MAYO CLINIC

An Emerging Role of Natriuretic Peptides: Igniting the Fat Furnace to Fuel and Warm the Heart

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Abstract

Natriuretic peptides are produced in the heart and have been well characterized for their actions in the cardiovascular system to promote diuresis and natriuresis, thereby contributing to maintenance of extracellular fluid volume and vascular tone. For this review, we scanned the literature using PubMed and MEDLINE using the following search terms: *beiging*, *adipose tissue*, *natriuretic peptides*, *obesity*, and *metabolic syndrome*. Articles were selected for inclusion if they represented primary data or review articles published from 1980 to 2015 from high-impact journals. With the advent of the newly approved class of drugs that inhibit the breakdown of natriuretic peptides, thereby increasing their circulation, we highlight additional functions for natriuretic peptides that have recently become appreciated, including their ability to drive lipolysis, facilitate beiging of adipose tissues, and promote lipid oxidation and mitochondrial respiration in skeletal muscle. We provide evidence for new roles for natriuretic peptides, emphasizing their ability to participate in body weight regulation and energy homeostasis and discuss how they may lead to novel strategies to treat obesity and the metabolic syndrome.

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Natriuretic peptides (NPs) have emerged as key regulators of metabolic processes, including activation of lipolysis, lipid oxidation, and mitochondrial respiration. Natriuretic peptides function as mediators in a cardiac-adipose tissue network, helping ensure an adequate supply of fuel for normal myocardial function. Although the cardiovascular effects of NPs are well known, this article focuses on the role of these peptides in regulating metabolism and energy expenditure. For this review, we scanned the literature through PubMed and selected recent data and review articles discussing the roles of NPs in adipose tissue function and metabolism. We first briefly discuss the traditional role of these peptides by citing seminal primary data and review articles (1980 to 1990s), and this is followed by descriptions of newly ascribed functions for NPs by limiting our inclusion to those primary data and review articles published after 2006 in high-impact journals.

TRADITIONAL ROLE OF NPs

Natriuretic peptides are members of a cardiac endocrine system that serves to regulate the fullness of the circulation. The initial discovery of this system came from studies demonstrating natriuresis after intravenous infusion of crude atrial extracts. The active factor in this response was localized to electron-dense secretory granules in the atria containing atrial natriuretic peptide (ANP). Atrial distention, brought about by acute volume expansion with saline, water immersion, postural changes, and salt feeding, all result in increased plasma ANP concentrations.[1,2] Although increased circulating volume is the most important stimulant of ANP release, various other factors can trigger the synthesis of ANP, including endothelin, platelet-activating factor, corticotropin-releasing factor, and glucagon-like peptide-1.[3-5] Brain natriuretic peptide (BNP) was originally described in porcine brain extracts but was later found to be highly expressed in the ventricular myocardium, and stress of the ventricular wall due to volume or pressure overload is the primary inducer of BNP synthesis.[6] Circulating ANP and BNP act on the kidney to promote diuresis and natriuresis and exert vasodilation on peripheral capacitance vessels.[7] Release of NPs can be viewed as both a sensing and an effector mechanism within

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the low-pressure atria and high-pressure ventricles serving to guard against adverse effects of intravascular volume expansion by stimulating the excretion of extracellular fluid volume and dampening the rise of mean arterial pressure that would otherwise occur (Figure 1).

