Hypoxic stimulus alters hypothalamic AMP-activated protein kinase phosphorylation concomitant to hypophagia

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Simler N, Malgoyre A, Koulmann N, Alonso A, Peinnequin A, Bigard AX. Hypoxic stimulus alters hypothalamic AMP-activated protein kinase phosphorylation concomitant to hypophagia. J Appl Physiol 102: 2135–2141, 2007. First published March 1, 2007; doi:10.1152/japplphysiol.01150.2006.—Acute exposure to hypobaric hypoxia is known to decrease food intake, but the molecular mechanisms of such alteration in feeding behavior remain unknown. We tested the hypothesis that hypothalamic AMP-activated protein kinase (AMPK) phosphorylation is affected by acute exposure to hypobaric hypoxia and thus would be involved in initial anorexia. To address this issue, male rats weighing 255–270 g were either submitted to hypobaric hypoxia (H, equivalent altitude of 5,500 m), maintained under local barometric pressure conditions (N), or pair-fed an equivalent quantity of food to that consumed by H rats (PF). In 6, 24, or 48 h. Daily food intake dropped by 73% during the first day of hypoxia (P < 0.01) and remained by 46% lower than in N rats thereafter (P < 0.01). Hypoxia per se, as estimated by comparing experimental data between the H and PF groups, increased ob gene transcription and plasma leptin concentration. A transient increase in glucose availability occurred in the H group compared with PF animals (P < 0.05). The hypoxic stimulus led to an early and transient decrease in hypothalamic AMPK and acetyl-CoA carboxylase (ACC) phosphorylation, concomitant with hypophagia and associated alterations in nutrients and hormones. An increase in NPY mRNA levels occurred from day 1, similarly in H and PF rats, and thus mainly related to food restriction alone (P < 0.05). In conclusion, the present study demonstrates that hypoxia per se inhibited AMPK and ACC phosphorylation in the hypothalamus, concomitant with profound anorexia. A powerful counterregulation occurs rapidly, mediated by NPY and devoted to avoid prolonged anorexia.

leptin; neuropeptide Y; insulin; altitude; acetyl-coenzyme A carboxylase

Many previous studies have reported weight loss in humans (6, 40) and depressed growth rates in animals (5, 36) submitted to low ambient oxygen, i.e., at high altitude. A variety of factors could contribute to decreased body weight, but reduced food intake is a major determinant of weight loss (14). During high mountain expeditions, many confounding factors that usually occur at altitude can cause weight loss (43). However, food intake is generally reduced in humans exposed to simulated high altitude in a hypobaric chamber, where stressful conditions are avoided (33, 39). These findings, together with results on animal models, clearly suggest that hypoxia per se induces a decrease in food intake. However, the molecular mechanisms of this alteration in feeding behavior remain largely unknown (14, 36, 39, 40).

The central nervous system, especially the arcuate nucleus and the paraventricular nucleus of the hypothalamus, has been shown to play an integrative role in appetite regulation (35). Neurons within the hypothalamus receive peripheral signals of whole body energy status via hormones and nutrients, such as leptin, ghrelin, insulin, glucose, and fatty acids. These complex signals are integrated in the arcuate nucleus by neurons that express orexigenic and anorexigenic neuropeptides (35). Thus the hypothalamus plays a key role in the control of food intake by integrating hormonal and nutrient signals.

Leptin, product of the ob gene, is a crucial component of this regulatory system that reduces anabolic and activates catabolic pathways and exerts an inhibitory effect on food intake (for review, see Ref. 1). Because ob gene expression is induced by hypoxia (8, 9), the role of leptin in the mediation of hypoxia-induced anorexia has been examined. Using a rat model of genetic leptin receptor deficiency, we recently demonstrated unequivocally that altitude-induced hypophagia cannot be ascribed to anorectic signals triggered by enhanced leptin production (36). Moreover, no detectable alterations of the main orexigenic and anorexigenic hypothalamic neuropeptides were reported early after hypoxia exposure.

