An Unsuspected Metabolic Role for Atrial Natriuretic Peptides

The Control of Lipolysis, Lipid Mobilization, and Systemic Nonesterified Fatty Acids Levels in Humans

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Abstract—In normal and obese humans, lipid mobilization and systemic nonesterified fatty acid levels are thought to be acutely controlled by catecholamines (ie, epinephrine and norepinephrine) and insulin. Natriuretic peptides (NPs) are known to play a key role in the regulation of salt and water balance and blood pressure homeostasis. They are involved in the pathophysiology of hypertension and heart failure. NPs have recently been found to exert potent lipolytic effects (ie, activating the breakdown of stored triacylglycerols) in isolated human fat cells and to promote lipid mobilization in vivo. Atrial natriuretic peptide increases the intracellular 3', 5'-cyclic guanosine monophosphate (cGMP) concentration which activates cGMP-dependent protein kinase leading to perilipin and hormone-sensitive lipase phosphorylation and lipolysis. NPs promote lipid mobilization when administered intravenously. NPs are also responsible for the residual lipid-mobilizing action observed under oral β-blockade in subjects performing physical exercise. NPs are therefore novel factors which may open promising research pathways to explain the control of lipid mobilization in physiological and pathological conditions. The metabolic impact of altered production and circulation of NPs remains to be established. The potential influence of NPs on the development of lipid disorders, obesity-related cardiovascular events, and cardiac cachexia will be discussed in this review. (Arterioscler Thromb Vasc Biol. 2005;25:0-0.)

Key Words: adipose tissue ■ adipocytes ■ lipolysis ■ natriuretic peptides ■ obesity ■ lipid metabolism ■ lipid mobilization ■ insulin

Obesity is increasing worldwide. It represents an underlying risk factor for cardiovascular disease and type 2 diabetes. Risk factors that predispose to type 2 diabetes and cardiovascular disease constitute the metabolic syndrome. It is now recognized as a clinical entity by the World Health Organization and the US National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP III). The NCEP definition of the metabolic syndrome includes 3 or more of the following: abdominal obesity, defined as waist circumference ≥102 cm in men and ≥88 cm in women; elevated triglyceride concentration ≥150 mg/dL, low HDL cholesterol (<40 mg/dL in men and <50 mg/dL in women); elevated fasting plasma glucose level (≥110 mg/dL); and elevated blood pressure (≥130/85 mm Hg).1 The presence of abdominal obesity is more closely associated with the metabolic risk factors than body mass index. The prevalence of the metabolic syndrome increases with age. Obesity interferes with many metabolic pathways which underlie numerous potential risk factors. It is very difficult to differentiate between the major and minor factors, and some remain to be discovered.2 This complexity leaves the area open and challenges basic and clinical researchers to uncover novel metabolic pathways. The discovery that cardiac natriuretic peptides (NPs) control human fat cell function is a provocative observation which reveals an unsuspected link between heart and adipose tissue. NPs exert potent lipolytic effects (eg, stimulation of hydrolysis of triacylglycerols stored in adipocytes) in isolated human fat cells.3 When administered intravenously, they potently increase plasma glycerol and nonesterified fatty acid (NEFA) levels; in other words they stimulate lipid mobilization.4 Before the discovery of the NP pathway, catecholamines and insulin were considered to be the major acute regulators of lipid mobilization in humans: they both act through a cAMP-dependent regulation of lipolysis. In contrast, NPs activate a 3', 5'-cyclic guanosine monophosphate (cGMP)-dependent pathway that is completely independent from the cAMP-dependent pathway.5 The NPs may represent a promising new pathway contributing to the control of lipid mobilization in humans in physiological and pathological conditions. The introductory section...
summarizes the role of circulating NEFAs and the regulation of lipolysis and lipid mobilizing pathways. The molecular mechanisms of action of NPs and their contribution to the physiological control of lipid mobilization are reviewed. The putative impact of NPs in the development of lipid disorders in obesity-related congestive heart failure and cardiac cachexia is also discussed.

