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17. The recombinant adenovirus was generated as described previously (*25*). Briefly, UL21.5 was amplified by PCR from the AD169 genome (nucleotides 27,108 to 27,499). The PCR fragment was sequenced and subcloned into pEYFP (Clontech, Palo Alto, CA) to generate pUL21.5-YFP through a Hind III–Sac II digest. The UL21.5-YFP coding sequence was excised with Bgl II–Not I and cloned into pAdShuttle (*25*). Homologous recombination of Pme I–linerized pAdShuttle 21.5-YFP DNA with the viral backbone vector pAd-Easy1 (*25*) was performed in *Escherichia coli* BJ5183 cells, yielding a replication-deficient adenovirus lacking the E1 and E3 transcription units (pAdShuttle UL21.5-YFP). Virus was cultured in 293 cells and isolated and purified as described (*25*).
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8 March 2000; accepted 20 April 2000

Malaria Parasite Development in a *Drosophila* Model

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Malaria is a devastating public health menace, killing over one million people every year and infecting about half a billion. Here it is shown that the protozoan *Plasmodium gallinaceum*, a close relative of the human malaria parasite *Plasmodium falciparum*, can develop in the fruit fly *Drosophila melanogaster*. *Plasmodium gallinaceum* ookinetes injected into the fly developed into sporozoites infectious to the vertebrate host with similar kinetics as seen in the mosquito host *Aedes aegypti*. In the fly, a component of the insect's innate immune system, the macrophage, can destroy *Plasmodia*. These experiments suggest that *Drosophila* can be used as a surrogate mosquito for defining the genetic pathways involved in both vector competence and part of the parasite sexual cycle.

Malaria is transmitted from one vertebrate host to another by a mosquito vector. Parasites of the genus *Plasmodium* undergo the sexual portion of their life cycle in a mosquito. The sequence begins when a mosquito, after biting a vertebrate, ingests a gametocyte-infected blood meal and ends with a second blood meal during which sporozoites are passed with the mosquito's saliva into a new vertebrate host (*1*, *2*). The parasites must overcome a number of barriers to develop in a mosquito. Immediately after ingestion, gametocytes emerge from red blood cells and differentiate into male and female gametes. These gametes are fertilized and the resulting zygotes develop into ookinetes within the gut. The ookinetes enter the body cavity (hemocoel) by crossing the gut lining (peritrophic matrix) and the midgut epithelium, where they subsequently lodge on the hemocoel side of the gut epithelium, beneath the basal lamina. The ookinetes then develop into oocysts. Oocysts undergo many rounds of nuclear division and produce thousands of sporozoites that are released into the hemocoel. Sporozoites invade the salivary glands to be injected into a vertebrate host about 2 weeks after the mosquito ingested the first infected blood meal.

The fruit fly *Drosophila melanogaster* has the potential to facilitate experimental analysis of insect stages of the parasite life cycle because the fly is genetically well defined and more easily manipulated than any mosquito. Though mosquitoes and fruit flies have obviously different lifestyles, they are members of the same phylogenetic order and, thus, are expected to share similarities in their physiology. To test whether *Plasmodia* could develop in the fruit fly, we attempted to reproduce *Plasmodium* sexual development in *Drosophila*. Fruit flies were fed blood infected with *Plasmodium gallinaceum*, which causes malaria in chickens. The flies ingested the blood meal, as evidenced by the blood visible in their abdomens, but the parasites were quickly cleared, as shown by the disappearance of *Plasmodium*-specific ribosomal RNA (rRNA) from the flies (Fig. 1). We wondered whether the parasite's failure to develop was caused by an inability of the fly to support *Plasmodium* gametogenesis. We therefore fed ookinetes to *Drosophila*. Ookinetes also failed to establish an infection when fed to the flies (Fig. 1). Again, the *Plasmodium* rRNA could no longer be detected in the parasite-fed flies, suggesting that the parasites were passed in the feces or digested.

The failure of *Plasmodia* ookinetes fed to *Drosophila* to establish an infection is likely to be a consequence of their inability to cross the midgut-associated barriers of the fly. To test this hypothesis, we prepared *P. gallinaceum* ookinetes in vitro from gametocytes obtained from infected chicken blood (*3*) and injected these directly into the hemocoel of adult flies. Under these conditions, *P. gallinaceum* rRNA could be detected in flies 9 days after injection, suggesting that the parasite survived in the fly hemocoel (Fig. 1).

