ternatively, sporozoites may be eliminated by either macrophages or by some other component of the immune system.

Our results suggest that the numerous genetic and biochemical tools available for *Drosophila* research can now be used to investigate *Plasmodium*-insect interactions. We have shown that *Drosophila* permits an ookinete to develop into an oocyst and can provide appropriate nutrients to sustain the growth of the oocyst. Further, the insect's cellular immune response is capable of affecting plasmodial development. Analysis of *Drosophila* mutants that alter any aspect of the *Plasmodium* life cycle should shed light on host requirements for *Plasmodium* development. By identifying factors that are critical to the survival of *Plasmodium*, these experiments may contribute to the development of new drugs, transmission-blocking vaccines, and engineered *Plasmodium*-resistant mosquitoes (*14*).

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Reduced Food Intake and Body Weight in Mice Treated with Fatty Acid Synthase Inhibitors

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With the escalation of obesity-related disease, there is great interest in defining the mechanisms that control appetite and body weight. We have identified a link between anabolic energy metabolism and appetite control. Both systemic and intracerebroventricular treatment of mice with fatty acid synthase (FAS) inhibitors (cerulenin and a synthetic compound C75) led to inhibition of feeding and dramatic weight loss. C75 inhibited expression of the prophagic signal neuropeptide Y in the hypothalamus and acted in a leptin-independent manner that appears to be mediated by malonyl–coenzyme A. Thus, FAS may represent an important link in feeding regulation and may be a potential therapeutic target.

Excess body weight is a major health problem in developed nations, affecting over 50% of the U.S. population (*1*), and is increasing both in prevalence and severity. This condition is associated with increased risk of type II diabetes, cardiovascular and cerebrovascular diseases, and increased mortality (*1*). The magnitude of this health problem and the recent difficulties with several weight-loss therapies emphasize the need for different approaches to weight-loss therapy.

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FAS catalyzes the reductive synthesis of long-chain fatty acids from acetyl–coenzyme A (acetyl-CoA) and malonyl-CoA (*2*). The mechanism through which two carbon units from malonyl-CoA are sequentially added to the growing fatty acid chain is unique among vertebrates, making FAS an attractive target for the design of therapeutic agents. Cerulenin, a natural FAS inhibitor, forms a well-characterized complex with the enzyme (*3*); however, its epoxide structure is thought to limit its utility as a drug. We synthesized a FAS inhibitor, C75. Intraperitoneal (ip) injection of mice with C75 leads to a 95% reduction in ^{14}C-acetate incorporation into fatty acids and to a 110% increase in the level of hepatic malonyl-CoA, the principal substrate of FAS (Web fig. 1) (*4*).

To investigate the physiological consequences of in vivo inhibition of fatty acid synthesis on global lipid metabolism, we administered cerulenin [60 mg/kg body weight per day (mg/kg/day) for 7 days] or C75 (single dose of 7.5 to 30 mg/kg) to mice by ip injection. We observed profound weight loss following treatment (Fig. 1). Weight loss occurred in a dose-dependent manner and persisted for a duration that increased with dose. In all cases, treated mice recovered lost body weight after the effect of the drug dissipated, arguing against induction of a persistent state of wasting. The treatment was well tolerated by the mice with no obvious toxicity. Necropsy and histological analysis of all major organs in treated mice revealed no adverse pathology and plasma alanine aminotransferase activity was unchanged (Web fig. 2A). In addition, C75-induced weight loss was observed in mice lacking interleukin-1r and tumor necrosis factor–α r1a receptors, suggesting that the weight loss is not mediated by an inflammatory response (Web fig. 2B) (*4*).

C75-induced weight loss was due primarily to inhibition of feeding. Intraperitoneal administration of C75 (15 mg/kg) reduced food intake by more than 90% over the first 24 hours (Fig. 1C). Feeding then returned to normal over the next 48 to 72 hours as the drug effect dissipated. Inhibition of feeding was observed within 20 min of treatment (Web fig. 3) (*4*). Forced feeding largely reversed the observed weight loss (*5*).

There was a 40% reduction in both water intake and urinary output in C75-treated mice (*6*). This is consistent with a change in osmotic balance resulting from decreased intake of salts and other solutes in the diet. However, we cannot exclude the possibility that some of the observed weight loss is due to water loss. The loss of adipose mass was

accompanied by a reduction of lean body mass typical of that observed in fasting (*6*).

To determine whether C75-induced weight loss is attributable entirely to suppression of feeding, we compared ip C75 treatment with fasting. C75-treated mice lost 45% more weight than did the fasted animals (Fig. 2A). Because the normal response to fasting is to reduce energy utilization (*7*), this result suggests that C75 treatment may allow maintenance of a normal energy utilization as well as inhibition of feeding. No gross changes in the animals' activity were observed (*6*).

