Blockade of fatty acid oxidation mimics phase II-phase III transition in a fasting bird, the king penguin

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Bernard, Servane F., Eliane Mioskowski, and René Groscolas. Blockade of fatty acid oxidation mimics phase II-phase III transition in a fasting bird, the king penguin. Am J Physiol Regulatory Integrative Comp Physiol 283: R144–R152, 2002. First published March 14, 2002; 10.1152/ajpregu.00011.2002.—This study tests the hypothesis that the metabolic and endocrine shift characterizing the phase II-phase III transition during prolonged fasting is related to a decrease in fatty acid (FA) oxidation. Changes in plasma concentrations of various metabolites and hormones in and lipolytic fluxes, as determined by continuous infusion of [2-3H]glycerol and [1-14C]palmitate, were examined in vivo in spontaneously fasting king penguins in the phase II status (large fat stores, protein sparing) before, during, and after treatment with mercaptoacetate (MA), an inhibitor of FA oxidation. MA induced a 7-fold decrease in plasma β-hydroxybutyrate and a 2- to 2.5-fold increase in plasma nonesterified fatty acids (NEFA), glycerol, and triacylglycerols. MA also stimulated lipolytic fluxes, increasing the rate of appearance of NEFA and glycerol by 60–90%. This stimulation might be partly mediated by a doubling of circulating glucagon, with plasma insulin remaining unchanged. Plasma glucose level was unaffected by MA treatment. Plasma uric acid increased 4-fold, indicating a marked acceleration of body protein breakdown, possibly mediated by a 2.5-fold increase in circulating corticosterone. Strong similarities between these changes and those observed at the phase II-phase III transition in fasting penguins support the view that entrance into phase III, and especially the end of protein sparing, is related to decreased FA oxidation, rather than reduced NEFA availability. MA could be therefore a useful tool for understanding mechanisms underlying the phase II-phase III transition in spontaneously fasting birds and the associated stimulation of feeding behavior.

protein sparing; lipolytic fluxes; isotopic tracers; mercapto-

acetic acid; seabirds

Prolonged fasting is characterized by the preferential utilization of lipid, with relative sparing of body protein (4, 6, 25). Previous studies indicated that protein sparing depends on the availability of lipid fuels (17). The conservation of body protein that characterizes the so-called phase II of fasting (9, 17) is no longer maintained when a lower threshold in fat stores is reached (6, 17, 22, 25). Then a metabolic shift occurs, with a simultaneous acceleration in the catabolism of body protein and a decrease in the contribution of lipid to energy production, the signature of the so-called phase III of fasting (6, 22, 25). Entrance into phase III is also accompanied by hormonal changes, such as an increase in the level of circulating glucocorticoids thought to contribute to the stimulation of protein breakdown (10). How fat store availability determines body protein sparing during phase II or accelerated catabolism during phase III is not well understood. Is protein sparing during phase II linked to the availability of nonesterified fatty acids (NEFA) mobilized from adipose tissue, or does it depend on their oxidation? Arguments suggest that NEFA may specifically modulate the breakdown of myofibrillar proteins independently of their oxidation as a fuel for muscle (26). This suggestion agrees with the finding of an inverse relationship between whole body proteolysis and NEFA availability (40). In contrast, results supporting the view that elevated plasma NEFA do not exert an independent effect on the overall balance of protein metabolism in skeletal muscle have been reported (44). Because a direct regulatory effect of the plasma level of ketone bodies on protein metabolism has been suggested (38), fatty acid (FA) oxidation could affect protein sparing through production of ketone bodies. However, although elevated plasma ketone bodies inhibit leucine oxidation and promote protein synthesis in humans (31), a direct effect on the rate of proteolysis has not been demonstrated in vivo (30, 31).

