

Cardiac-Secreted Factors as Peripheral Metabolic Regulators and Potential Disease Biomarkers

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In the United States alone, 1 person dies from a coronary event approximately every 1.5 minutes.¹ Cardiovascular disease (CVD) encompasses larger subclasses of diseases, such as hypertension, coronary/peripheral artery disease, endothelial dysfunction, and atrial fibrillation, and is associated with metabolic diseases such as diabetes and obesity. Although these diseases are distinctive and their etiologies are diverse, they share a common progressive end stage, which is heart failure (HF). As the heart fails, it begins to lose the ability to maintain energy balance and regulate metabolic processes.² Depending on the pathogenesis of CVD, the metabolic profile can be distinct. Further complicating diagnostic and prognostic assessments is the variable temporal progression of the disease.

Clinical Diagnosis of Cardiovascular Disease

The heterogeneous clinical manifestations of CVD range from subtle to life-threatening conditions. According to the National Heart, Lung, and Blood Institute, clinical confirmation relies on multiple tests, including evaluation of risk factors, physical exam, and family history, and results from tests and procedures such as electrocardiogram, echocardiogram, chest x-ray, and blood tests. Misdiagnosis is common with these methods, obscured by age, sex, complications from other conditions such as obesity (including edema and dyspnea), or erroneous baselines for circulating secreted

factors such as cardiac troponin, among others.^{3,4} Thus, many studies strive to identify novel biomarkers for risk stratification of CVD, diagnosis, and prognosis; however, progress is hindered by the heterogeneous origin of these disorders.

Progress and Issues With Current CVD Biomarkers

As defined by the National Institutes of Health, a biomarker is “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.”⁵ Progress in the CVD biomarker field has been slow, given that a good biomarker must satisfy stringent criteria including screening method, assay sensitivity, and the context for its assay. For example, the screening of bodily fluids, such as blood, urine, and saliva, are preferable to painful and invasive organ biopsies. Tests should be sensitive, yielding results quickly (in minutes or hours, not days). Candidate biomarkers are assessed by context-specific criteria such as their potential for diagnosis, prognosis, or treatment-guided therapy. Finally, new biomarkers should offer improvements over established biomarkers.

Current CVD biomarkers include many classes of molecules, including metabolites, lipids, proteins, and peptides. Blood-derived lipid levels are a good marker for CVD risk stratification (Table 1), and the general guidelines for analysis include: total cholesterol (ideally $\times < 200$ mg/dL), low-density lipoprotein (LDL; $\times < 130$ mg/dL), high-density lipoprotein (HDL; $\times > 60$ mg/dL) and triglycerides (TGs; $\times < 150$ mg/dL). High total cholesterol, LDL, and TG levels negatively correlate with patient outcomes whereas HDL levels have a positive correlation. However, a drawback to lipid analysis is that they are general markers for systemic health, not necessarily cardiac health. Assay advantages and disadvantages are listed in Table 1, and disadvantages include variable levels upon sample collection (postprandial, fasting, morning, night, etc) and many are not cardiac specific.

Circulating proteins and peptides are also used as biomarkers, and like lipids, many are not cardiac specific or

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Table 1. Summary of Common Circulating Biomarkers for CVD Diagnosis

Circulating Factor	Assay Advantages	Assay Disadvantages
BNP	High levels indicate heart damage.	High BNP level alone is not enough to diagnose a heart problem.
CRP	Increased levels indicate an inflammatory response to injury or infection.	Measuring CRP alone does not indicate risk for heart disease.
Fibrinectin	Excess protein can result in clot formation, leading to a heart attack or stroke.	Currently, no direct treatments to lower fibrinogen levels; test is also not universally standardized.
Glucose	High blood glucose level is a risk factor for insulin resistance, prediabetes, and type 2 diabetes mellitus. Untreated diabetes mellitus can lead to heart disease and stroke.	Timing of measurements is critical because levels can vary throughout the day (postprandial, fasting, circadian rhythm, AM VS PM).
HDL	HDL assists in removing LDL cholesterol, keeping arteries open, and increased blood flow.	Timing of measurements is critical because levels can vary throughout the day (postprandial, fasting, circadian rhythm, AM VS PM).
Lp (a)	Levels are genetically determined. High levels of Lp(a) may be a sign of increased risk of heart disease.	Research has not clarified risk levels.
LDL cholesterol	High blood levels cause accumulation of fatty deposits (plaques) in arteries (atherosclerosis), which reduces blood flow. Plaques can rupture and lead to major heart and vascular problems.	Timing of measurements is critical because levels can vary throughout the day (postprandial, fasting, circadian rhythm, AM VS PM).
TC	High levels increase risk of heart disease.	
TGs	High levels indicate more calories are consumed than metabolically burned. High levels increase risk of heart disease.	Timing of measurements is critical because levels can vary throughout the day (postprandial, fasting, circadian rhythm, AM VS PM).

Common diagnostic biomarkers for cardiovascular disease (CVD) are listed. These biomarkers are circulating factors assayed from human blood. Advantages and disadvantages for each assay are concisely stated. BNP indicates B-type natriuretic peptide; CRP, C-reactive protein; HDL, High-density lipoprotein; LDL, Low-density lipoprotein; Lp (a), Lipoprotein (a); TC, Total Cholesterol; TGs, Triglycerides.

only are detected after significant cardiac damage has occurred (Table 1). Proteins analyzed include: C-reactive protein (CRP), fibrinogen, and the neurohormones, B-type natriuretic peptide (BNP) and norepinephrine (NE).^{6,7} CRP is a general marker of inflammation, high fibrinogen levels indicate clot risk for stroke and atherosclerosis, NE concentration directly correlates to left ventricular dysfunction,⁸ and BNP levels are a strong predictor of mortality. From these examples, it is clear that the magnitude and rate of changes in circulating biomarker levels is critical for risk stratification, diagnosis, and disease treatment.

