Atrial Natriuretic Peptide Induces Postprandial Lipid Oxidation in Humans

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OBJECTIVE—Atrial natriuretic peptide (ANP) regulates arterial blood pressure. In addition, ANP has recently been shown to promote human adipose tissue lipolysis through cGMP-mediated hormone-sensitive lipase activation. We hypothesized that ANP increases postprandial free fatty acid (FFA) availability and energy expenditure while decreasing arterial blood pressure.

RESEARCH DESIGN AND METHODS—We infused human ANP (25 ng · kg^{-1} · min^{-1}) in 12 men (age 32 ± 0.8 years, BMI 23.3 ± 0.4 kg/m^2) before, during, and 2 h after ingestion of a standardized high-fat test meal in a randomized, double-blind, cross-over fashion. Cardiovascular changes were monitored by continuous electrocardiogram and beat-by-beat blood pressure recordings. Metabolism was monitored through venous blood sampling, intramuscular and subcutaneous abdominal adipose tissue microdialysis, and indirect calorimetry.

RESULTS—ANP infusion decreased mean arterial blood pressure by 4 mmHg during the postprandial phase ($P < 0.01$ vs. placebo). At the same time, ANP induced lipolysis systemically ($P < 0.05$ vs. placebo) and locally in subcutaneous abdominal adipose tissue ($P < 0.0001$ vs. placebo), leading to a 50% increase in venous glycerol ($P < 0.01$) and FFA ($P < 0.05$) concentrations compared with placebo. The increase in FFA availability with ANP was paralleled by a 15% increase in lipid oxidation rates ($P < 0.05$ vs. placebo), driving a substantial increase in postprandial energy expenditure ($P < 0.05$ vs. placebo).

CONCLUSIONS—Our data identify the ANP system as a novel pathway regulating postprandial lipid oxidation, energy expenditure, and concomitantly arterial blood pressure. The findings could have therapeutic implications. ***Diabetes* 57: 3199–3204, 2008**

A modest mismatch between energy intake and expenditure elicits major changes in body weight over years. Total daily energy expenditure comprises resting metabolic rate, physical activity, and postprandial thermogenesis. Measures that increase postprandial thermogenesis could prevent or treat obesity. Pharmacological strategies to augment energy expenditure have been unsuccessful because of side effects (1). Manipulation of adrenergic transmission is associated with increased thermogenesis, blood pressure elevations, and other cardiac side effects (2). Atrial natriuretic peptide (ANP) has recently been shown to promote adipose tissue lipolysis through cGMP-mediated, hormone-sensitive lipase activation (3). ANP-mediated lipolysis has only been observed in primates such as macaques and humans but not in other species (4). ANP increases circulating free fatty acid (FFA) levels in human subjects (5–8). Previous studies with adrenergic agonists suggested that increased circulating FFA concentrations can drive an increase in energy expenditure (9). In our earlier studies, ANP-mediated lipolysis did not alter energy expenditure in the fasted state, whereas lipid oxidation rate increased slightly (6,8). We now tested the hypothesis that ANP augments postprandial FFA availability, lipid oxidation, and energy expenditure while concomitantly decreasing blood pressure in healthy young men.

RESEARCH DESIGN AND METHODS

We recruited 12 healthy, nonoverweight, nonobese individuals aged 32 ± 0.8 years with BMI 23.3 ± 0.4 kg/m^2. Subjects did not ingest any medication. The institutional review board approved the studies, and written, informed consent was obtained before enrollment.

Forty-eight hours before the experiment, volunteers were asked to abstain from smoking, alcohol ingestion, caffeine-containing beverages, and vigorous exercise. Subjects were studied twice on separate days in a randomized, double-blind, placebo-controlled, and cross-over fashion. For that purpose, subjects were randomized when to receive ANP or placebo by an automated randomization program by a study nurse. On the study days, one of our coworkers, who was not involved in the analysis of the data, prepared the infusions. On one study day, subjects received an intravenous ANP (Clinalfa-Bachem, Läufelingen, Switzerland) infusion at a rate of 25 ng · kg^{-1} · min^{-1}. In previous studies, this dosage has been shown to yield venous ANP concentrations that are within the physiological meaningful range (6,7). Normal saline solution served as placebo. After 30 min of continuous infusion, subjects ingested a standardized fat-rich test meal containing 45% fat, 40% carbohydrate, and 15% protein. Infusions were continued for an additional 120 min after food intake. Two subjects received an ANP infusion without a meal on a third study day.