CHEMISTRY OF NPs

Atrial natriuretic peptide is synthesized as a 151-amino acid preprohormone and is stored in atrial myocytes as a 126-amino acid prohormone (pro-ANP).[8] Upon secretion, pro-ANP is cleaved, yielding N-ANP and the biologically active C-terminal hormone (ANP). Brain natriuretic peptide is synthesized as a 134-amino acid preprohormone, which is then split into a signal peptide and a 108-amino acid propeptide (pro-BNP). Upon secretion from the cardiomyocyte, pro-BNP is split into equimolar amounts to the physiologically active BNP (32 amino acids) and a biologically inactive 76-amino acid fragment NT-pro-BNP. Brain natriuretic peptide is structurally similar to ANP, with 17 of the 32 amino acids sharing a common amino acid sequence. C-type natriuretic peptide (CNP) is a third member of this family originally identified in porcine brain and is distributed throughout the central nervous system but is mainly produced by the vascular endothelium. It acts as a local paracrine factor in controlling the vascular tone, and it appears that this peptide is only weakly natriuretic.[9] Dendroaspis NP, isolated from the venom of the green Mamba snake *Dendroaspis angusticeps*, is a 38-amino acid peptide containing a 17-amino acid disulfide ring structure similar to that of ANP, BNP, and CNP. It is present in human plasma and is elevated in congestive heart failure.[10] Like ANP and BNP, this peptide also exerts natriuretic and diuretic effects linked to cyclic guanosine monophosphate (cGMP) in the renal tubule. Urodilatin is an NH_2 terminal—extended form of circulating ANP; however, unlike ANP, this peptide is not found in systemic circulation but rather is synthesized in the kidney, in which it acts as a paracrine factor. Similar to ANP, this peptide exerts a natriuretic effect and presumably participates in regulation of natriuresis under physiologic conditions.[11]

The NPs mediate their biologic effects through a family of particulate guanylyl cyclase receptors (natriuretic peptide receptor [NPR] A NPR-A, NPR-B, and NPR-C).[8] Affinity of ANP and BNP is greatest for NPR-A, whereas that of CNP is much higher for NPR-B. All 3 NPs bind to a third receptor (NPR-C) that does not contain guanylyl cyclase and functions predominately as a clearance receptor. The NPR-C is mainly present in adipose tissue and kidney. The affinity of the clearance receptor is much greater for ANP versus BNP, possibly accounting for the 7-fold longer half-life of BNP as compared with that of ANP.[12,13] Natriuretic peptides are also cleared by the neural endopeptidase neprilysin, a metalloproteinase richly found in the kidney and lung.[14]

ARTICLE HIGHLIGHTS

- With the approval of the Food and Drug Administration for the neprilysin inhibitor, there may be new off-target benefits associated with increasing the circulating levels of natriuretic peptides, including "beiging" of adipose tissue, improvements in glucose homeostasis, increased energy expenditure, and reductions in overall body weight. This review highlights potential mechanisms by which these beneficial metabolic effects may occur.
- Men and women differ with respect to their circulating levels of natriuretic peptides. These differences may be important in prescribing and dosing neprilysin inhibitors. This review highlights how estrogens potentiate the effects of natriuretic peptides and provides teleological explanations for the sexual dimorphism observed.
- Obesity incidence is on the rise, and transforming adipose tissues to be more metabolically active may provide a novel and important therapeutic advantage by increasing energy expenditure. This review highlights an important role for natriuretic peptides in shivering and nonshivering thermogenesis and energy expenditure.
- Heart failure is seen by all clinicians and is a leading cause of death. A diseased heart augments its fuel utilization in ways that are detrimental. This review discusses the role of natriuretic peptides in functioning as mediators in a cardiac-adipose tissue network, helping ensure an adequate supply of fuel for normal myocardial function.

NATRIURETIC PEPTIDES AND METABOLISM AND ENERGY EXPENDITURE

Natriuretic peptides have long been known to stimulate lipolysis in adipocytes in a manner

