analyzed tissue samples from brain. Brain tissue was minced, transferred to a 1.5-ml microcentrifuge tube, and washed twice with sterile phosphate-buffered saline (PBS). Tissue chunks were resuspended in 1.0 ml Dulbecco's MEM with 2% serum. The tissue was then homogenized and subjected to three freeze-thaw cycles. Particulate matter was pelleted and the supernatant was used for plaque assays.

17. J. DeFalco, unpublished observations.
18. J. C. Erickson, G. Hollopeter, R. D. Palmiter, *Science* **274**, 1704 (1996).
19. H. Higuchi, H. Y. Yang, S. L. Sabol, *J. Biol. Chem.* **263**, 6288 (1988).
20. C. Elias et al., *Neuron* **23**, 775 (1999).
21. BAC clones containing the *Npy* gene were identified by hybridization to a mouse BAC library filter array using a mouse *Npy* cDNA fragment. A pSV1 shuttle vector subclone containing *Npy* exons 1 and 2 was subsequently generated to give pSV1-*Npy*. Using PCR, we mutated the first ATG to ATT and introduced a Pac I site directly 3′ to the mutated ATG. PCR was used to generate an NLS-*Cre*-SV40 poly(A⁺) which was then inserted into the Pac I site of pSV1-*Npy*. Recombination into the BAC clone was performed as in X. W. Yang et al. [*Nature Biotechnol.* **15**, 859 (1997)].
22. D. R. Gehlert et al., *Synapse* **1**, 25 (1987).
23. L. Zaborsky, *Adv. Anat. Embryol. Cell Biol.* **69**, 1 (1982).
24. H. Fei et al., *Prod. Natl. Acad. Sci. U.S.A.* **94**, 7001 (1997).
25. J. P. Card et al., *J. Neurosci.* **10**, 1974 (1990).
26. F. C. Barone et al., *Brain Res. Bull.* **7**, 75 (1981).
27. H. Bester, J. M. Besson, J. F. Bernard, *J. Comp. Neurol.* **383**, 245 (1997).
28. G. D. Petrovich, P. Y. Risold, L. W. Swanson, *J. Comp. Neurol.* **374**, 387 (1996).
29. M. Yang et al., *J. Virol.* **73**, 4350 (1999).
30. Intracerebral virus injections were performed as in J. P. Card et al. [*J. Comp. Neurol.* **407**, 438 (1999)] with two exceptions: a 32-gauge cannula was used to minimize tissue trauma and fluorescent microspheres (Molecular Probes, Eugene, OR) were coinjected to mark injection site. Striatal injection coordinates were +0.38 mm bregma, 1.5 mm lateral of sagittal suture, and −3.5 mm dorsal-ventral. For cortex injections, coordinates were −1.2 mm bregma, 3.65 mm lateral and −2.65 dorsal-ventral. Coordinates for arcuate injections were: −1.9 mm bregma, 0.15 mm lateral, and −5.5 mm dorsal-ventral. A total of 100 nl of virus was injected at a rate of 10 nl/min. The needle was removed after 10 min.
31. Animals were killed by pentobarbital overdose and perfused with 4% paraformaldehyde in PBS. Brains were postfixed, equilibrated in PBS + 30% sucrose and sectioned serially on a cryostat. For immunofluorescence, sections were blocked in PBS with 2% goat serum, 3% bovine serum albumin, and 0.1% Triton X-100 and incubated with the respective primary antibody according to the supplier's recommendations. Antibodies against NPY were obtained from Peninsula Labs (San Carlos, CA). Monoclonal antibodies against gC were purchased from Chemicon (Temecula, CA). Sections were then washed and incubated in FITC or TRITC secondary antibody. Primary antibody dilutions used were as follows: against gC, 1:1000; against NPY, 1:10,000; against GFP, 1:350. Sections were examined using a Zeiss Axioplan microscope. Images were collected using a Princeton Graphics digital camera and processed using IPLab Powermicrotome deconvolution software from Scanolytics (Fairfax, VA).
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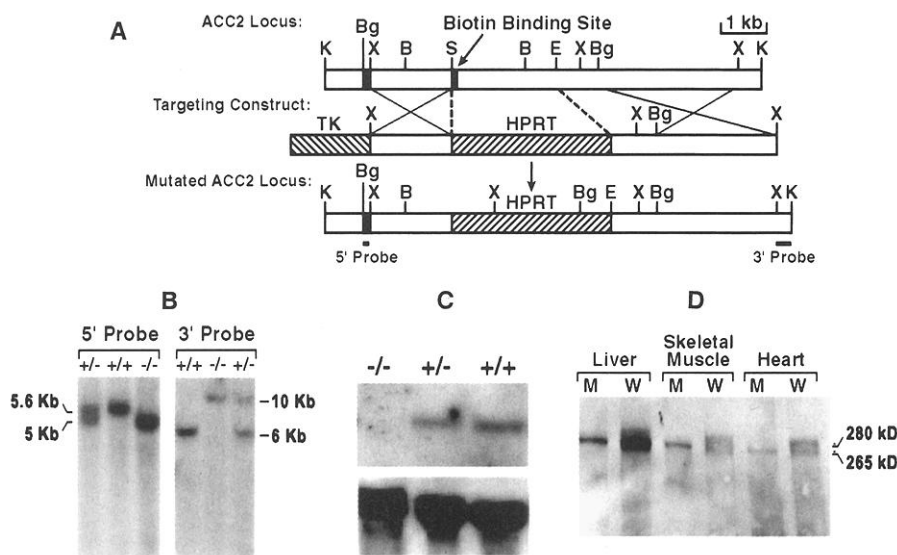
Continuous Fatty Acid Oxidation and Reduced Fat Storage in Mice Lacking Acetyl-CoA Carboxylase 2