AMP-activated protein kinase (AMPK) is a fuel-sensing enzyme that is activated by physiological and pathological metabolic stresses that alter the cellular energy status (for review, see Refs. 10, 43). There is emerging evidence that AMPK plays an important role in the regulation of whole body energy metabolism through a coordination of anabolic and catabolic pathways in various peripheral tissues. In addition to its role on peripheral tissues, AMPK is also thought to regulate food intake in the hypothalamus by integrating nutritional and hormonal signals and by modulating the expression of orexigenic and anorexigenic neuropeptides such as neuropeptide Y (NPY) and agouti-related protein (22). Enhanced AMPK activity in the hypothalamus increases food intake, whereas decreased AMPK activity reduces food intake and body weight (for review, see Refs. 31, 43). Changes in orexigenic and anorexigenic circulating hormones, and alterations in cellular energy levels and in nutrient cues, all factors which can be affected by hypoxia exposure, are known to modulate AMPK activity and thus to affect feeding behavior. Whether ambient hypoxia affects AMPK activity in the hypothalamus and contributes to explain the hypoxia-induced hypophagia has not been determined and needs to be examined.

The present study was undertaken, therefore, to test whether AMPK activity in the hypothalamus is affected by acute exposure to ambient hypobaric hypoxia and thus would be involved in the marked early hypophagia. Because AMPK in the hypothalamus is viewed as a central integrator of hormonal and metabolic signals involved in the control of food intake,
we hypothesized that hypoxia exposure would decrease AMPK activity in the hypothalamus and thus contribute to explain the initial marked decrease in food intake. Because AMPK is not only a cell-autonomous energy sensor, but also an integrative metabolic sensor, attention has been focused on the control of energy intake in normoxic animals. To address this issue and to differentiate the effects of hypoxia per se from those related to hypoxia-induced decrease in food intake, we used a group of rats maintained under local barometric pressure conditions and pair-fed quantities of food equivalent to those consumed by animals subjected to hypobaric hypoxia. Changes in AMPK phosphorylation were examined in parallel with changes in nutrient and hormone signals known to control its activity and with one of the downstream targets of AMPK, i.e., NPY gene.

MATERIALS AND METHODS

Animals. This study was performed in accordance with the Helsinki Accord for humane treatment of laboratory animals and the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Council of Europe no. 129, Strasbourg, 1985) and was approved by the Animal Ethics Committee of the Centre de Recherches du Service de Sante des Armees. Male Wistar rats obtained from Charles River Laboratories (L’Arbresles, France) were used in this experiment. All animals were housed individually in hanging wire-mesh cages and were kept on an artificial 12:12-h light-dark cycle with lights on at 0800. After 1 wk of acclimatization, rats weighing 255–270 g were randomly assigned to three experimental groups either submitted to hypobaric hypoxia (H), maintained under local barometric pressure conditions (normoxia, N), or pair-fed an equivalent quantity of food to that consumed by H rats (PF) for 6, 24, or 48 h. During all the experimental period, both N and H rats had free access to standard laboratory chow (AO3 UAR, Charles River, Les Oncins, France) and tap water. Spillage was considered negligible. Animals from H groups were housed in a hypobaric chamber (T.I.M., Marseille, France) and exposed to barometric pressure that was progressively reduced (20 min) until the equivalent of 5,500-m altitude was reached (barometric pressure 50.5 kPa, partial oxygen pressure 10.3 kPa), for periods of 6 (n = 6), 12 (n = 8), and 48 h (n = 8). Ambient temperature was maintained at 22 ± 2°C. Rats were weighed daily to monitor body weight gain, and the daily food intake was expressed either as absolute values or per 100 grams body weight.

Animals were anesthetized by intraperitoneal injection of pentobarbital sodium, between 2:00 pm and 3:00 pm, after 6, 24, or 48 h of hypoxia exposure. Rats from the H groups were anesthetized, and all biological materials were taken under hypoxic conditions. Blood samples were withdrawn from the abdominal aorta into a heparin-treated syringe, collected on EDTA, and kept in ice. Retroperitoneal samples were withdrawn from the abdominal aorta into a heparin-treated syringe, collected on EDTA, and kept in ice. Retroperitoneal white adipose tissue was collected and stored in an RNA stabilization buffer (RNAlater Stabilization Reagent, Ambion, Huntington, UK). Tissue samples in RNAlater were symmetric parts, and one portion was flash-frozen in liquid nitrogen as frozen hypothalamus was kept at −80°C whereas frozen hypothalamus was stored in RNAlater. These operations required only 5 min, 4°C) for 5 min, 4°C) and stored at −20°C whereas frozen hypothalamus was kept at −80°C until use.