Instrumentation. One catheter (Vasocan 20G; B. Braun, Melsungen, Germany) each was placed into large antecubital veins of both arms. We used one catheter for ANP infusion and the other one for blood sampling. A microdialysis probe was inserted into abdominal subcutaneous adipose tissue and another into skeletal muscle (quadriceps femoris and vastus lateralis). Oxygen consumption and carbon dioxide production were measured by indirect calorimetry using a ventilated hood (DeltatracII; Datex Ohmeda, Duisburg,

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Received 15 May 2008 and accepted 16 September 2008.
Published ahead of print at http://diabetes.diabetesjournals.org on 3 October 2008. DOI: 10.2337/db08-0649.
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Germany) to assess energy expenditure and respiratory quotient and lipid oxidation rates. Whole-body fat oxidation rates were calculated from each period of gas collection and estimated using stoichiometric equations (10). Heart rate was measured continuously by electrocardiogram (Cardioscreen; Medis, Ilmenau, Germany). Beat-by-beat blood pressure (Finapres; Ohmeda, Amsterdam, the Netherlands) and brachial arterial blood pressure (Dinamap; Critikon) were determined.

Microdialysis. Details of the microdialysis technique were described previously (11,12). Briefly, before insertion of the probes, we applied a local anesthetic (lidocaine) either as a cream for adipose tissue (EMLA, Astra, Germany) or as a subcutaneous injection for muscle (Xylocitin 1%; Jenapharm, Jena, Germany). After probe insertion, we started the tissue perfusion with lactate-free Ringer solution (Serumwerk, Bernburg, Germany) at a flow rate of 2 μl/min. The solution was supplemented with 50 mmol/l ethanol (B. Braun, Melsungen, Germany) for blood flow determinations. CMA/60 microdialysis catheters and CMA/102 microdialysis pumps (both from CMA Microdialysis, Solna, Sweden) were used. A 60-min period was allowed for tissue recovery and for baseline calibration before ANP infusion. Two 15-min dialysate fractions were collected at baseline.

Analytical methods. ANP concentrations were determined using a radioimmunoassay (Peninsula Laboratories, San Carlos, CA), and venous glycerol concentrations were determined with an ultrasensitive radiometric method (13). Venous FFAs were assayed with an enzymatic method (Wako kit; Unipath, Dardilly, France). Catecholamines were collected in EGTA tubes (Kabevette; Kabe Labortechnik, Nümbrecht-Elsenroth, Germany) and processed immediately in a refrigerated centrifuge. The plasma was stored at −80°C until analysis. Plasma epinephrine and norepinephrine were assayed by high-pressure liquid chromatography with electrochemical (amperometric) detection (14). Plasma renin concentration was measured by Immunoradiometric Assay provided by Cis Bio International (Gif-sur-Yvette, France). Within- and between-run coefficients of variation were 7.4 and 7.2% at 6.8 mU/l, 6.2 and 2.6% at 37.4 mU/l, and 1.3 and 4.7% at 217 mU/l. Reference values are 7–75 mU/l. β-Hydroxybutyrate was measured with an enzymatic method (Wako kit; Unipath). Insulin concentrations were measured using a radioimmunoassay (Sanofi Diagnostics, Pasteur, France). Ethanol concentrations in perfusate (inflow) and dialysate (outflow) were measured with a standard enzymatic assay (15). Dialysate glucose, lactate, pyruvate, and glycerol concentrations were measured with a CMA/600 analyzer (CMA Microdialysis).

Calculations and statistics. Changes in blood flow were determined using the ethanol dilution technique, which is based on Fick's principle (16,17). Accordingly, a decrease in the outflow-to-inflow ratio is equivalent to an increase in blood flow and vice versa. For simplicity, the term "ethanol ratio" is substituted for the term "ethanol outflow-to-inflow ratio." Changes in glycerol concentration were used to assess changes in lipolysis and/or lipid mobilization, and changes in glucose, lactate, and pyruvate concentrations were used to assess changes in carbohydrate metabolism (18,19). In situ recovery for glycerol, glucose, lactate, and pyruvate in the dialysate was assessed by near-equilibrium dialysis (20). For all four metabolites, we found recoveries of ~30% in adipose tissue and 50% in skeletal muscle. All data are expressed as means ± SE. Two-way ANOVA testing was used to compare ANP and placebo responses followed by Bonferroni post tests. In addition, we determined changes from baseline for each intervention with one-way ANOVA tests followed by Dunnett's post test when the ANOVA P value was <0.05. A value of $P < 0.05$ was considered significant.