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Malonyl–coenzyme A (malonyl-CoA), generated by acetyl-CoA carboxylases ACC1 and ACC2, is a key metabolite in the regulation of energy homeostasis. Here, we show that $Acc2^{-/-}$ mutant mice have a normal life span, a higher fatty acid oxidation rate, and lower amounts of fat. In comparison to the wild type, Acc2-deficient mice had 10- and 30-fold lower levels of malonyl-CoA in heart and muscle, respectively. The fatty acid oxidation rate in the soleus muscle of the $Acc2^{-/-}$ mice was 30% higher than that of wild-type mice and was not affected by addition of insulin; however, addition of insulin to the wild-type muscle reduced fatty acid oxidation by 45%. The mutant mice accumulated 50% less fat in their adipose tissue than did wild-type mice. These results raise the possibility that pharmacological manipulation of ACC2 may lead to loss of body fat in the context of normal caloric intake.

Acetyl–coenzyme A (acetyl-CoA) carboxylase (ACC) catalyzes the synthesis of malonyl-CoA, a metabolite that plays a pivotal role in the synthesis of fatty acids as the donor of "C_2 units" (1–3) and in the oxidation of fatty acid as the regulator of the mitochondrial shuttle system (4, 5). Hence, ACC links fatty acid and carbohydrate metabolism through the shared intermediate acetyl-CoA, the product of pyruvate dehydrogenase. Deciphering the roles of ACC in energy metabolism in lipogenic tissues (liver and adipose) and nonlipogenic tissues (heart and muscle) has become the focus of many studies (4–11). In animals, including humans, there are two isoforms of acetyl-CoA carboxylase, ACC1 [relative molecular mass (M_r) ~ 265,000] and ACC2 (M_r ~ 280,000), which are encoded by separate genes and display distinct tissue distributions (12–16). ACC1 is highly expressed in liver and adipose tissue, whereas ACC2 is predominantly expressed in heart and muscle, and to a lesser extent in liver (3, 13–17). ACC2 is localized in the mitochondria and ACC1 in the cytosol (6).

The carboxylases are highly regulated by diet, hormones, and other physiological factors. Food intake, especially fat-free diets, induces the synthesis of ACCs and increases their activities. Starvation or dia-

betes mellitus represses the expression of the ACC genes and decreases the activities of the enzymes. Malonyl-CoA, the product of ACC1 and ACC2, is the key metabolic signal for the control of fatty acid oxidation and synthesis in response to dietary changes. Among the critical unanswered questions are whether malonyl-CoA pools exist that differentially control fatty acid oxidation and synthesis, whether these putative pools can be independently manipulated, and if so, what would be the physiological consequences of such manipulation. To examine these issues, we generated ACC2-deficient mice.