Biochemical analysis. Plasma glucose, nonesterified free fatty acids (FFA), and glycerol concentrations were measured by enzymatic methods on an automated biomedical analyzer (Roche-Hitachi 912, Meylan, France). Plasma concentrations of insulin and leptin were assessed by immunoassays according to the manufacturer’s specifications [Insulin-CT (Cis Bio, Gif sur Yvette, France), Quantikine Mouse Leptin Immunoassay (R & D Systems, Lille, France), respectively].

RNA isolation and mRNA levels. Total RNA was extracted from retroperitoneal adipose tissue samples and from dissected one-half hypothalamus stored in RNAlater. White adipose tissue cDNA was synthesized from 1 μg of total RNA, using oligo(dT)15 and Reverse transcriptase (Euronegentec Reverse Transcription Core kit, Seraing, Belgium). Hypothalamic cDNA was generated from 0.5 μg of total RNA with oligo(dT)15 and random primers, using the QuantiScript reverse transcriptase (Quintecrit RT kit, Quiagen, Courtaboeuf, France). Real-time quantitative PCR analysis was performed in a final volume of 20 μl, using the LCFast Start DNA Master SYBR Green I kit in a Light Cycler detection system (Roche Diagnostics, Meylan, France) (Table 1). Specificity was checked for each sample by melting curve analysis. Transcription levels were normalized using housekeeping genes with a comparative threshold cycle method (20) by RelQuant software (Roche Diagnostics, Meylan, France). A pool of all samples was used as a calibrator, as described previously (28). Final standardization was performed by the geometric mean obtained from the amplification of two different housekeeping genes in adipose tissue [cyclophilin-A (CycA) and hypoxanthine-guanine phosphoribosyl transferase] and four different genes in hypothalamus [CycA, β-actin, RNA polymerase binding protein RBP1, and acidic ribosomal phosphoprotein P0].

Western blot analysis. Frozen hypothalamus samples were homogenized in 150 μl of lysis buffer at 4°C [1% Triton X-100, 50 mM Tris-HCl (pH 7.5), 250 mM mannitol, 1 mM EDTA, 1 mM sodium azide, 5 mM sodium pyrophosphate, 50 mM sodium fluoride, 1 mM sodium orthovanadate, 0.5 mM PMSF, 1 mM DTT and 1.6 μM/ml final solution of antiproteases cocktail (Protein Inhibitor Cocktail Set III, Merck Calibiochem, Fontenay-sous-bois, France)] by manual grinding. Insoluble material was removed by centrifugation for 20 min at 14,000 g at 4°C. The protein concentration of the supernatant was determined by the Bradford dye-binding method. One-hundred twenty milligrams of protein extract obtained from each tissue were separated by SDS-PAGE, transferred to nitrocellulose membranes, and blotted with anti-phospho-AMPKα(Thr172), anti-AMPK, anti-phospho-acetyl-CoA carboxylase(Thr79) (anti-phospho-ACC), and anti-ACC antibodies (nos. 2531, 2532, 3661, and 3662, respectively; Cell Signaling Technology, Danvers, MA).

Statistical analysis. All data are expressed as means ± SE. A two-way ANOVA was processed on the raw data to assess global effects of the experimental condition (N, H, or PF), time of hypoxia exposure (6, 24, or 48 h), and/or interaction between these two factors. When appropriate, differences between groups were tested with a Newman-Keuls post hoc test. Values of P < 0.05 were considered to be statistically significant.

RESULTS

Effect of hypoxia on food intake and body weight. Food consumption was almost stopped during the first 6 h of hypoxia exposure (Table 2). Daily food intake dropped by 73% during the first day of hypoxia exposure (P < 0.01) and remained 46% lower than in N rats thereafter (P < 0.01) (Table 2). When normalized per 100 g of body weight, food intake showed similar changes with a 70% and 40% decrease in energy intake at day 1 and day 2, respectively (P < 0.01) (data not shown).

Substantial weight loss was observed during the first 2 days of hypoxia exposure in H rats (8.5% at day 2 compared with the N group, P < 0.01) (Table 2). PF exhibited parallel decrease in body weight (7% at day 2 compared with the N group, P < 0.01), suggesting that body weight loss during

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exposure to high altitude can be mainly ascribed to the decrement of food intake.

Ob gene expression and plasma leptin concentration. The first 24 h of hypoxia were not associated with significant changes in adipose tissue leptin mRNA when compared with N rats (Fig. 1A). At day 2, mRNA levels in H animals decreased and were significantly lower than in N rats ($P < 0.05$). In contrast, a sharp decrease in leptin mRNA levels was shown in PF rats as soon as 6 h after food restriction ($P < 0.05$). When compared with PF rats, leptin mRNA levels were higher in adipose tissue of H animals during the first 24 h of hypoxia ($P < 0.05$).