RESULTS

ANP concentrations are within the clinically relevant range. Baseline plasma ANP concentrations were 77 ± 5 pg/ml with ANP and 63 ± 8 pg/ml with placebo (NS vs. placebo). With ANP infusion, ANP concentrations increased fourfold ($P < 0.001$ vs. placebo) throughout the experiment, whereas ANP concentrations remained unchanged with placebo (data not shown). In pathological conditions, such as heart failure, plasma ANP concentrations can exceed 500 pg/ml (21), suggesting that ANP concentrations in our study were in a physiologically meaningful range.

ANP infusion reduces postprandial blood pressure. Blood pressure and heart rate responses after ingestion of a standardized meal during placebo or ANP infusion are illustrated in Fig. 1. Baseline mean arterial pressure was 80 ± 2 mmHg before placebo and 81 ± 2 mmHg before ANP administration. Thirty minutes into the ANP infusion,

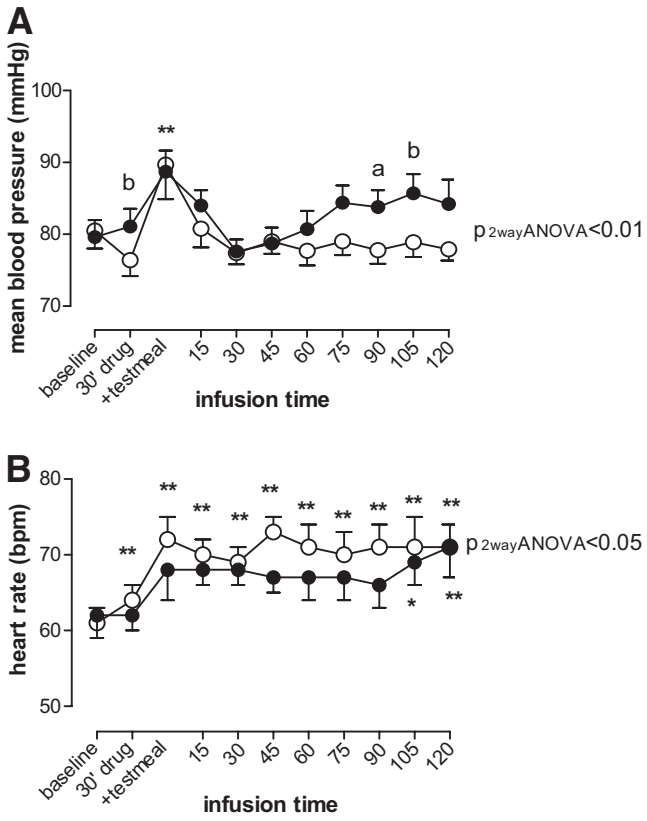

FIG. 1. Mean blood pressure (*A*) and heart rate (*B*) before and after ingestion of a test meal. On one day, subjects ingested the meal during a continuous ANP infusion; on another day, they ingested a meal during placebo (PLC) infusion. **P* < 0.05 vs. baseline; ***P* < 0.01 vs. baseline; a, *P* < 0.05 ANP vs. placebo; b, *P* < 0.01 ANP vs. placebo. ○, ANP; ●, PLC.

mean arterial pressure was 76 ± 2 mmHg ($P < 0.01$ vs. placebo). Blood pressure increased acutely during meal ingestion in both groups. In the postprandial phase, mean arterial pressure was significantly reduced with ANP infusion compared with placebo ($P < 0.01$, two-way ANOVA). Heart rate increased with food ingestion and remained elevated throughout the postprandial phase. The heart rate increase was slightly greater with ANP. Baseline plasma norepinephrine and epinephrine concentrations were 0.53 ± 0.07 and 0.09 ± 0.01 pmol/ml, respectively, with placebo. With ANP, the values were 0.47 ± 0.06 and 0.10 ± 0.01 pmol/ml, respectively (NS vs. placebo). At the end of the postprandial phase, norepinephrine concentrations with placebo were 0.56 ± 0.06 compared with 0.73 ± 0.12 pmol/ml with ANP (NS vs. placebo), and epinephrine concentrations with placebo were 0.13 ± 0.02 compared with 0.21 ± 0.07 pmol/ml (NS vs. placebo) with ANP. Plasma renin concentration at baseline was 9.4 ± 1.1 and 9.5 ± 1.2 mU/l with ANP and placebo infusion, respectively. Sixty minutes after the test meal, ANP decreased renin concentrations by 30 ± 5% ($P = 0.05$ vs. placebo).