**Obesity, NEFAs, and the Pathogenesis of Type 2 Diabetes Mellitus**

NEFAs are released by lipolysis of adipose tissue (AT) triacylglycerols (TAG) stored in the adipocytes. They serve as a source of energy during fasting and conditions of stress. Through its capacity to store NEFAs, the AT makes a major contribution to the control of daily lipid flux in the body. An imbalance between NEFA storage and release, commonly observed in obese subjects, has major metabolic consequences and increases cardiovascular risk. Fastig plasma NEFA concentrations are ~20% greater in obese men and women. The adipose tissue of obese persons releases more NEFAs into the circulation, and subjects with type 2 diabetes have high NEFA concentrations. A high plasma NEFA concentration is a risk factor for deterioration of glucose tolerance independent of the other insulin resistance or insulin secretion markers that characterize subjects at risk for type 2 diabetes. Day-long elevations in plasma NEFA levels can lead to aggravation of impaired glucose homeostasis in obese and type 2 diabetes individuals. Chronically elevated plasma NEFA concentrations stimulate gluconeogenesis, cause hepatic/muscle insulin resistance, and also impair insulin secretion in genetically predisposed individuals. NEFAs have been proposed to represent a major link between obesity and insulin resistance/type 2 diabetes. Excessive influx of NEFAs into skeletal muscle and hepatocytes leads to insulin resistance and elevated liver TAG content (fatty liver), respectively. NEFAs can alter insulin action directly by interfering with different steps of the insulin signaling cascade. NEFAs interfere with skeletal muscle insulin signaling via protein-kinase C–induced phosphorylation of IRS-1 and reduction of IRS-1–mediated actions. NEFAs also modulate vascular tone and tissue blood flow. Plasma NEFA levels are increased in cardiomyopathy and may contribute to alterations in insulin action and myocardial metabolism (ie, imbalance between glucose and NEFA oxidation in the failing heart). Increases in circulating NEFAs may play a central role in mediating myocardial insulin resistance because NEFAs are a predictive risk factor for sudden death.

The pattern of fat distribution is also an important variable for whole body NEFA metabolism and insulin sensitivity. Individuals with upper body and visceral fat accumulation have a higher risk of developing hyperinsulinaemia, insulin resistance, dyslipidemia, and type 2 diabetes. It has been hypothesized that visceral AT lipolysis releases excess NEFAs into the portal vein, exposing the liver to higher NEFA concentrations. The contribution of visceral adipose tissue lipolysis to hepatic NEFA delivery increases with expanding visceral fat in humans, and this effect is greater in women than in men. However, it represents a very small percentage of total NEFAs delivered to skeletal muscles. Given the major pathogenic role attributed to NEFA dysregulation, the mechanisms influencing NEFA release by fat cells and the major pathways controlling the lipolytic system will be briefly reviewed.

**The Lipolytic Pathways and the Regulation of Lipid Mobilization in Humans**

In nonobese man, the mean turnover rate of TAG in the total fat mass is ~100 to 300g of TAG per day. AT lipolysis, ie, the catabolic process leading to the breakdown of TAG into NEFAs and glycerol, is often thought of as a well established metabolic pathway. Nevertheless it is not completely known what truly fixes the basal rate of lipolysis in AT or what the identity of all the hormonal determinants of lipid mobilization actually are. Basically, during lipolysis, intracellular TAG undergoes hydrolysis after the activation of a neutral lipase, hormone-sensitive lipase (HSL). After TAG hydrolysis, NEFAs and glycerol are transported by the bloodstream to other tissues (mainly the liver for glycerol and skeletal muscle and heart for NEFAs). However, some NEFAs generated during lipolysis are re-esterified in a futile cycle back into the adipocytes as intracellular TAG. The amount of NEFA released into the circulation is the result of a balance between TAG breakdown and resynthesis. The glycerol formed during lipolysis is not reused by fat cells because they only contain minimal amounts of the enzyme glycerol kinase under standard conditions. Glycerol is mainly used as an important substrate for glucose synthesis by the liver. However, glyceroneogenesis may occur in adipocytes in some physiological conditions and providing the glycerol-3-phosphate required for NEFA reesterification.

The mobilization of TAG stored in AT is a major contributor to the supply of NEFAs to the working muscle. Altered lipolysis might predispose to obesity and interindividual variations in AT lipolysis influence the rate of weight loss. A low activity of sympathetic nervous system (SNS) is associated with the development of obesity in rodents and humans. Defective SNS signaling at the target cell could affect the regulation of lipolysis and thermogenesis. In man, the major hormones controlling lipolysis are catecholamines and insulin. Epinephrine and norepinephrine stimulate and/or inhibit lipolysis depending on the relative importance of the adrenergic receptors (AR) involved in the initiation of the response (see recent reviews). Lipolysis and fat mobilization are exquisitely sensitive to suppression by insulin. This hormone completely suppresses NEFA release from subcutaneous fat when infused at moderate physiological concentrations. The physiological role of other agents that regulate lipolysis in vitro or when administered intravenously remains to be elucidated. Growth hormone (GH) only has permissive and long-term effects on lipolysis. Other peptide hormones such as parathyroid hormone (PTH), tumor necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6) stimulate lipolysis in isolated fat cells. Nevertheless, a number of questions remain about their contribution to the physiological regulation of lipid mobilization in humans.
Catecholamines and Fat Cell Adrenergic Receptors