Most of our knowledge of the relationships between lipid and protein metabolism during fasting derives from studies in humans and laboratory animals. Undoubtedly, studies on wild birds and mammals that spontaneously fast for prolonged periods at certain stages of their annual cycle could lead to a better understanding of these relationships. Recently, we found that, in breeding-fasting king penguins (Aptenodytes patagonicus), entrance into phase III was not associated with a decrease in NEFA production by adipose tissue but, paradoxically, with an increase (unpublished data). This finding agrees with previous

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data showing a transitory increase in plasma NEFA concentration at this time (7, 8, 10). This led us to suggest that, during the phase II-phase III transition, the metabolic, endocrine, and behavioral shifts that were previously found in this species and other seabirds (22) are not related to a decreased NEFA availability but to decreased FA oxidation. The present study tests this hypothesis. The metabolic and endocrine response of phase II fasting king penguins to blockade of FA oxidation was investigated in vivo. This was done by measuring the plasma concentration of various metabolites and hormones and by determining lipolytic fluxes [rate of appearance (R_{a}) of NEFA and glycerol], using isotopic tracer infusion, before, during, and after treatment with mercaptoacetate (MA), an inhibitor of the β-oxidation pathway in mammals. The response to blockade of FA oxidation has been examined previously in mammals, including fasted rats (26), but not in birds or during spontaneous fasting. No study has examined the effect of inhibiting FA oxidation on in vivo lipolytic fluxes during prolonged fasting.

MATERIALS AND METHODS

**Animals.** This study was carried out at the breeding colony of the Baie du Marin (Possession Island, Crozet Archipelago; 46°25′ S, 51°52′ E) during January 2000. It was approved by the Ethical Committee of the Institut Français pour la Recherche et la Technologie Polaires. Seven male king penguins were identified from their song and caught while pairing, i.e., at the onset of the ~25-day breeding fast and after having been fasting ashore for 1–3 days. Their average body mass at capture was 12.20 ± 0.10 kg. Birds were then kept fasting in an outdoor fenced area (3 m × 3 m) next to the colony under natural climatic conditions and within their thermoneutral range. They were habituated to captivity for 6 days, a time period known to be sufficient to suppress the confinement stress and for daily body mass loss, body temperature, and plasma fuel level to reach a steady state in penguins (23).

The infusion experiment lasted a total of 9 h and was separated into basal condition (3 h of tracer infusion without MA), MA infusion (3 h of tracer plus MA infusion), and post-MA period (3 h of tracer infusion without MA). On the day before the infusion experiment, the unanesthetized birds were cannulated with a polyethylene catheter (50 mm long, 1.1 mm OD) inserted percutaneously into the marginal vein of each flipper and extended with a 2-m-long section of tubing. Catheters were kept patent by infusion of saline (12 ml/day) with a small peristaltic pump. Catheterization of an artery for blood sampling for lipolytic flux measurements could not be adequately performed under field conditions. We assumed that any particular metabolism of the flipper (essentially feathers, bones, and tendons) is low and that flipper venous blood reflects whole body metabolism. After catheterization, the birds were allowed to habituate to the experimental setup for 24 h. This setup was installed in the fenced area and consisted of a small wooden pen (70 cm × 70 cm) with one wall high enough to prevent us from being seen by the bird. Catheter extensions were placed into a balance lever system to avoid damage to the extensions or removal of catheters. It also allowed the bird to move freely (a few steps) inside the pen and even to lie on its belly or sleep with the bill under the shoulder, as was regularly observed during tracer infusion. The free ends of the catheter extensions were brought outside the pen to allow intravenous infusion of isotopic tracers and MA into one flipper and blood sampling from the other, from a distance, without disturbing the animal. On the day after the infusion experiment, the equipment was removed, and the penguins were weighed, marked on the chest with nyanzol dye to allow resighting, and released in the colony next to the beach. At release, body mass was 10.91 ± 0.09 kg, i.e., 1.3 kg higher than the 9.6-kg body mass corresponding to the phase II-phase III transition in this species (5). This indicates that, during the experiment, penguins were in the phase II metabolic status, having the capacity to fast for ~12 more days before entering phase III. All the birds used in the study were rested in the following weeks, caught, and weighed. All had restored their body mass, which indicates that they had been successfully feeding at sea and that the experiments had no impact on their health.

