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# Natriuretic Peptides, Heart, and Adipose Tissue: New Findings and Future Developments for Diabetes Research

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Natriuretic peptides (NPs) play a key role in cardiovascular homeostasis, counteracting the deleterious effects of volume and pressure overload and activating antitrophic and antihypertrophic pathways in the heart. N-terminal B-type NP (NT-proBNP) also is a promising biomarker of global cardiovascular risk in the general population, and there is increasing interest on its potential use in diabetic patients for screening of silent cardiovascular abnormalities, cardiovascular risk stratification, and guided intervention. Recently, both atrial NP (ANP) and B-type NP (BNP) have emerged as key mediators in the control of metabolic processes including the heart in the network of organs that regulate energy usage and metabolism. Epidemiological studies have shown that ANP and BNP are reduced in people with obesity, insulin resistance, and diabetes, and this deficiency may contribute to enhance their global cardiovascular risk. Moreover, ANP and BNP have receptors in the adipose tissue, enhance lipolysis and energy expenditure, and modulate adipokine release and food intake. Therefore, low ANP and BNP levels may be not only a consequence but also a cause of obesity, and recent prospective studies have shown that low levels of NT-proBNP and midregional proANP (MR-proANP) are a strong predictor of type 2 diabetes onset. Whether ANP and BNP supplementation may result in either cardiovascular or metabolic benefits in humans remains, however, to be established.

Natriuretic peptides (NPs) have gained great interest as indicators of the state of cardiovascular health across the spectrum of cardiovascular diseases (CVD) in diabetic subjects (1–4). Recently, NPs also have emerged as key mediators in the control of metabolic processes (5) and have been implicated in the development of diabetes (6,7), shifting current paradigms and opening novel avenues in diabetes research. This review will summarize new findings in the field and discuss their potential clinical relevance.

## **NPs AND THEIR RECEPTORS**

NPs are a family of genetically distinct and structurally related peptides, including atrial NP (ANP), B-type NP (BNP), and C-type NP (CNP). They share a similar structural conformation, characterized by a peptide ring with a cysteine bridge, which is well preserved throughout evolution, being the portion of the chain that binds to the receptor. ANP and BNP are predominantly secreted by cardiomyocytes, whereas CNP is mainly produced by the central nervous system, the endothelium, the bone, and the reproductive system (8–11).

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Human NP genes encode for long inactive peptides that are processed to active peptides and equimolar concentrations of inactive fragments, which have a longer plasma half-life and are thus more suitable biomarkers for clinical use (12). ProANP is processed into active ANP and inactive fragments (N-terminal BNP [NT-proBNP] and mid-regional proANP [MR-proANP]) predominantly by the enzyme corin (13). ProBNP, which is often posttranslationally O-glycosylated in the N-terminal region, is cleaved into active BNP and the inactive fragment NT-proBNP by corin and furin (9). ProBNP can be released into the bloodstream prior to processing (14), but has six- to eightfold lower activity than BNP. The CNP gene encodes for a 126 prepropeptide that contains the two active forms of CNP (CNP-22 and CNP-53) and the inactive NT-proCNP fragment (11).

The most important factor governing ANP secretion is mechanical stretching of the atria. ANP is stored in secretory granules and stretching induces both secretion of previously synthesized ANP and increased ANP gene transcription. Stress of the ventricular wall due to volume and/or pressure overload also is the main inducer of BNP transcription (10,15). Circulating BNP and NT-proBNP levels are quite low in the normal state but rise in pathophysiological conditions characterized by fluid retention, such as heart failure (HF) (16). In addition, hypoxia-response elements are present in the promoter region of the BNP gene and both myocardial ischemia and hypoxia are potent BNP inducers (17). This explains why high BNP levels are found in acute coronary syndrome or during exercise-induced ischemia despite the absence of ventricular dilatation. Finally, ANP and BNP secretion can be modulated by humoral factors, including endothelin-1, angiotensin II, sex steroids and thyroid hormones, glucocorticoids, and inflammatory cytokines (tumor necrosis factor- $\alpha$  [TNF- $\alpha$ ], interleukin [IL]-1, IL-6) (8–10,18). CNP expression is regulated by transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1) and other growth factors in a cell- and tissue-specific manner. In the endothelium, TGF- $\beta$ , TNF- $\alpha$ , and IL-1 enhance CNP expression, whereas platelet-derived growth factor-BB is the major CNP inducer in vascular smooth muscle cells (11).