As previously mentioned, a good biomarker must satisfy stringent criteria, including screening method, assay sensitivity, and the context for its assay. New biomarkers must improve upon the old, and a good example is cardiac-specific troponin T (cTnT). cTnT supplanted previous assays detecting creatine kinase/creatin kinase-MB (CK/CKMB) because they are not cardiac specific. Interestingly, troponin T is expressed in both cardiac and skeletal muscles; however, this protein is encoded by different genes and results in 2 immunologically distinct proteins. Also, assays detecting cTnT are more sensitive than CK/CKMB assays, resulting in a faster diagnosis. Hence, efforts to identify novel, cardiac-specific biomarkers could improve upon current clinical biomarkers.

Identification of “Cardiokines” for Mechanistic Studies

In addition to identifying novel biomarkers, it is important to understand how biomarkers fit into our mechanistic understanding of CVD. As the heart begins to fail, disruption in its metabolic processes can lead to the secretion of proteins from the heart into the circulation called “cardiokines.”⁹ Cardiokines are synthesized and secreted from multiple cell types in the heart, including cardiomyocytes, fibroblasts, smooth muscle (aortic or blood-derived progenitors), and vascular endothelial cells. An extensive list of cardiokines are in Table 2; also listed is their cellular origin, examples of stress-induced regulation, as well as method of secretion (classical or nonclassical). Cardiokines derived from cardiomyocytes and fibroblasts are emphasized in Table 2, because these cell types represent $\approx 56\%$ and 27% of cells in the heart, respectively.¹⁰

Many different types of stress, occurring at various stages of cardiac disease, can initiate cardiokine synthesis and secretion. These stressors include: ischemia/reperfusion, oxidative stress, hemodynamic stress, hypertrophy, etc, and are listed in Table 2. Cardiokines have multiple modes of action, including: autocrine, paracrine, and/or endocrine effects. The significance of these endocrine effects is of particular interest

Table 2. Reported Cardiokines

Cardiokine	Cellular Origin	Secretory Pathway	Stress-Mediated Regulation
Activin-A	Myocyte	Unknown	Ischemia/reperfusion injury ¹¹
ADM	Myocyte	Classical	Nitric oxide ¹²
APLN	Myocyte	Classical	Cardiac ischemia ¹³
Angiotensin II	Myocyte	Unknown	Oxidative stress ^{14–17}
Annexin V	Myocyte	Nonclassical	Myocardial infarction ¹⁸
ANP*	Myocyte*	Classical*	Hemodynamic stress ^{19*}
BNP*	Myocyte*	Classical*	Hemodynamic stress ^{20*}
CTRP9*	Myocyte*	Classical*	Ischemia/reperfusion injury ^{21*}
CGRP	Myocyte	Classical	Ischemia/reperfusion injury ²²
CT-1	Myocyte	Classical	Myocardial infarction ²³
C-C motif chemokine	Fibroblast	Classical	Ischemia-induced myocardial injury ^{24–26}
Clusterin	Myocyte	Classical	Myocardial infarction ²⁷
Collagen	Fibroblast	Classical	Cardiac fibrosis ^{23,28–31}
C-type natriuretic peptide	Myocytes	Classical	Myocardial infarction ^{32,33}
CypA	Myocyte	Nonclassical	Hypoxia ³⁴
ET-1	Myocyte	Classical	Myocardial infarction ³⁵
Enkephalin	Myocyte	Classical	Ischemia ³⁶
FSTL-1/FRP	Myocyte	Classical	Transverse aortic constriction, ischemia/reperfusion injury, and myocardial infarction ^{37,38}
FSTL-3	Neither	Unknown	Ischemia/reperfusion injury ³⁷
FGF-1	Fibroblast	Nonclassical	Ischemia/reperfusion injury ³⁹
FGF-2	Fibroblast	Nonclassical	Ischemia/reperfusion injury ⁴⁰
GDF-15/macrophage-inhibitory cytokine 1*	Myocyte*	Unknown*	Ischemia/reperfusion injury ^{41,42*}
HSP60	Fibroblast	Nonclassical	Myocardial infarction ^{43,44}
HMG-1	Fibroblast	Nonclassical	Ischemia/reperfusion injury ⁴⁵
IL-1 α	Neither	Nonclassical	Myocardial infarction ^{46,47}
IL-1 β	Neither	Nonclassical	Myocyte hypertrophy ⁴⁶
IL-6	Neither	Classical	Atherothrombosis ^{23,47,48}
IL-33	Fibroblast	Classical	Apoptosis ^{15,49,50}
MANF	Myocyte	Unknown	Myocardial infarction ²⁹
MIF	Neither	Nonclassical	Atherosclerotic vascular lesions ⁵¹
GDF-8*	Myocyte*	Classical*	Cardiac hypertrophy ^{41,42*}
Necrosis factor- α	Both	Classical	Hypertension ^{47,52,53}
NRG1		Nonclassical	Reactive oxygen species ^{54,55}
OPN	Myocyte	Classical	Cardiac hypertrophy, Ischemia/reperfusion injury ^{29,56,57}
PTX3	Fibroblast	Classical	Myocardial infarction ^{58,59}
PI16	Myocyte	Classical	Cardiac hypertrophy ⁶⁰
S100-A1	Myocyte	Nonclassical	Myocardial infarction ⁶¹
Sfrp2	Neither	Unknown	Myocardial infarction ^{28,62}
Thioredoxin	Myocyte	Unknown	Myocardial infarction/ischemia/reperfusion injury ⁶³
TNF α	Myocyte	Unknown	Ischemia/reperfusion injury ^{47,53}
TGF- β 1	Both	Unknown	Hypertrophy ^{16,64,65}