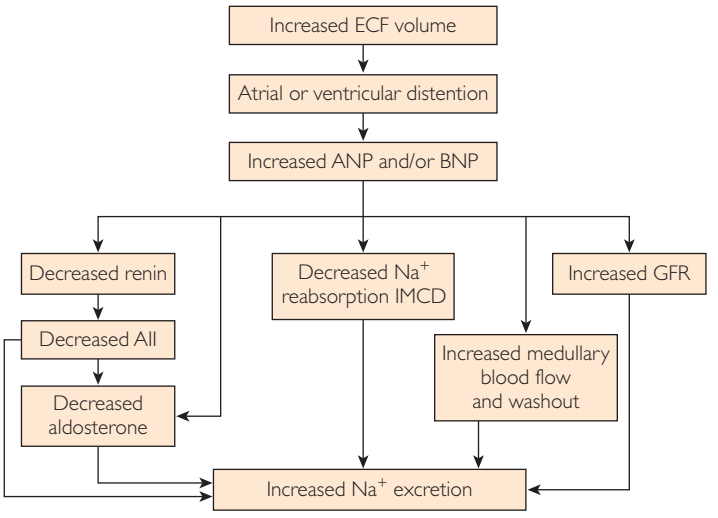

FIGURE 1. Effects of natriuretic peptides on renal function and mechanism by which urinary Na^+ excretion is increased. ANP = atrial natriuretic peptide; BNP = brain natriuretic peptide; ECF = extracellular fluid volume; GFR = glomerular filtration rate; IMCD = inner medullary collecting duct.

distinct from the lipolytic effect of catecholamines. Catecholamines bind to β-adrenergic receptors, leading to cAMP-dependent activation of hormone-sensitive lipase. Insulin antagonizes this effect by degrading cAMP through the activation of phosphodiesterase 3B.[15] In contrast, NPs bind to NPR-A on adipocytes and promote perilipin and hormone-sensitive lipase-mediating triglyceride degradation through a cGMP-dependent protein kinase G—activating pathway.[16] These distinct pathways account for an additive effect in promoting lipolysis in human adipocytes when ANP and β-agonists are combined.[17]

The NPR-C receptors on adipocytes reduce NPR-A—mediated lipolysis by lowering local natriuretic peptide bioavailability. In this regard, rodents that express NPR-C at high levels on adipocytes have a blunted lipolytic response to NPs in comparison to primates. Lipolysis is fully restored in mice when NPR-C is deleted.[18] In obese humans, circulating NP levels are suppressed when compared with those in lean subjects.[19] Studies in obese subjects with congestive heart failure show a graded inverse relationship between NP levels and body mass index (BMI; calculated as the weight in kilograms divided by the height in meters squared).[20]

Obesity is associated with increased expression of NPR-C in adipose tissue, implicating increased degradation of NPs as facilitating weight gain.[21] Hyperinsulinemia (commonly present in obesity) induces NPR-C expression in human adipocytes and monocytes.[22] In addition, neprilysin, the NP degrading endopeptidase, is expressed in human adipose tissue and at increased levels in obesity.[23] Taken together, these findings argue for accelerated NP clearance due to a shift in the NPR-A/NPR-C ratio toward NPR-C and increased neprilysin levels in obese humans.

A combination drug containing sacubitril (a neprilysin inhibitor) and valsartan (an angiotensin II receptor blocker) was recently approved to reduce the risk of death and hospitalization in patients with chronic heart failure.[24] It is interesting to speculate that although there are improvements in cardiovascular effects such as increased vasodilation and natriuresis after drug administration, additional clinical benefits may relate to enhanced NP activity in adipose tissue, particularly in perivascular and epicardial adipose depots. Perivascular and epicardial adipose tissues are metabolically active adipose tissue depots that secrete several bioactive proteins to include adiponectin, resistin, and various anti-inflammatory molecules.[25-27] Specifically, ANP influences epicardial/perivascular adipose tissue to stimulate adiponectin production, which, in turn, stimulates vasodilation and nitric oxide release and reduces inflammation.[28] As depicted in Figure 2, one can speculate that additional benefits of this combinatorial drug may be both through its effects on the renin-angiotensin system and through increasing the half-life of the NPs, which may, in turn, directly influence cardiac contractility through effects on perivascular and epicardial adipose tissue depots.

WHY DOES RELEASE OF NPs BY THE HEART RESULT IN LIPOLYSIS?