ANP increases systemic FFA availability. Figure 2 shows changes in venous insulin (Fig. 2*A*, *left*), glucose (Fig. 2*A*, *right*), FFA (Fig. 2*B*, *left*), and glycerol (Fig. 2*B*, *right*) concentrations after meal ingestion, with or without ANP infusion. Fasting glucose concentration was 4.3 ± 0.16 mmol/l (78 ± 2.8 mg/dl) before placebo infusion and 4.3 ± 0.12 mmol/l (78 ± 2.1 mg/dl) before ANP infusion (NS vs. placebo). In the postprandial phase, venous glucose concentration increased similarly with placebo or ANP, reaching a maximum ~30 min after the meal. Base-

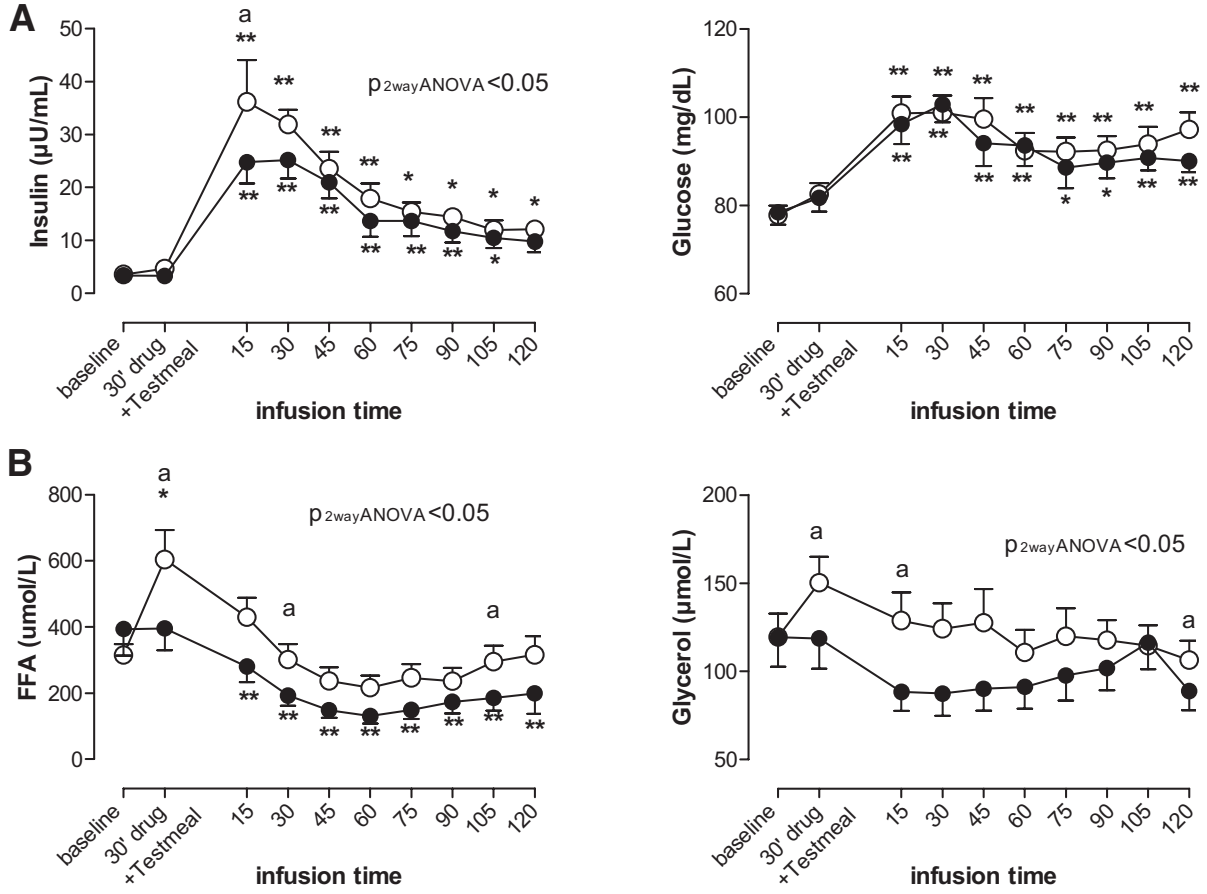

FIG. 2. Venous insulin (*A*), glucose (*B*), FFAs (*C*), and glycerol (*D*) concentrations before and after ingestion of a high-fat test meal. On one day, subjects ingested the meal during a continuous ANP infusion; on another day, they ingested a meal during placebo (PLC) infusion. **P* < 0.05 vs. baseline; ***P* < 0.01 vs. baseline; a, *P* < 0.05 ANP vs. placebo. ○, ANP; ●, PLC.

line insulin concentrations were 3.6 ± 0.5 and 3.4 ± 0.4 μU/ml on the ANP and placebo infusion days, respectively. The HOMA index, a surrogate for insulin sensitivity, was 0.68 ± 0.1 and 0.69 ± 0.1 on the ANP and placebo infusion days, respectively. Even though glucose concentrations throughout the study were similar, postprandial insulin concentration was augmented with ANP. Glycerol was produced during triglyceride hydrolysis. With placebo, venous glycerol concentrations decreased sharply in the postprandial phase. The phenomenon is explained by the antilipolytic action of insulin. In contrast, with ANP, venous glycerol remained elevated throughout the postprandial phase compared with placebo even though insulin levels were elevated. The observation suggests that ANP increased lipolysis in the postprandial phase. Baseline FFA concentrations were 315 ± 33 μmol/l on the ANP infusion day and 394 ± 80 μmol/l with placebo (NS). The ANP-mediated increase in postprandial lipolysis resulted in increased systemic FFA availability. We also tested the effect of ANP infusion without a test meal in two subjects. FFA concentrations were lowest during placebo with a test meal, higher with ANP and a test meal, and even higher when ANP was given without a meal (data not shown).

