Natriuretic peptides and cGMP signaling control of energy homeostasis

Cedric Moro\textsuperscript{1,2} and Max Lafontan\textsuperscript{1,2}

\textsuperscript{1}Institut National de la Santé et de la Recherche Médicale/UPS UMR 1048-I2MC-Institute of Metabolic and Cardiovascular Diseases, Toulouse France; and \textsuperscript{2}Paul Sabatier University-Toulouse 3, Toulouse, France

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Moro C, Lafontan M. Natriuretic peptides and cGMP signaling control of energy homeostasis. Am J Physiol Heart Circ Physiol 304: H358–H368, 2013. First published November 30, 2012; doi:10.1152/ajpheart.00704.2012.—Since the discovery of natriuretic peptides (NPs) by de Bold et al. in 1981, the cardiovascular community has been well aware that they exert potent effects on vessels, heart remodeling, kidney function, and the regulation of sodium and water balance. Who would have thought that NPs are also able to exert metabolic effects and contribute to an original cross talk between heart, adipose tissues, and skeletal muscle? The attention on the metabolic role of NPs was awakened in the year 2000 with the discovery that NPs exert potent lipolytic effects mediated by the NP receptor type A/cGMP pathway in human fat cells and that they contribute to lipid mobilization in vivo. In this review, we will discuss the biological effects of NPs on the main tissues involved in the regulation of energy metabolism (i.e., white and brown adipose tissues, skeletal muscle, liver, and pancreas). These recent results on NPs are opening a new chapter into the physiological properties and therapeutic usefulness of this family of hormones.

lipolysis; obesity; fat oxidation; skeletal muscle; type 2 diabetes

Natriuretic Peptides: Emerging Role in the Regulation of Energy Metabolism

Natriuretic peptides (NPs) are a family of structurally similar endogenous peptide hormones that are mainly secreted by cardiomyocytes in response to a stress on the cardiac wall [see recent reviews (67, 70)] (Fig. 1). The metabolic roles of atrial NP (ANP) and B-type NP (BNP) were unsuspected until the discovery in 2000 that they exert potent lipolytic effects in human fat cells (78) and increase plasma nonesterified fatty acids (NEFAs) levels when infused intravenously (27). Thus NPs display a far broader biological spectrum of action than the sole regulation of blood pressure and volume. Sex-related differences in NP production have been reported; sex hormones and sex hormone-binding globulin have been considered to play an important role in the regulation of NPs as extensively discussed by Clerico et al. (19, 21). Briefly, estrogens display a stimulating effect on the NP system. By contrast, several lines of evidence suggest that androgens may suppress the NP system, accounting for the lower NP levels in men compared with women. Further studies are warranted to elucidate how sex hormone-related modulation of the NP system may contribute to sex-related metabolic responses and cardiovascular risk. In addition, a number of recent results have highlighted putative relationships between plasma NP levels, obesity, and the risk of diabetes. Several epidemiological studies have revealed that plasma NP levels are suppressed in subjects with obesity, metabolic syndrome, and type 2 diabetes (6, 22, 86, 91, 92). Circulating plasma NP levels are negatively related to obesity and predict the development of type 2 diabetes (40, 74). Reduced BNP levels are also observed in obese heart failure patients compared with their nonobese counterparts (43). Along these lines, a genetic polymorphism in the BNP gene promoter region is associated with increased circulating BNP levels and lower risk of type 2 diabetes (44). Recent data show a protective role of NPs and the cGMP-dependent protein kinase (cGK-I) against high-fat diet-induced obesity and glucose intolerance in mice (46). There are likely multiple mechanisms underlying the protective role of NPs against obesity and type 2 diabetes (Table 1). This could involve a combination of reduced body weight concomitant to increased energy expenditure, enhanced insulin secretion, and improved peripheral insulin action. We will here review the role of NP/cGMP signaling in several important metabolic organs such as white and brown fat, skeletal muscle, pancreas, and liver. We will mainly focus on human studies, though diversion toward interspecies differences will be evoked when required.