Continued

Table 2. Continued

Cardiokine	Cellular Origin	Secretory Pathway	Stress-Mediated Regulation
UCN	Both	Classical	Ischemia/reperfusion injury ¹²
VEGF	Myocyte	Classical	Myocardial infarction ^{47,66}

This table is a comprehensive list of cardiokines reported in the literature, with emphasis on factors secreted by cardiomyocytes (abbreviated “myocyte” in the table) and fibroblasts. *There is a striking contrast between the number of cardiokines reported in the literature that act by autocrine and paracrine mechanisms and endocrine. These include 5 cardiokines with endocrine effects that modulate peripheral metabolism: atrial natriuretic peptide (ANP); B-type natriuretic peptide (BNP); growth differentiation factor-8 (GDF-8/myostatin) and -15 (GDF-15); and C1q/TNF-related protein 9 (CTRP9). This list reinforces the need to explore cardiokines in the context of endocrine actions, to identify novel stressors resulting in cardiokine secretion, and identification of cellular pathways leading to cardiokine secretion, including classical (endoplasmic reticulum [ER] dependent) and nonclassical (ER independent). ADM indicates Adrenomedullin; ANP, Atrial natriuretic peptide; APLN, Apelin; BNP, B-type or brain natriuretic peptide; CGRP, Calcitonin gene-related peptide; CT-1, Cardiostrophin-1; CTRP9, C1q/TNF-related protein 9; CypA, Cyclophilin A; ET-1, Endothelin-1; FGF-1, Fibroblast growth factor -1; FGF-2, Fibroblast growth factor-2; FRP, Follistatin Related Protein; FSTL-1, Follistatin-like 1; FSTL-3, Follistatin-like 3; GDF-8, Growth differentiation factor 8; GDF-15, Growth differentiation factor; HMG-1, High mobility group 1 protein; HSP60, Heat shock protein 60; IL-1 α , Interleukin-1 α ; IL-1 β , Interleukin-1 β ; IL-6, Interleukin-6; IL-33, Interleukin-33; MANF, Mesencephalic astrocyte-derived neurotrophic factor; MIF, Migration inhibitory factor; NRG1, Neuregulin 1; OPN, Osteopontin; PI16, Protease inhibitor 16; PTX3, Pentraxin-3; Sfrp2, Secreted frizzled-related protein; TGF- β 1, Transforming growth factor- β 1; TNF α , Tumor necrosis factor α ; UCN, Urocortin; VEGF, Vascular endothelial growth factor.

mechanistically, given that multiple organs outside the heart are affected by CVD, and organ cross-talk could potentially precipitate its origin and/or progression. Yet, few cardiokines are known to have endocrine effects (asterisk [*] symbol, Table 2), although these factors may hold tremendous promise in understanding CVD.

Communication between the heart and peripheral organs is essential to maintain an efficient metabolism and preserve normal cardiac function. Conversely, other organs can signal to the heart by secreting their own factors. Adipose tissue secretes adipokines; most are upregulated in the obese state, are proinflammatory, and contribute to metabolic dysfunction. Examples of these adipokines include leptin, tumor necrosis factor (TNF), and interleukin-6 (IL-6), as reviewed elsewhere.⁶⁷ In contrast, adiponectin (APN) is considered anti-inflammatory and beneficial in target tissues, where it regulates glucose homeostasis.⁶⁸ It has been proposed that metabolic dysfunction and other diseases result from an imbalance in these anti- and proinflammatory adipokines.⁶⁸ Adipokines serve as a striking example of how stress in one organ can significantly impact the metabolism of peripheral organs. Another example of a peripherally released factor is growth differentiation factor-11 (GDF-11). Loffredo et al. identified GDF-11 as a circulating factor possibly originating from the spleen that prevents age-dependent cardiac hypertrophy using parabiosis experiment with young animals.⁶⁹ Similar roles are beginning to be defined in the stressed heart and its secreted cardiokines.

The heart is susceptible to stress impacting energy homeostasis. To maintain cardiac contractility, the human heart requires a large energy supply in the form of ATP. The myocardial ATP pool is a balance between anabolic and catabolic processes and is turned over ≈ 6 times per minute.⁷⁰ It is estimated that the heart stores enough energy to sustain function for only a few beats. The adult heart relies on mitochondrial fatty acid oxidation (FAO) as its primary ATP energy output (60–90% of ATP production) whereas glycolytic

pathways contribute the remaining 10% to 40%.⁷¹ Underscoring the importance of FAO to cardiomyocyte function, $\approx 30\%$ of cellular volume consists of mitochondria.⁷² During CVD, cardiac mitochondrial function is diminished and the heart shifts from FAO to a glycolytic metabolic program.⁷³ The resulting metabolic switch reduces ATP production and impairs cardiac contractility.

Beyond ATP production, additional metabolic pathways are altered in disease, including autophagy, cell growth, and redox homeostasis, as reviewed here.⁷⁴ During the transition to HF, whole-body metabolism shifts to favor catabolism in a process called cachexia. Thus, cardiac metabolic changes can signify the transition from early to late cardiac disease and represent multiple points of intervention for novel therapeutics. In this review, we highlight cardiokines that regulate systemic metabolism, including atrial natriuretic peptide (ANP), B-type natriuretic peptide (BNP), growth differentiation factor-8 (GDF-8) or myostatin, growth differentiation factor-15 (GDF-15), and C1q/TNF-related protein 9 (CTRP9). We emphasize that understanding the type of stress that induces these cardiokines and regulates their secretion are key parameters to evaluate their efficacy as CVD biomarkers. Also, even cardiokines with limited clinical applications can become hypothesis generating in the context of basic research.