**Infusion and preparation of the infusates.** At 0800, a primed constant-rate infusion of labeled glycerol and palmitate was started using a calibrated syringe pump and continued for 9 h (see above). The tracer infusate was prepared daily as described by Wolfe (47) and Turcotte et al. (43) using [2-3H]glycerol (Amersham; 40.7 GBq/mlmmol) and [1-13C]palmitate (Amersham; 2.04 GBq/mlmmol). Delipidated bovine serum albumin (catalog no. A-3803, Sigma) was used as a palmitate carrier. Palmitate is one of the most commonly used FA for measuring NEFA kinetics in mammals: it is the second-most-abundant NEFA and shows low interindividual variability in its percent contribution to NEFA. The same was observed here for king penguins (average weight in NEFA = 23.8 ± 0.6%), and we determined previously that using palmitate to measure NEFA kinetics in penguins gives realistic estimates of R_{a} NEFA (unpublished data). Infusion rates of [2-3H]glycerol and [1-13C]palmitate were 218,900 ± 6,600 and 111,000 ± 3,900 dpm·kg⁻¹·min⁻¹, respectively (n = 7), which corresponded to trace amounts of <0.02% of basal R_{a} glycerol and <0.03% of basal R_{a} palmitate. The priming dose was equivalent to 210 min of isotope infusion. MA (sodium salt; catalog no. 10900-2, Sigma-Aldrich) dissolved in sterile saline was infused at 25 mg·kg⁻¹·min⁻¹ using a calibrated syringe pump and the same catheter used for tracers. This dose was chosen from similar studies in mammals (11, 26, 37) after adjustment for body mass. In preliminary trials, we verified that it had no side effects in penguins.

A delay of 120 min separated the beginning of the tracer infusion and the first blood sampling (time ~60 min in Figures 1–5) to ensure that a steady state had been reached. During the basal period, four blood samples were taken at 20-min intervals. Thereafter, during MA infusion (3 h) and after MA infusion (3 h), blood sampling was performed every 30 min. Five milliliters of blood were collected at each sampling time, with EDTA used as an anticoagulant. Immediately after blood was sampled, it was centrifuged and the plasma was separated and stored at −20°C until analysis.

Determination of glycerol and palmitate specific activities. Plasma glycerol and NEFA specific activities were determined as previously described (3). A 1-ml aliquot of plasma was mixed with chloroform-methanol (2:1, vol/vol). After extraction and evaporation, an aqueous extract and an organic extract were obtained and resuspended in ethanol-water (1:1, vol/vol) and hexane-isopropanol (3:2, vol/vol), respectively. A volume of aqueous extract equivalent to 300 µl of plasma was used to determine the glycerol concentration. It was dried under nitrogen and resuspended in hydradine buffer. Glycerol concentration was measured enzymatically. Total tri- tium activity was counted on another aliquot of aqueous extract equivalent to 150 µl of plasma using scintillation
fluid (EcoScint A, National Diagnostics) and a Wallac 1409 counter. At this step of analysis, tritium activity is found only in glycerol and glucose. The percent activity in glycerol was obtained by separating glucose from glycerol using thin-layer chromatography with chloroform-methanol (40:24, vol/vol) as the developing solvent (3). The glycerol and glucose fractions were resuspended in scintillation fluid for counting. The specific activity of glycerol was calculated as total tritium activity times the fraction of activity in glycerol divided by glycerol concentration.