Regulation of Fat Cell Metabolism

The lipolytic effect of ANP involves binding to the NP receptor type A (NPR-A) and activation of cGMP production and lipases (78). Other peptides such as the brain NP, dendroaspis NP (DNP), exhibit potent lipolytic effects in human fat cells (51). Plant signaling molecules (i.e., plant NPs) secreted into the apoplast particularly under conditions of biotic...
and abiotic stress (93) also exert lipolytic effects (M. Lafontan, unpublished results). Stimulation of human fat cells with ANP, BNP, and DNP promotes a rapid and sustained increase in intracellular cGMP levels followed by a rapid activation of cGK-I (51, 78).

The signaling pathway activated by ANP only relies on intracellular cGMP kinetics, and ANP-induced lipolysis operates through the serine phosphorylation of hormone-sensitive lipase (HSL) and perilipin 1 by cGK-I (79) (Fig. 2). The ANP-mediated lipolytic response and NPR-A expression are increased in large versus small adipocytes located within the same fat depot (96). NP clearance systems are also expressed in human fat cells. The neutral endopeptidase neprilysin (NEP 24.11) and the NP receptor type C (NPR-C), involved in NPs degradation, have been identified in human fat cell membranes. The phosphodiesterase type 5 (PDE-5), which plays a specific role in hydrolyzing cGMP, has also been identified in adipocytes (4, 52). Although functional PDE-5A and NEP activities are present in adipocytes, these enzymes do not seem to play a major role in the regulation of ANP-mediated lipolysis in mature human subcutaneous adipocytes from healthy donors. The highest levels of PDE-5A protein were detected in preadipocytes, and these levels decreased with adipocyte maturation (52). A similar pattern of differentiation-dependent PDE-5A transcripts was observed in human visceral adipocytes (4). Insulin, the main antilipolytic hormone, counteracts the lipolytic effect of catecholamines. Both hormones act in the opposite direction to the cAMP-PKA pathway. Conversely, treatment of human fat cells with insulin has no effect on ANP-induced lipolysis. ANP-induced lipolysis is completely unaffected by an acute treatment of fat cells with insulin (51, 78), a result that was confirmed in vivo (58). The ANP-dependent lipolytic pathway undergoes desensitization in vitro in isolated adipocytes and in situ after acute infusion of ANP.

Table 1. Summary of the main metabolic effects of natriuretic peptides and cGMP

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Biological Effect</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>White fat</td>
<td>Lipolysis</td>
<td>(49, 78)</td>
</tr>
<tr>
<td></td>
<td>Adiponecin secretion</td>
<td>(9, 84)</td>
</tr>
<tr>
<td></td>
<td>Leptin secretion</td>
<td>(26, 53)</td>
</tr>
<tr>
<td></td>
<td>IL-6, TNF-α, MCP1</td>
<td>(53)</td>
</tr>
<tr>
<td></td>
<td>UCP1, PGC-1α</td>
<td>(13)</td>
</tr>
<tr>
<td>Brown fat</td>
<td>UCP1, PGC-1α</td>
<td>(13)</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>PGC-1α</td>
<td>(45, 46, 63)</td>
</tr>
<tr>
<td>Liver</td>
<td>Oxidative capacity</td>
<td>(25)</td>
</tr>
<tr>
<td></td>
<td>Inflammation</td>
<td>(34)</td>
</tr>
<tr>
<td>Pancreas</td>
<td>Insulin secretion</td>
<td>(12, 72)</td>
</tr>
<tr>
<td></td>
<td>β-Cell mass</td>
<td>(72)</td>
</tr>
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MCP1, monocyte chemoattractant protein 1; UCP1, uncoupling protein 1; PGC-1α, peroxisome proliferator-activated receptor-γ coactivator-1α.
NP-induced lipid mobilization in humans