The heart has a very high energy demand and must continually generate adenosine triphosphate (ATP) to sustain contractile function, basal metabolic processes, and ionic homeostasis. The heart acts as an "omnivore" and can use various carbon substrates as energy sources, including fatty acids, glucose, lactate, ketones, pyruvate, and amino acids. Under normal circumstances, fatty acid β-oxidation is the preferred substrate for the heart, accounting for 50% to 70% of ATP production, with the remainder primarily

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coming from the oxidation of pyruvate derived from glycolysis and lactate oxidation.[29]

Under physiological conditions, the release of free fatty acids (FFAs) from adipose is well regulated; therefore, appropriate amounts of FFAs are released to meet energy requirements. With exercise, release of NPs along with sympathetic nerve activation contributes to increased lipolysis in adipocytes to ensure delivery of FFAs in an amount necessary to meet myocardial energy demands. In addition, plasma levels of ANP have been shown to significantly increase after both short-term and endurance exercise.[30] Intravenous administration of ANP increases lipid oxidation acutely and increases postprandial energy expenditure in healthy men.[31] The NPs enhance lipid oxidation and mitochondrial respiration in human skeletal muscle and may contribute to exercise training–induced improvements in skeletal muscle fat oxidation.[32]

When energy intake exceeds expenditure, as in obesity, circulating levels of FFAs and triglyceride are elevated. Increased cardiac fatty acid β-oxidation rates, as seen in obesity or diabetes, have been implicated in the development of impaired cardiac function. In this setting, an increased reliance on fatty acids relative to glucose as a metabolic fuel decreases cardiac efficiency for any given level of left ventricular work.[33,34] This adverse effect may be related to higher oxygen requirements for fatty acid metabolism as compared to glucose. Research is now being focused on inhibiting fatty acid β-oxidation in obesity, diabetes, heart failure, and ischemic heart disease as an approach to improve heart function.[29] Circulating levels of NPs are suppressed in obesity, and reduced levels are viewed as an appropriate response to limit further mobilization of fatty acids from adipocytes when circulating levels are already in excess (Table 1).

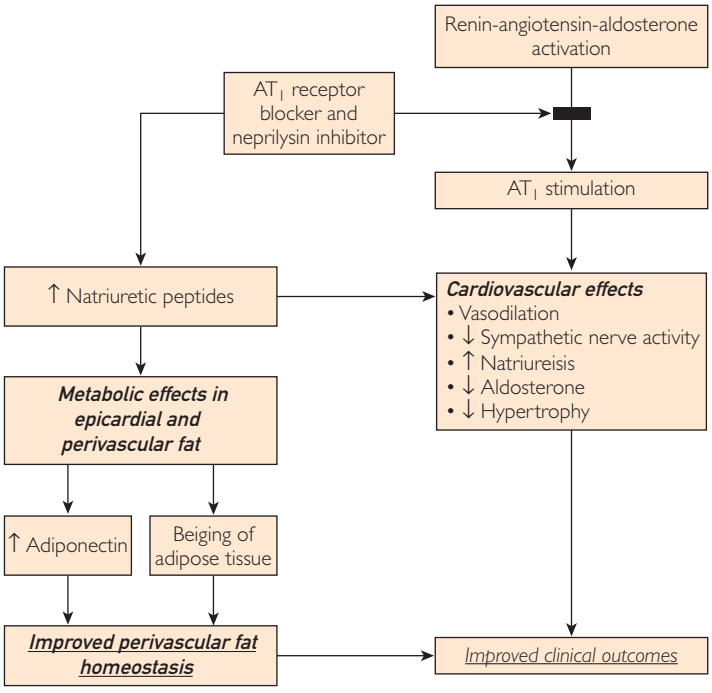

FIGURE 2. Mechanisms by which combined angiotensin receptor blockade and neprilysin inhibition may provide clinical benefits in patients with congestive heart failure. In addition to well-recognized cardiovascular functions, increased natriuretic peptides may lead to improvements in metabolic homeostasis in epicardial and perivascular fat depots providing local beneficial effects on myocardial function. AT_1 = angiotensin receptor subtype 1.