Atrial and B-Type Natriuretic Peptides

Previously, de Bold et al. demonstrated that ANP is a cardiokine that modulates a peripheral organ (the kidney).⁷⁵ Subsequently, the ANP, BNP receptor atrial natriuretic peptide receptor-A (NPR-A) was identified in multiple tissues, including adipose tissue, suggesting other actions in addition to regulating blood volume. It was subsequently determined that cardiac-derived natriuretic peptides communicate with adipose tissue to initiate “browning,” a thermogenic process with therapeutic potential for treating obesity and reducing the risk of CVD.⁷⁶

Discovery of ANP and BNP

The first evidence suggesting a cardiac-secreted factor existed was published in 1963 when secretory granules were identified by electron microscopy in the atria.^{77,78} The first indication that the heart is capable of releasing cardiokines affecting peripheral organs came in 1981 when it was demonstrated that infusing atrial extracts into rats resulted in a rapid natriuresis and diuresis from the kidney.⁷⁵ The active molecule was purified in 1983, and named ANP.^{79,80} In 1988, a second cardiac natriuretic peptide, BNP, was isolated in pig and subsequently human atria.^{81–84}

Induction of Natriuretic Peptide Synthesis and Secretion

ANP and BNP are members of the natriuretic peptide family of proteins and expressed from the *NPPA* and *NPPB* genes, respectively, on chromosome 1. ANP and BNP undergo regulated secretion, and a focus of previous studies is defining factors regulating their synthesis and secretions.⁸⁵ The synthesis and release of ANP and BNP are highly regulated processes underscoring important aspects of cardiokine biology. Mechanical strain, including hemodynamic stress, is an established inducer of ANP and BNP expression.²⁰ Other signals that induce ANP, BNP production include: vasoconstrictors (endothelin-1,¹⁹ angiotensin II, and α -adrenergic agonists⁸⁶), hormones (glucocorticoids,⁸⁷ thyroid hormone^{88–90}), growth factors, and proinflammatory cytokines.⁹¹ Conversely, secretion of ANP, BNP is reduced by nitric oxide (NO) and leptin.^{92,93} These signals are linked to CVD, indicating a diverse, highly regulated mechanism controlling secretion of these important signaling molecules.

Enzymatic Processing of Mature ANP and BNP

ANP, BNP are initially synthesized as prohormones and stored as secretory granules before secretion.⁸⁵ The cardiokine action of ANP, BNP is largely regulated through prohormone cleavage, which generates the mature and active peptides. ANP, BNP are cleaved into their mature form by corin, a serine protease expressed in heart, kidney, and blood.⁹⁴ Interestingly, multiple ANP and BNP peptides are found in circulation. Detection of these processed fragments is important for their utility as biomarkers.

Full-length ANP (153 amino acids [aa]) is cleaved by signal peptidase in the sarcoplasmic reticulum (SR) to generate pro-ANP (126 aa). Pro-ANP is stored in secretory granules for regulated secretion (see Table 2). Once secreted, extracellular pro-ANP is cleaved into inactive peptide NT-pro-ANP (aa 1–98) and 28 aa active ANP (aa 99–126) by the cell-surface protein, corin.⁹⁵ For BNP processing, the signal peptide (26 aa) is

cleaved from preproBNP (134 aa) while it is cotranslated in the SR. The resulting proBNP (108 aa) is secreted by the conventional (ER-Golgi) pathway⁹⁶ and regulated by O-glycosylation at threonine residue 71 (Thr71).⁹⁷ Modification at Thr71 prevents cleavage, yielding glycosylated proBNP (108 aa); no modification at Thr71 enables cleavage into NT-proBNP (aa 1–76) and BNP-32 (aa 77–108). Distinct substrate sequence preferences yield BNP (1–32) with furin cleavage and BNP (4–32) with corin cleavage, peptides detected both in vitro and in vivo.^{98,99} Another form detected in plasma, BNP (3–32), could result from dipeptidyl peptidase cleavage.¹⁰⁰

ANP and BNP Signaling

After cleavage, active peptides bind atrial natriuretic peptide receptors A (NPR-A) or C (NPR-C), encoded by the genes, *NPR1* and *NPR3*, respectively. When bound to the “clearance receptor,” NPR-C,¹⁰¹ the peptide is cleared from circulation; to NPR-A, blood pressure is reduced and body fluid homeostasis is altered, among other effects. NPR-A is a cell-surface receptor and a guanylate cyclase family member. ANP, BNP ligand binding activates its catalytic function, converting GTP into the second messenger, cyclic GMP.^{102,103} In contrast, NPR-C is called the “clearance receptor” because it lacks a guanylyl cyclase domain; it is an atypical G-protein-coupled receptor coupled to inhibitor G proteins (G_i). It both inhibits adenylyl cyclase and activates phospholipase C.¹⁰⁴

To determine novel target organs of ANP and BNP, expression patterns for both receptors are being defined at different physiological and pathological states. Under physiological conditions, NPR-A is expressed in heart and adipose tissue,¹⁰⁵ kidney and vascular tissue,^{106,107} adrenal gland,¹⁰⁸ aortic smooth muscle and endothelial cells,¹⁰⁹ and brain,¹¹⁰ among others. NPR-C is expressed in brain and choroid plexus¹¹¹ and adipose tissue.^{112,113} Under pathological conditions, such as pressure overload, rats have upregulated ligand (ANP and BNP) and receptor (NPR-A and NPR-C) expression in the left ventricle.¹¹⁴