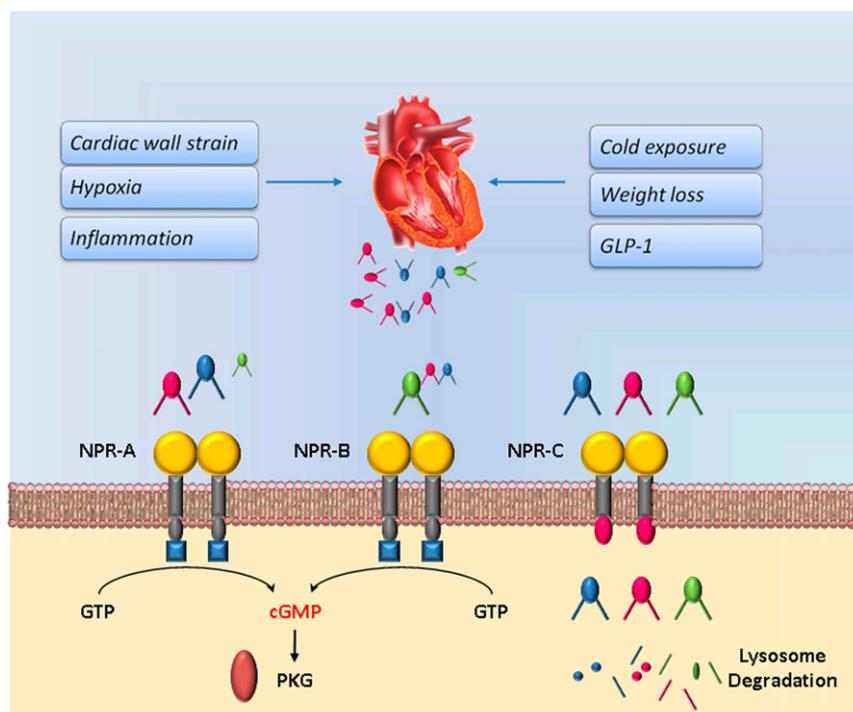
ANP and BNP plasma levels are higher in women than in men and increase with age (19). There is preliminary evidence that estrogens induce ANP and BNP production, whereas androgens suppress it; this may account for the sex differences in plasma concentrations (20). Plasma CNP levels are greater in men than in women, decline from adolescent to the fifth decade, and tend to increase thereafter, particularly in men (21). This is likely because both testosterone and growth hormone are potent CNP inducers (11).

NPs bind to high-affinity transmembrane NP receptors (NPR)-A, NPR-B, NPR-C that share a relatively common molecular configuration. NPR-C binds to all three peptides with similar affinity, whereas NPR-A and NPR-B differ in ligand specificity (NPR-A: ANP>BNP>>CNP; NPR-B: CNP>>>ANP $\approx$ BNP); this, together with NPR tissue distribution, affects NP peripheral activity. Binding to both NPR-A and NPR-B causes a conformational change in the receptor molecule that, in turn, activates particulate guanylyl-cyclase and increases intracellular cGMP that represents a second

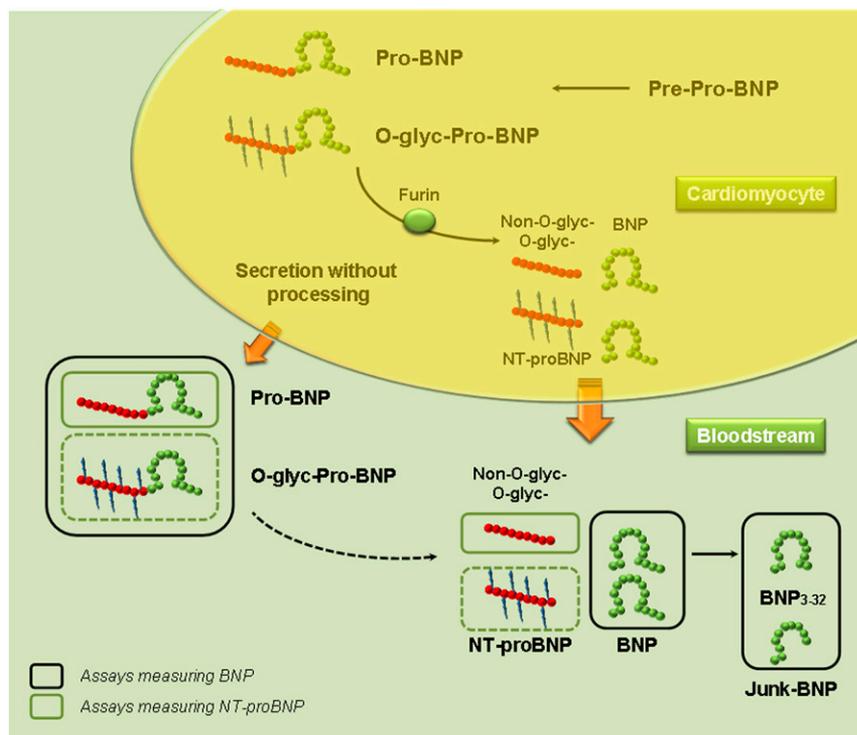
messenger critical to NP activity. By contrast, binding to NPR-C results in NP internalization and enzymatic degradation, and NPR-C plays a key role in NP clearance (9,22) (Fig. 1). In addition to undergoing receptor-mediated degradation, NPs can be degraded by extracellular proteases, including neutral endopeptidase (NEP) and insulin-degrading enzyme (8,23). Moreover, cleavage of two N-terminal amino acids from BNP by the enzyme dipeptidyl peptidase-4 (DPP-4) yields BNP3–32, which retains the physiologic effects of BNP on the heart but has lower activity on both the kidney and the vessel wall (24). Other aminopeptidases can further digest the N-terminal region of BNP and BNP3–32, leading to formation of breakdown products (junk-BNP) of unclear biological significance (25) (Fig. 2).

#### BIOLOGICAL ACTIONS OF NPs AND METABOLISM

Both ANP and BNP are important in maintaining cardiovascular homeostasis and counteracting the deleterious effects of volume and pressure overload.