The current view of regulation of human fat cell lipolysis by catecholamines and insulin and the recently discovered ANP-dependent pathway is illustrated in Figure 1. HSL regulation is under the potent control of cAMP and cAMP-dependent protein kinase (protein kinase A [PKA]). PKA phosphorylates perilipin, an important lipid droplet-associated protein essential for lipolysis regulation, and HSL which is thereby activated and hydrolyzes TAG. In the human fat cell, adenylyl cyclase activity and cAMP production are mainly under the control of β1-/β2-AR–dependent stimulation and β2-AR–mediated inhibition. Through Gs and Gi heterotrimeric GTP binding protein activation, β1-/β2- and α2-ARs control plasma membrane adenylyl cyclase activity and cAMP production in human fat cells. Although β2-adrenergic receptor agonists have been shown to exert lipolytic effects in human fat cells, the physiological role of these receptors remains controversial.34–37 A lack of β2-adrenergic action in the control of lipolysis, energy expenditure, and lipid oxidation has been found during isoproterenol infusion in humans.38 In vitro studies of human fat cells have demonstrated that the fine-tuning of cAMP levels and lipolysis by catecholamines is mainly dependent on the balanced cross-talk between β1-/β2- and α2-AR–dependent pathways.

The adrenergic control of human fat cell lipolysis is complex because of the heterogeneity of α2/β1-2AR distribution in various fat deposits.21 A number of in vitro studies have clearly established that the repertoire and the expression level of human adipocyte adrenergic receptors largely differ according to the anatomic location and the extent of AT depots, the size of the adipocytes, the sex and age of the subjects, and genetic determinants. Moreover, lipolytic resistance to catecholamines has been shown in human subcutaneous AT, the major fat depot in obese subjects. Conversely, fat cells from visceral deposits exhibit the highest...
β-adrenergic responsiveness. Blunted adrenaline-induced lipolysis has been reported in the AT of obese subjects. Differences in the lipolytic response to catecholamines in obese compared with lean subjects are associated with variations in HSL expression and the functional balance between β- and α2-ARs. Other perturbations in catecholamine signal transduction pathways might also explain lipolytic defects (see reviews).

Insulin: a Major Antilipolytic Agent

Insulin plays an important role in the control of NEFA release. Insulin is a major regulator of lipolysis. It inhibits lipolysis and NEFA efflux and stimulates glucose uptake by the fat cell and fat storage (ie, increases the rate of resynthesis of TAG from NEFA; the re-esterification effect). The supply of NEFAs from AT to other tissues is rapidly and strongly inhibited by an elevation of the plasma insulin concentration. The cellular mechanisms involved in the inhibition of lipolysis by insulin have been delineated.

In the postprandial situation or when insulin is infused intravenously using the euglycemic hyperinsulinemic clamp technique, lipolysis is rapidly and strikingly suppressed. Reduction of plasma insulin levels, either during fasting, physical exercise, or even after acute somatostatin administration, leads to a dramatic increase in the lipolytic rate. A number of circulating factors (such as TNF-α, ILs, insulin itself, NEFAs, and glycation products) have been shown to influence insulin action at the target cell level and could lead to hyperglycemia and type 2 diabetes when their action is altered.

It seems reasonable to propose that the well-known upper-body obesity-related metabolic disturbances may be linked to regional variations in lipolysis regulation and NEFA production by insulin. It is clear that moderate changes in fasting insulin levels or insulin sensitivity will alter fat cell lipolysis and fasting plasma NEFA concentrations. Striking adipose-depot–related differences, modulated by obesity, have been found in fat cell responsiveness to insulin. Insulin-induced suppression of lipolysis and activation of NEFA re-esterification are blunted in omental compared with subcutaneous fat cells. Various functional differences have been identified at the insulin receptor level and the postreceptor level of the insulin signaling cascade. Other partners of the insulin signaling cascade such as PDE-3B and protein-tyrosine phosphatases (PTPase) involved in the dephosphorylation of the insulin receptor could also contribute to the modulation of insulin action. Endogenous PTPase activity, including PTPase-1B, is increased in omental adipose tissue and may contribute to the relative insulin resistance of this fat depot.

Increases in baseline systemic NEFA flux have been reported in upper body obese women. They have partly been attributed to a decreased sensitivity to the antilipolytic effect of insulin, independent of fat cell size, and to increased lipolytic rates associated with subcutaneous fat cell hypertrophy. Subcutaneous abdominal adipocytes are more resistant to the antilipolytic effect of insulin than gluteal adipocytes, independently of cell size. In vivo results have confirmed the regional heterogeneity of insulin-regulated NEFA release in vivo. Visceral adipose tissue is more resistant to the antilipolytic effects of insulin than is leg and nonsplanchnic body fat. Nevertheless, visceral fat may be a marker for, but not the source of, excess postprandial NEFAs in obesity because the increased postprandial NEFA release observed in upper body obese women and type 2 diabetics originates from the nonsplanchnic upper body fat, not visceral fat.