NATRIURETIC PEPTIDES AND THERMOGENESIS

On exposure to cold, metabolic and physiologic processes are activated to reduce rates of heat loss and increase rates of heat production to maintain core body temperature at approximately 37°C. Shivering is an involuntary rhythmic tremor of skeletal muscle that can be elicited within minutes and can progress to severe shivering, involving generalized involuntary movement of all muscle groups. Maximum heat production can increase up to 5 times above baseline values in association with increased expenditure of energy, consumption of oxygen, and production of carbon dioxide.[35] The ATP required for skeletal muscle contraction is initially generated from the oxidation of glucose, followed by lipids. Once depletion of glycogen stores occurs, and depending on the intensity of shivering, protein oxidation can begin to make a substantial contribution to ATP generation.

In contrast, nonshivering thermogenesis is a mechanism of cold-induced heat production *not* associated with muscle activity induced by shivering and is linked to metabolic activity in brown adipose tissue (BAT). The mass of BAT increases and regresses as a function of cold exposure. Although heat production is quantitatively greater with shivering, nonshivering thermogenesis provides a first-line defense

TABLE 1. Functions of Natriuretic Peptides

A. Maintenance of extracellular fluid
a. Increased urinary sodium excretion
b. Vasodilation to minimize increases in blood pressure
B. Contribution to availability of preferred fuel substrate for myocardial metabolism
C. Enhancement of skeletal muscle exercise performance
a. Enhances lipolysis and provides fatty acids for fuel
b. Enhances lipid oxidation and mitochondrial respiration
D. Maintenance of body temperature
a. Increases nonshivering thermogenesis through beige cell recruitment
b. Enhances shivering thermogenesis by providing fuel source through lipolysis
c. Stimulates appetite through inhibition of leptin release
E. Contribution to brown fat accumulation of newborn
F. Contribution to the greater amount of brown fat in women versus men
G. Contribution to adaptation in hypoxia
a. Attenuates pulmonary vasoconstriction
b. Mobilizes fatty acids for myocardial use

for the maintenance of body temperature in the cold, with far less expenditure of metabolic fuel.

The ability to maintain core body temperature after exposure to cold is important to the heart. Chronic exposure to cold causes a volume-overloaded hypertrophy of both ventricles, which is associated with deterioration in cardiac performance.[36,37] Cold exposure also leads to increased circulating levels of ANP.[38,39] The NPs mobilize fatty acids and enhance lipid oxidation and mitochondrial respiration in human skeletal muscle, helping ensure maintenance of body temperature for optimal cardiac function.

NATRIURETIC PEPTIDES AND NONSHIVERING THERMOGENESIS

White adipose tissue (WAT) primarily functions as an energy storage depot within the body. Breakdown of triglycerides in white adipocytes delivers FFAs into the circulation to serve as a fuel to other organs. Under conditions of nutrient excess or surplus, excess energy is stored in the form of triglycerides initially by increasing adipocyte storage capacity, hyperplasia, or size through hypertrophy. Once the maximum storage capacity is reached, a population of preadipocytes and/or progenitor cells can be induced to differentiate into mature adipocytes to accommodate the incoming energy.[40]

As previously mentioned, BAT is another type of adipose tissue that functions by dissipation of energy through the production of heat.[41,42] It is densely packed with mitochondria and generates heat through an uncoupling protein called UCP-1 located on the inner mitochondrial membrane. The UCP-1 short-circuits the mitochondrial proton gradient, so that oxygen consumption is no longer coupled to ATP synthesis. The UCP-1 uncouples mitochondrial oxidative phosphorylation from ATP production and dissipates chemical energy as heat. Mice lacking UCP-1 are severely compromised in their ability to maintain normal body temperature when acutely exposed to cold, and they are also prone to become obese.[43] The BAT cells are derived from progenitors expressing myogenic factor 5, indicating they are derived from the same or similar progenitors as skeletal muscle.[44] Brown adipose tissue is richly innervated by sympathetic nerve efferent fibers and is highly vascularized to allow dissipation of generated heat. Brown adipose tissue is abundant in small mammals or newborns who have a small body volume-to-body surface ratio and therefore have difficulty maintaining an adequate core body temperature through muscle shivering alone.