**Figure 1**—ANP (red), BNP (blue), and CNP (green) NPs are secreted by the heart in response to various inducers and bind to high-affinity NPR-A, NPR-B, and NPR-C. NPR-C binds to all three peptides with similar affinity, whereas NPR-A and NPR-B differ in ligand specificity (NPR-A: ANP>BNP>>CNP; NPR-B: CNP>>>ANP $\approx$ BNP). Binding to both NPR-A and NPR-B activates particulate guanylyl cyclase and increases intracellular cGMP leading to PKG activation. Binding to the clearance NPR-C leads to NP internalization and enzymatic degradation. GTP, guanosine-5-triphosphate.



**Figure 2**—Schematic representation of proBNP processing and cross-reactivity of assays measuring BNP and NT-proBNP. ProBNP can be posttranslationally O-glycosylated (O-glyc) within the Golgi apparatus. Both O-glycosylated and non-O-glycosylated proBNP are cleaved to BNP and NT-proBNP in equimolar fashion by the enzyme furin and then secreted into the circulation. In the bloodstream, BNP can be further degraded to BNP3–32 and other breakdown products (junk-BNP). ProBNP can be released prior to processing, particularly when O-glycosylated at position Thr71 interferes with cleavage by furin, and may undergo processing into the bloodstream. Assays measuring BNP also recognize proBNP, BNP3–32, and junk-BNP (black square). Assays measuring NT-proBNP cross-react with proBNP (green square) but have poor reactivity for O-glycosylated NT-proBNP and proBNP (dotted green square).

Indeed, they act on the kidney to promote diuresis and natriuresis, induce vasodilation, and protect the heart from high preload and afterload pressures, which can result in hypertrophy and fibrosis by activating antifibrotic and antihypertrophic pathways (26–28). In addition, ANP and BNP reduce the sympathetic tone and suppress both renin and aldosterone secretion; therefore, they not only counterbalance but also lower the activity of systems with opposing renal and cardiovascular effects (29). Endothelial CNP, which is known to induce vasorelaxation by hyperpolarization of underlying vascular muscle cells, is a potential endothelium-derived hyperpolarizing factor, participating in the paracrine action of other endothelial vasorelaxant mediators, such as nitric oxide (NO) and prostacyclin (11).

Our understanding of the physiology of the NP system has dramatically increased in the past decades, and today the key role of NPs in cardiovascular

homeostasis is well established (see previous reviews [26–29]); however, emerging evidence suggests that the effects of NPs extend beyond the cardiovascular system. As detailed below, NPR are expressed in human adipose tissue, and NPs can stimulate lipolysis, promote browning of adipocytes, and also may modulate adipokine secretion and food intake (5,30–35). This previously unsuspected role of NPs in the regulation of metabolism is important as it raises the possibility that the heart belongs to the network of endocrine organs that regulate energy usage and metabolism. Furthermore, it may provide new targets for therapeutic intervention in the fight against obesity and insulin resistance.

The lipolytic effect of ANP was first shown almost 20 years ago in physiological studies in humans. Intravenous infusion of ANP acutely increases plasma concentrations of both glycerol and free fatty acids (FFA) (36). Furthermore, ANP

infusion in human subcutaneous abdominal adipose tissue induces lipolysis and increases local blood flow, resulting in enhanced lipid mobilization (37). Elegant studies performed in humans suggest that FFA mobilized in response to ANP may undergo mitochondrial oxidation in the liver, the skeletal muscle, and the adipose tissue itself (37–39). In vitro studies on adipocytes have partially clarified the underlying mechanism of NP lipolytic activity. Activation of the NPR-A receptor induces a rise in cGMP levels, followed by activation of protein kinase G (PKG). PKG, in turn, phosphorylates perilipin, and this induces a physical alteration of the lipid droplet surface that facilitates activation of both adipose triglyceride lipase and hormone-sensitive lipase, thus triggering lipolysis (35). The clearance receptor NPR-C reduces NPR-A-mediated lipolysis by lowering local NP bioavailability. Indeed, rodents, which express NPR-C at high levels on adipocytes, have a blunted lipolytic response to NPs, and NPR-C deletion rescues their responsiveness (32).

A recent breakthrough in our understanding of role of NP on fat tissue metabolism has been the demonstration that in adipocytes NPR-A activation induces transcription of genes enhancing energy expenditure and heat production (adipocyte browning) (32). The most important gene encodes the mitochondrial transporter uncoupling protein 1 (UCP1) that enables the separation of lipid oxidation from ATP production, allowing the conversion of nutritional energy to heat. NPR-A activation elicits this response by increasing p38 $\alpha$  signaling and phosphorylation of ATF2, directly increasing PPAR $\gamma$  coactivator-1 $\alpha$  [PGC-1 $\alpha$ ] and UCP1 transcription. PGC-1 $\alpha$  and UCP-1 promote mitochondrial biogenesis and both coupled and uncoupled respiration, and this results in enhanced energy expenditure thereby limiting white fat mass expansion. In line with these findings, NPR-A knockout mice have an increased fat mass, whereas BNP infusion results in enhanced energy expenditure in mice (32).