Natriuretic Peptides Contribute to the Control of Lipolysis and Lipid Mobilization in Humans

Natriuretic Peptides

The first member of the NP family, atrial natriuretic peptide (ANP) was discovered when de Bold et al showed, for the first time, that infusion of atrial tissue extracts in rats strongly stimulated natriuresis and diuresis. Isolation and purification procedures led to the identification of ANP in heart atria. Ten years later, a second compound of the family was isolated from porcine brain and was called brain natriuretic peptide (BNP). In fact, it is also a cardiac hormone mainly secreted from the cardiac ventricles. A third natriuretic peptide, C-type NP is expressed primarily in the central nervous system as well as endothelial cells and chondrocytes. It is not found in cardiac tissue or blood and it is not stored in granules. ANP is synthesized by the heart atrium and released in response to atrial stretch. BNP is synthesized primarily by ventricle cardiomyocytes in response to increased ventricular volume expansion and pressure overload, and its circulating concentrations are significantly elevated in severe congestive heart failure. Both NPs function as true circulating counter-regulatory hormones whereas CNP is thought to act locally in a paracrine way. The structure and chromosome location of the genes in humans, the size of mRNA transcripts, and biosynthetic pathways leading to the production of the active peptides are quite well characterized. ANP and BNP regulate a variety of physiological events; they have natriuretic, vasodilating, and lusitropic properties. They also influence SNS activity and the renin–angiotensin system. ANP and BNP are apparently antagonists to vasopressin, endothelins, and the renin–vasopressin–aldosterone system. Most of their effects are mediated by the stimulation of cGMP production in target cells. Recent studies have also focused on a role in the control of myocardial function and remodeling. In vitro studies suggest a role for BNP as a regulator of myocardial structure via control of cardiac fibroblast function and cardiomyocyte hypertrophy. The role of CNP in vivo is less well defined. Although CNP might not be a modulator of diuresis and natriuresis, it is a vasodilator expressed by endothelial cells. CNP induces vasodilatation by stimulation of natriuretic peptide receptor-B, but also by activation of Gi-coupled natriuretic peptide receptor-C receptors. In pharmacological experiments, the potency of CNP for vasodilation or reduction of blood pressure is much lower as compared with
Lipolytic Effect of Natriuretic Peptides

The original finding that was the impetus for subsequent studies on the metabolic role of NPs was the discovery of the lipolytic action of NPs in human isolated fat cells. NPs exert potent lipolytic effects similar to those induced by the β-AR agonist, isoproterenol. The relative order of lipolytic potency of the peptides (ANP>BNP>CNP) suggested the presence of a NPR-A receptor in human fat cells. This point was confirmed by the binding studies performed on human fat cell membranes using [125I]ANP as a radioligand and various peptide competitors.

NPs promoted a strong and sustained increase in intracellular cGMP in human fat cells without any change in cAMP. This effect is unrelated to inhibition of the cGMP-inhibitable phosphodiesterase PDE-3B of the adipocytes. In fact, although PDE3–3B catalytic sites have a similar high affinity for cAMP and cGMP, the $V_{max}$ for cAMP is much higher (4 to 10×) than for cGMP. ANP-induced lipolysis is associated with an increase in the serine phosphorylation of HSL in mature human adipocytes as well as in adipocyte precursors differentiated into adipocytes. The signal transduction pathway stimulated by ANP to promote lipolysis in human fat cells is strictly connected to an increase in intracellular cGMP concentrations. The nonhydrolysable analogue of cGMP, 8-bromo-cGMP, mimicked the lipolytic effects of ANP. It was verified that ANP does not stimulate PKA activity and that inhibition of PKA by the PKA inhibitor H-89 does not affect ANP-induced lipolysis. ANP-mediated lipolysis does not involve cross-talk between cGMP and PKA. It is PKG (ie, cGK-I was the unique form identified in human fat cells) that promotes perilipin and HSL phosphorylation and that underlies ANP-induced lipolysis. This was confirmed using the cGMP analogue inhibitor of cGK-I, 8-pCPT-cGMPS, which inhibited both HSL phosphorylation/activation and lipolysis. This finding in isolated human fat cells confirms early data from a rat cell-free system where the phosphorylation of HSL by PKG was reported. Recently, studies have shown a role for the MAP kinases in the control of lipolysis by TNF-α in human fat cells. ANP does not modulate the phosphorylation of extracellular signal regulated kinase (ERK)-1/2 and p38 MAP kinase. These kinases are not involved in the ANP-mediated HSL phosphorylation because MAP kinase inhibitors did not affect the ANP-induced HSL phosphorylation.