To determine whether the appearance of *Plasmodium* rRNA was due to the development of the parasites or to the presence of arrested ookinetes, we injected ookinetes into flies and into the laboratory host mosquito, *Aedes aegypti*, and compared the parasites by microscopy. We observed *Plasmodium* oocysts in flies 9 days after injection of ookinetes, and the resulting oocysts were similar to those found in *Ae. aegypti* infected by a blood meal (Fig. 2, A, B, C, and D). The *Drosophila* oocysts were found attached to a variety of tissues, including the gut, salivary glands, and fat body. Sporozoites were detected at about the same time in the two hosts (9 days in flies; 11 days in mosquitoes) and were morphologically similar (Fig. 2, E and F). Mosquitoes produced seven times as many sporozoites as

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flies. The sporozoites in *Drosophila* also reacted with monoclonal antibodies to the circumsporozoite surface protein (CSP) (*4*), producing a similar immunofluorescence pattern as that seen in the mosquito (Fig. 2, G and H). These experiments demonstrate that *Drosophila* supports the development of *Plasmodium* oocysts into sporozoites that are morphologically and biochemically similar to those produced in a mosquito.

We next determined whether the *Drosophila*-generated sporozoites were infectious to their vertebrate host. Homogenates of infected insects were injected into 4-week-old white leghorn chickens. Infection of the chickens was monitored by daily blood smears. Chickens injected with mosquito-produced sporozoites became infected in 1 week. In initial experiments, no infection was observed in chickens infected with *Drosophila*-produced parasites even 3 weeks after sporozoite injection. We suspected that the parasites were present in these chickens but at an undetectable level, perhaps due to an immune response of the host (*5*). In addition, a preliminary examination showed that the sporozoites do not enter the *Drosophila* salivary glands (*6*). It has been demonstrated previously that hemolymph-stage sporozoites are less virulent than salivary gland–stage parasites (*7*). Serial passage of blood to a second and third naïve bird resulted in successful infections, demonstrating the infectivity of the *Drosophila* sporozoites (Fig. 2I). This experiment was performed four times with similar results. When *Ae. aegypti* mosquitoes were allowed to feed on these chickens infected with *Drosophila*-produced sporozoites, they became infected with *Plasmodium*, as assayed by production of oocysts (*6*).

In *Anopheles gambiae* mosquitoes, which are competent vectors for human malaria parasites, as few as 1 in 30,000 gametes develops into an oocyst, suggesting that even natural hosts are effective at combating the parasite, most likely through an immune response (*8*). To test whether the *Drosophila* immune system might also respond to a *Plasmodium* infection, we measured sporozoite levels over the course of an infection in *Drosophila*. We observed that sporozoite levels rise rapidly 9 days after injection and then drop (Fig. 3A). The rapid clearance of sporozoites from the circulation is consistent with an attack by the immune system. To test this hypothesis, we examined the effect of known *Drosophila* immune responses on a plasmodial infection.

Using real-time quantitative reverse transcription–polymerase chain reaction (RT-PCR), we assayed transcript levels for four

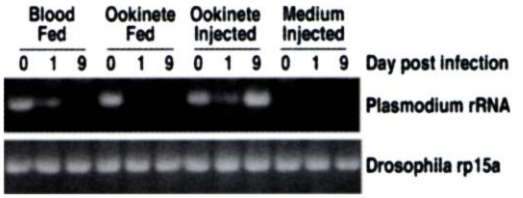


Fig. 1. *Plasmodia* are infectious to *Drosophila* when injected but not when they are ingested. Flies were either fed or injected with parasites and then incubated at 25°C for the times marked in (*14*, *15*). Flies were injected in a rostral, ventrolateral position on the abdomen with a hand-held glass capillary and a PLI 100 microinjector (Medical Systems Corp., Greenvale, New York). Total RNA was extracted from five flies on day 0, 1, or 9 post infection. RT-PCR was performed with Tth1 polymerase and the following primers: *Plasmodium* rRNA (5′-GAACGAGATCTTAACCTGC-3′, 5′-TATTGATAAAGATTACCTA-3′) (*15*, *16*) and *Drosophila* ribosomal protein 15a (5′-CGTTTGCGTGACGGTCGTGT-3′, 5′-GCCGAGAATTTTGCCTCCCAA-3′) as a loading control. DNA products were run on an agarose gel, were stained with ethidium bromide, and were photographed.