Total NEFA concentration was measured on 10 μl of plasma with an analytic test kit (NEFA C, Wako Chemicals). Palmitate concentration was obtained by multiplying NEFA concentration by the fractional contribution of palmitate to total NEFA, as determined by gas-liquid chromatography. Briefly, an aliquot of the lipid extract was separated by thin-layer chromatography using hexane-diethyl ether-acetic acid (70:30:1, vol/vol/vol) as the developing solvent. The NEFA fraction was isolated and converted to methyl esters using 14% boron trifluoride in methanol. FA methyl esters were separated in the gas-chromatograph (Chrompack CP 9001) equipped with a capillary column (AT-WAX) and a flame ionization detector. The total 14C activity was counted on an aliquot of the lipid extract, and its distribution in plasma lipids [triaclyglycerols (TAG), diacylglycerols, NEFA, and phospholipids] was determined after separation of lipids by thin-layer chromatography, as described above. Each fraction was resuspended in ethanol-water (1:1, vol/vol) and counted in scintillation fluid (EcoScint A). Because no 14C is incorporated in FA other than palmitate, palmitate activity was calculated by multiplying total 14C activity in the lipid extract by the fraction of activity in NEFA. Palmitate activity divided by palmitate concentration yielded palmitate specific activity.

Other plasma metabolites and hormones. Plasma glucose and β-hydroxybutyrate (β-OHB) were determined on deproteinized plasma by enzymatic methods (Test-Combination, Boehringer-Mannheim). Uric acid and TAG levels were estimated by enzymatic colorimetric methods using commercial kits (UA plus and Peridochrom triglycerides GPO-PAP, respectively, Boehringer-Mannheim). Radioimmunoassay was used to measure plasma glucagon (GL-32K kit, Linco), insulin (insulin-CT kit, CIS Bio International), and corticosterone (DA 200T kit, ICN). All the measurements were made in the same run, and the intra-assay coefficient of variation was 5–8% depending on the hormone. Glucagon and insulin were measured at the end of each infusion period (basal, MA, and after MA). Corticosterone was measured throughout the experiment.

Calculations and statistics. During the basal period, physiological and isotopic steady states were maintained. Glycerol and palmitate Ra were therefore calculated with the steady-state equation of Steele (39): Ra = tracer infusion rate (dpm/min)/specific activity (dpm/mmol). During and after MA infusion, the isotopic steady state was not significantly disrupted, but significant changes in plasma glycerol and palmitate concentrations were observed. In this case, the Ra and the rate of disappearance (Rd) of glycerol and palmitate were calculated using the non-steady-state equations of Steele. Because the distribution volume of glycerol and palmitate is unknown in penguins, calculations were made using the various values reported for mammals. Irrespective of distribution volume (150–325 and 40–50 ml/kg for glycerol and palmitate, respectively), at all times of the experiment Ra and Rd were not significantly different from each other or from flux rate values (Rf) calculated using the steady-state equation (P > 0.38). Consequently, all fluxes are presented as Rf calculated with the steady-state equation and expressed per unit of body mass. Rd, NEFA was determined by dividing Ra, palmitate by the fractional contribution of palmitate to total NEFA. This contribution did not change significantly during the experiment (P > 0.28) and averaged 23.8 ± 0.6%.

The absolute and relative rates of primary TAG-FA cycling (i.e., where FA are reesterified in adipose tissue without entering the circulation) were calculated according to Wolfe et al. (48). It is known that substantial and significant rates can be obtained only if the ratio of Ra, NEFA to Ra glycerol is substantially < 3 (47).

Ra and Rd were compared with the Wilcoxon signed rank test. The identity of Ra and Rd was determined by verifying that the slope of the linear regression between them was not statistically different from unity, according to Tomassone et al. (42). In all other cases, statistical differences were assessed using two-way analysis of variance (ANOVA) or Kruskal-Wallis ANOVA on ranks (when populations were not normal or homoscedastic), with time and penguins as the main factors. When significant changes were detected with ANOVA, the Student-Newman-Keuls method was used to determine which means were different from basal values. Values are means ± SE. The criterion of significance was P < 0.05.

RESULTS

Plasma metabolites and hormones. Blockade of FA oxidation induced a sevenfold decrease in the concentration of plasma β-OHB (P < 0.001): from 2.64 ± 0.63 mmol/l under basal conditions to 0.60 ± 0.17 mmol/l at the end of MA infusion and to 0.38 ± 0.07 mmol/l during the post-MA period (Fig. 1A). The depression in plasma β-OHB was significant from 90 min after the onset of MA infusion and was maintained after MA.