Importantly, it was observed that the lipolytic effect of NPs is completely independent from the major antilipolytic hormone, insulin. Insulin is well known for its antilipolytic effects in human fat cells leading to inhibition of β-AR agonist- (ie, isoproterenol) and catecholamine-induced lipolysis. Insulin treatment of human fat cells has no effect on ANP-induced lipolytic response. Because the antilipolytic effects of insulin are mediated by the reduction of intracellular cAMP levels initiated by PDE-3B activation, it is understandable why this hormone does not interfere with cGMP-dependent NP effects. Delineation of mechanisms involved in the downregulation of NP action merits attention. Chronic stimulation with NPs and pathological conditions associated with overproduction of NPs could limit fat cell responsiveness by desensitization of NPR-A activity. Homol-
ogous desensitization of the ANP-dependent pathway, subse-
quently to prior exposure of the adipose cells to ANP, has been shown in isolated fat cells in vitro.87

Occurrence of NP-induced lipolysis is specific to primate fat cells. NPs do not stimulate lipolysis in fat cells of other species including rats, mice, rabbit, and dogs. ANP increased basal cGMP-production 300-fold in human fat cells, whereas it was only stimulated 3-fold in rat adipocytes. One of the major explanations of such striking species-related differences in fat cell responsiveness to NPs is that adipocytes from species nonresponsive to NPs present express mainly plasma membrane clearance NPR-C and a very low expression of the biologically active NPR-A. Stimulation of the small NPR-A population on these cells is not sufficient to promote the increment in cGMP levels required to reach the set-point for HSL activation.88 The fact that only primates possess this new lipolytic pathway is noteworthy. However, the lack of animal models will not facilitate future studies on the role and the regulation of this system.

Induction of Lipid Mobilization by Local or Systemic Administration of ANP

The lipolytic action demonstrated in isolated human subcu-
taneous fat cells in vitro was confirmed in vivo with the administration of ANP in a microdialysis probe implanted in human subcutaneous abdominal AT (SCAAT). In situ microdialysis offers several opportunities for clinical investigation of vascular and metabolic effects initiated in subcutaneous AT by local or systemic administration of drugs or exercise.89 ANP infusion in the microdialysis probe raised the extracellular concentration of glycerol in AT and vasodilatated the vessels draining the fat depot.90 Both events contribute to the coordinated enhancement of lipid mobilization in SCAAT.

Earlier studies in normal humans showed that administration of pharmacological doses of human-ANP (h-ANP) af-
fected sodium intake, modulated insulin secretion and/or metabolism, and elicited a possibly baroreflex-mediated sympathetic activation and lipolysis.91 At that time, a direct effect of ANP on fat cells was not considered. We studied the lipid-mobilizing effect of an intravenous infusion of h-ANP as well as various metabolic and cardiovascular parameters, in normal weight and young obese subjects. Microdialysis probes were inserted into SCAAT to measure modifications of the extra cellular glycerol concentrations occurring during intravenous h-ANP administration. Spectral analysis of blood pressure and heart rate oscillations, recorded using digital photoplethysmography, were used to assess changes in autonomic nervous system activity. h-ANP (50 ng/kg/min) in-
fused intravenously for 60 minutes induced a marked increase in plasma glycerol and NEFA and a weak increase in insulin plasma levels in lean and obese men. Plasma norepinephrine concentrations rose weakly and to the same extent during h-ANP infusion in lean and obese men. The effects of h-ANP infusion on the autonomic nervous system were similar in both groups, with an increase in the spectral energy of the low-frequency band of systolic blood pressure variability and a decrease in the spectral energy of the high-frequency band of the heart rate. In SCAAT, intravenous infusion of h-ANP increased extracellular glycerol concentration and increased blood flow similarly in both groups. The increase in extracellular glycerol observed during h-ANP infusion was not modified when propranolol was added to the microdialysis probe perfusate to prevent fat cell β-AR activation. These in vivo studies showed that ANP is a potent lipid mobilizing hormone which acts independently of the activation of the sympathetic nervous system. Apparently obesity did not modify the lipid-mobilizing effect of ANP in young obese subjects.4 In these studies, plasma ANP concentrations sub-
stantially exceeded the levels that are encountered clinically. Before arriving at a final answer concerning the role of the ANP lipid-mobilizing pathway in obese subjects, it is neces-
sary to expand this kind of approach to a larger number of obese patients and to pay special attention to the anatomic distribution of AT, sex, age, and the duration of the obese state (and the existence or not of metabolic disorders related to the obese state). A recent study where h-ANP was infused at rates of 6.25, 12.5, and 25 ng/kg/min has shown that ANP briskly stimulates lipid mobilization and oxidation at plasma concentrations that are encountered in conditions such as heart failure.92 Confirming the in vitro studies, desensitization of ANP action also occurred in vivo after ANP infusion in a microdialysis probe.93 Desensitization of ANP-dependent responses and mechanisms has been previously reported in other cell types. The phenomenon could be attributed to a decrease in the intrinsic GC activity of NPR-A or to downregulation of plasma membrane NPR-A.94,95 Further investigations need to be designed to clarify the mechanisms of the homologous desensitization and possible heterologous desen-
sitization affecting the NP pathway in fat cells.