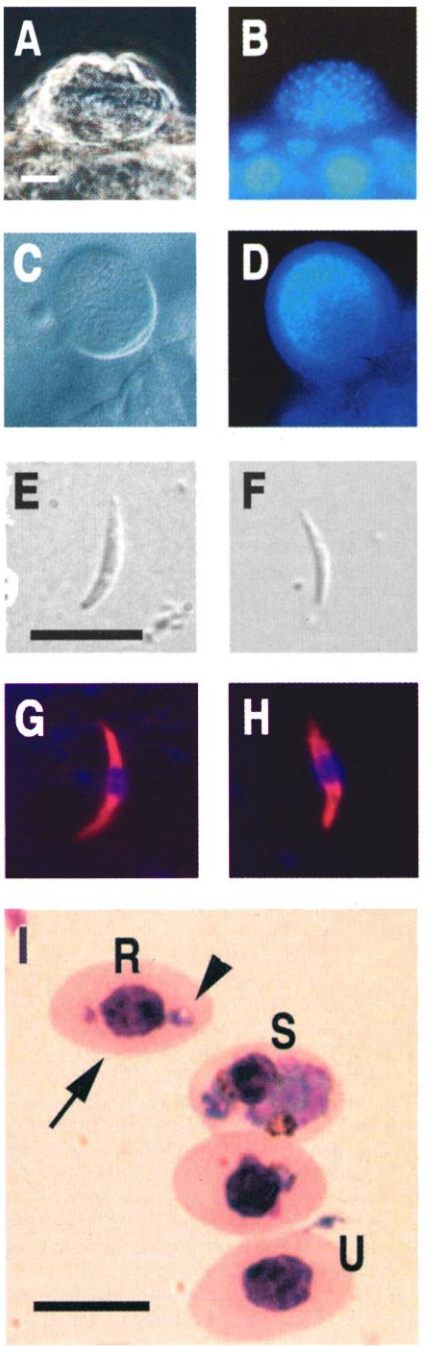
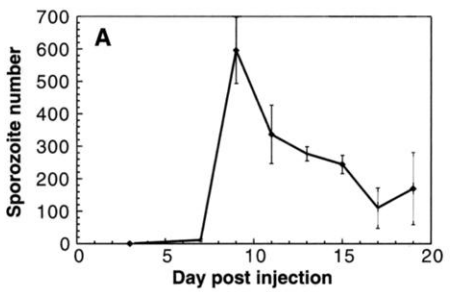