Intravenous infusion of human ANP acutely increases plasma concentrations of glycerol and NEFAs (27). It was demonstrated that infused ANP acted independently of an activation of the sympathetic nervous system. When ANP was infused at doses that raised its plasma concentrations to levels similar to those found in advanced heart failure, a potent lipid mobilization was observed (10). ANP rapidly induced both the mobilization of lipids and their oxidation (10, 11). When directly infused into human subcutaneous abdominal adipose tissue (scAT), ANP increased extracellular glycerol concentration (i.e., lipolysis) and enhanced adipose tissue blood flow (78), contributing to an increase in lipid mobilization. Interestingly, we observed a large decrease in ANP-mediated lipolysis in vivo in the scAT of young overweight/obese (mean age, 26.0 ± 1.4yr) subjects compared with lean matched-controls (mean age, 22.3 ± 1.5yr) (C. Moro and M. Lafontan, unpublished data). Furthermore, endurance training has been shown to improve lipid mobilization and adipose tissue blood flow induced by ANP infusion into the scAT of overweight subjects (57) and in women with polycystic ovary syndrome (54).

After the discovery of the lipid mobilizing effects of intravenous infusions of ANP (10, 27), a putative implication of NPs in the physiological control of lipid mobilization was considered. The fact that NP-dependent lipolytic pathways occur only in primates considerably limits the opportunities for preclinical studies (80). Until recently, exercise-induced lipid...
mobilization in humans was widely accepted to rely mainly on catecholamine and insulin action (29). Nonetheless, ANP and BNP are released from the heart during exercise (35, 60) concomitantly with sympathetic nervous system activation, an increase in plasma concentrations of epinephrine, and a decrease in plasma insulin levels. A noticeable level of lipid mobilization has been shown to persist under β-adrenergic receptor blockade because of enhanced exercise-induced NPs release (49). A greater contribution of ANP in exercise-induced lipid mobilization was found in women compared with men (56). Outside exercise-based protocols, ANP is also involved in lipid mobilization induced by head-down bed rest, a condition known to increase plasma levels of ANP (55). However, the lack of a selective NPR-A antagonist for clinical studies does not facilitate investigations to determine the relative contribution of the various lipid mobilizing pathways to exercise-induced lipid mobilization. Efficient antagonists are expected for further studies. To conclude, the increase in circulating ANP and BNP during exercise contributes, concomitantly with catecholamines, to energy supply (i.e., activation of lipolysis and increment of circulating plasma NEFA levels) (Fig. 4).

Control of Cytokine and Adipokine Production in Adipose Tissue

In addition to their impact on lipolysis, NPs are able, via the cGMP/cGK-I pathway, to modulate the secretion of adipokines and cytokines by AT. Increased expression of several cytokines has been observed in the AT of obese individuals. Plasma levels of numerous adipokines (leptin and retinol binding protein-4) and proinflammatory cytokines such as interleukin-6 and -8 (IL-6, IL-8), TNF-α, and monocyte chemoattractant protein (MCP-1) are increased during obesity. Macrophages infiltrating the AT of overweight and obese individuals are a noticeable source of inflammatory cytokines. The increased secretion of adipokines and cytokines has been suggested to play a role in the etiology of the chronic state of low-grade inflammation and insulin resistance associated with obesity (14).

Incubation of isolated human adipocytes with ANP inhibits leptin release (26). Studies based on the use of protein arrays to measure the secretion of adipokines and cytokines after a 24-h culture period of scAT explants have expanded the discovery of the effects of ANP. ANP modulates both the secretion of several adipokines (derived from adipocytes) and also that of cytokines (derived from resident AT macrophages) (53). ANP was previously known to inhibit proinflammatory systems in blood-derived macrophages (23, 34). Using adipose tissue explants, we have shown that ANP inhibits the secretion of TNF-α, IL-6, MCP-1, MCP-2, macrophage inflammatory protein-1β, growth-related oncogene-α, and leptin. The mechanism involves either a direct action on adipocytes and macrophages or an indirect action through the production of active lipolysis-derived by-products (53). If the effects revealed in vitro exist in vivo, ANP may exert important functions within human AT to regulate the production of adipokines and cytokines involved in immune cell recruitment and activation, as well as in the pathogenesis of insulin resistance (Fig. 4). Further studies are necessary to demonstrate the physiological relevance of these in vitro results.