Contribution of ANP to Physiological Control of Lipid Mobilization in Humans

In humans, acute exercise-induced lipid mobilization is con-
sidered to mainly depend on SNS activation and the action of catecholamines on fat cells and local blood flow in AT. Catecholamine-dependent inhibition of insulin release also contributes to the lipid mobilizing effect during exercise. Considering the results showing that ANP/BNP exert lipoly-
tic and lipid-mobilizing effects in humans, a putative physi-
ological contribution of ANP/BNP in exercise-induced lipid mobilization was hypothesized. Strenuous endurance exercise is followed by increases in plasma BNP. During exercise, the SNS is activated and, concomitantly, ANP/BNP are released from the exercising heart.67,69,94 Exercise-induced lipid mobilization challenges were performed using in situ microdialysis in SCAAT in healthy young men during 2 successive exercise bouts performed at 35% and 60% V02max after placebo, local, or oral nonselective β-antagonist (tertatolol) treatment. In placebo-treated subjects, as expected, exercise raised extracellular glycerol concentration (EGC). This in-
crease was only partially suppressed by a local administration of a β-antagonist at a concentration known to promote complete β-AR blockade. The infusion of propranolol (non-
selective β-antagonist) in the microdialysis probe only par-
tially reduced the increase in EGC promoted by exercise (Figure 2A). Such a result suggests the potential contribution of another lipid-mobilizing pathway independent of catechol-
amines. Indeed, oral β-AR blockade (tertatolol given 1 hour
before the beginning of exercise) did not prevent exercise-induced lipid mobilization in SCAAT (Figure 2B). Because blockade of fat cell β-ARs was verified, the existence of another partner and the possible contribution of ANP was strengthened. The exercise-induced increase in plasma ANP was potently amplified by oral tertatolol administration (Figure 3A). A positive correlation was found between EGC and plasma ANP levels but also between extracellular cGMP levels and EGC in SCAAT (Figure 3B).95 Exercise-induced lipid mobilization, resistant to local propranolol, is partly related to the action of ANP. Oral β-AR blockade promotes strong exercise-related ANP release by heart, which explains the lipid mobilization in SCAAT. The physiological contribution of ANP, concomitantly with SNS, to the control of lipid mobilization is supported by these results. Other than ANP, it is impossible to propose another reliable candidate in the context of the biological factors released during exercise and that is known to exert lipolytic effects in human. Moderate exercise had no action96 or slightly increased the PTH concentration in blood.97 GH is known to be secreted during the type of exercise for which ANP action was proposed.95,98 However, GH is a less potent lipolytic agent than catecholamines or ANP; moreover, the time-course of appearance of the lipid mobilizing effect of GH appears at least 2 hours after intravenous injection of GH.99 Thus, involvement of GH-related lipolytic effects, if any, cannot account for the acute and short-term lipid mobilization promoted by exercise. High concentrations of IL-6 appear to be necessary to stimulate lipolysis, and a lipolytic effect of this agent is only seen after several hours.100 Glucagon and corticotropin (ACTH), which are known to stimulate lipolysis in rodent fat cells, are not active in human fat cells. Other putative agents, released by exercise, such as prostaglandins, adenosine, and neuropeptide Y,101 are known to exert antilipolytic effects on human fat cells.

**Figure 2.** Comparison of the mean changes in extracellular glycerol concentrations in abdominal subcutaneous adipose tissue during 2 successive exercise bouts performed at 35% (exercise 1) and 60% (exercise 2) of VO₂max and during recovery. A, Effect of local β-adrenergic receptor blockade: The control microdialysis probe was perfused with Krebs-Ringer buffer. For the study of local β-adrenergic receptor blockade, a microdialysis probe was supplemented with propranolol (100 μmol/L). Propranolol partially blocked exercise-induced EGC increment. β-adrenergic responsiveness of fat cells was fully blocked.95 B, Effect of oral β-adrenergic receptor blockade with tertatolol: Control (placebo) microdialysis probe was perfused with Krebs-Ringer buffer. Tertatolol (5 mg) was given 1 hour before exercise. Tertatolol treatment has no effect on exercise-induced EGC increment. It was verified that β-adrenergic responsiveness of the adipocytes was fully blocked.95

**Figure 3.** Comparison of the mean changes in plasma ANP and cGMP levels as well as extracellular glycerol (EGC) and cGMP concentration during 2 successive exercise bouts performed at 35% (exercise 1) and 60% (exercise 2) of VO₂max in placebo and tertatolol (β-adrenergic receptor antagonist)-treated patients.95 A, Correlation between plasma ANP concentrations and the increase in the concentration of extracellular glycerol in the probes perfused with propranolol in placebo- and tertatolol-treated subjects. ANP and EGC values were determined at the 10th and the 20th minute of each exercise bout. B, Correlation between EGC and extracellular cGMP concentrations. Plasma cGMP, EGC, and extracellular cGMP concentrations were determined at rest and during each exercise bout.
Physiological Questions and Future Trends