NPs also have a direct effect on the skeletal muscle, the major site of fuel oxidation and energy expenditure. Transgenic mice overexpressing BNP and fed with a high-fat diet display higher expression of mitochondrial

oxidative genes in skeletal muscle (40). Furthermore, cultured human skeletal muscle myotubes respond to both ANP and BNP with a significant increase in mitochondrial fat oxidative capacity through transcriptional activation of PGC-1 $\alpha$  and subsequent induction of oxidative phosphorylation (OXPHOS) genes and mitochondrial respiration (39). ANP also induces energy uncoupling, possibly via overexpression of UCP-3 and adenine nucleotide translocase 1, which accounts for 50% of basal (noninduced) proton leak (41). Notably, an upregulation of NPR-A, PGC-1 $\alpha$ , and OXPHOS genes is observed in the skeletal muscle of obese subjects in response to an aerobic exercise training program, suggesting that some of the metabolic adaptations of skeletal muscle in response to chronic exercise may be mediated by ANP and BNP (39).

Data on the interplay between the NP system and cytokines released by the fat tissue are still limited. However, in vitro in human adipocytes, ANP inhibits leptin release and both ANP and BNP enhance adiponectin secretion via NPR-A (33). In addition, in adipose tissue explants, ANP reduces IL-6, TNF- $\alpha$ , MCP-1, and leptin secretion (35). Finally, ANP infusion enhances adiponectin plasma levels in humans (42). Collectively, these findings suggest that NPR-A activation may modulate adipokine secretion and has a beneficial effect on low-grade inflammation of the adipose tissue and thus insulin resistance.

Recent studies suggest that NPs are involved in food intake control. A study performed in healthy fasting volunteers has shown that BNP infusion decreases hunger and increases satiety, possibly by lowering circulating ghrelin levels (34). Ghrelin also has direct cardiovascular effects (43), and this supports the existence of a bidirectional heart-gut cross talk. In addition, the high expression of NPR-B, the main CNP receptor, in the arcuate nucleus of the hypothalamus suggests a role of CNP in the control of food intake. Consistently, a recent experimental study has shown that central administration of CNP reduces food intake and body weight, partially through activation of the hypothalamic melanocortin pathway, which also is engaged by leptin and serotonin (44). Recently, Kim et al. (45) have described an entirely new mechanism in the control of ANP release from the heart by the

gut-released hormone GLP-1, revealing a new gut-heart connection. Activation of GLP-1 receptors in the atrial myocardium induces ANP secretion, resulting in vasodilation, natriuresis, and reduced blood pressure. Therefore, an increase in nutrients after a meal triggers a gut-mediated response, leading not only to effective regulation of insulin and glucagon release through a direct GLP-1 effect but also to indirect metabolic and hemodynamic changes mediated by ANP. ANP-induced vasodilation may be advantageous in this setting as it may promote an increased blood supply to the gut and adipose tissue, thus resulting in better absorption and distribution of nutrients (45,46).

The key role of insulin in metabolism has prompted studies on the potential interplay between NPs and  $\beta$ -cells. In vitro ANP induces insulin secretion in an NPR-A-dependent manner by increasing Ca<sup>2+</sup> influx through blockade of ATP-sensitive K<sup>+</sup> channels. Furthermore, ANP increases the insulin content of isolated islets, and NPR-A knockout mice have lower insulin levels in freshly isolated islets, reduced  $\beta$ -cell mass, and higher fasting blood glucose levels (47). In keeping with these data, ANP infusion enhances insulin secretion in humans in the fasted state and in response to a meal test (37,48).

Only a few studies have assessed the effect of NP infusion on glucose homeostasis. In fasting healthy men, an acute high-dose ANP infusion induces a modest increase in blood glucose that is likely due to enhanced FFA mobilization leading to increased gluconeogenesis (38). Short-term BNP infusion in healthy volunteers during an intravenous glucose tolerance test reduces the rise in blood glucose levels as compared with saline. This occurs without changes in insulin secretion and sensitivity and probably results from enhanced glucose volume distribution (49). These physiological studies do not, however, address the far more relevant issue of the long-term effects of chronic NP treatment on glucose metabolism. This has not yet been investigated in humans; however, a recent study in *db/db* mice has shown that chronic BNP infusion induces a significant reduction in blood glucose levels (50).

Obviously there is much additional work to be done in both basic and

clinical research to fully understand the function of the NP system in metabolic processes. However, emerging evidence indicating that NPs are the mediators of a novel "heart-adipose axis" has opened the way to an entirely novel and exciting area of research with potentially high clinical impact.

## NPs AND OBESITY, DIABETES, AND DIABETES COMPLICATIONS

### Obesity

Obesity is associated with decreased circulating levels of ANP and BNP, and a negative linear relationship between BMI and NP plasma values has been consistently reported in epidemiological studies (5,35,51). Conversely, weight loss increases NT-proBNP levels (52). There also is evidence that a reduction in NP levels is associated to a less favorable adipose tissue distribution profile. Recent data from the Dallas Heart Study show that both BNP and NT-proBNP are inversely associated with visceral and liver fat and positively associated with lower gluteofemoral body fat, independent of confounders including insulin resistance (53).

Central obesity is a key feature of the metabolic syndrome (MetS); it is, however, still matter of debate if BNP and NT-proBNP levels are reduced in MetS, with studies reporting either lower or unchanged values. This is likely due to differences among examined populations in the relative frequencies of MetS components with opposite effects on BNP. Consistently, a recent longitudinal study performed on 3,019 African Americans has shown that both lower and higher BNP levels were predictors of incident MetS in a nonlinear U-shape relationship, likely a reflection of lower BNP relation to obesity and higher BNP relation to blood pressure (54).