Infusion of MA resulted in a significant 2.2-fold increase (P < 0.001) in the plasma level of NEFA: from 0.53 ± 0.07 mmol/l under basal conditions to 1.18 ± 0.73 mmol/l after MA (Fig. 1B). This increase was detected 60 min after the onset of MA treatment and was significant from 90 min. Plasma TAG was maintained during MA infusion (0.66 ± 0.11 mmol/l) and then progressively increased to reach a 2.5-fold higher level at the end of the post-MA period (P < 0.001; Fig. 2A). Plasma glycerol remained low and unchanged (0.04 ± 0.01 mmol/l) during MA infusion but increased thereafter, reaching a 1.9-fold higher level (P < 0.001) at the end of the post-MA period (Fig. 2B).

As shown in Fig. 3A, plasma uric acid progressively increased from the end of MA infusion, reaching a level fourfold higher (P < 0.001) at the end of the post-MA period than under basal conditions: 0.76 ± 0.31 vs. 0.19 ± 0.08 mmol/l. Plasma glucose did not change significantly throughout the experiment (P = 0.68; Fig. 3B).

Plasma insulin did not change significantly during the experiment (Table 1; P = 0.22). At the end of MA treatment as well as at the end of the post-MA period, the plasma glucagon level was twice as high as basal levels (P < 0.05). Plasma corticosterone significantly increased by 2.5-fold (P < 0.001) after MA infusion: from 9.9 ± 1.0 ng/ml under basal conditions to an average maximum value of 24.4 ± 3.0 ng/ml at 30–120 min after MA treatment (Fig. 4). Plasma corticosterone
had returned to basal levels at the end of the post-MA period.

**NEFA and glycerol kinetics.** As illustrated in Fig. 5A, \( \text{R}_a \) NEFA remained unchanged at 15.9 ± 2.6 \( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \) under basal conditions and during the 1st hour of MA infusion. Thereafter, it significantly increased by 60% \((P < 0.001)\) and was maintained at 25.7 ± 4.8 \( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \) throughout the post-MA period. These changes were identical to those in \( \text{R}_a \) palmitate (not shown). \( \text{R}_a \) glycerol remained unchanged at 5.6 ± 0.3 \( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \) under basal conditions and during MA infusion (Fig. 5B). Then it progressively increased and reached 10.7 ± 1.6 \( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \) at the end of the post-MA period, a rate 90% higher than basal values \((P < 0.001)\). Under basal conditions and during the 1st hour of MA infusion, intracellular TAG-FA cycling corresponded to ~10% of reesterification. Thereafter, TAG-FA cycling was so low (with sometimes negative values) that a significant average value could not be obtained.