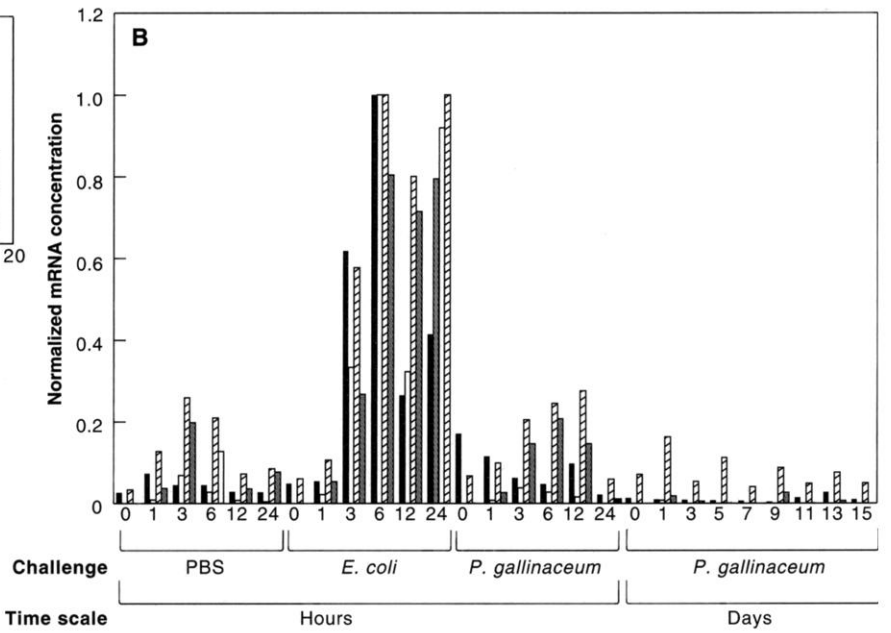
Fig. 2. Parasites that develop in *Drosophila* are similar to those in mosquitoes. *Ae. aegypti* mosquitoes were fed infected blood meals and their midguts were dissected, fixed, and stained with 4′,6′-diamidino-2-phenylindole (DAPI) 7 days after feeding. (**A**) Darkfield illumination. (**B**) DAPI immunofluorescence. The oocyst nuclei are characteristically numerous and tiny compared with the large nuclei of the mosquito gut. Ookinetes (n = 350 in 35 nl of medium M199) were injected into the abdomens of *D. melanogaster* Oregon R females, and the flies were dissected 9 days after infection. (**C**) Differential interference contrast (DIC) optics. (**D**) DAPI immunofluorescence. The *Drosophila*-raised oocyst has the same characteristic abundant and small nuclei found in oocysts developing in a mosquito. Ookinetes (n = 350 in 35 nl of medium M199) were injected into the abdomens of virgin *D. melanogaster* females (Oregon R) and the thoraces of *Ae. aegypti* females. *Aedes*-grown (**E**) and *Drosophila*-grown (**F**) sporozoites were isolated from their hosts 13 and 10 days after infection, respectively. Sporozoites were isolated by teasing apart three insects, washing the carcasses in 50-μl of PBS, and collecting a crude filtrate by centrifugation through a pinprick in a 0.5-ml tube (*17*). Sporozoites were observed in wet mounts with DIC optics. Alternatively, 10-μl sporozoite samples from mosquitoes (**G**) and flies (**H**) were air-dried onto a microscope slide and processed for indirect immunofluorescence with an antibody to circumsporozoite (anti-CSP) (*4*) and DAPI. Red, CSP; blue, DAPI. (**I**) *Drosophila*-raised sporozoites are infectious to chickens. Single infected flies were homogenized and injected subcutaneously into 4-week-old white leghorn chickens. Parasitemia was not observed during 3 weeks of culture in the first infected chicken. Blood (0.5 ml) from this animal was inoculated into a second naïve chicken. The parasitemia in this animal peaked at 2%, 12 days after infection. On day 14 after infection, 0.5 ml of blood from this chicken was injected into a third naïve chicken. A parasitemia of 80% was recorded in this chicken 7 days after infection. Blood smears were stained with geimsa. The nuclei of the chicken blood cells stain darkly (arrowhead) and the *Plasmodium* stain light blue (arrow). Two erythrocytic stages of the *P. gallinaceum* life cycle are visible in this photograph of a blood smear from the third chicken. U, uninfected; R, ring-stage parasite; S, a multinucleate schizont (replicating erythrocytic form). Scale bars in (A), (E), and (I), 10 μm.

Drosophila antimicrobial genes (defensin, diptericin, drosomycin, and metchnikowin) that respond to a variety of immune challenges. We found no reproducible up-regulation of any of these genes during the course of a *Plasmodium* infection (Fig. 3B). The response of the fly to phosphate-buffered saline (PBS) and *Plasmodia* was small and indistinguishable, whereas *Escherichia coli* induced a 10- to 10,000-fold increase in the transcription of these genes. Further, mutations in Toll and immune defective (imd), known to alter the expression of antimicrobial genes in fungal and bacterial infection models (*9*, *10*), did not change the course of plasmodial infections in *Drosophila* (*6*). No difference in survival of mutant flies during plasmodial infections was observed.

Mosquitoes express a variety of proteins in response to a plasmodial infection (*11*, *12*). Likewise, a distinct set of genes transcribed in response to bacterial or fungal infections has been identified in *Drosophila*. There is little overlap between the *Plasmodium*-induced gene expression profile in the mosquito and that observed in *Drosophila* in response to bacterial or fungal infections. The majority of *Plasmodium*-induced antimicrobial gene expression in the mosquito occurs in tissues that are invaded. Because no epithelia are crossed in a plasmodial infection in the fly, it is perhaps not surprising that we see no expression of known antimicrobials. Also, it is clear that fungi and bacteria elicit only partially overlapping immune responses in the fly, and therefore anti-protozoan responses may use different signal transduction cascades and antimicrobial peptides from the bacterial and fungal responses we were able to test.