Before adding a new lipolytic agent to the list of proven lipid mobilizing hormones, it is necessary to determine whether the circulating or local (ie, at the adipocyte level) concentration of the lipolytic agent is able to initiate lipid mobilization in vivo during a lipid-mobilizing challenge. ANP results fit with this rule although the lack of a suitable NPR-A antagonist limits the interpretation. Investigations into the role of ANP using microdialysis were performed in SCAAT for practical reasons related to the use of the microdialysis technique. The action of ANP on other fat deposits must be expanded in the near future to verify whether fat cell sensitivity to ANP/BNP differs according to the anatomic location of the fat deposit as reported for other regulatory pathways.21

The administration of h-ANP has clearly demonstrated the lipid-mobilizing effect of the hormone and its ability to reach fat cells when administered intravenously.4,91 The human fat cell possesses all the elements for NP action and regulation. NPR-A and NPR-C are expressed in human fat cells. Moreover, NEP, the enzyme which is involved in NP degradation, has also been identified in human fat cell membranes (Moro et al, unpublished data; Figure 4). Modulation of its activity could interfere with the effects of NPs in fat cells. The putative relevance of circulating NPs in physiological conditions remains to be settled. The physiological relevance of this pathway was revealed in young subjects performing physical exercise.95 During exercise, in addition to catecholamines, ANP plays a noticeable role in the control of lipid mobilization in SCAAT. Elevated plasma levels of ANP were found during prolonged exercise (eg, long distance running) and exaggerated release of ANP has been reported in older individuals.102 One of the positive and specific effects of ANP during prolonged exercise might be to sustain lipid mobilization from AT. ANP may contribute, by elevating circulating NEFA levels, to the huge energy demand during long distance running. An enhancement of the lipid-mobilizing potencies of NPs has been reported in obese women submitted to a 28-day low calorie diet associated with significant weight loss. It is possible to speculate that during periods of low calorie diet, a reduction in NPR-C receptor density occurs. According to the classical paradigm, clearance of ANP through uptake by NPR-C could be reduced while interactions with NPR-A would be facilitated to enhance ANP-related effects (Figure 4). Variation in the promoter region of the Npr3 gene (encoding for NPR-C receptor) was associated with lower plasma ANP and higher blood pressure in hypertensive obese patients who have a reduced NPR-A-to-NPR-C expression ratio (based on mRNA determinations) in adipose tissue.103 An epidemiological study has recently shown that homozygosity for the A(−55) allelic variant of the Npr3 gene is associated with lower BMI. Male subjects carrying the A(−55)A NPR-C genotype had a significantly lower prevalence of overweight, obesity, and abdominal obesity. They also had a lower 20-year rate of overweightness compared with CC individuals.104 It will be of interest to expand this study with functional investigations for the determination of ANP/BNP release and NPR-A and NPR-C receptor expression and function in fat cells. Preliminary studies in young obese subjects have not revealed major differences between lean and obese responses to ANP administration.4 However, experiments should be continued to evaluate the impact of the NPs in older obese subjects of both sexes with greater attention to AT distribution and metabolic disorders related to the obese state.

An epidemiological study on the Framingham Heart Study offspring cohort has shown that plasma ANP and BNP levels are reduced in obese subjects.105 In addition, reduced BNP levels occur in the obese individuals with heart failure.106 Occurrence of an altered distribution of fat cell NPR-A/C distribution and altered NP responsiveness has been proposed in the obese.107,108 These epidemiological studies must be followed by functional explorations to delineate the mechanisms leading to altered NP pathways.
Pathological Questions and Future Trends

**β-AR Antagonist Treatment**

The importance of the ANP-dependent lipid mobilizing pathway is observed when subjects are submitted to treatment with a β-AR antagonist which, as expected, blocked β-adrenergic effects at the fat cell level and inhibited lipolysis. Administration of a β-AR antagonist along with exercise is a prerequisite to promote enhanced ANP release. Subjects receiving β-AR antagonist medication (ie, metoprolol, bisoprolol, atenolol, propranolol, and sotalol) are characterized by substantially elevated ANP, BNP, and cGMP plasma concentrations. Through ANP release, a substantial amount of NEFAs is released by adipose tissue while β-ARs are blocked by a β-blocker. This is the situation for millions of people taking β-AR antagonist treatment for hypertension and coronary artery diseases. The lipid mobilizing activity remaining in AT when performing exercise, in the subjects treated with a β-AR antagonist, is essentially attributable to the ANP-dependent pathway. The efficiency of this ANP-dependent lipid-mobilizing loop could be of interest in β-blocker-treated patients to maintain some lipid-mobilizing capacities and to avoid accumulation of fat. Lipid mobilization was followed by an increased whole body lipid oxidation and reduced carbohydrate oxidation at plasma ANP concentrations encountered in such patients. Regular exercise will be particularly beneficial in such patients to limit the deleterious action of fat cell β-AR blockade on fat accumulation. Possible relationships between ANP/BNP increase and alterations of plasma NEFA levels have never been considered in detail in patients with cardiac dysfunction (and treated or not by β-blockers). In subjects with congestive heart failure and/or ventricle hypertrophy, plasma levels of NPs (especially BNP) are very high. Type 2 Diabetes