Natriuretic Peptides in Organ Cross-talk

Metabolic disorders such as obesity can accelerate CVD, and recent evidence with cardiokines suggests the heart communicates with adipose tissue to regulate systemic metabolism. NPR-A is expressed in the adipose tissue of rats¹¹⁵ and humans,¹⁰⁵ and exogenous ANP, BNP application onto human adipocytes results in lipolysis, that is, lipid breakdown by hydrolysis of TG into free fatty acids and glycerol.¹¹⁶ ANP and BNP also induce expression of brown adipocyte markers, including uncoupling protein-1 (*UCP1*), peroxisome proliferator-activated receptor gamma coactivator or PGC-1 α (*PPARGC1A*), cytochrome c (*CYCS*), and PRD1-BF1-RIZ1

homologous domain containing 16 (*PRDM16*).⁷⁶ Activation of these factors indicates a “browning” of white fat to a thermogenic tissue, because brown/beige fat increases energy expenditure through uncoupled respiration. It also suggests that the diseased heart may release ANP, BNP in an attempt to provide itself with an increased energy supply in the form of free fatty acids.

ANP, BNP ligand availability is determined by NPR-C expression. NPR-C levels fluctuate based on nutrient availability, and fasting mice have reduced NPR-C levels in adipose tissue.¹¹⁷ In contrast to human adipocytes, rodent cells do not activate lipolysis when exogenously treated with ANP and BNP.¹¹⁸ Humans have lower NPR-C levels relative to NPR-A; specifically, rodent NPR-C levels are 100-fold greater than in humans.^{118,119} By comparing lipolysis in white adipocytes cultured from *Npr3*^{+/+} and *Npr3*^{-/-} animals, it was found that cells lacking NPR-C could respond to ANP and initiate lipolysis.⁷⁶ These results indicate that NPR-C levels are a key mediator of lipolytic response by regulating ligand availability (ANP, BNP) in both rodent and human adipose tissue.

Clinical Relevance of Natriuretic Peptides to Cardiovascular Health

Finally, genetic manipulation of this peptide system results in cardiovascular stress or disease phenotypes in mice. Corin deficiency results in cardiac hypertrophy and hypertension.¹²⁰ ANP knockouts (*Nppa*^{-/-}) have salt-sensitive hypertension, elevated blood pressure, and cardiac hypertrophy at baseline.^{121,122} BNP knockouts (*Nppb*^{-/-}) have ventricular fibrotic lesions at baseline, but no ventricular hypertrophy or systemic hypertension.¹²³ Cardiomyocyte-specific NPR-A knockout results in hypertrophy and hypotension.¹²⁴ NPR-C knockout mice (*Npr3*^{-/-}) have reduced blood pressure, reduced ability to concentrate urine, and are called “long john” (lgj) stemming from skeletal overgrowth resulting in a longer body.^{119,125} These genetic models underscore the importance of natriuretic peptide (ANP, BNP) secretion to maintain normal cardiac structure and function during stress.

A combination of basic science and clinical testing of ANP and BNP has yielded excellent data concerning plasma levels, stable peptides to bioassay, as well as genetic abnormalities or single-nucleotide polymorphisms, leading to altered plasma levels of these peptides.¹²⁶ In humans, elevated plasma levels of both N-terminal (NT)-proANP and NT-proBNP correlate with cardiovascular stress and mortality.¹²⁷ Yet, ANP and BNP plasma levels are differentially regulated in patients with CVD,¹²⁸ and NT-proBNP is considered a more robust biomarker.¹²⁹ BNP and NT-proBNP measurements are more consistent than ANP because of a longer half-life (ANP half-life is ≈ 2.5 minutes,^{130,131} BNP is ≈ 20 minutes, and NT-proBNP is ≈ 24.8 minutes¹³²). Patient BNP and NT-proBNP levels are

detected by commercially available antibody-based bioassays, but a drawback is erroneous antibody binding, such as to other BNP cleavage products, and results vary by manufacturer.¹³³ Overall, agreement between these NT-proBNP immunoassays is superior to BNP, as determined by the CardioOrmoCheck study.¹³⁴ Another drawback is elevated circulating levels of BNP and NT-proBNP is not specific to CVD and elevated levels are reported in: acute ischemic stroke,¹³⁵ cancer patients without volume overload,¹³⁶ and end-stage renal disease.¹³⁷ Overall, however, BNP and NT-proBNP levels are widely used as a good prognostic marker in CVD and in biomarker-guided therapy, where a BNP algorithm has been developed to aid in clinical decision making, such as drug selection and dose.¹³⁸

GDF-8 and GDF-15

GDF-8 and GDF-15 are members of the transforming growth factor- β (TGF- β)/bone morphogenetic protein cytokine superfamily. TGF- β family members have diverse functions in cellular proliferation, differentiation, growth, inflammation, and extracellular matrix deposition. Activation of this signaling pathway has contradictory effects on cardiovascular health, and circulating levels of TGF- β can have cross-reactivity issues with other TGF- β family members, thus limiting their application as biomarkers. In this section, we discuss these circulating factors and their diverse effects on systemic metabolism, primarily focusing on GDF-8/myostatin.