Neuropeptide Y (NPY) acts in the hypothalamus as a central member of a coordinated group of neuropeptides, regulated by adiposity and feeding status, that control feeding and energy utilization. NPY promotes feeding (*8*) and its expression increases in the fasted state (*9*). To ascertain whether C75 affects NPY expression in the hypothalamus, we performed Northern blot analysis of hypothalamic tissue from fed, fasted, and C75-treated mice (Fig. 2). As expected, fasting markedly up-regulated NPY mRNA. However, hypothalamic NPY mRNA levels in C75-treated mice were even lower than those in fed control mice, even though the C75-treated mice had not eaten. This indicates that C75 inhibits feeding, at least in part, by blocking NPY-induced feeding.

We next examined whether NPY could reverse C75-induced anorexia. Intracerebroventricular (ICV) injection of NPY (2.5 μg) into mice pretreated with ip injection of either vehicle or C75 (C75-NPY) rapidly led to voracious feeding, whereas ICV injection of vehicle had no effect on feeding. Total food intake within 1 hour by C75-NPY–treated mice was similar to that by mice treated with NPY alone, and intake was nine times greater than that by C75-treated mice (Fig. 2C). This result indicates that the pathways downstream of NPY are functional in C75-treated mice and that C75 acts upstream of NPY. C75 also suppresses voracious feeding in fasted animals that have already up-regulated their endogenous NPY (Web fig. 3) (*4*), suggesting that C75 must have additional feeding regulatory effects.

Leptin is elevated in the fed state and inhibits NPY production and feeding (*10*) in a manner similar to that observed with C75 treatment. Because leptin is synthesized primarily in white adipose tissue (*11*), a primary site of fatty acid synthesis, we tested whether it mediates the effects of C75. Serum leptin levels were reduced rather than elevated in C75-treated mice (Fig. 3A), indicating that leptin does not mediate the C75 signal. Northern blot analysis of leptin mRNA levels in white adipose tissue supported this observation (*6*).

If C75 acts by a leptin-independent mechanism, it should reduce the obesity of leptin-deficient *ob/ob* mice (*11*). A 2-week course of ip C75 treatment was found to reduce body weight by 10 g (Fig. 3, B and C). Histological examination of the liver from C75-treated mice revealed a dramatic normalization of the fatty liver that is characteristic of *ob/ob* mice (Fig. 3D). There was no evidence of histological abnormality. In addition, C75 treatment corrected the hyperglycemia observed in control *ob/ob* mice, leading to a nearly threefold reduction in serum glucose levels. A 24-hour ip treatment of wild-type mice had no effect on serum glucose beyond that attributable to fasting (Web fig. 4) (*4*).

The role of metabolism in controlling feeding is well established. The infusion of physiological fuels such as glucose (*12*) or fatty acids [reviewed in (*13*)] has long been known to inhibit feeding. Furthermore, inhibitors of glucose or fatty acid oxidation (e.g., 2-deoxyglucose or mercaptoacetate) stimulate feeding. However, the FAS inhibitors described here operate by a distinct mechanism because they induce a feeding-inhibitory signal in the absence of an added physiological fuel.

A physiological link between feeding inhibition and fatty acid synthesis is consistent with the fact that synthesis occurs only during energy surplus, when excess physiological fuels are channeled into energy storage. However, the observation that FAS inhibition produces the same response predicted for active fatty acid synthesis argues against an effect of the end product of the pathway. Rather it suggests that an intermediate, preceding FAS in the pathway, mediates the metabolic signal. We postulate that elevation in the level of the FAS substrate, malonyl-CoA, during fatty acid synthesis may be linked to feeding control (Fig. 4A). It is unlikely that inhibition of fatty acid synthesis per se causes feeding inhibition. In previous studies (*14*), treatment of mice with inhibitors of acetyl-