A slight increase in circulating leptin levels was shown 6 h after hypoxia exposure compared with N rats, while plasma leptin concentration decreased by nearly two- and fourfold in both H and PF group, consistent with food restriction. Insulin concentrations were decreased by nearly two- and fourfold in both H and PF rats at day 2, respectively (Fig. 2B). This decrease was early in PF rats, from 6 h of hypoxia (38%, $P < 0.05$), and only significant after 24 h of hypoxia in H rats (45%, $P < 0.05$).

Plasma FFA and glycerol concentrations increased in both H and PF rats from the first hours of hypoxia and/or food restriction (Fig. 3). A progressive decrease in plasma FFAs occurred in H rats from 24 h so that plasma FFA concentration recovered control values at day 2. However, plasma glycerol remained three- to fivefold higher in PF and H rats than in control normoxic animals, providing evidence of fatty acid mobilization from triacylglycerol (Fig. 3B).

Table 2. Food intake and body weight of H, N, and PF rats

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NPY, neuropeptide Y; POMC, proopiomelanocortin; CycA, cyclophilin-A; RBP1, RNA polymerase binding protein; HPRT, hypoxanthine-guanine phosphoribosyltransferase; ARBP0, ribosomal phosphoprotein P0; S, sense primer; AS, antisense primer.

Table 2. Food intake and body weight of H, N, and PF rats

<table>
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<tr>
<th>Food Intake, g</th>
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<th>6 h</th>
<th>24 h</th>
<th>48 h</th>
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<td>N rats</td>
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<td>4.1±0.3</td>
<td>26.9±1.8</td>
<td>26.5±0.8</td>
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<td>0.3±0.1*</td>
<td>7.1±0.8*</td>
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<tr>
<td>PF rats</td>
<td>26.9±1.7</td>
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<td></td>
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</tr>
<tr>
<td>Body weight, g</td>
<td>263±2</td>
<td>266±3</td>
<td>277±3</td>
<td>277±3</td>
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<tr>
<td>N rats</td>
<td>257±3</td>
<td>236±2*</td>
<td>235±2*</td>
<td>235±2*</td>
</tr>
<tr>
<td>H rats</td>
<td>249±2*</td>
<td>250±2*</td>
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</tr>
</tbody>
</table>

Values are means ± SE. H, rats submitted to hypobaric hypoxia; N, rats maintained under local barometric pressure conditions (normoxia); PF, rats pair-fed an equivalent quantity of food to that consumed by H rats. Food intake values in PF groups at 6, 24, and 48 h were the same as in H groups.

*Significantly different from N values, $P < 0.05$.
in H group recovered values similar to N groups at day 2, and although slightly lower, did not significantly differ from PF rats.

**NPY and POMC mRNA levels in the hypothalamus.** Expression of mRNA encoding the orexigenic NPY increased in H rats from day 1 compared with the N group (Fig. 5A). These changes in NPY mRNA levels were similar in H (51% and 61%, $P < 0.05$, at day 1 and day 2, respectively) and PF rats (38% and 76%, $P < 0.05$, at day 1 and day 2, respectively). Expression of the anorexigenic neuropeptide POMC was not significantly affected by food restriction alone but had a tendency to be downregulated in H rats from day 1 (Fig. 5B).

**DISCUSSION**

The primary goal of this experiment was to study the phosphorylation state of AMPK in the hypothalamus of rats exposed to a simulated altitude of 5,500 m in a hypobaric chamber. We investigated the hypothesis that the initial marked anorexia consistently observed after high-altitude exposure would be dependent on reduced AMPK phosphorylation in the hypothalamus. The most striking observation is that hypoxia per se, as estimated by comparing experimental data between the H and PF groups, leads to a rapid decrease in AMPK phosphorylation within 6 h of hypoxia exposure and returned to control levels at 48 h. Because AMPK is considered as a master regulator of food intake (2, 15, 22), our results suggest that the decreased hypothalamic AMPK phosphorylation observed early in hypoxic rats may account for the initial marked hypophagia. However, reduced energy intake continues during prolonged exposure to hypobaric hypoxia to a lesser extent, and the role played by hypothalamic AMPK activity remains to be investigated.

