Atrial natriuretic peptide contribution to lipid mobilization and utilization during head-down bed rest in humans

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Moro C, Pillard F, de Glisezinski I, Crampes F, Thalamas C, Harant I, Marques M-A, Lafontan M, Berlan M. Atrial natriuretic peptide contribution to lipid mobilization and utilization during head-down bed rest in humans. Am J Physiol Regul Integr Comp Physiol 293: R612–R617, 2007. —Head-down bed rest (HDBR) increases plasma levels of atrial natriuretic peptide (ANP) and decreases norepinephrine levels. We previously demonstrated that ANP promotes lipid mobilization and utilization, an effect independent of sympathetic nervous system activation, when infused into lean healthy men at pharmacological doses. The purpose of the present study was to demonstrate that a physiological increase in ANP contributes to lipid mobilization and oxidation in healthy young men. Eight men were positioned for 4 h in a sitting (control) or in a HDBR position. Indexes of lipid mobilization and hormonal changes were measured in plasma. Extracellular glycerol, an index of lipolysis, was determined in subcutaneous adipose tissue (SCAT) with a microdialysis technique. A twofold increase in plasma ANP concentration was observed after 60 min of HDBR, and a plateau was maintained thereafter. Plasma norepinephrine decreased by 30–40% during HDBR, while plasma insulin and glucose levels did not change. The level of plasma nonesterified fatty acids was higher during HDBR. SCAT lipolysis, as reflected by interstitial glycerol, as well as interstitial cGMP, the second messenger of the ANP pathway, increased during HDBR. This was associated with an increase in blood flow observed throughout HDBR. Significant changes in respiratory exchange ratio and percent use of lipid and carbohydrate were seen only after 3 h of HDBR. Thus the proportion of lipid oxidized increased by 40% after 3 h of HDBR. The rise in plasma ANP during HDBR was associated with increased lipolysis in SCAT and whole body lipid oxidation. In this physiological setting, independent of increasing catecholamines, our study suggests that ANP contributes to lipid mobilization and oxidation in healthy young men.

lipolysis; lipid oxidation

THE REGULATION OF LIPOLYSIS in human adipose tissue has for a long time been attributed to the effect of catecholamines and insulin. Catecholamines activate lipolysis through β-adrenergic receptors (AR) and inhibit it through α2-AR stimulation, while insulin potently inhibits lipolysis by well-characterized cellular mechanisms. The activation of AR modulates the intracellular cAMP concentration and activates cAMP-dependent protein kinase, leading to the phosphorylation and activation of hormone-sensitive lipase (HSL) (11, 12).

The recent discovery that natriuretic peptides (NPs) are activators of lipolysis in human fat cells raises new questions about the physiological control of lipid mobilization in humans (20). NP stimulates fat cell plasma membrane receptors (NPR-A subtype) that have an intrinsic guanylyl cyclase activity, and this raises intracellular levels of cGMP that activate a cGMP-dependent protein kinase (PKG). PKG-dependent phosphorylation of HSL stimulates lipolysis (21, 22). NPs are potent lipolytic agents when they act on isolated fat cells from subcutaneous adipose tissue (SCAT) (21). Intravenous infusion of pharmacological doses of human ANP in humans promotes a strong lipolytic effect that is independent of a reflex activation of the sympathetic nervous system (SNS) since it is not blocked by propranolol administered in a microdialysis probe directly implanted in SCAT (9). We have also demonstrated that a physiological release of ANP during acute endurance exercise contributes to the stimulation of lipid mobilization and the supply of nonesterified fatty acids (NEFA) for the working muscle. This lipid-mobilizing effect of exercise largely depends on ANP release since it is only partly blocked by propranolol infusion in a microdialysis probe implanted in SCAT. Moreover, oral β-AR blockade did not prevent exercise-induced lipid mobilization while exerting fat cell β-AR receptor blockade; exercise-induced increase in plasma ANP was amplified (16). Moreover, within a physiological range, ANP stimulates adipose tissue lipolysis and fat oxidation and increases circulating levels of NEFA (3). The remaining challenge is, in conditions of physiological release of ANP other than exercise, to study the effects of ANP on lipid mobilization/utilization independent of increasing catecholamines. The idea is to evaluate the lipolytic effects of endogenously released ANP under physiological conditions in which the SNS is not activated to further dissociate potential lipolytic effects of ANP from SNS activation.