**DISCUSSION**

**Response of lipid and glucose metabolism to MA.** MA is a blocker of FA oxidation that acts through inhibition of long-chain acyl-CoA dehydrogenase activity in the mitochondrial matrix (2). Whether it also inhibits microsomal \( \beta \)-oxidation is unknown. The intensity and the kinetics of the response to MA obviously depend on the dose and the duration of treatment. Our results show that MA infused at 25 mg⋅kg\(^{-1}\)⋅min\(^{-1}\) for 3 h in the king penguin strongly and rapidly inhibits FA oxidation, as reflected by the sevenfold decrease in plasma \( \beta \)-OHB, a product of hepatic FA oxidation. This inhibitory effect of MA is long lasting, since the plasma \( \beta \)-OHB level remained maximally depressed for ≥3 h after MA infusion. A similar potent and prolonged inhibitory effect has been reported in cattle (11) and rats (37), with a more than twofold depression of plasma \( \beta \)-OHB for ≥6 h after an intraperitoneal MA injection in the latter species. In accordance with previous findings in mammals (11, 36, 37), blockade of FA oxidation also resulted in an increase in plasma NEFA. This increase could initially be the result of an accumulation in the plasma of nonutilized FA. Indeed, the increase in plasma NEFA was concurrent with the drop in the plasma \( \beta \)-OHB level and was detected ~30 min before the increase in \( \text{R}_a \) NEFA. Thereafter and as indicated by the parallel changes in plasma NEFA and \( \text{R}_a \) NEFA, the increase in plasma NEFA was mostly, if
not entirely, the result of an increase of NEFA release by the adipose tissue. A stimulatory effect of MA on NEFA release from adipose tissue has been previously reported in vitro in the rat and was attributed to an increase in lipolysis and a reduction of NEFA reesterification (35). In our study, a significant increase in Ra NEFA was observed 60 min before a significant increase in lipolysis (Ra glycerol), suggesting that, in the king penguin, MA stimulates NEFA release from adipose tissue, first by depressing primary TAG-FA cycling and then by stimulating lipolysis. Because significant rates of TAG-FA cycling could not be calculated during the major part of the infusion experiment because of their low levels, no data are available to confirm this suggestion. Thus, in king penguins, MA could have stimulated lipolysis by acting directly on adipose tissue. An indirect action through induction of metabolic or hormonal changes can also be postulated. Because /H9252-OHB appears to inhibit lipolysis (28), the MA-induced increase in lipolysis might be related to the decrease in the circulating level of this lipid fuel.

The impairment of FA oxidation has been shown to increase glucagon release from isolated Langerhans islets in mammals (13). Accordingly, here MA induced a twofold increase in plasma glucagon, the main lipolytic hormone in birds, including penguins (20). Because insulin has no antilipolytic effect in penguins (20) and did not change significantly during the experiment, its contribution to the stimulation of lipolysis is not retained. Whatever the underlying mechanism, the increased NEFA disposal observed after MA infusion might be considered a means of compensating for the reduction in NEFA oxidation through increased availability and mass action.

Inhibition of FA oxidation by MA also resulted in a progressive increase in the plasma TAG level with a time lag of ~3 h relative to the changes in plasma NEFA and /H9252-OHB. Such an increase has been previously observed in mammals after treatment with inhibitors of FA oxidation (27, 32), although not in cattle injected with MA (11). The increase probably resulted from changes in the metabolic fate of FA in the liver, with a reorientation toward reesterification (secondary recycling) to handle the excess NEFA resulting from the reduction in FA oxidation and the increase in Ra NEFA. Indeed, an increased secretion of very-low-density lipoprotein TAG after inhibition of FA oxidation has been reported in isolated rat liver (24). Hepatic very-low-density lipoprotein secretion and plasma TAG concentration are known to increase in vivo in response to an elevation of plasma NEFA (45). The same probably applies here, since the increase in plasma TAG actually started after plasma NEFA had increased.

Table 1. Changes in the plasma concentration of glucagon and insulin induced by MA infusion in phase II fasting king penguins

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<th>Basal</th>
<th>MA</th>
<th>Post-MA</th>
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<tr>
<td>Glucagon, pg/ml</td>
<td>45 ± 9</td>
<td>98 ± 13*</td>
<td>104 ± 23*</td>
</tr>
<tr>
<td>Insulin, μIU/ml</td>
<td>10 ± 1</td>
<td>9 ± 1</td>
<td>14 ± 2</td>
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Values are means ± SE (n = 7) obtained at the end of each 3-h period. MA, mercaptoacetate (25 mg·kg⁻¹·min⁻¹). *Significantly different from basal, P < 0.05.
stabilized at an elevated level. Hepatic reesterification of FA requires glycerol-3-phosphate, a substrate originating from glycerol and glucose. The observation that the plasma glycerol concentration increased in parallel with plasma TAG concentration, whereas glycemia remained unchanged during the whole experiment, suggests that the increase in hepatic FA reesterification had been facilitated by the maintenance of an adequate supply of glycerol-3-phosphate precursors. Inhibition of FA oxidation has been shown to result in a hypoglycemia in diabetic rats (32) but not in rats fed a carbohydrate-free, high-fat diet (12). The maintenance of plasma glucose in MA-treated penguins agrees with the transient increase in plasma NEFA concentration reported by others (7, 8, 10).