Contribution of adipose tissue to ANP-induced changes in metabolism. We used microdialysis to follow changes in adipose tissue and in skeletal muscle metabolism. To monitor changes in blood flow, we applied the ethanol dilution technique (17). Adipose tissue ethanol ratio was similar before placebo and before ANP infusion. The ratio decreased with both ANP ($P = 0.05$, one-way ANOVA) and placebo ($P < 0.001$, one-way ANOVA; data not shown), suggesting an increase in local blood flow with both treatments. Microdialysate glycerol in adipose tissue was increased with ANP but shows a two-phase response with a decrease 60 min after meal ingestion and an increase late into the postprandial phase (Fig. 3, *top*). Because local blood flow is increased in subcutaneous adipose tissue with ANP, more substrate escapes from the interstitial space. Therefore, the real increase in glycerol with ANP might be even higher than the one we were able to detect. With placebo, subcutaneous adipose tissue glycerol concentrations slightly decreased after the test meal (Fig. 3, *top*). In skeletal muscle, the ethanol ratio was similar with ANP and placebo and did not change with both treatments. Postprandial glycerol changes in skeletal muscle decreased to the same extent with placebo or ANP (Fig. 3, *bottom*).

ANP increases postprandial thermogenesis through lipid oxidation. Figure 4 illustrates changes in energy expenditure, relative lipid oxidation rates, and systemic concentrations of the ketone body β-hydroxybutyrate with meal ingestion during placebo or ANP. With placebo, energy expenditure increased substantially in the postprandial phase. The respiratory quotient did not change with placebo, suggesting that the increase in energy expenditure resulted from raised combined carbohydrate and lipid oxidation. ANP augmented the postprandial increase in energy expenditure. On ANP, the respiratory quotient decreased from 0.771 ± 0.016 at baseline to 0.735 ± 0.018 30 min after the meal ($P < 0.05$ compared