GDF-8/Myostatin

The secreted factor myostatin is expressed in skeletal muscle where it is a striking regulator of muscle mass. Elevated skeletal myostatin levels reduce skeletal muscle hypertrophy and hyperplasia, whereas decreased myostatin levels increase skeletal muscle mass.^{139,140} Recently, low levels of myostatin were reported in cardiac muscle. Surprisingly, this cardiac-derived myostatin acts in an endocrine fashion on skeletal muscle to reduce muscle mass.¹⁴¹ This finding is important in HF, because an endpoint of many disorders is muscle wasting and cachexia. Here, we discuss the significance of cardiac-secreted myostatin for CVD treatments in the prevention of cachexia.

Discovery of myostatin

Discovered in 1997, myostatin or GDF-8 is a member of the TGF- β superfamily.¹⁴² Myostatin/GDF-8 is expressed from the *MSTN* gene, primarily in skeletal muscle, but also at lower levels in heart muscle and adipose tissue.^{142,143} Loss of function *Mstn* mutations leads to a doubling of skeletal muscle mass in the cattle breeds Belgian Blue and Piedmontese.^{139,140} *Mstn*^{-/-} mice have increased skeletal muscle

mass and significantly reduced fat accumulation relative to controls, whereas cardiac muscle mass is unaltered.^{140,144–146} Humans with myostatin mutations are also exceptionally strong and lean¹⁴⁷ and/or capable of building greater muscle mass with exercise.¹⁴⁸ Myostatin level is therefore a key determinant of skeletal muscle growth in multiple species. Whether this increase in skeletal muscle mass stems from hyperplasia or hypertrophy is a point of contention and may result from a combination thereof.^{142,149,150} One hypothesis posits the mechanism of skeletal growth is dependent on myostatin protein levels, and *Mstn*^{-/-} induces both hypertrophy and hyperplasia, whereas low-moderate levels of myostatin induce hypertrophy.¹⁵¹

Maturation, receptor binding, and signaling

Myostatin is synthesized as a prepropeptide (376 aa) and proteolytically cleaved by the Ca²⁺-dependent protease, furin, a ubiquitously expressed proprotein convertase family member localized to the trans-Golgi network.¹⁵² Myostatin forms a dimer through its C-terminus that serves as the functional ligand for heterodimeric activin receptors. The activin A receptor type II B (ACTRIIB), encoded by the *ACVR2B* gene, forms a stable complex with the type I receptor to activate activin signaling. Receptor activation is antagonized by binding myostatin propeptide or follistatin, an activin-binding protein, among others. Follistatin transgenic mice and *Acvr2b* dominant-negative mice are hypermuscular relative to controls, similar to myostatin knockout mice.

Activation of ACTRIIB stimulates the small mothers of decapentaplegic (Smad)-dependent TGF- β signaling pathway, specifically Smad2/3. Afterward, downstream growth pathways, such as protein kinase B (Akt)/mammalian target of rapamycin complex 1/p70S6K are suppressed, resulting in inhibition of muscle cell differentiation and growth.¹⁵³ Myostatin is also implicated in a TGF- β -independent pathway during the induction of cachexia. Myostatin inhibits AKT phosphorylation, resulting in forkhead box O1 upregulation of ubiquitin proteasome genes.¹⁵⁴

Clinical relevance of myostatin to cardiovascular health

Myostatin mRNA and protein levels are upregulated after cardiac hypertrophy or injury. Humans with advanced heart failure have increased cardiac myostatin levels.^{155,156} Similar to humans, myostatin synthesis and secretion is increased in murine heart failure models. During late-stage heart failure, whole-body metabolism favors catabolism, culminating in cachexia. Because of the observed increase in myostatin after cardiac stress, and its known functions regulating muscle growth and differentiation, one hypothesis is that heart failure increases cardiac-secreted myostatin to reduce peripheral muscle mass, thus decreasing the cardiac burden. A recent

article by Heineke et al. tested this hypothesis. To model cardiac-induced skeletal muscle atrophy, a long-term pressure overload model was used. After this stress, circulating myostatin levels were increased in wild-type mice, but not in cardiomyocyte-specific myostatin knockout mice. Conversely, cardiomyocyte-specific myostatin transgenic mice have a 3- to 4-fold increase in circulating myostatin that is sufficient to reduce both skeletal and cardiac muscle mass. Together, these data supports a role for cardiomyocyte-derived myostatin in regulation of peripheral skeletal muscle mass.¹⁴¹

To prevent the loss of skeletal muscle mass, Heineke et al. infused an antimyostatin antibody (JA-16) after onset of HF. The goal was to inhibit binding of myostatin to ACTRIIB. Whereas a 6-week JA-16 treatment (administered 8 weeks post-TAC [transverse aortic constriction] or sham) successfully maintained muscle mass relative to controls, treatment did not improve survival or cardiac performance. The authors concluded the antibody intervention was not as effective as a genetic deletion (*Mstn*^{-/-}) model of myostatin; interestingly, *Mstn*^{-/-} mice lacked myostatin before TAC.¹⁴¹ These results suggest earlier inhibition of myostatin for optimal therapy. In humans, multiple myostatin inhibitory antibodies (MYO-029¹⁵⁷ and AMG 745) have been developed to increase muscle mass in muscular dystrophy; however, their application in the treatment of HF-induced cachexia is unknown.

Further mechanistic insight into what signaling cascades induce the synthesis and secretion of myostatin in cardiomyocytes may yield more-promising avenues for the prevention of cachexia during progressive HF. Thus, the possible endocrine functions of myostatin is an interesting new area of research that warrants further investigation; however, as a cardiac biomarker, myostatin is not ideal because it is not cardiac specific, but may prove useful in a multimarker approach. For example, coronary HF patients have increased plasma myostatin concentrations correlating with NT-proBNP.¹⁵⁸

GDF-15

GDF15 was cloned by multiple laboratories as early as 1997 and is known by many names, including macrophage inhibitory cytokine 1,¹⁵⁹ nonsteroidal anti-inflammatory drug-activated gene-1,¹⁶⁰ and placental bone morphogenetic protein B.¹⁶¹ These diverse names are reflective of just a few of GDF-15's reported functions, including metabolism and cardiovascular health.