The stimulation of protein catabolism induced by inhibiting FA oxidation might have been mediated directly by the decrease in the production and circulating levels of ketone bodies and/or secondarily by hormonal changes associated with MA treatment. Although it has been proposed that protein conservation during fasting is related to ketosis (4), data concerning a direct role of ketone bodies in protein sparing are inconsistent. Elevated plasma levels of ketone bodies have been shown to inhibit leucine oxidation and to promote protein synthesis in humans (31), suggesting a direct positive effect on protein conservation. On the other hand, infused β-OHB failed to decrease proteolysis in vivo (30). Because β-OHB is known to affect the secretions of a variety of hormones such as insulin and glucose through the stimulation of gluconeogenesis and glycogenolysis, the latter having been observed in fasting penguins (20). Independent of the exact mechanism, our results support the view that active FA oxidation is not essential for maintaining a normal plasma glucose concentration in spontaneously fasting penguins.

**FA oxidation and protein catabolism.** Several studies have provided supporting arguments for the idea that NEFA availability may directly modulate protein metabolism in mammals. For example, elevation of plasma NEFA has been shown to suppress the levels of plasma amino acids (14, 46) and their release by resting muscle (46). By inhibiting lipolysis or FA oxidation in phase II fasting rats, Lowell and Goodman (26) obtained results suggesting that NEFA may specifically attenuate the breakdown of myofibrillar proteins in muscle independently of their oxidation as muscle fuel. They concluded that NEFA, and not ketone bodies, may be responsible for protein sparing during prolonged starvation. In contrast, the results of the present study support the view that the conservation of body protein during phase II requires the continued oxidation of lipid fuels at a high rate, and not only an adequate availability of these fuels. Indeed, by blocking NEFA oxidation, the resulting increase in NEFA availability was associated with an increase in protein catabolism, as reflected by the marked and rapid increase in the concentration of circulating uric acid. Uric acid is the end product of protein degradation in birds, and its plasma level has been shown to be a good index of this degradation in penguins (34). Thus our results agree with the finding that elevated plasma NEFA levels in fed humans do not exert an independent effect on the overall balance of protein metabolism in skeletal muscles (44). However, Walker et al. (44) suggested that NEFA availability itself might have a more chronic and adaptive effect in situations such as starvation. Present and previous results do not support such a possibility. We recently demonstrated that the acceleration of protein catabolism at the entrance into phase III in king penguins is associated with an increased production of NEFA by adipose tissue (unpublished data), which agrees with the transitory increase in plasma NEFA concentration reported by others (7, 8, 10).

![Fig. 5. Rate of appearance (R_{a}) of NEFA (A) and glycerol (B) before, during, and after MA infusion (25 mg·kg^{-1}·min^{-1}) in phase II fasting king penguins. Values are means ± SE (n = 7). *Significantly different from basal values, P < 0.05. See Fig. 1 legend for further explanation.](http://ajpregu.physiology.org/)
glucagon in mammals (29), the alteration of protein catabolism caused by MA might be secondary to hormonal changes. In mammals, plasma insulin has been shown to decrease after MA treatment (11), and insulin is known to stimulate protein synthesis and inhibit protein breakdown in muscle (16). Here, MA did not affect the plasma insulin level, which is similar in the basal state and during recovery, i.e., when the metabolic changes were the most pronounced. As a consequence, it is unlikely that insulin intervened in the increase in protein breakdown induced by the MA treatment. Stimulation of protein catabolism was accompanied by a marked increase in circulating corticosterone, in agreement with the previous observation that inhibition of FA oxidation in phase II fasting rats resulted in accelerated protein breakdown and increased circulating levels of glucocorticoids (26). Glucocorticoids have been reported to decrease protein synthesis (16) and to accelerate protein breakdown when reaching elevated plasma concentrations (41). Thus the increase in plasma corticosterone level observed here after MA infusion has likely contributed to the stimulation of body protein catabolism, suggesting that, in penguins, glucocorticoids might constitute a link between FA oxidation and protein catabolism. This suggestion is consistent with the idea that the protection of protein stores by NEFA in fasting humans might be through the ability of these fuels to prevent excessive cortisol secretion (15). However, it must be noted that in the present study there is substantial deviation in the time courses of plasma uric acid and corticosterone levels after MA infusion, notably in the post-MA period. Whether this deviation reflects a time lag between changes in plasma corticosterone and protein catabolism or whether it indicates that factors other than corticosterone also intervene in the control of proteolysis is unknown.