The cause of the relative ANP and BNP deficiency seen in obesity (natriuretic handicap) is poorly understood, but it is of clinical relevance as it may expose obese patients to enhanced cardiovascular risk and represent a potential mechanism behind the frequent clustering of obesity and hypertension. Almost two decades ago, researchers interested in the relationship between obesity and hypertension noted that levels of the clearance NPR-C were markedly elevated in obese subjects. The underlying

mechanism remains elusive; however, food intake and insulin resistance are likely involved as adipose tissue NPR-C expression is increased by a high-fat diet, correlates with fasting insulin, and is reduced by pharmacological strategies improving insulin sensitivity (5,40). Regardless of the mechanism, the observation that NPR-C is overexpressed in obese subjects fueled speculation that increased NP clearance could explain the “natriuretic handicap.” However, both NT-proBNP and NT-proANP, which are not cleared by NPR-C, show a similar inverse relationship with BMI, making this hypothesis less likely. In addition, recent data indicate that NPs are mainly cleared by NEP.

Alternatively, ANP and BNP levels may be reduced in obesity because of a lower synthesis and/or release from the heart. In keeping with this hypothesis, cardiac mRNA expression of both ANP and BNP is reduced in obese Zucker fatty rats and *db/db* mice (50,55). Data in humans are limited; however, a study in subjects who underwent cardiac catheterization has shown that cardiac BNP release is inversely and independently related to BMI (56). Obese individuals may be prone to a relative NP deficiency, stemming from suppressive effects of circulating androgens on ANP and BNP synthesis (57) and possibly from the deleterious systemic and local effects of cardiac ectopic fat on NP synthesis and release (58,59). Consistently, downregulation of cardiac ANP and BNP expression is paralleled by a rise in cardiac triglycerides content in animal models of obesity, and oleic acid reduces ANP mRNA levels in cultured cardiomyocytes (60).

On the other hand, the recently discovered effects of NPs on metabolism and energy expenditure raise the possibility that NP deficiency may directly contribute to the development and worsening of obesity and dysmetabolism. Indeed, in obese and insulin-resistant subjects, reduced ANP and BNP levels and upregulation of the clearance NPR-C in the adipose tissue may hamper the ability of the local NP system to enhance lipolysis and energy expenditure (5). In keeping with this hypothesis, targeted deletion of the NPR-C gene in rodents results in a lean phenotype, transgenic BNP mice fed with a high-fat diet are

protected from obesity and insulin resistance, and chronic BNP infusion improves the metabolic profile of *db/db* mice, including a reduction in fat content (32,40,50).

### Diabetes

Growing evidence of a role of NPs in obesity and insulin resistance raises the question of whether NP deficiency is implicated in the development of type 2 diabetes (T2D). Recently, two large and well-designed prospective studies have substantiated this hypothesis. In the Malmö Diet and Cancer Study (6), MR-ANP and NT-proBNP levels were measured in 1,828 nondiabetic individuals who subsequently underwent a 16-year follow-up exam, including an oral glucose tolerance test. Results showed that low MR-ANP plasma levels predict development of future diabetes. After full adjustment, the odds ratio for incident diabetes in the bottom compared with the top quartile of MR-ANP was 1.65 (95% CI 1.08–2.51), independent of confounders. An inverse, but not statistically significant, association was found between NT-proBNP and diabetes risk. In the same cohort, the variant rs5068 within the ANP gene, which results in enhanced plasma ANP levels, was associated with a 12% reduced adjusted-risk of incident diabetes (61). Furthermore, other studies have reported that the rs5068 variant is associated with lower BMI, waist circumference, and prevalence of obesity and MetS (62,63).

In the longitudinal Atherosclerosis Risk in Communities Study (ARIC) (7), which examined 7,822 nondiabetic individuals, with respect to NT-proBNP values in the highest quartile (>114 pg/mL), values in the lowest quartile (<31 pg/mL) were associated with higher risk of incident diabetes over a 12-year follow-up period, independent of confounders and risk factors (hazard ratio 0.75 [95% CI 0.64–0.87]). A significant linear trend across quartiles also was evident, even after stratification by BMI (7). Furthermore, a common genetic BNP variant (rs198389), which results in a 20% increase in plasma BNP levels, was found to be associated with a 15% reduced risk of T2D (64). See Table 1 for a summary of human studies (6,7,61,64,65).

Insight on the mechanisms whereby NP deficiency may enhance diabetes risk

is still insufficient. In the Malmö and ARIC studies, the prospective relationship between MR-ANP and NT-proBNP and diabetes remained significant after adjustment for insulin resistance, indicating that alternative mechanisms, such as NPR-A–induced energy expenditure, adiponectin secretion, and improved  $\beta$ -cell function, may be involved (Fig. 3). Regardless of the mechanism, the evidence that low NP levels are implicated in the development of diabetes together with the well-established role of low NPs in hypertension and CVD suggests that NP deficiency may be a link between the relevant clinical problem of shared propensity to diabetes, hypertension, and CVD. Moreover, it suggests that NT-proBNP and MR-ANP may be used as biomarker for diabetes prediction. Further studies are, however, required to establish clinical relevance.