The aim of this study was to examine the contributions of ANP in the control of lipolysis and lipid oxidation in humans in the head-down bed rest (HDBR) position. HDBR promotes a shift in fluids to the thorax, with an increase in central venous pressure (5, 10). Previous studies have shown that HDBR induces a rapid and sustained increase in plasma ANP (10). Additionally, HDBR inhibits SNS activity and does not alter plasma insulin levels. HDBR represents a suitable physiological model to study the specific effects of ANP on lipid mobilization and oxidation in humans. In the present study, we...
randomly assigned eight healthy male subjects to 6° HDBR for 4 h or to a control study (sitting). To assess the specific role of ANP in the control of lipolysis, lipid mobilization was measured in SCAT with microdialysis. The microdialysis method is useful to monitor local lipid mobilization in SCAT. It is the most suitable method available to perform mechanistic explorations of adipose tissue function in vivo (1, 23, 24).

**MATERIALS AND METHODS**

*Subjects.* Eight healthy young men aged 23 ± 0.6 yr (range: 22–27 yr) with a mean body mass index of 24 ± 0.7 kg/m² (range: 21.6–24.9 kg/m²) who had not been enrolled in any pharmacological or nutritional protocol before the study were recruited. Selection of the subjects was based on detailed medical history, a physical examination, and several blood chemistry analyses. The Ethical Committee of the Faculty of Medicine, Toulouse University, approved the study. All subjects gave their informed consent for the experimental conditions after detailed explanation.

*Experimental protocol.* Dietary intake was controlled during the 2 days before the beginning of the study to limit the impact of diet on the determination of carbohydrate/fat oxidation parameters. The subjects were told to have equilibrated meals adapted to their body weight (1 g/kg body wt protein, 1 g/kg body wt lipid, 5 g/kg body wt carbohydrates, and 6 g NaCl/day) designed by a dietitian. The subjects entered the hospital at 8:00 AM after an overnight fast and were maintained in a sitting position.

An indwelling polyethylene catheter was inserted into the antecubital vein for blood sampling. At 8:30 AM, two microdialysis probes (Carnegie Medicine, Stockholm, Sweden) 20 × 0.5 mm and molecular weight cutoff of 20,000, were inserted percutaneously after epidural anesthesia (200 μl of 1% lidocaine; Roger-Bellon, Neuilly-sur-Seine, France) into the SCAT at a distance of 10 cm from the umbilicus. The probes were connected to a microperfusion pump (Harvard apparatus, S.A.R.L., Les Ulis, France) and perfused with Ringer solution (in mmol/l: 139 sodium, 2.7 potassium, 0.9 calcium, 140.5 chloride, 2.4 bicarbonate). Ethanol (1.7 g/l) was added to the perfusate in order to estimate changes in the adipose tissue blood flow, as previously described (8). After a 60-min equilibration period, fractions of dialysate were collected at a flow rate of 2.5 μl/min for the remaining experimental period. Subjects were then positioned either in a sitting position or in a −6° HDBR position for 240 min. The days for experiments were separated by 1 wk according to a randomized procedure.

For each probe, a 30-min fraction of the outgoing dialysate was collected for determination of dialysate glycerol concentrations. Before the positioning, 10 ml of blood was collected from an indwelling polyethylene catheter inserted into an antecubital vein for plasma determinations. Venous fasting blood samples were taken with the patient resting quietly in a sitting position and then during the HDBR position. For both positions, blood was collected every 30 min up to 120 min and then every 60 min up to 240 min. Blood was collected into 50 μl of an anticoagulant and antioxidant cocktail (Immunotech, Marseilles, France), to prevent catecholamine oxidation, and processed immediately in a refrigerated centrifuge. The plasma was stored at −80°C until analysis. For ANP determinations, blood samples were drawn in ice-cold tubes containing ethylenediaminetetraacetic acid (1 mg/ml) and aprotinin (Trasylol, Bayer, 3,500 kallikrein inhibitory units). Blood specimens were centrifuged immediately in a refrigerated centrifuge at 4°C, and the plasma was stored at −80°C without freeze-thaw cycles until ANP was measured.

Heart rate was continuously monitored by telemetry with a heart rate monitor (Ergocard, Jaeger, Germany). Breath-by-breath measurements were taken throughout the experimental positioning period to assess air flow, and O₂ and CO₂ concentrations in expired gases were measured with a computerized ergospirometer (Oxycon Pro, Jaeger, Wuerzberg, Germany). The respiratory exchange ratio (RER) was calculated during the last 20 min of each hour for both sessions. O₂ concentration was analyzed by a paramagnetic analyzer and CO₂ concentration by an infrared analyzer. Certified calibration gases were used to calibrate the analyzers every day before the beginning of the assay and before the experimental period.