A recent study has focused attention on the impact of NPs on the control of adiponectin production, an important adipokine in the regulation of metabolism and prevention of insulin resistance (30). This hormone is primarily produced by adipocytes and its plasma levels are inversely correlated to body mass index and the percentage of body fat (2). Both ANP and BNP dose-dependently enhanced the expression of adiponectin mRNA and its secretion in human adipocytes in vitro (84). This effect appears to be specific since it is blunted by pretreatment of adipocytes with a selective NPR-A antagonist. Moreover, the plasma adiponectin level was increased at 4 days after administration of human ANP (84) and 7 days after administration of saralasin (i.e., synthetic human ANP) (95) to patients with congestive heart failure (CHF). Similarly, we have recently shown that intravenous infusion of human ANP increases total and high molecular weight plasma adiponectin in lean healthy volunteers (9).

**Action of NPs on Brown Fat**

The potential role of brown adipose tissue (BAT) in the regulation of energy dissipation and energy balance in adult humans has been recently reconsidered by the scientific community. BAT is highly specialized to generate heat in rodents and numerous small mammals. Brown adipocytes are located in BAT, whereas a population of “brown like” adipocytes has been identified in white adipose tissue. It is considered that the ability of mammals to expand the number and the activation state of brown-like adipocytes within white fat depots provides resistance to fat mass expansion (71). The recent (re)discovery of the existence of BAT deposits and the demonstration that BAT acts as a nonshivering thermogenesis effector in humans have boosted the interest in BAT in humans (66).

The involvement of NP-activated particulate guanylyl cyclases (i.e., NPR-A and NPR-B) in BAT has been recently demonstrated. Bordicchia et al. (13) demonstrated a thermogenic effect of NPs in mice and also in adipocytes differentiated from human multipotent adipose-derived stem cells. The authors highlighted a molecular mechanism involving activation of p38 MAPK and of the activating transcription factor-2 for the induction of uncoupling protein-1. This mechanism is common to both NP/cGMP and β-adrenergic signaling pathways as previously described in mouse adipocytes (16, 17). Interestingly, the authors described a browning of white fat depots in NPR-C knockout mice (NPR-C−/−) as well as an upregulated thermogenic response in the white and brown fat of wild-type mice infused with BNP for 1 wk (13). The physiological relevance was investigated in mice in which chronic BNP infusion for 7 days increased the browning of white fat and enhanced oxygen consumption and energy expenditure. However, the robust increase in oxygen consumption under BNP treatment could be confounded by significant effects in skeletal muscle. Wild-type mice placed at 4°C had elevated plasma levels of BNP and cardiac NP expression in parallel with BAT thermogenesis. It is important to mention at this point that cold-induced NP secretion and thermogenesis in BAT could be independent of each other. Future studies using transgenic animals will be required to assess the contribution of NP to cold-induced thermogenesis in vivo. These results add another dimension to the metabolic role of NPs and to the regulation of BAT (Fig. 3). With the rediscovery of the BAT...
and the browning of adipocytes in humans on one hand and the unquestionable metabolic role of NPs in the control of lipid mobilization on the other hand, these results open interesting perspectives. Initially, it will be important to validate the physiological relevance of these findings in the regulation of human BAT. It will also be interesting to assess whether obesity-related NP deficiency impacts on BAT function in humans.