To determine whether the cellular immune response of *Drosophila* could clear *Plasmodia*, we developed a method of inhibiting macrophages in the fly. Injection of 60 nl of a 2% suspension containing polystyrene beads into the hemolymph of the fly blocks *Drosophila* macrophage phagocytosis (*13*). When macrophages were inhibited 2 days before ookinete injection, we found a fourfold increase in sporozoite numbers on day 9 after infection (Fig. 3C). This suggests that macrophages destroy many injected ookinetes and prevent them from developing into oocysts. In contrast, we found that the inhibition of macrophages at day 8 after injection (post injection), immediately before sporozoites were released, had no significant effect on sporozoite profiles (Fig. 3C). These experiments suggest that macrophages alone are not responsible for the drop in sporozoite numbers. One possibility is that macrophages are more efficient at destroying ookinetes as compared to sporozoites. Al-





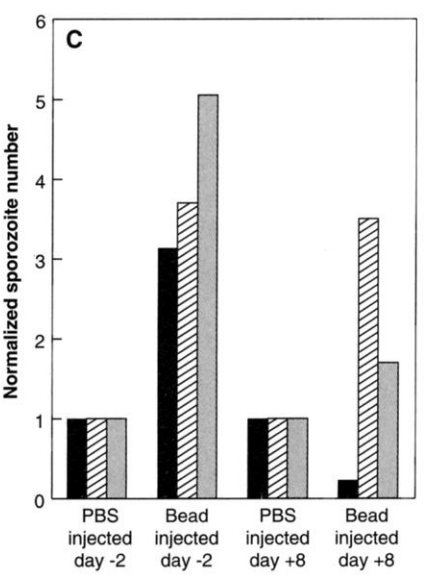


Fig. 3. *Plasmodia* are affected by the cellular immune response of the fly. (**A**) Ookinetes (n = 350 in 35 nl of M199 medium) were injected into virgin female *Drosophila* (Oregon R). Sporozoites were prepared and counted every 2 days, starting 3 days post injection. Sporozoites were prepared as described in Fig. 2 and stained with anti-CSP. Data were gathered by counting 10 of the ×40 fields on three separate slides. (**B**) Antibacterial and antifungal peptides are not expressed in response to *P. gallinaceum* development in the fly. Ookinetes (n = 350 in 35 nl of M199 medium) were injected into female *Drosophila*. A similar volume of *E. coli* was injected into female flies as a control for the induction of antimicrobial genes. PBS was injected to control for any potential wounding response. Two groups of five flies were frozen for each time point. RNA was prepared from these flies and was subjected to real-time RT-PCR analysis. Antimicrobial peptide transcript concentrations were normalized to the expression of the ribosomal protein 15a transcript in each sample. The values recorded are the averages from two RNA preparations. Oligonucleotides (oligos) used (listed 5′ to 3′) are as follows: rp15a, (5′ oligo) TGGACCACGAGGAGGCTAGG, (3′ oligo) GTTGGTTGCATGGTCGGTGA, and (hybridization oligo) 6FAM-TGGGAGGCAAAATTCTCGGCTTC-TAMRA; defensin, (5′) TTCTCGTGGCTATCGCTTTT, (3′) GGAGAGTAGGTCGCATGTGG, and (hybridization) 6FAM-AGGATCATGTCCTGGTGCATGAGGA-TAMRA; diptericin, (5′) ACCGCAGTACCCACTCAATC, (3′) CCCAAGTGCTGTCCATATCC, and (hybridization) 6FAM-CAGTCCAGGGTCACCAGAAGGTGTG-TAMRA; metchnikowin, (5′) TCTTGGGAGCGATTTTTCTGG, (3′) AATAAATTGGACCCGGTCTTG, and (hybridization) 6FAM-ACATCGTCACCAGGGACCCATTTTC-TAMRA; drosomycin, (5′) GTACTTGTTCGCCCTCTTCG, (3′) CTTGCACACACGACGACAG, and (hybridization) 6FAM-TCCGGAAGATACAAGGGTCCCTGTG-TAMRA. Solid bars, defensin; open bars, diptericin; diagonal-striped bars, drosomycin; gray bars, metchnikowin. (**C**) Flies were injected with 60 nl of PBS or with PBS containing 2% amine-coated polystyrene 0.2-μm diameter beads (Molecular Probes, Eugene, Oregon) to inhibit phagocytosis 2 days before infection or 8 days post infection. On day 0, female flies (Oregon R) were injected with 350 ookinetes in 35 nl of M199. Nine days after infection, sporozoites were prepared and counted. Solid bars, trial 1; diagonal-striped bars, trial 2; gray bars, trial 3.

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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28 February 2000; accepted 10 May 2000

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