Most patients with type 2 diabetes are overweight or obese. They experience day-long elevations in plasma NEFA concentrations escape to the normal suppression by meals or glucose load. Because elevated circulating NEFAs can cause/aggravate insulin resistance in muscle and liver and can increase the risk for sudden death, it is important to know whether NPs, in addition to insulin resistance, operate as partners in disorders related to the metabolic syndrome. Controversies exist concerning plasma levels of ANP/BNP in diabetic patients. Congestive Heart Failure and Cardiac Cachexia

Weight loss in heart failure is associated with a poor prognosis. Heart failure is associated with a number of metabolic and neurohormonal dysfunctions. It is unknown whether NPs contribute to this disease. Congestive heart failure was associated with a higher prevalence of type 2 diabetes or was a risk factor for its development. Elevated NEFA concentrations were suspected to play a pivotal role. It will be interesting to see whether such high levels of NPs are associated with increased plasma NEFA or TAG levels and modifications of lipid metabolism (mobilization/oxidation). Moreover, the lipolytic responsiveness of fat cells to NPs in such patients is unknown. Severe heart failure is a ketone-prone state, and NEFA concentrations and lipid oxidation are elevated in congestive heart failure. One likely mechanism proposed is increased stress hormone–related lipolysis. In addition to the putative hormonal factors previously proposed (ie, norepinephrine, IL-6, and TNF-α), increased endogenous ANP/BNP concentrations in congestive heart failure may promote lipid mobilization, enhance lipid oxidation, and contribute to cardiac cachexia. Cachexia also appears in the presence of cachexia-inducing tumors which produce a lipid-mobilizing factor that causes immediate release of glycerol by murine adipocytes. This is an ANP-independent phenomenon; induction of lipolysis by the lipid-mobilizing factor was associated with an increase in intracellular cAMP levels.

Homologous desensitization of ANP-dependent effects on lipolysis and lipid mobilization occurs consecutively to acute exposure to ANP. Considering the excess release of NPs observed in congestive heart failure, the body may become acclimated to increased levels of endogenous BNP through downregulation of NP signaling pathways in adipose tissue. Moreover, an upregulation of the clearing pathways (ie, increased clearance NPR-C expression and/or enhanced activity of NEP) cannot be excluded.

**Pharmacological Treatments**

Medications that interfere with the natriuretic peptidergic system (ie, NPR-A agonists and NEP 24.11 inhibitors) could cause metabolic changes. BNP (Nesiritide) has now been approved for treating acute heart failure because it has beneficial effects on central hemodynamics and urinary excretion of Na⁺. Compared with ANP, BNP seems to be less susceptible to degradation by NEP 24.11 and could be a more potent natriuretic agent than ANP. It will be essential to verify whether infusion of BNP promotes, like intravenous administration of ANP, a potent and sustained lipid mobilizing effect and increases plasma NEFA levels which could alter heart function and counteract some beneficial actions of the compound at other tissues. This is still an important and open question, and therefore all the drugs known to activate NPR-A/B-dependent pathways should be evaluated for their metabolic side effects.

**Conclusions**

To conclude, there are only 2 hormone systems, namely catecholamines and ANP, which have acute stimulatory effects on lipolysis and fat mobilization in humans. GH has only permissive and chronic effects. Adipose tissue (eg, fat cells and the vascular bed of adipose tissue) is a new and unsuspected target organ of NPs. The NP pathway is mainly operative in primate fat cells. Induction of lipolysis and lipid mobilization must be now included in the numerous physiological actions of NPs. Other NP/cGMP-dependent regulatory mechanisms will probably be discovered in fat cells in the near future. ANP, like β-AR agonists, has been recently shown to reduce leptin production in human fat cells. Further fundamental and clinical studies will be required to answer the numerous questions raised by the discovery of the metabolic effects of NPs. The effect of β-AR antagonist treatment on NP release merits further study. Moreover, it
must be determined whether, in addition to the well-known SNS activity alterations, a chronic and sustained increase in NP production influences cachexia in patients with congestive heart failure or other diseases related to altered NP production. Finally, all the drugs known to modulate NP-dependent pathways should be evaluated for their putative metabolic side effects when given for management of cardiovascular disease.

References