Maturation, receptor binding, and signaling

GDF-15 is a divergent member of the TGF- β superfamily.¹⁵⁹ Encoded by the *GDF15* gene as an immature 308 aa, \approx 34 kDa protein, it is cleaved at N²⁵²-M²⁵³ into a \approx 6 kDa C-terminal fragment by membrane type 1 matrix metalloproteinase and a

≈30 kDa N-terminus that homodimerizes to form an active peptide.¹⁶² GDF-15 binds activin receptor-like kinase type 1 receptors (1–7) and is involved in many cellular processes, such as tumorigenesis, proliferation, differentiation, apoptosis, and inflammation. As such, it is a circulating factor of particular interest to many fields, including diabetes mellitus and CVD research. Importantly, a sensitive immunoradiometric sandwich assay detects GDF-15 in patient blood with minimal cross-reactivity with other TGF-β family members.¹⁶³

Clinical relevance of GDF-15 to cardiovascular health

CVD patients with cardiac hypertrophy or chronic HF have elevated, circulating GDF-15 levels correlating with disease severity.¹⁶⁴ *Gdf15* is induced during cardiac stress and released from the mouse myocardium; cardiac-specific *Gdf15* transgenic mice have some resistance to hypertrophy induced by phenylephrine, angiotensin, and pressure overload. Conversely, *Gdf15* null mice develop normally, but increased hypertrophy occurs in response to TAC.¹⁶⁵ These results reinforce that GDF15 is an antihypertrophic factor. Other studies indicate that GDF-15 is cardioprotective by activating the phosphoinositide 3-kinase/AKT/endothelial nitric oxide synthase/NO pathway.¹⁶⁶ In general, TGF-β signaling activation was previously viewed as detrimental to cardiac function, resulting in cardiac remodeling, including hypertrophy and fibrosis. However, GDF-15 downstream pathway activation is through SMAD2/3¹⁶⁵ and is antihypertrophic. Additional research is needed to clarify this discrepancy; it is tempting to speculate that this avenue could yield novel targets for drug treatment.

Screening of circulating GDF-15 levels is a promising prognostic marker for metabolic disorders, including obesity, insulin resistance, and type 2 diabetes mellitus (T2DM), where its circulating levels are also increased.^{167,168} Using a global *Gdf15* transgenic mouse fed high-fat diet versus control, and despite equivalent food intake, the transgenic mice have less white and brown adipose tissue. These transgenics also have improved glucose tolerance and lower insulin levels.¹⁶⁹ Treatment of obese C57BL/6 mice with *Gdf15* expressing xenografts had less adipose tissue, with increased expression of the lipolytic genes, adipose triglyceride lipase, and hormone-sensitive lipase.¹⁶⁹ Interestingly, circulating GDF-15 levels are also being investigated for mitochondrial disorders, where it is a promising biomarker.¹⁷⁰ As a result of these studies, GDF-15 is an emerging biomarker for both cardiovascular and cardiometabolic disorders.

CTRP9

Newly recognized paralogs of adiponectin (APN), a major adipokine, are the CTRPs, with CTRP9 having the greatest

degree of amino acid identity. CTRP9 is a recently identified cardiokine (2009), and studies suggest that it regulates prosurvival cardiac pathways. It also appears to affect metabolism, given that its circulating levels are inversely correlated with fasting glucose and insulin resistance. Because of its recent discovery, many aspects of this protein's regulation and functions are uncharacterized.

Maturation, Receptor Binding, and Signaling

CTRP9 is encoded by the *C1QTNF9* gene on chromosome 13 (human) or chromosome 14 (mouse). While highly expressed in adipose tissue, it is also abundantly expressed in the heart.¹⁷¹ Emerging evidence suggests CTRP9 acts as a cardiokine rather than specifically an adipokine like APN. The active form of CTRP9 requires proteolysis of the full-length (fCTRP9) into a globular domain isoform (gCTRP9). CTRP9 circulates and can be measured in the plasma as gCTRP9. It has been shown that cardiac tissues possess the ability to cleave fCTRP9 into gCTRP9, thus releasing it into the circulation. The cardiokine action of CTRP9 has been shown to be similar to that of APN. Treated adult cardiomyocytes with gCTRP9 elicited the activation of survival pathways, including Akt, AMP-activated protein kinase (AMPK), and eNOS.¹⁷²

Clinical Relevance of CTRP9 to Cardiovascular Health

Interestingly, *Ctrp9* levels are decreased in diabetic animals¹⁷³ and after an acute myocardial infarction.¹⁷⁴ Administration of *Ctrp9* in a mouse model of myocardial infarct led to a decrease in myocyte apoptosis and activation of the AMPK pathway.²¹ These findings open up the possible therapeutic potential of CTRP9, and serum and plasma levels of CTRP9 can be easily measured in humans. In a study comparing human subjects with normal glucose tolerance with prediabetic/T2DM serum, CTRP9 levels are lower in older subjects with metabolically unhealthy profiles. Additionally, serum CTRP9 levels are inversely correlated with age, blood pressure, fasting glucose, insulin resistance, and APN level.¹⁷⁵ Serum CTRP9 levels also correlate with arterial stiffness in T2DM patients.¹⁷⁶ These studies identify CTRP9 as a novel cardiokine with roles in metabolic and CVDs.