Action of NPs on Skeletal Muscle

Transgenic mice overexpressing BNP and cGK-I are protected from diet-induced insulin resistance. This phenotype could be explained by multiple metabolic adaptations. A first explanation could be that NP/cGMP signaling indirectly improves insulin sensitivity through lower body weight gain under high-fat feeding. In agreement with this hypothesis, transgenic mice overexpressing BNP and cGK-I have higher whole body energy expenditure and fat oxidation, lower fat mass, and higher expression of mitochondrial oxidative genes in skeletal muscle when on a high-fat diet, compared with their wild-type controls. Similarly, high fat-fed mice chronically treated with sildenafil (i.e., a PDE-5 selective inhibitor) for 12 wk show reduced fat mass, higher energy expenditure, and insulin sensitivity (5). The reduced total fat mass could explain lower lipid deposition in the liver and skeletal muscle since body fat is a major determinant of ectopic fat (50). The lower ectopic fat deposition in the liver and skeletal muscle can improve insulin signaling and action (48). Importantly, these effects could be due to an upregulation of fat oxidation and mitochondrial biogenesis in skeletal muscle. A role of cGMP signaling in mitochondrial biogenesis had been previously suggested in skeletal muscle in response to calorie restriction in mice (64) and humans (18). It was shown that NP/cGMP turns on mitochondrial biogenesis in vitro in C2C12 myotubes and reverses mitochondrial dysfunction induced by high glucose and high insulin (45). Similarly, the nitric oxide/cGMP-dependent pathway controls mitochondrial biogenesis in vivo in mice deficient in endothelial nitric oxide synthase (eNOS<sup>−/−</sup>) mice (62). Interestingly, reduced mitochondrial content and oxygen consumption were also observed in skeletal muscle, brain, kidneys, liver, and heart of eNOS<sup>−/−</sup> mice, and subsarcolemmal mitochondria were more dramatically depleted than intermyofibrillar mitochondria in these mice.

An important point is the recent demonstration of the physiological relevance of these findings in human skeletal muscle by our group (25) (Fig. 3). Chronic NP treatment increases oxidative capacity and fat oxidation in human primary myotubes. The molecular mechanism involves a transcriptional activation of PGC-1α and subsequent induction of oxidative phosphorylation genes and mitochondrial respiration (25). It was also shown that NP/cGMP signaling activates the uncoupling protein-3 and the ATP/ADP translocase adenine nucleotide translocase 1 known to increase basal proton leak and energy uncoupling (36). These data are in agreement with those of Nisoli et al. (63) reporting an increased uncoupling in L6 myotubes chronically treated with 8-bromo-cGMP for 6 days (63). Most notably, a concomitant upregulation of NPR-A, PGC-1α and oxidative phosphorylation genes was observed in the skeletal muscle of obese subjects in response to an 8-wk aerobic exercise training program (25). These data suggest that NP/cGMP signaling, in addition to its lipid mobilizing effects, could contribute to exercise-induced mitochondrial biogenesis in human skeletal muscle. The two complementary processes may operate toward increasing insulin sensitivity (Fig. 4).

Finally, there is evidence of a direct effect of cGMP signaling on muscle glucose uptake. In support of this, activation of cGMP signaling (i.e., using the nitric oxide donor, spermine...
NONOate) ex vivo in human muscle strips was shown to increase cGMP levels and to induce insulin-independent glucose transport and glycogen synthesis. The mechanisms could involve AMPK-α1 activity (24). Future studies should investigate whether direct activation of NP signaling in human skeletal muscle promotes glucose uptake.

**Action of NPs on Pancreas and Liver**

Ropero et al. (72) have recently shown that ANP increases insulin secretion in mice islets in an NPR-A-dependent manner by increasing Ca²⁺ influx through blockade of ATP-sensitive K⁺ channels. They also reported that NPR-A⁻/⁻ mice have lower β-cell mass and higher fasting blood glucose levels compared with their wild-type littermates. These data are in agreement with in vivo data showing that ANP infusion enhances insulin secretion in healthy humans in the fasted state (85) and in response to a meal test (12). ANP was also shown to increase the insulin content of isolated islets, whereas NPR-A knockout mice have lower insulin levels in freshly isolated islets (72). Together, these data suggest that ANP/cGMP signaling in pancreas increases β-cell mass and insulin secretion (Fig. 3).

Some studies have also suggested that ANP may exert hepatoprotective actions by inhibiting oxidant injury (7) and Kupffer cell-mediated TNF-α release in response to LPS or ischemia-reperfusion without altering the vital host defense function of these cells. This effect is mediated via NPR-A and cGMP and involves a reduction in NF-κB binding activity (32). In addition, in Kupffer cells, ANP specifically induced heme oxygenase-1, a known protective mediator in ischemia-reperfusion injury (33). The anti-inflammatory action of ANP in liver could preserve hepatic insulin sensitivity. Additionally, Lutz et al. (39) reported that genetic ablation of cGK-I in nonneuronal cells results in liver inflammation, fasting hyperglycemia, and reduced insulin tolerance (39). This phenotype is accompanied by a selective reduction in hepatic insulin signaling and induction of phosphoenol pyruvate carboxyl-kinase, the key limiting enzyme of liver gluconeogenesis. The hepatoprotective action of NPs should be considered when investigating their metabolic impact (Fig. 3).