*Drugs and analytical methods.* Glycerol in the dialysate and in plasma was analyzed by an enzymatic method (Sigma, St. Louis, MO). Ethanol in the dialysate and the perfusate was determined with an enzymatic method (2). cGMP in the dialysate was determined with a cGMP enzyme immunoassay kit (SPL-BIO, Montigny, France). Plasma glucose and NEFA were determined with a glucose-oxidase technique (Biotrol, Paris, France) and an enzymatic procedure (Wako, Unipath, Dardilly, France), respectively. Plasma insulin concentrations were measured with enzyme immunoassay kits from ICN Pharmaceuticals (Merckodia, Sweden). Plasma epinephrine and norepinephrine were assayed in 1-ml aliquots of plasma by high-pressure liquid chromatography using electrochemical (amperometric) detection. The detection limit was 10 pg/sample. ANP was measured from unextracted plasma with RIA kits from Peninsula Laboratories (San Carlos, CA). All measurements were made in duplicate and averaged. The average interassay coefficients of variation were 9.6% for ANP. ANP concentrations were determined by exactly the same procedure, which in practical terms means that a comparison between absolute values can be justified.

*Statistical analysis.* All values are means ± SE. The responses to the two postures were analyzed by a repeated-measurement analysis of variance (ANOVA). ANOVA with or without repeated-measures models were fitted to test for differences between the postures. The response curves of the plasma and extracellular concentrations were calculated as the total integrated changes over the baseline values. Significance values are quoted in Figs. 1–5 and Tables 1 and 2. *P* < 0.05 was considered statistically significant.

**RESULTS**

*Effect of posture on plasma ANP, catecholamine, NEFA, glycerol, glucose, and insulin concentrations.* Plasma ANP and norepinephrine concentrations were quite similar at rest (sitting position) in both sessions (Table 1). When the subjects were maintained in the sitting position, no significant changes were observed for these two parameters (Fig. 1). On the contrary, HDBR induced a significant increase in plasma ANP concentrations, with the peak values being observed after 60 min HDBR and then remaining at a constant level for the remaining 180 min. Plasma norepinephrine levels decreased after 30 min of HDBR, and this decrease was significantly stabilized during
the remaining 210 min of HDBR (Fig. 1). No changes in epinephrine were observed in either of the two situations (Table 2).

Before the different postures were established, NEFA, glycerol, glucose, insulin, and plasma levels were similar. No significant changes were observed in plasma insulin and glucose plasma values regardless of posture (Table 2). In the sitting position, plasma NEFA tended to increase progressively until the end of the experimental procedure, but the increase was not significant (Fig. 2). However, there was a significant increase in plasma NEFA levels during the first 90 min of the HDBR position, which increased significantly at 180 and 240 min. The plasma glycerol concentration decreased regularly in the sitting position and was stable after 60 min. In the HDBR position, plasma glycerol was maintained at the initial concentration during the first 30 min and then decreased steadily with time (Fig. 2).

**Effect of posture on extracellular glycerol concentration, change in blood flow, and cGMP in dialysate from adipose tissue.**

Before the postures were established, the baseline dialysate glycerol concentrations (DGC) in the probe were similar between the two sessions (89.4 ± 110.006 13.5 and 99.0 ± 110.006 14.5 mol/l for sitting and HDBR, respectively). In the sitting position, no significant changes were observed throughout the 240 min. HDBR induced a transitional increase in DGC during the first 60 min, and then the values tended to be higher than pre-HDBR values until the end of the experiment (Fig. 3). The ethanol outflow-to-inflow ratio measured before the postures was similar in both cases (70.6 ± 110.006 3.2% and 71.7 ± 110.006 3.0 71% for sitting and HDBR, respectively). During the sitting session, no significant changes were observed.
change in the ethanol ratio was observed. During the first 60 min of HDBR, the ethanol ratio was significantly reduced at about 60 min and was maintained until the end of the experimental period (Fig. 3).

Before the experimental periods, the baseline cGMP concentrations in the dialysate were similar between groups. In the sitting position no significant changes were observed, while HDBR induced a significant increase in cGMP throughout the 240 min (Fig. 4).

The analysis of results for repeated measures for the time factor and posture tended to be significant for the increase in DGC ($P = 0.07$). cGMP increase and ethanol ratio decrease were significant ($P < 0.003$ and $P = 0.03$, respectively) (Figs. 3 and 4).