In adults, BAT had long been considered to be absent or of no relevance. This view changed when a series of articles using positron emission tomographic (PET) images and molecular analysis determined that there are significant amounts of metabolically active BAT in humans.[45-48] Studies that predispose subjects to cold temperatures for 1 to 2 hours increase the prevalence of BAT-positive scans from a baseline value of less than 15% to as high as 96%.[46] Although short-term exposure to cold activates existing depots of BAT, repetitive exposure or more prolonged exposure leads to expansion of BAT mass.

In human autopsy studies, BAT can be identified around the aorta, common carotid artery, brachiocephaclic artery, paracardial mediastinal fat, epicardial coronary artery and veins, internal mammary artery, and intercostal artery and veins.[49-51] In the past several years, using magnetic resonance imaging, several laboratories were able to detect intrascapular BAT mass in thermoneutrality as well as in response to cold stress in humans.[48,49]

An additional type of fat is called "beige" or "brite" cells and is derived from progenitors different from the classical BAT myogenic factor 5 lineage.[52] These cells are defined by their multilocular lipid droplet morphology and

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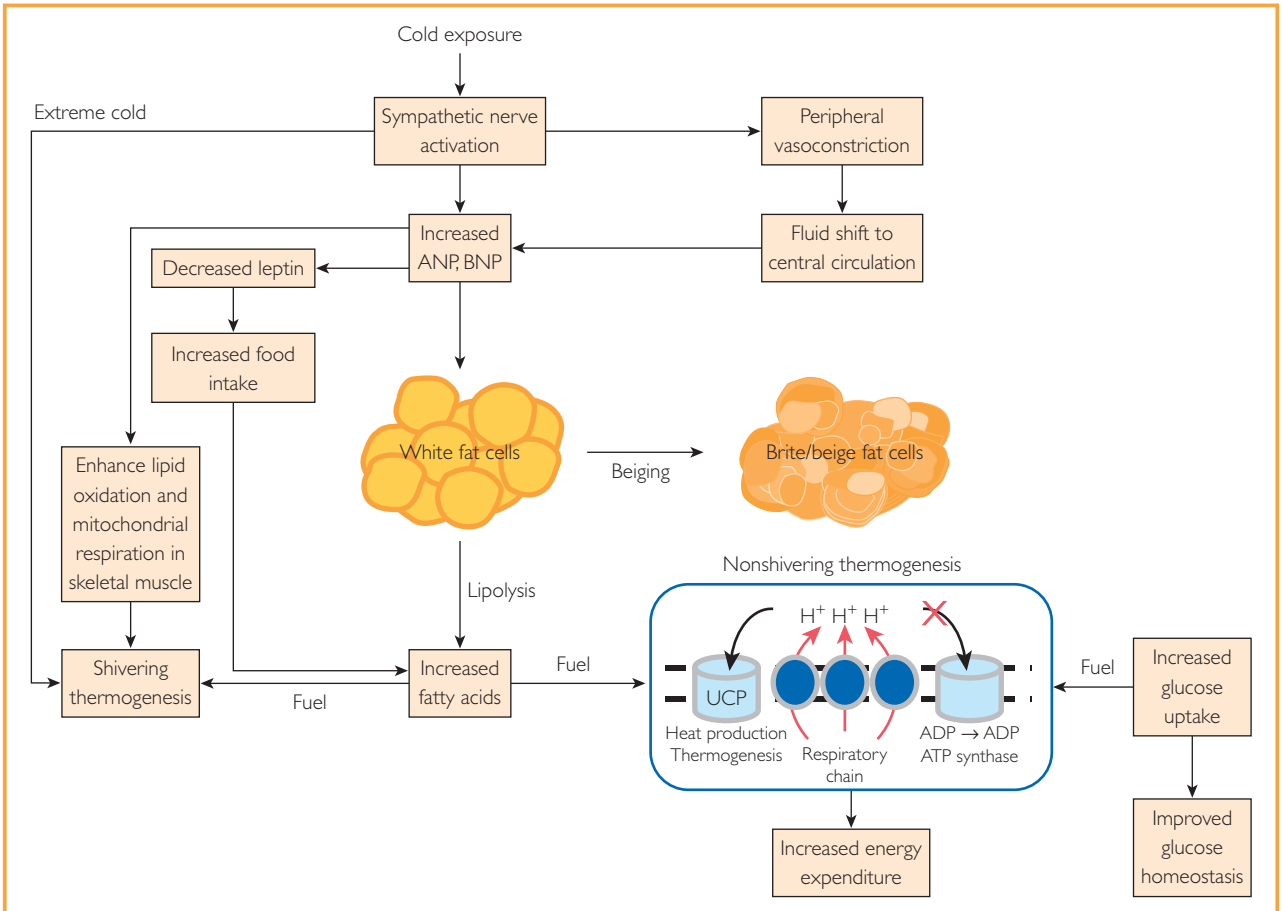

