Smoking history and body mass index (BMI) were determined for all participants. Blood samples were collected after overnight fasting, and plasma was frozen in aliquots at −80°C immediately after centrifugation (1600g for 15 min at 4°C).

We measured ghrelin with an enzyme immunoassay (Phoenix Pharmaceuticals). The intraassay CV was <5%, the interassay CV was <14%, and the lower detection limit was 0.1 μg/L. The participants smoked 1 filtered cigarette containing 0.8 mg of nicotine under highly regulated conditions. Every 15 s, a puff lasting 5 s was taken, and the whole cigarette had to be smoked within 5 min. Plasma ghrelin concentrations were determined at 0, 2, 5, 15, and 60 min after the initiation of smoking.

We used the Student t-test to compare the mean values for the studied groups. Significance between groups was set at P = 0.05. The mean (SD) plasma concentrations for baseline and at 2, 5, 15, and 60 min are shown in Fig. 1. We observed significant increases (P <0.001) from baseline at 2, 5, and 15 min after smoking. A separate analysis revealed that increases occurred in both smokers and nonsmokers. In general, peak values were observed at 2 min (Fig. 1). Baseline mean values for smokers did not differ significantly from those for nonsmokers. We observed no significant difference in mean (SD) BMI between men [25.8 (2.5) kg/m²] and women [23.4 (5.1) kg/m²; P = 0.73] or between smokers [25.8 (3.2) kg/m²] and nonsmokers [23.7 (3.3) kg/m²; P = 0.1].

This study demonstrates the acute effect of cigarette smoking on plasma concentrations of the novel orexigenic hormone ghrelin. The most interesting finding of this study was the unexpected increase in ghrelin concentrations shortly after smoking. The effect of smoking 1 cigarette continued for at least 15 min and was independent of the BMI of each individual. The most intense increase was noted 2 min after smoking initiation. Given the known anorexic action of smoking (6), a decrease in ghrelin concentrations might be expected as an acute effect of smoking. Alcohol has also exhibited an unexpected acute inhibitory effect on ghrelin secretion in healthy individuals, despite its strong orexigenic influence in humans (7).

We speculate that this acute increase of ghrelin during smoking is related to adverse effects on gastric mucosa, gastric motility, mucosal blood flow, and concentrations of free radicals (8). Enhancement of ghrelin secretion could also be an indirect influence mediated by other factors, including growth hormone (nicotine increases the growth hormone concentration) (9), leptin (inverse correlation with ghrelin concentrations) (10), and vagal nerve stimulation (smoking affects vagal nerve activity) (11). Our finding that smoking is more likely to produce an acute release of intracellular ghrelin into the circulation rather than a time- or dose-dependent stomach secretion is partially in agreement with the hypothesis of investigators (4, 5) that circulating ghrelin concentrations are related to the time elapsed after smoking. Rather than being de-

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**Fig. 1.** Mean (SD) plasma ghrelin concentrations (μg/L) after cigarette smoking for all study participants.

**+, P = 0.0005; *, P <0.001; NS, not significant.**
In conclusion, the findings of this study provide evidence that smoking affects hormones that influence the appetite, specifically ghrelin. Further studies are warranted to clarify the effect of smoking on various gut hormones to explain the weight disturbances associated with smoking cessation.

We have no financial relationship with any commercial entity that has an interest in the subject of this letter. We are grateful to George Zacharis, MD, for valuable assistance in enrolling healthy volunteers for our project.

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Effect of Plasmodium falciparum Parasitemia on Erythrocyte Zinc Protoporphyrin

To the Editor:

In regions holoendemic for malaria, both iron deficiency and asymptomatic malaria parasitemia are common (1). Measurements of transferrin receptor and erythrocyte zinc protoporphyrin IX (ZnPPIX) concentrations have been suggested as screening tools to detect iron deficiency (1,2). Chronic iron-deficient erythropoiesis leads to a relative increase in the insertion of zinc rather than iron into erythrocyte PPX.

Anemia of chronic disease and hyperbilirubinemia can decrease the specificity of an iron deficiency diagnosis based on hematofluorometry-determined increases in the ratio of ZnPPIX to heme. The influence of malaria parasitemia on front-face hematofluorometric ZnPPIX determinations is controversial. Increased ZnPPIX has been statistically associated with malaria, and interference by increased bilirubin from hemolysis has been postulated (3). In a study by Asobayire et al. (4), malaria did not increase ZnPPIX in washed blood, but in that study only 5%–8% of malaria-positive persons had >5000 parasites/μL. In different studies using unwashed blood, increased ZnPPIX concentrations were associated with >5000 parasites/μL (5,6). These increases in ZnPPIX could either be real, caused by true iron deficiency and/or malaria-related anemia of chronic disease, or false, caused by hyperbilirubinemia, mostly from hemolysis. Bilirubinemia influencing front-face ZnPPIX determinations depends on the instrument (7,8), use of intact vs lysed specimens (9), or washing of intact cells before use (10). Although jaundice can be present in malarial disease, bilirubinemia is loosely correlated with disease severity (11). Asymptomatic parasitic children are likely to have lower concentrations and incidence of hyperbilirubinemia.

Another potential reason for malarial interference with ZnPPIX determination is hemozoin, the black birefringent intracellular heme crystal. Intra-erythrocytic Plasmodium accumulates more than one half of its heme into hemozoin (12). The heme crystal has a much lower absorbance at 400 nm, suggesting that high malarial parasitemia might lead to increased estimations of erythrocyte protoporphyrin by hematofluorometer (13).

To ascertain whether the intraerythrocytic parasite influences erythrocyte fluorescence, we prepared a thin blood film smear of synchronized Plasmodium falciparum trophozoite-infected erythrocytes, fixed it with methanol for 1 min, and obtained images on a Zeiss LSM 510 microscope with excitation at 410 nm and emission above 575 nm. These emission and excitation wavelengths correspond to those used on the AVIV front-face hematofluorometer. The individual trophozoite-infected erythrocytes showed much less fluorescence than did uninfected erythrocytes (Fig. 1). The synchronized