A mouse Acc2 genomic clone was isolated using an Acc2 cDNA probe, and a targeting vector was constructed to generate embryonic stem cells with one mutant copy of the $Acc2^{tm1AE}$ allele (Fig. 1A). Heterozygotes did not have any obvious abnormalities. Genotype analysis of offspring from heterozygous matings were consistent with Mendelian inheritance [24% $Acc2^{-/-}$ ($n = 72$), 54% $Acc2^{+/-}$ ($n = 162$), and 22% wild type ($n = 66$); (Fig. 1B)]. Northern blot (Fig. 1C) and Western blot (Fig. 1D) analyses indicated that the $Acc2^{tm1AE}$ allele was null. The $Acc2^{-/-}$ mutants are fertile and appear to have a normal life span.

Because malonyl-CoA is generated by both ACC1 and ACC2, we investigated whether ACC1 can compensate for the absence of ACC2. ACC activities in wild-type and mutant livers were the same (1.3 and 2.4 nmol/min/mg of protein in the absence and presence of citrate, respectively), suggesting that malonyl-CoA in the mutant liver is pro-

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duced mainly by ACC1 (Fig. 2). In contrast, the ACC activities in the absence and presence of citrate were 5.5 and 6.3 nmol/min/mg of protein, in heart and skeletal muscle, respectively, for the wild type and 2.0 and 2.3 nmol/min/mg of protein, respectively, for the mutant. Malonyl-CoA levels in heart and skeletal muscle were about 10- and 30-fold lower, respectively, in the $Acc2^{-/-}$ mice compared to the wild type, suggesting that

malonyl-CoA in skeletal muscle is produced primarily by ACC2 (Fig. 2). During fasting, the levels of malonyl-CoA dropped comparably in the liver, heart, and muscle of wild-type and $Acc2^{-/-}$ mice, confirming that ACC1 is regulated by diet (Fig. 2). Because malonyl-CoA in muscle is generated primarily by ACC2 (3), and because there is a 70% reduction of malonyl-CoA in fasted wild-type mice, ACC2 must also be regulated by diet.

Because ACC is the rate-determining step in fatty acid synthesis (2) and the levels of malonyl-CoA in wild-type and $Acc2^{-/-}$ livers were similar, we expected that fatty acid synthesis would also be similar. Indeed, the synthesis of palmitate, as measured by the incorporation of [^{14}C]acetyl-CoA (18) was the same for both groups. However, the livers of wild-type mice were lighter in color than the mutant livers, suggesting that they contained more fat (Fig. 3A). To confirm this, we stained liver sections with Oil Red-O to detect lipids and to estimate their lipid and triglyceride contents. Wild-type livers contained abundant lipid droplets (Fig. 3B), which are primarily triglycerides, whereas $Acc2^{-/-}$ livers contained significantly fewer lipid droplets (Fig. 3C). Extraction and analysis of the total lipids by thin-layer chromatography showed that the mutant livers contained 20% less lipid than wild-type livers, and the triglyceride content of the lipid was 80 to 90% lower than wild type (18).

Since the activities of ACC and fatty acid synthase in liver extracts of the wild type and the $Acc2^{-/-}$ mutants were the same (18), the difference in the liver lipid content must be secondary to uncontrolled mitochondrial fatty acid oxidation in the $Acc2^{-/-}$ livers, rather than due to suppression of fatty acid synthesis. Also, because malonyl-CoA is a negative regulator of the mitochondrial carnitine palmitoyl-CoA shuttle system (5), its absence in $Acc2^{-/-}$ livers would be expected to increase fatty acid translocation across the mitochondrial membrane and subsequent β-oxidation. Thus, these results suggest that malonyl-CoA, synthesized by ACC2, affects the accumulation of fat in the liver by controlling fatty acid oxidation. Since ACC1-generated malonyl-CoA, which is abundant in the livers of both groups of mice, apparently did not inhibit the β-oxidation of fatty acids, we conclude that the malonyl-CoA produced by ACC1 and ACC2 exists in two distinct compartments of the cell, the cytosol and the mitochondria, respectively, and carries out distinct functions in these compartments. Because both ACC1 and ACC2 are present in both the periportal (zone 1) and perivenous (zone 3) hepatocytes of rat liver (19), it is unlikely that the two pools of malonyl-CoA derive from differential expression of ACC1 and ACC2 in these discrete regions of the liver.