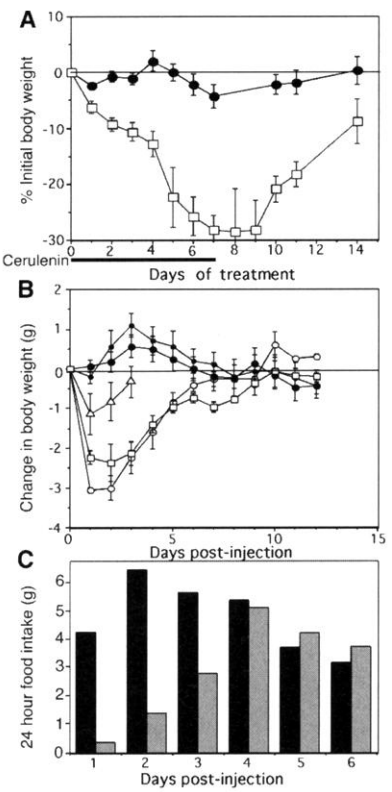

Fig. 1. Effect of FAS inhibitors on body weight and food intake. (**A**) Female BALB/c mice were treated by ip injection of RPMI medium vehicle (●) or cerulenin at 60 mg/kg/day (□) for 7 days. (**B**) Female BALB/c mice were given a single ip injection of C75 at doses of 7.5 (△), 15 (○), or 30 (□) mg/kg or (●) RPMI vehicle. Mice were housed in metabolic cages and monitored for body weight, food and water intake, and urine and feces output. Mean change from initial body weight is expressed ± SEM. (**C**) Total food intake per day by mice treated with vehicle (black bars) or C75 (15 mg/kg) (gray bars) from (B). Experiments consisted of three animals per group and were repeated four times.

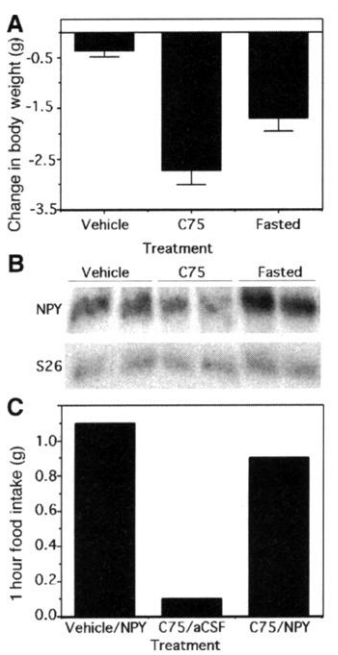

Fig. 2. Role of NPY in regulation of feeding by C75. (**A**) Male BALB/c mice were preweighed and fasted or were treated with vehicle or C75 (30 mg/kg) and fed ad libitum. After 24 hours, mice were weighed (n = 6 mice, repeated seven times). Change from initial body weight is expressed as mean ± SEM. (**B**) Hypothalamic RNA from mice in (A) was analyzed by Northern blot using random primed probes for transcripts encoding NPY and the ribosomal protein S26 (which was used as a loading control) (n = 6 mice). (**C**) Mice were pretreated for 4 hours with C75 (30 mg/kg) or with RPMI by ip injection, anaesthetized by inhaled metofane, and given an ICV injection of 2.5 μg of NPY (2.5 μl) or artificial cerebrospinal fluid vehicle. Mice were observed for feeding behavior and were monitored for food intake for 1 hour (three repetitions of three mice per group, nine mice total).

CoA carboxylase (ACC), the enzyme preceding FAS in the pathway, inhibited fatty acid synthesis but not feeding. Administration of ACC inhibitors would block malonyl-CoA production and, thus, would not be expected to inhibit feeding. In contrast, inhibition of FAS by C75 results in elevation of malonyl-CoA levels in vivo that may mimic fatty acid synthesis and, thus, the fed state.

This model predicts that feeding inhibition by FAS inhibitors should be attenuated by inhibitors of ACCs. To test this, we pretreated mice with either the ACC inhibitor TOFA or vehicle by ICV injection and examined the ability of an ip injection of C75 to inhibit feeding. TOFA largely restored food intake in C75-treated mice (Fig. 4B), supporting the hypothesis that malonyl-CoA mediates feeding inhibition. In addition, the efficacy of centrally administered TOFA argues for a central nervous system (CNS) mechanism of action. ICV administration of C75 inhibited feeding by 82% (Fig. 4C), supporting the central target action of C75.

We have observed expression of both FAS and ACC in select neurons in the brain, most notably in the arcuate nucleus of the hypothalamus (*15*). Our studies with [5-^{3}H]-C75 indicate that the drug enters the brain (Web fig. 5) (*4*). Thus, it is conceivable that these inhibitors act directly on the brain to control feeding, either in NPY-producing neurons or in neurons that act on them.

Fatty acid synthesis regulates fatty acid oxidation by a well-characterized regulatory mechanism (*16*). In this paradigm, malonyl-CoA levels rise during fatty acid synthesis and result in inhibition of carnitine palmitoyl transferase 1–mediated uptake of fatty acids into the mitochondrion. This results in elevation of cytoplasmic long-chain fatty acyl CoAs and diacylglycerol, molecules that may play a role in signaling, which leads to the proposal that malonyl-CoA levels act as a signal of the availability of physiological fuels (*17*). One such role proposed for malonyl-CoA is the mediation of nutrient-stimulated insulin secretion in the beta cell. Glucose-sensing neurons that regulate feeding in the hypothalamus share many features with the beta cell, including expression of glucokinase and the adenosine triphosphate–sensitive potassium channel (*18*). Our data support the prediction (*17*) that malonyl-CoA may signal fuel status in hypothalamic neurons.

Our studies provide evidence that FAS has a role in the control of feeding behavior and, thus, may represent a therapeutic target for the control of appetite and body weight.