### Diabetes Complications

Both BNP and NT-proBNP are well-established diagnostic and prognostic biomarkers of HF in both diabetic and nondiabetic subjects (16). Although BNP and NT-proBNP levels appear elevated in patients with HF, the fraction of active BNP is relatively small. Current assays cross-react with poorly active BNP-related peptides (proBNP, BNP3–32, junk-BNP) (Fig. 2), whose levels are increased in HF, and they thus overestimate plasma and serum BNP and NT-proBNP values (25). Altered cardiac processing of proBNP to BNP is a likely explanation for the rise in circulating proBNP levels in HF. Notably, a recent study in humans has shown that cardiac proBNP release was greater in patients with HF and that the only variable positively associated with enhanced proBNP secretion was diabetes (14). This suggests that HF, particularly in diabetes, affects either the expression and activity of proBNP cleaving enzymes or proBNP glycosylation, a modification that changes proBNP susceptibility to cleavage by furin, particularly when it occurs at Thr71 (25). On the other hand, studies in *db/db* mice suggest that both enhanced BNP3–32 formation and resistance to mechanisms of proBNP clearance from the circulation also may contribute to increase circulating BNP immunoreactivity in diabetes (50).

BNP and NT-proBNP are not always reliable biomarkers for HF diagnosis as

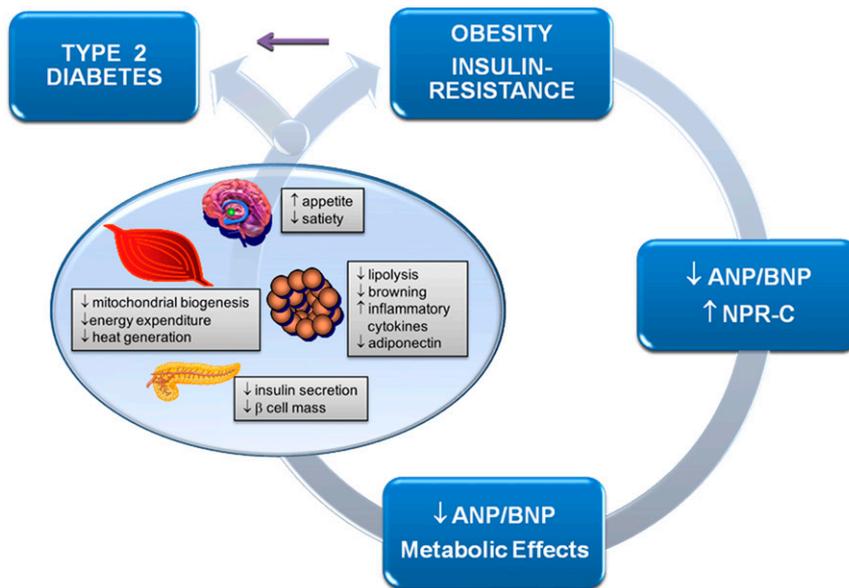
**Table 1—NPs and the risk of T2D in human epidemiological studies**

Study	Study design	Study population	N	Biomarkers/gene variant	Event	Adjusted OR/HR (95% CI)
Malmö Study (6)	Prospective study (16-year follow-up)	Swedish, no history of diabetes, * CVD, HF	1,828	MR-ANP, NT-proBNP	Incident diabetes (301 events: OGTT)	MR-ANP, OR 0.85 (0.73–0.99) per SD; NT-proBNP, OR 0.92 (80–1.06) per SD
ARIC Study (7)	Prospective study (12-year follow-up)	U.S., no history of diabetes, * CVD, HF, CKD	7,822	NT-proBNP	Incident diabetes (1,740 events: self-reported, medications)	4th NT-proBNP quartile vs. 1st quartile, HR 0.75 (0.64–0.87)
FINRISK97 Study (65)	Prospective study (10.8-year follow-up)	Finnish, no history of diabetes, * 5% known CVD	7,827	NT-proBNP	Incident diabetes (417 events: national registers)	NT-proBNP, HR 0.82 (0.73–0.93) per SD
Pfister et al. (64)	Case-cohort study nested in the EPIC-Norfolk study	Norfolk, U.K., no history of diabetes, CVD	440 cases, 740 control subjects	NT-proBNP	T2D (440 cases: self-reported, medications, national registers)	HR 0.79 (0.64–0.97) per SD
Pfister et al. (64)	Mendelian randomization study: meta-analysis of 11 case-control studies	European descent	23,382 cases, 57,898 control subjects	C allele rs198389 in BNP locus	T2D (23,382 cases)	OR 0.94 (0.91–0.97) per allele
Malmö Study (61)	Mendelian randomization study (14-year follow-up)	Swedish, no history of diabetes**	27,307	One copy of the G allele rs5068 in the ANP locus	Incident diabetes (2,823 events: national registries)	HR 0.88 (0.78–0.99) per allele

CKD, chronic kidney disease; HR, hazard ratio; OGTT, oral glucose tolerance test; OR, odds ratio. \*Fasting glucose available at baseline. \*\*Fasting glucose available in a subgroup.