To examine whether the loss of ACC2 affects the level of glycogen, an important regulator of energy homeostasis, we resected livers from wild-type and $Acc2^{-/-}$ mutant mice, and frozen sections were stained for glycogen (Fig. 3, D and E). In the nourished state, the wild-type livers contained abundant amounts of glycogen


Fig. 1. Targeted mutation of the *ACC2* locus. (**A**) Genomic region [2.5 kilobases (kb)] containing the biotin-binding motif, (Met-Lys-Met), was replaced with a hypoxanthine phosphorylribosyltransferase (HPRT) expression cassette to generate the targeting construct. The 5′ and 3′ probes used to identify the targeted events by Southern blot analysis are indicated. B, Bam H1; Bg, Bgl II; K, Kpn 1; E, Eco R1; S, Sma 1; X, Xba 1. (**B**) Southern blot analysis of genomic DNA extracted from mouse tails. DNA digested with Bgl II was probed with the 5′ probe; DNA digested with Bam H1 and Kpn 1 was probed with the 3′ probe. DNA from the wild-type (+/+), heterozygous (+/−), and $Acc2^{-/-}$ mutant (−/−) mice gave the expected fragment sizes. (**C**) Northern blot of total RNA prepared from skeletal muscle of wild-type (+/+), heterozygous (+/−), and $Acc2^{-/-}$ mutant (−/−) mice was probed with the [^{32}P]-labeled 362–base pair cDNA fragment, which was used to screen the genomic library. The probe detected a 10-kb transcript in the $Acc2^{+/-}$ and wild-type mice but not in the $Acc2^{-/-}$ mice (upper panel). Hybridization of the same filter (after stripping) with a mouse β-actin cDNA probe confirmed that equal amounts of RNA were loaded (lower panel). (**D**) Absence of ACC2 protein in the $Acc2^{-/-}$ mice. Extracts (50 μg each) from liver, skeletal muscle, and heart were subjected to SDS–polyacrylamide gel electrophoresis (6%). The proteins were transferred onto a nitrocellulose filter and probed with avidin-peroxidase to detect biotin-containing proteins. The positions of the 280-kD ACC2 carboxylase and the 265-kD ACC1 carboxylase are indicated.
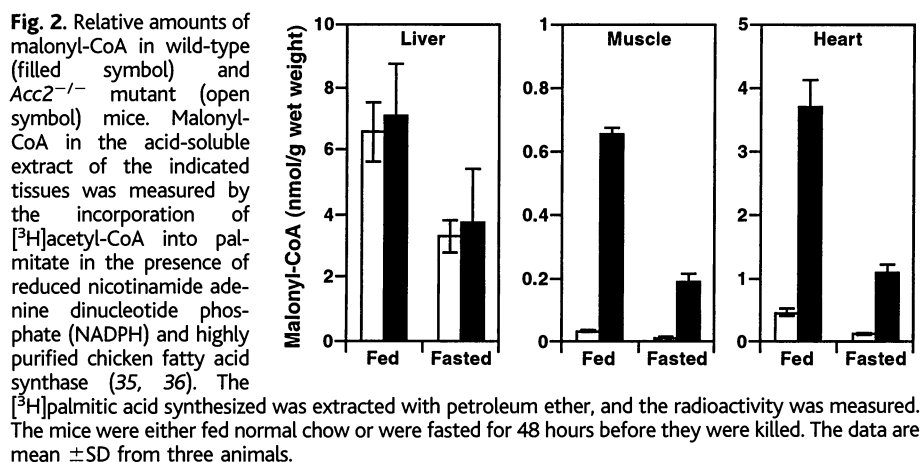
Fig. 2. Relative amounts of malonyl-CoA in wild-type (filled symbol) and $Acc2^{-/-}$ mutant (open symbol) mice. Malonyl-CoA in the acid-soluble extract of the indicated tissues was measured by the incorporation of [^{3}H]acetyl-CoA into palmitate in the presence of reduced nicotinamide adenine dinucleotide phosphate (NADPH) and highly purified chicken fatty acid synthase (35, 36). The [^{3}H]palmitic acid synthesized was extracted with petroleum ether, and the radioactivity was measured. The mice were either fed normal chow or were fasted for 48 hours before they were killed. The data are mean ±SD from three animals.