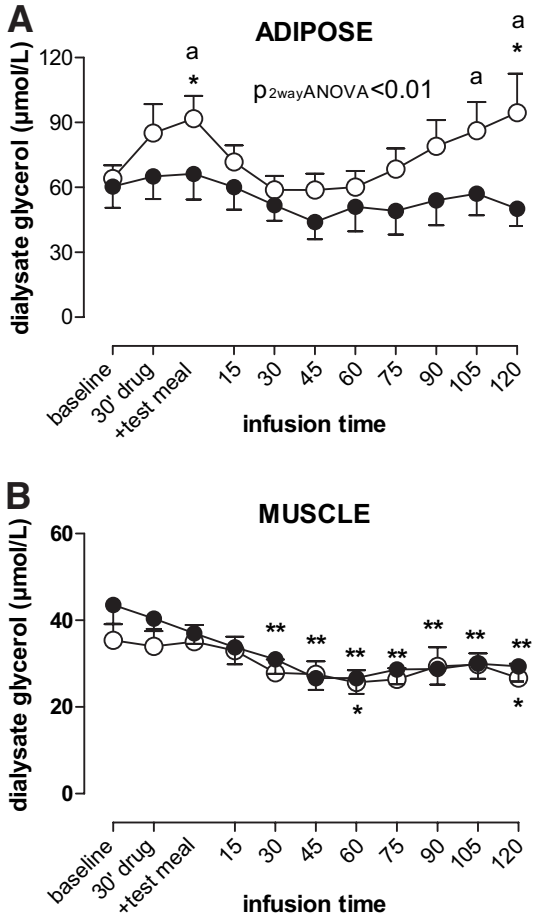

FIG. 3. Changes in microdialysis subcutaneous abdominal glycerol concentration (*A*) and skeletal muscle glycerol concentrations (*B*) before and after ingestion after a test meal. On one day, subjects ingested the meal during a continuous ANP infusion; on another day, they ingested a meal during placebo (PLC) infusion. **P* < 0.05 vs. baseline; ***P* < 0.01 vs. baseline; a, *P* < 0.05 ANP vs. placebo. ○, ANP; ●, PLC.

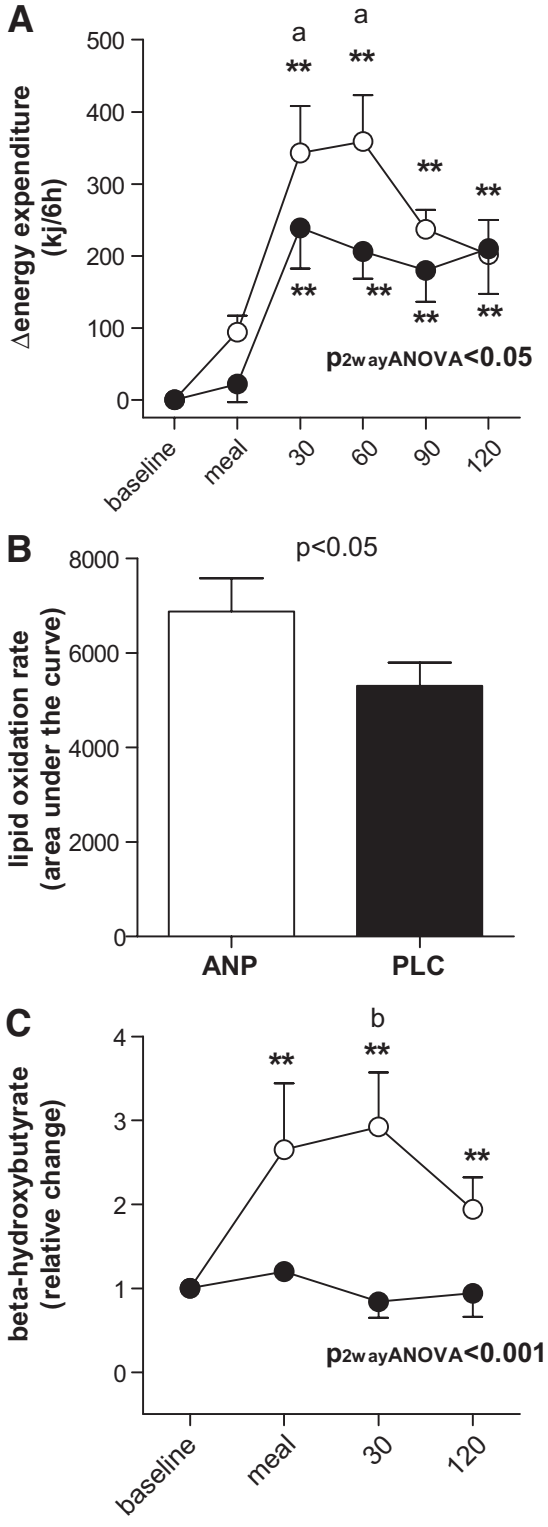

FIG. 4. Changes in energy expenditure (*A*) and lipid oxidation rates (*B*) and relative changes in systemic β-hydroxybutyrate concentrations (C) with ANP or placebo (PLC) infusions. **P* < 0.05 vs. baseline; ***P* < 0.01 vs. baseline; a, *P* < 0.05 ANP vs. placebo; b, *P* < 0.01 ANP vs. placebo. ○, ANP; ●, PLC.