30% of symptomatic HF patients with preserved ejection fraction (HFPEF) have normal BNP and NT-proBNP levels (66). This is of relevance to diabetes as HFPEF prevalence is very high in T2D and the relative risk of cardiovascular death or HF hospitalization conferred by diabetes is significantly greater in HFPEF than in HF with reduced ejection fraction (HFREF) (67,68). Recently, studies performed on myocardial tissue from patients with HFPEF and left ventricular (LV) diastolic dysfunction have given new insight on the potential relationship between diabetes, HFPEF, and BNP. According to a novel proposed paradigm for the pathogenesis of HFPEF, a systemic inflammatory state induced by obesity, diabetes, and other comorbidities causes oxidative stress in the coronary microvascular endothelium, reducing myocardial NO bioavailability and leading to reduced PKG activity in cardiomyocytes, which therefore become stiff and hypertrophied (69). As BNP gene expression is regulated by diastolic LV wall stress, which is lower in HFPEF because of prevailing concentric LV remodeling, this is not compensated by an enhanced BNP production. Consistently, myocardial BNP expression is lower in patients with HFPEF than in those with HFREF (70). This raises the possibility that drugs increasing BNP levels may represent a novel therapeutic strategy in patients with HFPEF and the PARAGON-HF trial is under development to test this hypothesis.

Besides HF, BNP and NT-proBNP can provide quantitative information about the state of cardiovascular health across the full spectrum of CVD, and there is increasing interest on their potential role as predictors and biomarkers of complications and cardiovascular mortality in diabetes. A prospective study performed on 315 T2D patients showed that NT-proBNP levels were greater in patients with albuminuria. Furthermore, NT-proBNP was a strong predictor of all-cause and cardiovascular mortality independent of potential confounding risk factors and had a predictive value for all-cause mortality comparable to that of microalbuminuria (1). Consistently, we have recently reported that NT-proBNP was strongly and independently associated with cardiovascular mortality in a longitudinal study performed on a large cohort of 1,825 T2D



**Figure 3**—Potential link between NPs, obesity, insulin resistance, and diabetes. Obesity and insulin resistance are associated with both impaired ANP and BNP cardiac release and over-expression of the clearance NPR-C, leading to reduced NP-mediated beneficial effects on target organs of metabolism (adipose tissue, skeletal muscle, pancreatic  $\beta$ -cells, central nervous system). This results in further weight gain and worsening of insulin resistance and also may favor diabetes development.

patients from the population-based Casale Monferrato Study (71), and in the same cohort, NT-proBNP also was a negative confounder in the association between central obesity and cardiovascular death (72). Similarly, in type 1 diabetes NT-proBNP is associated with micro- and macrovascular complications and is a predictor of cardiovascular mortality (73,74). Recent studies also have demonstrated that NT-proBNP predicts cardiovascular events in T2D patients and has a greater predictive value than other well-established cardiac risk markers (2,75,76). Collectively, these data suggest the possible use of NT-proBNP as a tool for risk stratification, and preliminary data support this approach. In the Action in Diabetes and Vascular Disease: Preterax and Diamicon MR Controlled Evaluation (ADVANCE) study, NT-proBNP greatly improved the accuracy with which the risk of cardiovascular events or death could be estimated in T2D patients and provided much more prognostic information than cholesterol or C-reactive protein (77). An additive effect of NT-proBNP and albuminuria in predicting cardiovascular mortality was, however, observed in the Casale Monferrato Study, suggesting that the parallel assessment of both may

provide additional information for risk assessment (71).

NT-proBNP and BNP levels also mirror clinically silent processes in the cardiovascular system that gradually shift the homeostatic balance from health to disease far before cardiac events occur, and they are both markers and predictors of earlier cardiac abnormalities in diabetic patients. In the Hoorn Study (3), a slight increase in baseline BNP levels was a strong predictor of both LV hypertrophy and diastolic LV dysfunction, and this association was particularly evident in T2D patients. In asymptomatic T2D patients, NT-proBNP was associated with silent myocardial ischemia independent of microalbuminuria. Furthermore, a 10-year longitudinal study performed on asymptomatic high-risk diabetic patients found that NT-proBNP  $\geq 38$  pg/mL was the only independent predictor of silent coronary artery disease (4). Hypoxia and myocardial wall strain are likely inducers of BNP in these settings. In addition, inflammatory cytokines also may be involved as both TNF- $\alpha$  and IL-6 induce BNP secretion in cultured cardiomyocytes, their plasma levels rise prior to that of NT-proBNP in hypertensive subjects (78), and the association between NT-proBNP and vascular complications was

dependent on TNF- $\alpha$  in the EURODIAB study (73). A strategy that selects asymptomatic T2D patients, based on BNP and NT-proBNP levels, for further investigation (e.g., echocardiography, stress testing) is very attractive. Supporting data are still scarce; however, Nadir et al. (79) have recently reported that in a community cohort BNP measurement was an optimal screening test to identify individuals with silent cardiac damage for further assessment. In addition, NT-proBNP may be proposed as a tool to guide intervention for primary prevention. In the Casale Monferrato study (71), NT-proBNP was prospectively associated with cardiovascular mortality even in normoalbuminuric patients without CVD at baseline, indicating that NT-proBNP may be useful for identification and treatment of patients with a hitherto unknown high risk of cardiovascular death. A prospective randomized controlled trial has recently substantiated this hypothesis by showing a remarkable 65% reduction in the risk of the primary end point of hospitalization or cardiac death in T2D patients with NT-proBNP levels greater than  $>125$  pg/mL and no known cardiac disease randomized to a biomarker-guided “intensified” treatment (80).