Blockade of FA oxidation with MA: a tool to mimic the phase II-phase III transition. The metabolic and endocrine changes induced by inhibiting FA oxidation with MA are strongly reminiscent of spontaneous changes observed at the entrance into phase III in penguins. With regard to lipid metabolism and as presently observed, these changes include a progressive decline in plasma β-OHB (8, 10, 18, 33), a transitory increase in plasma NEFA (7, 8, 10; unpublished data) and glycerol (7, 8, 10), and an increase in lipolysis (Ra glycerol/kg body fat) and NEFA availability (Ra NEFA/kg body mass; unpublished data). However, the present post-MA increase in plasma TAG contrasts with the maintenance of this parameter at the entrance into phase III (19). As observed here during inhibition of FA oxidation, an acceleration of protein catabolism at the entrance into phase III has been demonstrated by the increase in plasma uric acid and urea (7, 8, 10, 33, 34) and an increase in the plasma concentration of most amino acids (21). Whereas glycemia is generally maintained during phase III (7), a slight increase (10, 18) or slight decrease (unpublished data) has also been observed. Finally, with reference to hormonal changes, the maintenance of insulinemia and the increase in plasma glucagon and corticosterone levels induced by MA treatment are also in agreement with observations in phase III fasted penguins (7, 10; unpublished data). Together, and in accordance with our suggestion (unpublished data), these strong similarities support the view that entrance into phase III in penguins is related to a depression in FA oxidation. Moreover, they suggest that the experimental blockade of FA oxidation by MA might be a useful tool to explore the mechanism of the metabolic and endocrine shift characterizing the phase II-phase III transition, which should be relevant for understanding the relationships between protein sparing and FA metabolism during fasting. Because entrance into phase III has been shown to be associated with a refeeding signal that redirects the activity of breeding-fasting penguins toward food searching (33), MA should be also a useful tool for studying the role of fat oxidation in the regulation of feeding behavior in these seabirds.

Conclusions and perspectives. In conclusion, this study is the first to demonstrate that MA inhibits FA oxidation in birds and stimulates lipolysis in vivo during fasting. The high susceptibility of fasting penguins to MA-induced inhibition of FA oxidation is likely related to their strong dependence on FA as a metabolic substrate for energy metabolism. The observed stimulation of protein catabolism in the face of an increase in NEFA availability suggests that protein conservation is related to FA oxidation, rather than availability, during phase II of fasting in birds. This relationship could be mediated by hormones, FA oxidation being interrelated with lipolysis by glucagon and with protein catabolism by corticosterone. The strong similarities between MA-induced metabolic and hormonal changes and those that spontaneously develop at the entrance into phase III of fasting suggest that MA could be a useful tool for understanding these interrelationships and the behavioral changes associated with the phase II-phase III transition. How FA oxidation could be turned off at the entrance into phase III when FA availability remains high should also be determined. It has previously been shown that, in the rat, entrance into phase III is associated with a rapid decrease in the total hepatic activity of two enzymes involved in FA oxidation: carnitine palmitoyltransferase and FA oxidase (1). Is this decrease in the capacity to oxidize FA triggered by a central signal that senses fuel stores, or do the cells somehow determine that it is time to reduce FA utilization and switch to protein?

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