**Pathophysiological Relevance of NPs in Metabolic Diseases**

Disruption of NP/cGMP signaling in metabolic diseases due to NPR-A/NPR-C imbalance. The physiological and pathological changes in the various components of the NP/cGMP signaling pathway must be more thoroughly investigated, particularly in humans. The biological effect of NPs could be dependent on changes in cardiac NP secretion; alterations in NPR-A function; imbalance between NPR-A and NPR-C signaling; increases in PDE-5 or nephrilysin activity; or some other downstream mechanism in adipocytes, brown fat cells, and skeletal muscle. Expression of NPR-C has been shown to be reduced in the AT of rats by fasting and appeared to be linked to an increased biological activity of ANP (76). The ablation of NPR-C in mice leads to a remarkable increase in ANP half-life (41). In mice, a downregulation of NPR-A and NPR-B was observed in skeletal muscle, white and brown fat during high-fat feeding in parallel with a strong upregulation of the clearance receptor NPR-C (46). We have similarly observed an increased expression of NPR-C in the white fat of genetically obese diabetic mice (db/db mice) compared with their wild-type littermates (C. Moro and M. Lafontan, unpublished data).

A low ratio of NPR-A to NPR-C in target tissues could reduce NP/cGMP signaling and/or NP concentrations in plasma and within the target tissues. Insulin was recently shown to regulate the expression of NP receptors in AT. NPR-C expression was dramatically downregulated in an insulin-deficient mouse model (59). In another study, NPR-C mRNA strongly correlated with fasting insulin levels in visceral AT and scAT, independently of other anthropometric and glycemic traits.
suggesting a major contribution of insulin per se. In conditions of both clamped euglycemia and hyperglycemia, insulin increased the expression of NPR-C in human scAT and slightly reduced circulating levels of midregional pro-ANP independently of glucose concentrations (68). Chronic upregulation of adipose tissue NPR-C expression may increase NP clearance, reduce circulating NP levels, and contribute to obesity-related cardiovascular and metabolic disorders. The potential relevance of insulin-induced upregulation of NPR-C on circulating NP levels in humans is still unclear and needs to be investigated in further long-term observations. Genetic approaches have revealed a common variant of NPR-C that was associated with low plasma ANP levels and hypertension in obese subjects (75). Conversely, individuals carrying an A(-55)A NPR-C genotype have a significantly lower prevalence of overweight, obesity, and abdominal adiposity (77). This observation could be clinically meaningful since a drop in plasma NP levels and/or tissue response is observed in various pathological conditions such as obesity, type 2 diabetes, and the metabolic syndrome.