**Effect of position on heart rate, RER, and lipid and carbohydrate utilization.** No differences were observed in heart rate before positioning (57.0 ± 2.0 and 56.8 ± 2.1 beats/min), and no significant changes occurred during the whole investigation in either posture (59.5 ± 2.4 and 57.8 ± 1.8 beats/min during sitting and HDBR, respectively). RER and lipid and carbohydrate utilization were similar before the positioning (Fig. 5). In the sitting position, there was no change in RER or lipid or carbohydrate utilization. After 180 min of HDBR the RER fell progressively, and this decrease was significant at the end of the experimental period. As a consequence, the calculated utilization of lipid progressively increased significantly while carbohydrate utilization simultaneously decreased (Fig. 5). Total energy expenditure was similar in both conditions.

**DISCUSSION**

The initial response to HDBR is a rapid shift in fluids to the thorax with an increase in plasma volume and central venous...
pressure. This is responsible for a rapid increase in stroke volume and cardiac output (10, 5). The activity of the renin-angiotensin-aldosterone system is reduced, and the plasma concentration of NPs increases (15). Similar changes were also demonstrated during spaceflight (14). Plasma ANP concentration increases after a rise in central venous pressure and cardiac filling pressure (10). In the present study we found a rapid increase (60 min) in plasma ANP level after placing the subjects in the HDBR position (Fig. 1). This was accompanied by a sustained inhibition of sympathetic activity, reflected by the fall in plasma norepinephrine values (with no changes in epinephrine concentration, suggesting that adrenal activation was not affected). We used HDBR as a suitable model to discriminate the effect of norepinephrine from those of ANP on lipid mobilization in lean healthy subjects.

The aim of the study was to evaluate how a physiological increase in plasma ANP might influence adipose tissue lipolysis. In this setting, we found that ANP is a good candidate to explain the enhanced lipid mobilization from adipose tissue leading to a direct or indirect increment in lipid oxidation. We previously demonstrated the metabolic impact of ANP administration in humans. We also showed that the local infusion of ANP into SCAT increased lipolysis and blood flow (20). Another study showed that intravenous infusion of ANP, within a concentration range observed in humans, increased lipid mobilization from adipose tissue as assessed by an increase in circulating NEFA, and also increased lipid utilization (3). When comparing the results obtained in perfusion studies (3) and the present HDBR results, it could be questioned whether the rise in ANP induced by HDBR is sufficient to promote a stimulation of lipolysis. Direct comparison of results obtained with ANP infusion (3) and HDBR is not easy. ANP infusion promotes a mild but significant increase of SNS activity and heart rate that does not exist in HDBR. The part played by the SNS when ANP is infused has recently been discussed (4). In this new set of experiments performed with the same experimental paradigm as in Ref. 3, glycerol increase in adipose tissue was clearly observed at 6.25 ng·kg⁻¹·min⁻¹. ANP infusion increases cardiac sympathetic activity, which is abolished by β-AR blockade. However, the level of SNS activation promoted by ANP does not reach the level necessary to impact on lipolysis since the ANP-dependent increase on NEFA and glycerol concentrations was not suppressed by propranolol (4, 9). To conclude, selected cardiovascular effects of ANP infusion are mediated by β-AR stimulation while ANP-induced changes in lipid mobilization are mediated by another mechanism (presumably stimulation of NP receptors).

In HDBR, where a reduction of SNS tone was observed (i.e., decrease in plasma norepinephrine levels), SNS impact on lipolysis cannot be proposed and ANP is the reasonable candidate to explain the effects. In situ lipolysis is the result of a complex functional balance between stimulatory (i.e., catecholamines, ANP) and inhibitory (i.e., insulin, α-adrenergic drive, adenosine, prostaglandins) factors that could also affect local blood flow modifications; it is not excluded that the interpretation of the responses between the two studies could be complicated by the fact that, in addition to ANP, other local regulatory factors interfere with the lipolytic response initiated during HDBR.