$(410 \pm 10 \mu\text{mol/g of wet liver})$, whereas the livers of $Acc2^{-/-}$ mice $(325 \pm 14 \mu\text{mol/g of wet liver})$ contained 20% less glycogen. We speculate that, compared to the wild-type liver, more glucose is used in the synthesis of fatty acids and their subsequent oxidation in the $Acc2^{-/-}$ liver, thus depleting glycogen. In the 24-hour-fasted wild-type liver, glycogen was clearly present (Fig. 3D), whereas it was undetectable in the $Acc2^{-/-}$ mutant liver (Fig. 3E).

We next analyzed the serum levels of cholesterol, glucose, triglycerides, free fatty acids, and ketone bodies in wild-type and $Acc2^{-/-}$ mice fed a standard diet. Cholesterol levels were similar in both groups of mice $(92.8 \pm 3.1$ and 95.1 ± 7.4 mg/dl), and glucose levels were 20% lower in mutant mice $(176.6 \pm 6.5$ versus 136.2 ± 5.4 mg/dl). Fatty acid levels were lower in mutant mice $(1.37 \pm 0.31$ versus 0.84 ± 0.12 mM), whereas triglyceride levels were 30% higher in mutant mice $(35.1 \pm 2.5$ versus 45.2 ± 5.9 mg/dl), possibly due to mobilization of triglycerides and fatty acids from liver and/or adipose for their delivery to the heart and muscle as substrate for oxidation. Serum levels of the ketone bodies (β-hydroxybutyrate) were nearly undetectable in both the wild type and the mutants. However, an overnight fast (10 to 12 hours) increased the blood β-hydroxybutyrate concentration of the $Acc2^{-/-}$ mice fourfold over that of the wild type $(2.5 \pm 0.6$ mM versus 0.7 ± 0.5 mM, $n = 5$), consistent with a higher degree of fatty acid oxidation in the mutant mice.

We also measured fatty acid oxidation in the soleus muscle, which is hormonally regulated (10, 11, 19–21). Oxidation of [^{3}H]palmitate was 30% higher in the muscle from $Acc2^{-/-}$ mice than in those from the wild type. Addition of insulin, known to activate both ACC1 and ACC2, thereby inducing fatty acid synthesis and suppressing fatty acid oxidation, respectively, did not affect the rate of fatty acid oxidation in the $Acc2^{-/-}$ muscle. However, it did suppress palmitate oxidation by 45% in wild-type muscle, suggesting that insulin-mediated inhibition of β-oxidation occurs through the activation of ACC2, possibly by dephosphorylation (7–10, 22–29). Thus, the mitochondrial β-oxidation of fatty acids occurred in the $Acc2^{-/-}$ mice in an unregulated, yet sustained, manner.

To investigate the role of this type of fatty acid β-oxidation and its effect on food consumption and weight gain, we carried out feeding experiments involving three groups of mice (each group consisting of five wild-type and five $Acc2^{-/-}$ mice) that were fed a weighed standard diet *ad liberatum*. Food consumption for each group was measured every week for 27 weeks, and the