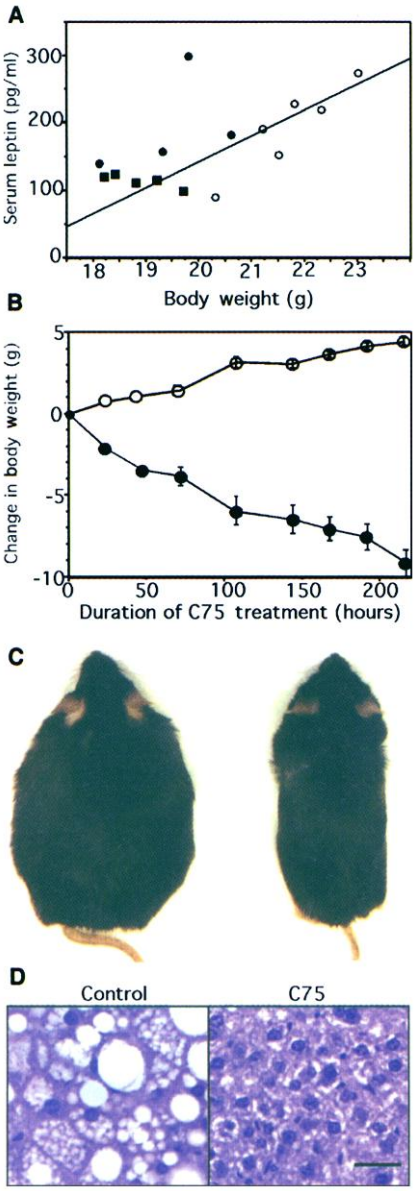

Fig. 3. Leptin independent of action C75. (**A**). Male BALB/c mice (n = 5) were fasted (●) or received ip injections of RPMI (○) or of C75 (30 mg/kg) (■) and were free-fed for 24 hours, weighed, and then exsanguinated. Serum leptin was determined with a Quantikine murine leptin enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, Minnesota) and plotted against total body weight. (**B**) Male *ob/ob* (C57BL/6OlaHsd-Lep^{ob}) (Harlan, Oxon, England) mice (n = 3) were treated with ip injections of RPMI (○) or of C75 (22 mg/kg) (●) every third day (representative of two experiments). Change in body weight is displayed as mean ± SEM. (**C**) Representative vehicle- (left) and C75-treated (right) mice from (B) after 14 days of treatment. (**D**) Livers from vehicle- (control) and C75-treated mice were formalin fixed and paraffin embedded. Tissue sections (4 μm) were stained with hematoxylin and eosin. Bar, 50 μm.

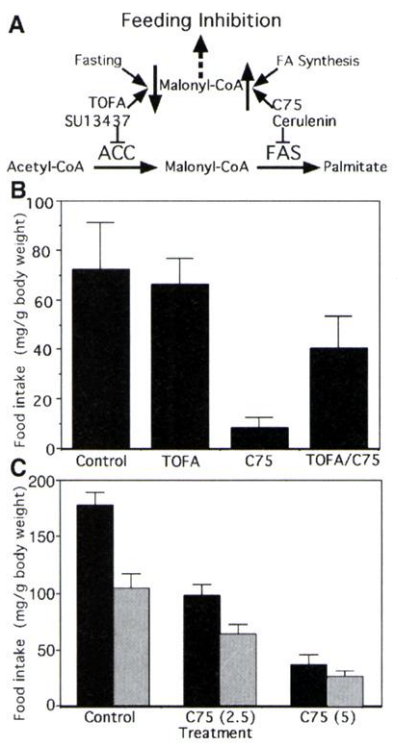

Fig. 4. Feeding regulation by malonyl-CoA. (**A**) Model of feeding regulation by inhibitors of FAS via malonyl-CoA. (**B**) BALB/c mice were anesthetized with metofane and received ICV injections of 2 μg of TOFA or dimethyl sulfoxide vehicle. After 2 hours recovery, mice received ip injections of C75 (15 mg/kg) or of RPMI vehicle and were monitored for total food intake over 2 hours. (**C**) Mice were anesthetized and received ICV injections of RPMI (2 μl) or of C75 (2.5 or 5 μg/μl), and food intake was monitored over 2 and 4 hours (gray and solid bars, respectively). (B) and (C) combine results from three experiments with n = 3 mice for each experiment (nine mice total).

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19. Experimental animals were treated in accordance with institutional animal usage guidelines. Except for where noted, all mice were obtained from Charles River Labs (Wilmington, MA). We thank R. Mains for the NPY probe. Supported by NIH grants DK09623 (T.M.L.) and DC02979 (G.V.R.), the NIDDK (M.D.L.), the W. M. Keck Foundation (G.V.R.), the Stewart Trust, and the Department of Defense (F.P.K.).

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