Taken together, epidemiological evidence summarized here indicates that BNP and NT-proBNP are important biomarkers of CVD complications in patients with diabetes. The “clinical paradox” of BNP and NT-proBNP being negative predictors of diabetes and positive predictors of diabetes complications is unexplained; however, a possible interpretation is that changes in BNP and NT-proBNP levels reflect different pathophysiological processes in subjects at risk for diabetes and in those at risk for diabetes complications. In obese subjects, reduced BNP and NT-proBNP levels may mirror a deficiency in BNP synthesis, contributing to dysmetabolism and diabetes onset. On the contrary, in diabetic patients an increase in BNP and NT-proBNP levels may reflect proBNP accumulation that masks the linear relationship between residual active BNP and metabolic parameters, but being the result of altered cardiac processing is a valuable biomarker of early cardiac and hemodynamic abnormalities and of the inability of the heart to effectively counteract

them by enhancing active BNP production. In keeping with this scenario, recent data from the Multi-Ethnic Study of Atherosclerosis (MESA) (81) have shown that when subclinical CVD develops and NT-proBNP levels rise, the inverse relationship between NT-proBNP and both BMI and insulin resistance is completely lost.

### FUTURE PERSPECTIVES

In the past decade our understanding of the role of the NP system in diabetes has significantly improved; however, many questions still need to be answered. In particular, more research is required to clarify the mechanisms of the natriuretic handicap in obesity, the pathogenetic link between NP deficiency and diabetes onset, and the regulation of proBNP processing both in the heart and the bloodstream.

Regarding the use of NPs for treatment, preclinical and epidemiological data support the hypothesis of a role of ANP and BNP in the development of obesity and diabetes, but it is still unknown whether ANP and BNP may be effective in inducing weight loss and preventing diabetes, and there are concerns that NP-induced excessive lipid mobilization may promote muscular and hepatic ectopic fat storage and, thus, insulin resistance. To address these issues, development of appropriate therapeutic tools targeting the NP system and intervention trials in humans is needed and some effort has already been made. Studies looking at the effect of chronic NP infusion on metabolism are ongoing (82) and long half-life BNP formulations are under development (83). Agents that selectively either block NPR-C or activate NPR-A may be even more desirable as NPR-C overexpression may reduce the efficacy of BNP infusion. An alternative strategy is to enhance ANP and BNP levels by inhibiting the catalytic enzyme NEP. However, NEP degrades other vasoactive peptides, such as angiotensin I, bradykinin, and endothelin-1, leading to undesirable hemodynamic effects. Combined inhibition of NEP and ACE is not a suitable strategy because of a significant increased risk of angioedema; however, the use of angiotensin receptor blockade with NEP inhibition (ARNi) is a potential alternative option (23). In this regard, it is noteworthy that the PARADIGM-HF trial, testing efficacy

and safety of the ARNi LCZ696 as compared with enalapril in patients with HFREF (34% with diabetes), has been stopped early based on evidence of compelling efficacy, and these promising incoming results may open the way to future trials in obese and diabetic patients.

Recent studies also suggest ancillary effects on the NP system of drugs currently used for diabetes treatment. In mice, liraglutide induces the cardiac release of ANP. If results are confirmed in humans, physicians may take advantage of this gut–heart link and use long-acting GLP-1 receptor agonists to compensate for NP deficiency and gain indirect beneficial hemodynamic and metabolic effects mediated by ANP (45). DPP-4 inhibitors reduce BNP degradation to BNP3–32 (24) and thus may enhance full-length BNP bioavailability, potentially resulting in advantageous effects. However, the Saxagliptin Assessment of Vascular Outcomes Recorded in Patients with Diabetes Mellitus–Thrombolysis in Myocardial Infarction 53 (SAVOR-TIMI 53) trial has unexpectedly shown increased rates of hospitalization for HF in diabetic patients treated with saxagliptin. Although treatment neither increased nor decreased the composite cardiovascular primary end point, further studies are needed to clarify this issue and to rule out the unlikely possibility of a BNP-related effect (84).

Finally, the use of more reliable and specific assays for the measurement of both BNP and NT-proBNP is required. Although poor specificity of current assays may not hamper the clinical value of BNP and NT-proBNP as a predictor and biomarker of CVD, it significantly limits our possibility to understand underlying pathophysiological mechanisms and to envision novel therapeutic strategies. Recently, a new antibody that binds to the cleavage site of proBNP (Hinge76) has been developed, allowing accurate proBNP measurement (85). Furthermore, new NT-proBNP assays using antibodies directed against non-glycosylated epitopes are under development to overcome the problem of poor reactivity of current assays for O-glycosylated NT-proBNP (25). Whether these new assays will result in better clinical applicability awaits further evaluation.

### CONCLUSIONS

Recent studies showing a role of NPs in metabolism and T2D onset have extended the interest of NP-related research beyond CVD and raised the possibility that new intervention strategies targeting the NP system may be effective in lowering not only blood pressure and sodium retention but also body weight and the risk of diabetes. The use of NT-proBNP as a tool for early diagnosis, risk assessment, and guided intervention in CVD holds great promise for improving the care of patients with diabetes.

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