Fig. 3. Histological analyses of livers of 32-week-old male mice fed standard diet. (A) Livers of wild-type (left) and $Acc2^{-/-}$ mutant (right) mice after 24 hours of starvation. Frozen sections of the livers were stained with Oil Red-O to detect lipid droplets and counterstained with Mayer's hematoxylin. The wild-type liver (B) shows an abundance of lipid droplets (stained red) in comparison to the mutant liver (C). Frozen sections from the same livers were stained for glycogen using the periodic acid–Schiff method and counterstained with hematoxylin. The wild-type liver (D) contains glycogen (stained pink) and unstained lipid vacuoles, whereas the mutant livers (E) have very little or no glycogen and lipid vacuoles. Bar, 50 μm.
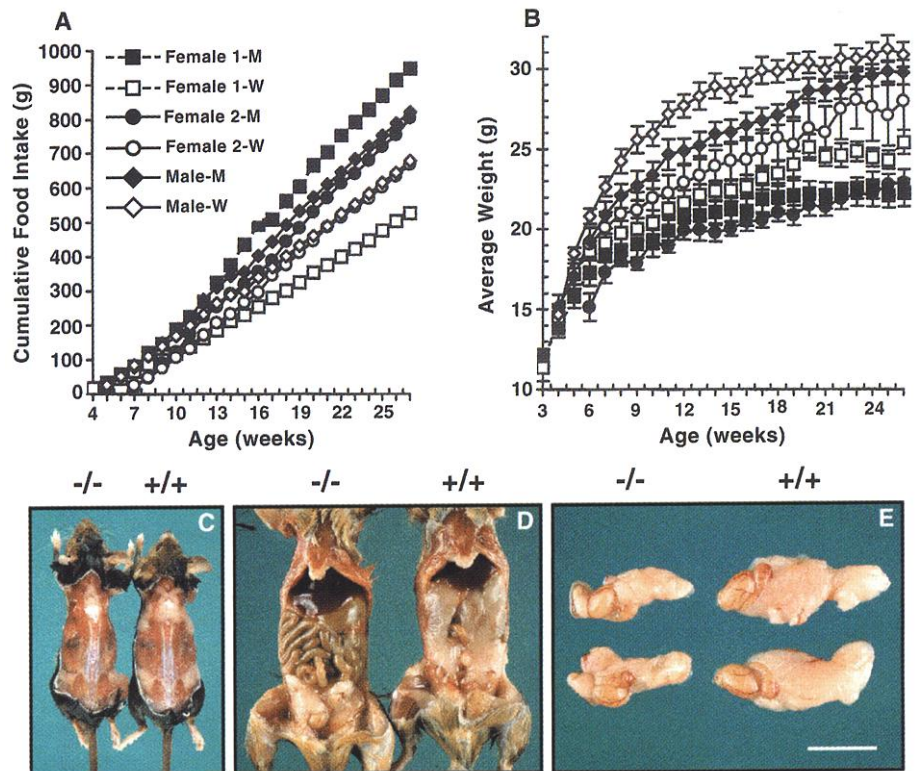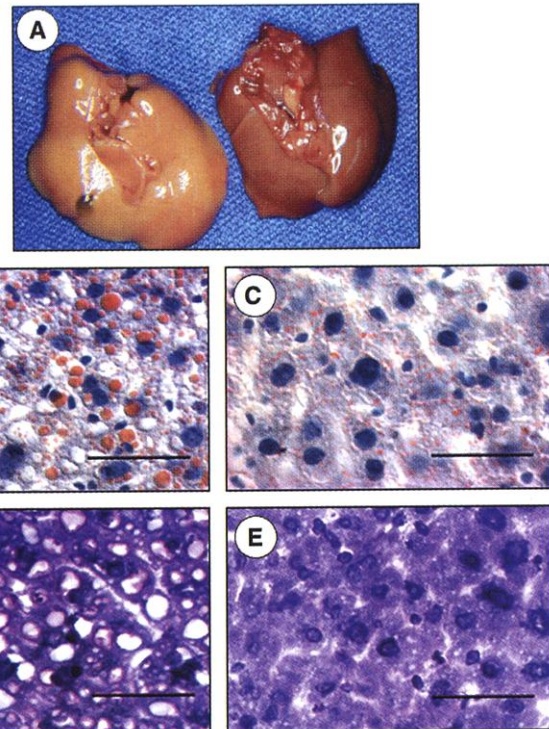
Fig. 4. Food intake, growth (body weight), and adipose tissue in $Acc2^{-/-}$ and wild-type mice. Two groups of female mice (numbered 1 and 2; 3 and 6 weeks old, respectively) and one group of 5-week-old males—with each group consisting of five $Acc2^{-/-}$ mutant (M, filled symbols) and five wild-type mice (W, open symbols)—were fed a standard diet for 27 weeks. (A) Food intake was measured every week and was expressed as cumulative food intake per mouse over the 27-week period. (B) The weight of each mouse within each group was measured weekly; the data are presented as means ± SD. (C) Dorsal view of male littermates, aged 32 weeks, fed with standard diet. The amount of white fat under the skin of the $Acc2^{-/-}$ mouse (33.6 g weight) was much less than that in the wild-type (+/+) mouse (34.2 g weight). (D) Abdominal view of the fat pad under the skin. (E) Epididymal fat pads isolated from the mutant (0.75 g) and the wild-type mice (1.4 g). Bar, 1 cm.