**Metabolic effects of NPs in CHF.** CHF is emerging as a major public health concern. In subjects with CHF, plasma levels of NPs (especially BNP) are very high (28). Increases in cardiac ANP expression and in ANP plasma levels have been extensively used as markers of left ventricular hypertrophy (61). Heart failure is associated with a number of metabolic and neurohormonal dysfunctions (31, 90). It is unclear whether the newly discovered metabolic effects of NPs may contribute to or worsen the disease. Weight loss in CHF is associated with a poor prognosis. It will be interesting to see whether such high levels of NPs are associated with increased plasma NEFA or triacylglycerol levels and modifications of lipid metabolism (lipid mobilization and lipid oxidation). NEFA concentrations and lipid oxidation are elevated in patients with CHF, and one likely mechanism was attributed to increased stress hormone-related lipolysis at that time; CHF represents a ketone-prone state (37). In addition to the putative hormonal factors previously proposed by the authors (i.e., norepinephrine, IL-6, and TNF-α), increased endogenous ANP/BNP concentrations in CHF may contribute to the development of cardiac cachexia. AT responsiveness is upregulated in cachectic patients. Catecholamine- and NP-induced lipolysis was increased 2- to 3-fold in human adipocytes coming from cachectic patients compared with controls (1). This effect is thought to be mediated through an upregulation of HSL mRNA and protein levels since it was completely abolished by selective inhibition of HSL. Results of a recent study, in accordance with results of previous acute studies (10), have confirmed the contribution of ANP to lipid mobilization and plasma NEFA levels (47). In response to the excess of plasma NPs in CHF patients, it is also possible that the body may adapt through the downregulation of NP signaling pathways. In fact, a single study has revealed that the NP-signaling system in AT does not desensitize in heart failure patients; the lipolytic response to ANP was preserved in isolated fat cells (8) and the in vivo lipolytic effect of BNP was also maintained (69). In contrast, attenuation of cardiovascular and renal responses to NPs has been reported in experimental and clinical heart failure studies. Upregulation of the clearance pathways (i.e., increased NPR-C expression and/or enhanced activity of nephrilysin) cannot be excluded to explain such tissue-dependent discrepancies. The pathological weight loss often observed in patients with CHF, associated with high levels of NPs in the circulation (but also with increased norepinephrine levels), may involve increased energy dissipation by BAT and skeletal muscle. Further work is needed to establish the validity of these hypotheses in humans. The BNP transgenic mice (46), which did not exhibit a cachectic phenotype, is not a suitable model to investigate the role of NPs in cachexia because of the lack of NP-induced lipolytic response in mice fat cells (80).

**Therapeutic potential of NP pathways.** The discovery of multiple metabolic effects of NPs (i.e., increased lipid mobilization, enhanced oxidative capacity and lipid oxidation in skeletal muscle and activation of brown fat cells thermogenic activity) opens new perspectives toward the possible clinical use of NPs to control energy balance and insulin sensitivity. Improved lipid mobilization and lipid oxidation may be beneficial in overweight and obese patients for weight loss purposes. In addition, the recent discovery of an appetite-suppressive effect of NPR-A agonism strengthens the potential role of NPs in the control of energy balance. Acute administration of BNP in healthy volunteers decreased total ghrelin and hunger, while increasing the feeling of satiety (87). Exercise and diet interventions, as well as pharmacological treatments enhancing circulating NP levels and/or NP signaling in skeletal muscle could improve the fat oxidative capacity of skeletal muscle and alleviate obesity-related metabolic disorders (Fig. 4). However, poorly controlled NP-mediated lipid mobilization could promote ectopic fat storage, insulin resistance, and eventually lead to cardiac cachexia (90).

Whatever investigations are carried out to elucidate the power of NPs in clinical practice, more research is necessary to unravel the appropriate clinical uses of NPs and related compounds. A limited number of pharmacological tools exist and have been used in various clinical protocols. Synthetic analogs of NPs (i.e., the human recombinant ANP, carperitide since 1995 in Japan, and human recombinant BNP, neseritide, in the United States since 2001) have been approved for intravenous treatment of acutely decompenated CHF (3, 65, 82). A number of questions still prevail concerning neseritide and its routine use in a population of patients with acute heart failure (65, 83). Novel NP-related molecules have been designed with the idea of creating agents with an improved pharmacological profile (89). NP drug leads from snake venoms are under consideration to overcome receptor-mediated clearance (88). Cenderitide (CD-NP) is a novel chimeric NP obtained by fusion of the 22 amino acid sequence of mature C-type natriuretic peptide with the 15 amino acid COOH-terminus of DNP. CD-NP is a new candidate drug peptide that can coactivate both NPR-A and NPR-B with increased resistance to peptidase degradation and weaker hypotensive actions than DNP. CD-NP is currently in phase I trial to target the heart, kidney, vasculature, and the endocrine system in heart failure to reduce rehospitalization following acute decompenated heart failure (42). This innovative therapeutic protein deserves a study of its effects in the emerging metabolic context of NPs action in obesity and type 2 diabetes.

In the same vein, pharmacological inhibition of the neutral endopeptidase nephrilysin could be beneficial in metabolic diseases. Chronic inhibition of nephrilysin could protect NPs from increased degradation as observed in obesity and preserve the beneficial actions of NP signaling in metabolic tissues (73).
Alternatively, nephrilysin inhibition could also lower blood glucose by increasing insulin secretion (94). This effect could be mediated by the parasympathetic nervous system.