Regulation of AMPK activity is complex but mainly related to covalent modification of its $\alpha$-subunit through phosphorylation of threonine-172 by upstream kinases (7, 11, 41). As previously reported in many studies, changes in AMPK activity are paralleled by associated changes in [Thr$^{172}$]AMPK phosphorylation (2, 16, 24, 38). Moreover, ACC phosphorylation is commonly coupled to AMPK phosphorylation, leading to inhibition of ACC activity (21, 23, 43) and thus to mitochondrial carnitine palmitoyltransferase (CPT1) activation (13). The early and significant decrease in AMPK phosphorylation, representative of AMPK activity, together with decreased ACC phosphorylation in the hypothalamus of rats submitted to ambient hypoxia, is consistent with the abrupt initial reduction in food intake and with the role played by AMPK in feeding responses to anorexic stimuli (2, 22, 25). Comparison of hypothalamic AMPK phosphorylation levels between rats with similar energy intake, i.e., PF and H groups, clearly supports a direct role of hypoxia on AMPK activity.

A number of factors may be involved in the hypoxia-induced changes in hypothalamic AMPK, including hormones and nutrients. Macronutrients such as glucose and fatty acids con-
tribute to regulate food intake, both by the control of circulating levels of leptin and insulin (42) and/or directly via metabolic pathway (19). Hyperglycemia inhibits hypothalamic AMPK activity (22), and a recent report provided direct evidence that glucose regulates food intake through the AMPK pathway (18). The significant increase in blood glucose levels observed early after hypoxia exposure, independently of food restriction, is consistent with previous findings (29, 30) and is in parallel with the anorexigenic effects of this nutrient through the AMPK pathway (18, 22). Because physiological changes in AMPK activity have been shown to vary with hypothalamic regions, our approach in whole hypothalamus could constitute a methodological weakness. However, a rise in plasma glucose is known to decrease AMPK activity in all hypothalamic regions (22), and changes in AMPK phosphorylation can be examined in whole hypothalamus (for example, 2, 15, 16, 32). Increased sympathetic activity resulting from exposure to hypoxia might be involved in the transient hyperglycemia observed in rats after acute exposure to hypoxia (3, 37). The inverse relationship shown between plasma glucose and AMPK phosphorylation, in both PF and H rats at any time, suggests a role of plasma glucose in the control of AMPK activity under ambient hypoxic conditions and supports the notion that AMPK is an important glucose sensor (18).

Circulating fatty acids may signal nutritional status on selective neurons in the brain, and α-lipoic acid, a naturally occurring fatty acid, has been shown to elicit anorexigenic effects through decreased AMPK activity and phosphorylation (16). We did not measure plasma α-lipoic acid concentrations in the present study, but the concomitant increase in plasma fatty acid concentration early after hypoxia exposure and decrease in food intake could be consistent with the satietogenic effects of lipids (26). The increased level of circulating fatty acids and glycerol observed in PF rats is also consistent with the effects of caloric restriction on enhanced fat mobilization from triacylglycerol, in part through decreased plasma insulin (4). However, plasma fatty acid availability was lower in H than PF rats at day 1 and day 2, while AMPK phosphorylation only decreased in H rats. It is thus unlikely that in contrast with glucose, circulating fatty acids could account for the hypoxia-induced anorexia through AMPK activity.

Hypothalamic AMPK activity is also responsive to other anorexigenic stimuli such as leptin and insulin. It has been shown that leptin decreased AMPK activity in specific hypothalamic nuclei (2), and furthermore, suppression of AMPK activity is necessary for the anorexic effects of leptin (22). The hypoxia-induced changes in plasma leptin reported in H rats compared with the PF group are consistent with decreased AMPK activity in the hypothalamus (2). Comparison of parallel changes in leptin mRNA and protein between normoxic

Fig. 3. Plasma free fatty acid (A) and glycerol (B) concentrations in H, N, and PF rats. Values are means ± SE. *Significantly different from N values, $P < 0.05; $Significantly different from PF values, $P < 0.05.