with placebo). The decrease in the respiratory quotient corresponds to an increase in lipid oxidation rate ($P < 0.05$ compared with placebo; Fig. 4*B*) in subjects receiving ANP infusions. β-Hydroxybutyrate, a marker of hepatic lipid oxidation, decreased slightly with placebo. With ANP, β-hydroxybutyrate concentrations increased threefold in the postprandial phase ($P < 0.01$ vs. placebo; Fig. 4*C*). The area under the curve for the relative change in FFA concentrations throughout the experiment inversely correlated with the area under the curve for relative respiratory quotient changes with ANP ($r^2 = 0.44$, $P = 0.05$). Taken together, our observations indicate that ANP is a potent stimulator of postprandial lipid oxidation and energy expenditure and that the liver is a major organ of ANP-induced lipid oxidation.

DISCUSSION

We identified the ANP system as a novel pathway that regulates postprandial lipid oxidation. In particular, we showed that ANP attenuates the postprandial decline in lipid mobilization leading to increased FFAs, both before and after ingestion of a standardized fat-rich test meal. The increase in circulating FFAs was associated with increased lipid oxidation driving an increase in postprandial energy expenditure. ANP decreased blood pressure in the postprandial phase with minimal reflex-mediated tachycardia.

The depressor response to ANP in our study can be attributed to its well-established cardiovascular actions, for example through modulation of the renin-angiotensin system (22). ANP decreased postprandial renin concentrations compared with placebo. Interestingly, the depressor response to ANP was more pronounced late into the

postprandial phase. Possibly, ANP reduced the ability of the cardiovascular system to cope with postprandial vasodilatation in the splanchnic tract (23). The mechanism could contribute to postprandial hypotension in the elderly (23). Near complete adrenergic receptor blockade by propranolol does not abrogate the metabolic actions of ANP (5,7), suggesting that ANP-mediated metabolic effects are not related to reflex-activated adrenergic stimulation. Vasodilatation per se has not conclusively been shown to affect lipolysis in the absence of release of a lipolytic factor or withdrawal of antilipolytic mechanisms (24,25).

In accordance with previous studies (5–7,26), augmented lipolysis with ANP resulted in a sustained increase in circulating FFAs throughout the experiment. The observation suggests that the increase in FFA release was not completely compensated by FFA re-esterification or lipid oxidation. Yet, compared with placebo, ANP induced an increase in postprandial lipid oxidation rate. Plasma ketone concentrations, which reflect hepatic lipid oxidation, increased sharply with ANP infusion. Circulating free carnitine concentrations have been reported to decrease with ANP infusion (26). Carnitine is a critical factor for fatty acid intramitochondrial transport by carnitine palmityl transferase I and thus β-oxidation (27).

The mechanisms increasing lipid oxidation rate with ANP are not fully understood. In human subjects, increased lipolysis can drive an increase in lipid oxidation rate. Systemic β-1 and β-2 adrenoreceptor agonist infusions increase lipolysis and lipid oxidation rate (9). Lipolysis inhibition with acipimox reduces adrenoreceptor-mediated lipolysis and lipid oxidation. In the present study, FFA concentrations were inversely correlated with the respiratory quotient, suggesting that FFAs drive, at least in part, the ANP-mediated increase in lipid oxidation rates. The increase in FFAs may have led to secondary changes in skeletal muscle metabolism (28). Increased postprandial insulin with unchanged systemic and muscular glucose is consistent with an ANP-induced state of insulin resistance favoring lipid rather than glucose utilization. Perhaps ANP also exerts a direct effect on fatty acid uptake and β-oxidation in peripheral tissues: ANP-induced lipolysis is mediated by cGMP formation. Recently, cGMP has been shown to increase mitochondrial biogenesis, ATP synthesis, and oxidative phosphorylation in cultured myotubes (29). Chronic inhibition of cGMP hydrolysis with a phosphodiesterase-5 inhibitor reduced weight and fat mass through increased energy expenditure in high-fat–fed mice (30).