Perspective: The Potential of Cardiokines for CVD Research

This review began by emphasizing the need for new and consistent biomarkers of CVD, in particular, cardiac-specific factors. Because its origin is diverse, we focused on the hypothesis that activation and/or perturbation of metabolic pathways leads to CVD.

Efforts to define cardiokines in the stressed heart led to the discovery that they can have endocrine effects on the liver and adipose tissue, thus altering systemic metabolism. Although the highlighted cardiokines are not necessarily cardiac specific, or secreted after significant damage has occurred, these factors are beginning to refine our understanding of when and how the heart initiates communication with peripheral organs. The rigorous testing of stress-induced expression and processing of these cardiokines, and identification of target receptor expression, could aid in the identification of novel pathways involved in CVD. Ideally, cardiokines will be identified that are uniquely cardiac specific, released at the earliest stages of CVD, and predict disease progression for clinical application and prognosis. Peripheral organs are, in turn, able to release their own circulating factors to regulate cardiac function. Also, understanding reciprocal organ cross-talk during CVD could narrow down potential biomarkers involved in its temporal progression.

As previously mentioned, key readouts of the stressed heart are metabolic changes, particularly insulin resistance, and mitochondrial abnormalities leading to altered substrate preference, reduced ATP production, and reduced cardiac contractility. Currently, cardiokines secreted in response to stress, such as ischemia/reperfusion, hypoxia, myocardial infarction, and TAC, in animal models are being defined as they contribute to different etiologies of CVD. Again, these cardiokines are listed in Table 2, and an extensive review of cardiokines can be found elsewhere.¹⁷⁷ Table 2 is intended as a platform for novel biomarker discovery. Many of these factors are inflammatory and not cardiac specific; however, even these factors have potential for risk stratification. Efforts to expand and refine this list are ongoing, and basic research studies are pushing this field forward.

Multiple model systems are used in basic science research to expedite the identification of novel cardiac-secreted factors or cardiokines and define their effects on systemic metabolism. Two commonly utilized approaches are cell culture and conditional genetic mouse models. As previously discussed, to test the effect of cardiokine secretion on peripheral organs, animal models are ideal. Multiple Cre lines are available for conditional genetic manipulation in cardiac tissue, and the alpha-myosin heavy chain promoter is a popular cardiomyocyte-specific Cre. Additional Cre lines for cardiovascular research are reviewed elsewhere.¹⁷⁸

Although animal models can be time-consuming and costly, they are optimal for testing hypotheses related to endocrine and paracrine factors. This approach has recently yielded exciting data that could lead to the identification of a novel cardiokine. MED13/THRAP1/TRAP240 is a subunit of the Mediator complex that regulates transcription by bridging transcription factors with the RNA polymerase II machinery. In

the heart, and other organs, thyroid hormone signaling is an essential mediator of energy homeostasis. MED13 is hypothesized to modulate thyroid-hormone-dependent transcription, as reviewed here.¹⁷⁹ Cardiac-specific *Med13* transgenic mice (MED13cTg) exposed to a high-fat diet are resistant to obesity and show improved glucose tolerance.¹⁸⁰ Conversely, *Med13* knockout mice have increased susceptibility to high-fat-diet-induced obesity. Heterotypic parabiosis experiments between wild-type and MED13cTg mice strongly suggest that a secreted factor promotes the lean phenotype.¹⁸¹ The target organs for this suspected cardiokine are liver and adipose tissue, given that lipid oxidation is increased in these tissues, as well as their mitochondria number and metabolic gene expression. Future experiments are aimed at identifying this cardiokine and the receptor it binds.

As previously mentioned, cardiokines are synthesized and secreted from multiple cell types in the heart, including cardiomyocytes, fibroblasts, smooth muscle (aortic or blood-derived progenitors), resident immune cells, and vascular endothelial cells.¹⁸² Defining novel cardiokines by cell culture is therefore advantageous because this approach uses homogeneous cell populations that can be processed under controlled conditions. However, because of these diverse cellular interactions, a major drawback to this method is the difficulty modeling mixed cellular interactions. Paracrine interactions, particularly between cardiomyocytes and fibroblasts, are key mediators of cardiac stress responses. For example, cardiac fibroblasts secrete paracrine factors to cardiomyocytes that reduce cardiac conduction velocity and action potential upstroke velocity.¹⁸³ Thus, cell-culture approaches are ideal for modeling autocrine factors, not necessarily paracrine or endocrine factors, although technical issues are addressable and reviewed here.¹⁸⁴

Finally, the secretomes of cardiac cells are being defined under physiological and pathological conditions by multiple approaches, including gene expression arrays and cloning. Secreted proteins can enter classical (endoplasmic reticulum [ER]-dependent) or nonclassical (ER-independent) routes in cells. Regulated secretion is an active process that occurs in healthy and stressed cells. Apoptotic or necrotic cells, however, do not regulate protein secretion. Thus, although cardiac troponins are excellent biomarkers, they are not useful for mechanistic or pharmaceutical purposes.

Conclusions

Investigations into cardiac-specific regulation of metabolic processes could expand our understanding of organ cross-talk under physiological and pathological conditions. In addition to defining novel cardiokines for mechanistic and biomarker studies, future reports should address their biology including: synthesis and secretion, receptor expression and ligand

binding, and activation of downstream signaling cascades. The information in this review advocates for metabolic intervention during CVD. Ultimately, the search for novel cardiokines should reveal pharmacological targets at the level of target tissues, including receptor expression studies, and at the level of transcriptional regulation of stressed cardiac tissue.

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Disclosures

None.

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