Fig. 4. Western blot analysis of phosphorylated AMP-activated protein kinase (pAMPK) and total AMPK (totAMPK) (A) and of phosphorylated acetyl-coenzyme A carboxylase (pACC) and total ACC (totACC) (B) in the hypothalamus of H, N, and PF rats. Values are means ± SE. *Significantly different from N values, $P < 0.05; $Significantly different from PF values, $P < 0.05.
and hypoxic groups shows high plasma protein compared with transcript levels after hypoxia exposure. This finding could be explained by hypoxia-induced reduction in water intake (12), leading to acute hemoconcentration, decrease in leptin protein degradation, and/or posttranscriptional events that have been suggested as regulators of leptin protein expression under ambient hypoxia conditions. Moreover, it has been previously shown that mRNA translation efficiency of hypoxia-inducible factor-I target genes, such as the * ob * gene, is improved during hypoxia exposure (8, 9, 17). Our results demonstrate that ambient hypoxia per se, independently of energy intake, has specific effects on the * ob * gene transcription and leptin production. These compensate the decline of leptin levels related to food restriction as shown in PF rats. However, the lack of increase in circulating leptin levels from day 1 after hypoxia exposure, compared with normoxic rats fed ad libitum, is consistent with the hypothesis that this anorexigenic hormone does not play a major role in the mediation of hypoxia-induced anorexia (36). Insulin is also a potent anorexigenic hormone that is known to inhibit AMPK activity and decrease AMPK phosphorylation after intracerebroventricular administration (22). The early decrease in AMPK phosphorylation observed in H rats was not related to changes in circulating insulin, and the decrease in plasma insulin after 24 h of hypoxia is consistent with the recovery of AMPK and ACC phosphorylation to N values and thereafter to PF levels.

Decreased AMPK activity and phosphorylation is known to inhibit food intake through changes in intracellular levels of malonyl-CoA and CPT1 activity but also by affecting the transcription of genes encoding orexigenic neuropeptides such as NPY (22, 24). In the present study, the early and transient decrease in AMPK phosphorylation observed in hypoxic ani-

mals did not affect the expression of mRNA encoding the orexigenic NPY. This finding could be consistent with the hypothesis that decreased hypothalamic AMPK would decrease food intake through increased hypothalamic malonyl-CoA and cellular accumulation of long-chain fatty acid-CoA, independently of alteration in expression of orexigenic neu-

ropeptides (26). In contrast, a rise in hypothalamic NPY gene expression occurred in both H and PF groups 24 h after hypoxia exposure. This upregulation in H rats is delayed after an initial period of marked anorexia and is concomitant with food intake resumption. This timing favors the hypothesis that NPY participates in mechanisms stimulating food consumption, independently of ambient hypoxia, reminiscent of the increase in hypothalamic NPY mRNA driven by fasting (4, 34). Moreover, increase in NPY mRNA in hypothaluminus of hypoxic rats could be seen as a biochemical determinant of food consumption allowing acclimatization to hypoxia. However, because the regulation of NPY expression within the arcuate nucleus may differ from that within other hypothala-

mus nuclei, it would be of great interest to determine in future studies whether NPY mRNA expression is differentially regu-

lated in distinct hypothalamic nuclei after acute and pro-

longed exposure to hypobaric hypoxia.

Many previous studies showed that the recovery of food intake during prolonged exposure to hypobaric hypoxia is incomplete and energy intake remains lower than in normoxic age- and weight-matched animals. This involves likely the establishment of a new energy homeostasis, with lower rates of energy intake and energy expenditure. Additional studies are thus warranted to determine whether changes in hypothalamic AMPK activity and transcription of orexigenic and anorexi-

genic hypothalamic peptides could account for this new energy balance.

In conclusion, the present study demonstrates that the hy-

poxic stimulus per se inhibited AMPK and ACC phosphory-

lation in the hypothalamus, concomitant with profound an-

orexia. When compared with animals fed an equivalent quanti-

ty of food, the results show that hypoxic stimulus per se decreased AMPK and ACC phosphorylation, mainly due to increased circulating glucose and leptin levels. However, the specific effects of hypoxia on the phosphorylation states of both AMPK and ACC were very transient and relevant to the crucial role of hypothalamic AMPK in the regulation of food intake only during acute exposure to hypobaric hypoxia. The transient pattern of alterations in AMPK and ACC phosphory-

lation, despite persistence of the hypoxic stimulus, indicates that a powerful counterregulation takes place, presumably as part of physiological mechanisms devoted to avoid prolonged anorexia. Moreover, a rise in hypothalamic NPY gene expression occurred early after the initial period of marked anorexia, concomitant with food intake resumption, supporting the notion that this orexigenic peptide could participate in mecha-

nisms stimulating food consumption, independently of ambient hypoxia, such as during fasting.

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GRANTS

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