weight of each mouse was recorded weekly. On average, the $Acc2^{-/-}$ mice consumed 20 to 30% more food than did the wild-type mice and attained an average body weight of 21 g per mouse compared to 23 g per wild-type mouse. The $Acc2^{-/-}$ mutant mice were generally leaner, weighing about 10% less than the wild-type mice throughout the feeding periods (Fig. 4B), and they accumulated less fat in their adipose tissues (Fig. 4, C and D). For example, the epididymal fat pad tissue in an $Acc2^{-/-}$ male weighed 0.75 g compared to 1.4 g in a wild-type male littermate (Fig. 4E). The decrease in the adipose size resulted in a decrease in the leptin release to the plasma from 53 ± 9 ng/ml in the wild type to 36 ± 3 ng/ml in the mutant mice (30, 31). Thus, mitochondrial oxidation of fatty acids regulates fat storage in the adipose tissue.

In conclusion, our analysis reveals that mice lacking malonyl-CoA show increased oxidation of fatty acids, decreased fat in adipose tissue and liver, and decreased storage of glycogen in the liver; however, they are still morphologically normal, grow at the expected rate, and breed normally. The $Acc2^{-/-}$ mice consumed 20 to 30% more food than did the wild type, yet lost or simply maintained body weight. In the absence of ACC2, the rate of fatty acid oxidation increased in heart and muscle, as well as the rest of the body. We speculate that adipose and liver triglycerides are mobilized, and their fatty acids are made available to the various tissues for oxidation. Hence, the reduction in fat contents and size of the adipose (Fig. 4E) led to a reduction by about 30% of leptin released to the plasma (30, 31), similar to that occurring in fasted mice, and signaled the hypothalamus to produce the appetite-stimulating neuropeptide Y, which promotes feeding (32, 33). We feel this is the most plausible explanation for the observation that $Acc2^{-/-}$ mice have smaller fat stores even as they consumed more food than did the wild type (Fig. 4). It has been suggested that malonyl-CoA may play a role in signaling the availability of physiological fuel by acting through the hypothalamic neurons (34). This suggestion was based on the inhibition of ACC by 5-(tetradecyloxy)-2 furoic acid that increased food intake in mice treated with fatty acid synthase inhibitors (34). Although this possibility could not be ruled out in the $Acc2^{-/-}$ mice, the lower leptin levels in the plasma may be sufficient to increase appetite. Moreover, the $Acc2^{-/-}$ mice appear to be normal, with no obvious neurological abnormalities.

Maintenance of high levels of fatty acid oxidation results in reduced fat accumulation and storage, a physiological state that humans try to attain through exercise. If the results shown here for mice hold true for humans, then pharmacological inhibition of ACC2 might allow individuals to lose weight while maintaining normal caloric intake.