Increased lipolysis with ANP increased circulating FFA levels throughout the experiment. Pancreatic β-cells are affected by FFAs depending on the duration of exposure. Acutely, FFAs together with an increase in glucose concentrations induce an exaggerated insulin response (31). In accord with previous studies (6,26), insulin concentrations increased with ANP infusion in the early postprandial phase. Insulin regulates lipolysis by modulating cAMP concentrations through type 3B phosphodiesterase (PDE-3B).

ANP-induced lipolysis is mediated by cGMP formation. Previously, radioligand binding assays using [^{123}I]-labeled ANP as the ligand showed high-affinity binding sites on human adipocytes (32). ANP increased intracellular cGMP concentrations 187-fold, whereas cAMP concentrations remained unchanged. The increase in cGMP activates cGMP-dependent protein kinase G, which phosphorylates and activates hormone-sensitive lipase. In noncellular systems, cGMP inhibits PDE-3B (33). However, pharmacological PDE-3B inhibition or PDE-3B stimulation through insulin did not affect ANP-induced lipolysis in isolated adipocytes (32,34). In our study, the ANP-mediated lipolysis was attenuated early in the postprandial phase. In subcutaneous adipose tissue, lipolysis decreased sharply after food ingestion and increased again late into the study. Lipolysis inhibition coincided with the peak in circulating insulin concentrations. This pattern might suggest a discrepancy between in vitro and in vivo actions of ANP on metabolism.

ANP induced lipolysis in subcutaneous adipose tissue but not in skeletal muscle. The molecular mechanism for this difference is unknown. In skeletal muscle, only combined hyperinsulinemia and hyperglycemia suppress lipolytic activity (35). Lipoprotein lipase activity, which hydrolyzes lipoproteins to FFAs and glycerol mainly in the epithelium of capillary beds, is reduced by insulin in skeletal muscle (36). However, consistent with the present study, ANP did not increase glycerol concentrations in skeletal muscle under basal conditions (6). This finding argues against a specific insulin effect on the ANP-mediated metabolic effect in skeletal muscle. The vastus lateralis muscle consists of mixed muscle fibers. Skeletal muscles show different degrees of lipolytic response to adrenergic stimuli according to their fiber type composition (37). In contrast to adipose tissue, glycerol can be taken up and reused in muscle after triglyceride hydrolysis. Postprandially, the effect can account for up to 50% of the released glycerol (38). Finally, increased blood flow could possibly wash out interstitial glycerol concentrations. We did not observe a change in local skeletal muscle blood flow with ANP. However, the ethanol ratio is a qualitative rather than quantitative method of determining blood flow and therefore not sensitive toward minor changes in blood flow (39). We cannot rule out the possibility that we missed subtle changes in blood flow and lipolysis.

Together, our findings suggest that the natriuretic peptide system is an important regulator of postprandial metabolism. The system may be amenable to therapeutic intervention. For example, neutral endopeptidase inhibitors are in clinical development for the treatment of arterial hypertension and heart failure (40). Neutral endopeptidase inhibition may promote lipid mobilization and oxidation. Our findings may also be important in terms of human pathophysiology, both in conditions associated with increased ANP and with decreased ANP availability. ANP availability is increased in heart failure. With induction of β-adrenoreceptor blocker therapy, natriuretic peptide release increases further (41). Possibly, increased lipid mobilization through natriuretic peptides sustains substrate supply to the failing heart (42). Cardiac cachexia is a complication of heart failure that worsens prognosis. The cause of cardiac cachexia is unknown. Our findings suggest that increased ANP-mediated lipid mobilization and oxidation could predispose to cardiac cachexia (43).

ANP availability is decreased in obesity and states of insulin resistance presumably through upregulation of the natriuretic peptide-C clearance receptor (44,45). BMI and circulating ANP and BNP concentrations are inversely correlated (46). Reduced ANP availability may provide a pathophysiological link between obesity and arterial hypertension (44,46). The novel findings presented here could have therapeutic implications for both cachexia and obesity.

ACKNOWLEDGMENTS

J.J. is part of the Klinische Forschergruppe (KFG) 192. The Deutsche Forschungsgemeinschaft supported this work

through a grant to J.J. and KFG 192. A.L.B. receives a fellowship from the Deutsche Forschungsgemeinschaft.

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