (7). Lipid class standards were spotted on the outside lanes of the chromatography plate to enable localization of the sample lipid classes. The gel bands corresponding to the standards were scraped off the plates, transferred to fresh tubes and then transmethylated in 14% methanolic boron trifluoride (Sigma) at 100°C for either 10 or 30 minutes (phospholipids and triglycerides). The content of resulting fatty acid methyl esters was determined by means of gas-liquid chromatography (8). The content of ceramides was determined as described previously (9) with the exception that N-palmitoyl-D-*erythro*-sphingosine (C17 Base; a gift from Dr. Zdzislaw Szulc, Medical University of South Carolina) was used as an internal standard.

FIGURE LEGENDS

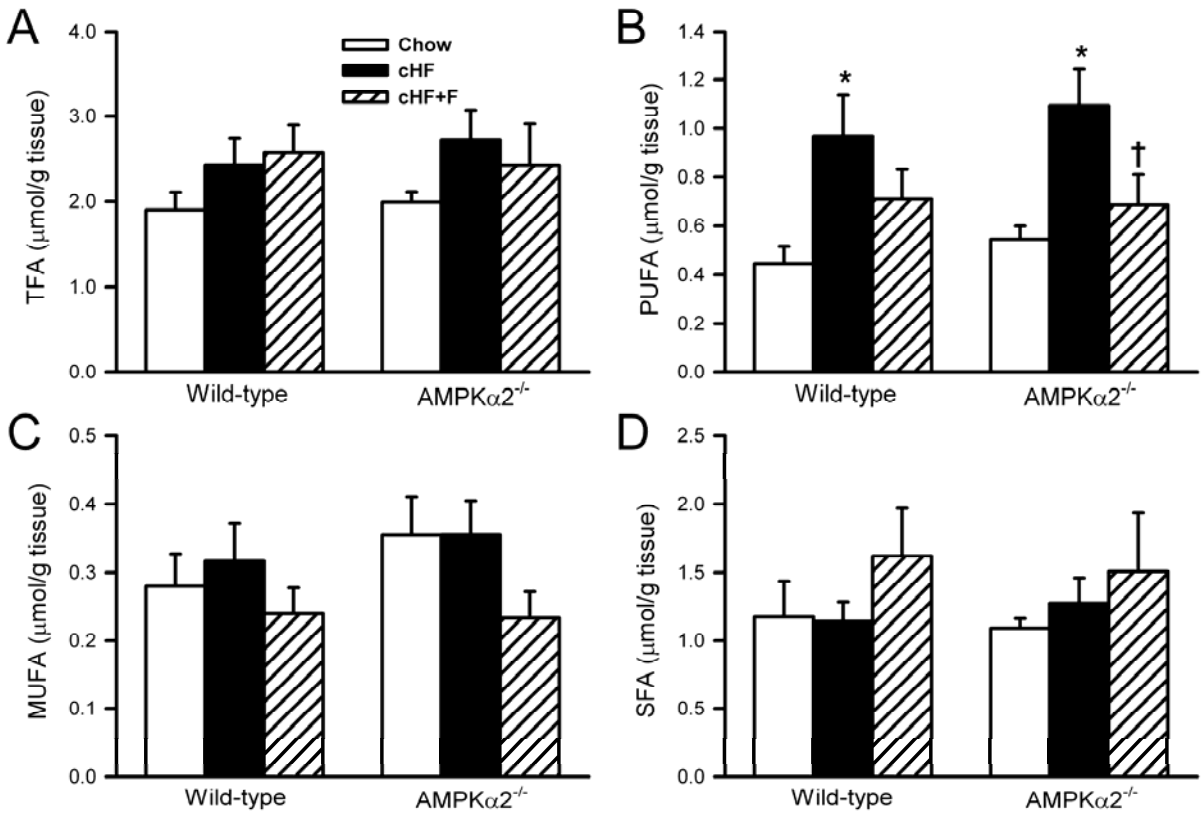
Supplemental Figure. 1. Body weight curves of wild-type (WT) and AMPKα2$^{-/-}$ (KO) mice fed either a Chow diet or corn oil-based high-fat diets without (cHF) or with 15% of the lipids in the form of n-3 LC-PUFA concentrate (cHF+F) for 9 weeks. The data are the means ± SE (n = 27-30).



Supplemental Fig. 2. Adiponectin levels in plasma of wild-type and AMPKα2$^{-/-}$ mice fed either a Chow diet or corn oil-based high-fat diets without (cHF) or with 15% of the lipids in the form of n-3 LC-PUFA concentrate (cHF+F) for 9 weeks, and killed in ad libitum fed state. The total adiponectin levels and the distribution of adiponectin multimeric complexes were determined using Western blotting (10). The data are the means ± SE (n = 13-15). **P* < 0.05 vs. genotype Chow; †*P* < 0.05 vs. genotype cHF; ‡*P* < 0.05 vs. wild-type on respective diet. Significance evaluated for the total adiponectin levels. A.U., arbitary units; LMW – low molecular weight; MMW – medium molecular weight; HMW – high molecular weight.



Supplemental Fig. 3. The composition of fatty acids in hepatic diacylglycerol fraction in wild-type and AMPKα2$^{-/-}$ mice subjected to hyperinsulinemic-euglycemic clamp: total fatty acids (TFA; *A*), polyunsaturated fatty acids (PUFA; *B*), monounsaturated fatty acids (MUFA; *C*), saturated fatty acids (SFA; *D*). Animals were fed either a Chow diet or corn oil-based high-fat diets without (cHF) or with 15% of the lipids in the form of n-3 LC-PUFA concentrate (cHF+F) for 9 weeks, and then subjected to hyperinsulinemic-euglycemic clamp. Liver tissue was collected immediately after the completion of the clamp. Data are means ± SE (n = 5-8). **P* < 0.05 vs. genotype Chow; †*P* < 0.05 vs. genotype cHF.



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