Concluding Remarks and Future Trends

The discovery of the lipolytic properties of NPs in human fat cells has brought these hormones into the club of hormones playing a major role in the regulation of metabolism such as epinephrine and norepinephrine (lipolytic) and insulin (antilipolytic). Recent results have expanded the metabolic interest in these hormones (Table 1); an important role in the control of energy dissipation and expenditure has been discovered. Parallel improvement of lipid mobilization, NEFA use, and energy expenditure may be beneficial in overweight and obese patients with respect to energy balance. NPs are able to activate the thermogenic program in human and rodent adipocytes. They can also activate the conversion of white to brown-like adipocytes, a process leading to the appearance of new thermogenic cells in fat deposits. At the moment, the study of the long-term effects of NPs on energy balance and body weight remains to be done in rodents. Moreover, if the conversion of white to brown-like adipocytes operates under physiological conditions in humans, NPs could be included in the list of factors contributing to the physiological control of energy dissipation. In addition, the very recent demonstration that NPs increase oxidative capacity and energy uncoupling in human skeletal muscle opens interesting perspectives. Ideally, NP signaling in skeletal muscle, enhanced by exercise, may trigger favorable metabolic adaptations to increase fat oxidation, alleviate lipotoxicity, and enhance insulin sensitivity.

To summarize, in normal weight subjects, under physiological conditions (i.e., during physical exercise), the sequence of metabolic events in response to a rise in plasma NPs may be summarized as follows: 1) initiation of increased triacylglycerol breakdown in adipocytes (and in brown adipocytes if present in healthy individuals) and release of NEFAs by fat cells followed by 2) a rise in NEFA levels in the bloodstream coupled with 3) enhanced fatty acid uptake/utilization by skeletal muscle, heart, and brown fat cells and followed by 4) increased oxidative capacity and energy uncoupling in human skeletal muscle and also in brown fat cells which may increase energy dissipation (Fig. 4). The thermogenic role of NPs in human BAT still remains to be demonstrated. Nevertheless, when disturbed, the NP-dependent pathways could also initiate some metabolic complications. The dual stimulation of lipid mobilization and fat oxidation in skeletal muscle could contribute to the wasting syndrome observed in heart failure patients developing cachexia. Increased fatty acid uptake by the heart could contribute to cardiac pathology and induce deleterious shifts in glucose and fatty acid oxidation processes (38).

In addition to the impact of NPs on AT function (i.e., lipolysis and adipokine secretion), an original cross talk between AT (via adipokines or cytokines) and the endocrine function of cardiomyocytes is highly suspected (20). Feedback pathways between AT and the heart deserve further investigation. It is questionable whether the increased production of leptin (and concomitant reduced secretion of adiponectin) by adipocytes from obese subjects could be at the origin of the decreased production of ANP/BNP by cardiomyocytes and the decrease in plasma levels reported in obese patients (92). In support of this idea, studies in leptin-deficient obese mice models have shown a downregulation of cardiac NP production (6, 15). The previously reported increased expression of the NPR-C in AT to explain reduced plasma NP levels may not be the main factor to explain the decreased NP levels observed in obesity. A putative increase in PDE-5 activity, which metabolizes cGMP, may also reduce the biological effect of ANP at the fat cell level, though this hypothesis has not been studied in human fat cells. The specific roles of adipokines in the control of NP production/secretion processes needs to be further investigated.

Collectively, these findings leave a number of key issues open. More studies in animal models as well as in humans are required to establish the causal links existing between dysregulation of NP/cGMP signaling pathways and the development of obesity and insulin resistance. The metabolic impact of various NP mimetics remains largely unknown or underestimated. The fact that NP-mediated lipolytic actions occurs only in primates limits the use of rodent models in preclinical studies.

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AUTHOR CONTRIBUTIONS

C.M. and M.L. prepared figures; C.M. and M.L. drafted manuscript; C.M. and M.L. edited and revised manuscript; C.M. and M.L. approved final version of manuscript.

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