References and Notes
1. S. J. Wakil, E. B. Titchener, D. M. Gibson, Biochim. Biophys. Acta 29, 225 (1958).
2. S. J. Wakil, J. K. Stoops, V. C. Joshi, Annu. Rev. Biochem. 52, 537 (1983).
3. K. G. Thampy, J. Biol. Chem. 264, 17631 (1989).
4. J. D. McGarry, G. P. Mannaerts, D. W. Foster, J. Clin. Invest. 60, 265 (1977).
5. J. D. McGarry, N. F. Brown, Eur. J. Biochem. 244, 1 (1997).
6. L. Abu-Elheiga et al., Proc. Natl. Acad. Sci. U.S.A. 97, 1444 (2000).
7. G. Lopaschuk, J. Gamble, Can. J. Physiol. Pharmacol. 72, 1101 (1994).
8. N. Kudo et al., J. Biol. Chem. 270, 17513 (1995).
9. J. R. Dyck et al., Eur. J. Biochem. 262, 184 (1999).
10. D. Vavvas et al., J. Biol. Chem. 272, 13255 (1997).
11. N. Alam, E. D. Saggerson, Biochem. J. 334, 233 (1998).
12. L. Abu-Elheiga, A. Jayakumar, A. Baldini, S. S. Chirala, S. J. Wakil, Proc. Natl. Acad. Sci. U.S.A. 92, 4011 (1995).
13. L. Abu-Elheiga, D. B. Almarza-Ortega, A. Baldini, S. J. Wakil, J. Biol. Chem. 272, 10669 (1997).
14. J. Ha et al., Proc. Natl. Acad. Sci. U.S.A. 93, 11466 (1996).
15. K. G. Thampy, S. J. Wakil, J. Biol. Chem. 263, 6447 (1988).
16. A. Bianchi et al., J. Biol. Chem. 265, 1502 (1990).
17. A. J. Iverson, A. Bianchi, A. C. Nordlund, L. A. Witters, Biochem. J. 269, 365 (1990).
18. L. Abu-Elheiga, M. M. Matzuk, K. A. H. Hashema, S. J. Wakil, data not shown.
19. J. L. Evans, B. Quistorff, L. A. Witters, Biochem. J. 259, 821 (1989).
20. I. H. Chaudry, M. K. Gould, Biochim. Biophys. Acta 177, 527 (1969).
21. A. Ibrahimi et al., J. Biol. Chem. 274, 26761 (1999).
22. K. H. Kim et al., FASEB J. 3, 2250 (1989).
23. K. G. Thampy, S. J. Wakil, J. Biol. Chem. 263, 6454 (1988).
24. G. M. Mabrouk et al., J. Biol. Chem. 265, 6330 (1990).
25. A. H. Mohamed et al., J. Biol. Chem. 269, 6859 (1994).
26. D. G. Hardie, Prog. Lipid Res. 28, 117 (1989).
27. B. B. Rasmussen, W. W. Winder, J. Appl. Physiol. 83, 1104 (1997).
28. W. W. Winder, D. G. Hardie, Am. J. Physiol. 270, E299 (1996).
29. B. B. Rasmussen, R. R. Wolfe, Annu. Rev. Nutr. 19, 463 (1999).
30. M. W. Schwartz et al., Diabetes 45, 531 (1996).
31. Y. Zhang et al., Nature 372, 425 (1994).
32. D. O'Shea et al., Endocrinology 138, 196 (1997).
33. M. Schwartz, J. Erickson, D. Baskin, R. Palmiter, Endocrinology 139, 2629 (1998).
34. T. M. Loftus et al., Science 288, 2379 (2000).
35. R. Bressler, S. J. Wakil, J. Biol. Chem. 236, 1643 (1961).
36. J. D. McGarry, S. E. Mills, C. S. Long, D. W. Foster, Biochem. J. 214, 21 (1983).
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Conservation Conflicts Across Africa

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There is increasing evidence that areas of outstanding conservation importance may coincide with dense human settlement or impact. We tested the generality of these findings using 1°-resolution data for sub-Saharan Africa. We find that human population density is positively correlated with species richness of birds, mammals, snakes, and amphibians. This association holds for widespread, narrowly endemic, and threatened species and looks set to persist in the face of foreseeable population growth. Our results contradict earlier expectations of low conflict based on the idea that species richness decreases and human impact increases with primary productivity. We find that across Africa, both variables instead exhibit unimodal relationships with productivity. Modifying priority-setting to take account of human density shows that, at this scale, conflicts between conservation and development are not easily avoided, because many densely inhabited grid cells contain species found nowhere else.

Efforts to stem the ongoing loss of biodiversity are hampered by conflicts between conservation and development (1–3). Priority-setting exercises for birds have shown that such conflicts may be frequent because areas of high conservation value are often characterized by rapid habitat clearance or high human population density (4, 5). In contrast, Huston (6) has suggested that species richness generally decreases and agri-cultural productivity generally increases with primary productivity and that therefore conservation and development needs can be readily reconciled. Here we test these contradictory hypotheses using 1°-resolution databases summarizing the distribution of birds, mammals, snakes, amphibians, people, habitat conversion, and net primary productivity (NPP) across sub-Saharan Africa (7).