It is seen in the present study that during the first 60 min of HDBR an increase in glycerol concentration in the dialysate occurs. Since ANP levels are increased concomitantly, glycerol increment could be the reflection of the ANP effect on SCAT lipolysis. During the following 60 min of HDBR, dialysate glycerol levels returned toward the initial values (Fig. 3) while plasma ANP and dialysate cGMP levels remained elevated. Apparently, the lack of parallelism between changes for ANP, dialysate glycerol, and cGMP makes the support of a causative relationship difficult. Interpretation of such kinetics is difficult, but some explanations are possible. Glycerol concentration determined in the dialysate probe results from glycerol release (lipolysis) and glycerol washout (related to changes in local adipose tissue blood flow). HDBR promotes a sustained increment of blood flow assessed by the ethanol ratio (Fig. 3). In addition to a reduction of sympathetic tone, this effect could also be related to the impact of ANP on adipose tissue vessels as previously shown (20); the contribution of both events could be additive and could explain the sustained action. There is no report showing the relationship between the rise of ANP in systemic circulation and its occurrence in adipose tissue. When the microdialysis technique is used, it is known that sustained vasodilatation in adipose tissue vessels can mask the effect of lipolytic agents on lipolysis (8). Under our experimental conditions, the lipoid-mobilizing effect of ANP was probably lessened and even became undetectable in the long term because of the potent and sustained increase in blood flow and the adipose tissue washout. We previously showed (17) that the ANP-dependent lipolytic pathway is desensitized in vitro in adipocytes and in vivo after infusion of ANP directly in microdialysis probes in humans. The desensitization process is not a sustainable explanation since cGMP concentrations in the dialysate continuously increased during HDBR. However, concerning the cGMP kinetics, it is not excluded that, in addition to fat cell-originating cGMP, cGMP of vascular origin could also contribute to changes. A combination of the various mechanisms could explain the glycerol and cGMP results even if the ANP plasma level remained elevated. It is certain that perfusion of subcutaneous adipose tissue with an ANP antagonist in the microdialysis probe will solve the dilemma. However, there is no drug available for such clinical investigations. This explanation could also be proposed to explain the increase in plasma NEFA during the first 90 min of HDBR followed by a decrease 120 min after HDBR (Table 1).

Plasma kinetics of NEFA and glycerol also require comment. After NEFA increase (compared with controls) during the first 90 min, the NEFA changes in HDBR and control subjects are strictly superimposed (Fig. 2). Evolution of plasma NEFA could be related to the observed progressive decrease in the RER, indicating an increase in lipid oxidation during HDBR (Fig. 5). It was previously shown that adrenergic mechanisms modulate substrate utilization during ANP infusion (4). This adrenergic component, which is missing in the present study, could explain the kinetic differences existing between the results in perfusion studies (3, 4) and HDBR. The decrease in plasma glycerol levels was more rapid when compared with plasma NEFA concentrations. These observations could be interpreted by considering liver gluconeogenesis and blood flow changes initiated by HDBR. Previous studies showed that alanine concentrations in blood reflect the rate of hepatic gluconeogenesis during HDBR (25) and also that hepatic blood flow increased during the first hour but not after 24 h of HDBR (18). During fasting there is also rapid removal...
of glycerol from the circulation and its reutilization mainly by the liver for gluconeogenesis and to a lesser extent by skeletal muscle and the kidneys (6, 13). Recent studies showed that fasting rapidly increased expression of aquaglyceroporins (aquaporin 9) which served as a membrane channel in hepatocytes to maximize glycerol influx required for increased gluconeogenesis (7) Finally, Rashed et al. (19) demonstrated that ANP increased hepatic gluconeogenesis via an elevation of cGMP production. Since glycerol is an important substrate in humans, this regulation may also explain why the reduction in plasma glycerol concentration is more rapid than that of NEFA.

Previous studies have suggested that ANP is an important factor involved in lipid mobilization in another physiological situation: endurance exercise (16). However, in this situation lipolysis may involve other biological factors, and the effect of ANP could be confounded with those of catecholamines or the fall in insulin. In fact, during exercise, and after block of β-AR with tertatolol, the lipolysis in SCAT (evaluated by microdialysis) was maintained. However, under these conditions, a decrease in insulin secretion also occurred. Thus the specific involvement of ANP in the control of lipid mobilization has not so far been fully demonstrated. HDBR represents a valuable physiological model to study the effect of ANP on lipid mobilization and oxidation associated with reduced sympathetic activation and maintained insulinemia. In the present results, some of the fluctuations occurring in plasma parameters (glycerol and NEFA) could be due to modification of local blood flow in adipose tissue, to liver neoglucogenesis, and to an increased NEFA utilization. Putative release, during HDBR, of biological compounds known to exert lipolytic or antilipolytic effects in human fat cells and interfering with ANP action should also be considered. Nevertheless, it is difficult to propose a reliable compound or hormone. Despite these questions and the practical limitations inherent in the physiological approach used in this study and discussed previously, the results suggest that the mobilization of lipids could be associated with an increase in ANP during HDBR, and they confirm that this mobilization was associated with a progressive increase in lipid oxidation.

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GRANTS

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