FIGURE 3. The role of natriuretic peptides in shivering and nonshivering thermogenesis with cold exposure (see text for detailed discussion). ADP = adenosine diphosphate; ANP = atrial natriuretic peptide; ATP = adenosine triphosphate; BNP = brain natriuretic peptide; UCP = uncoupling protein.

high mitochondrial content and exhibit the same properties as classical BAT with respect to UCP-1–mediated thermogenesis. There is a unique pattern of gene expression differentiating beige adipocytes from BAT. Transformation of classical white adipocytes into beige adipocytes is referred to as "browning," and these pluripotent cells can revert to white adipocytes after exposure to heat. Following a second period of cold adaptation, these same cells regain the typical multilocular morphology and specific gene expression profile of beige adipocytes.[53-55] The presence of brown, beige, and white adipocytes highlights the heterogeneity of adipose tissues and links their diverse functions to energy metabolism.

Activation of the sympathetic nervous system plays a key role in the ability of cold exposure to activate and expand BAT in both rodents and humans. Stimulation of β-adrenergic receptors on adipocytes through cAMP-dependent protein kinase A activates p38 MAPK, which, in turn, mediates a transcriptional response cascade resulting in increased expression of UCP-1 and peroxisome proliferator-activated receptor-gamma coactivator (PGC-1α).[56,57] The UCP-1 dissipates the proton gradient along the inner mitochondrial bilayer such that lipid oxidation is redirected from ATP synthesis and toward generation of uncoupling, thereby releasing heat, and PGC-1α activates mitochondrial biogenesis and thereby thermogenesis.[58]

Natriuretic peptides participate in cross talk with sympathetic nerve activity after a drop in ambient temperature. As previously discussed, NPs have been implicated in promoting nonshivering thermogenesis and increasing energy

TABLE 2. Characteristics of Natriuretic Peptides in Various Conditions

Parameter	Obesity	Hypoxia	Premenopausal women as compared with men	Roux-en-Y gastric bypass
Natriuretic peptide (NP) levels	Reduced Low levels can be viewed as appropriate because circulating levels of fatty acids are already in excess	Increased Hypoxia-inducible factor increases the transcription of NPs High levels serve to attenuate pulmonary vasoconstriction and enhance the mobilization of fatty acids as a fuel source for myocardium	Increased Estrogens transcriptionally regulate NPs NPs may participate in brown fat accumulation in newborns	Increased Increased GLP-1 levels may stimulate release
Ratio of the clearance receptor NPR-C to active receptor NPR-A	Increased Hyerinsulinemia typically present in obesity upregulates NPR-C; neprilysin expression (NP degrading endopeptidase) is also increased in adipose tissue	Reduced Reduced NPR-C expression with subsequent increased NP levels in chronic hypoxia attenuates pulmonary vasoconstriction	Reduced Estrogen directly increases NRP-A expression	Reduced
Brown and beige fat	Reduced Low NP levels along with catecholamine resistance may contribute to decrease in brown fat	Not well studied Likely increased in part driven by lactate[72] No change detected in animal model of intermittent hypoxia[73]	Increased quantity Teleologically may be due to the greater risk of hypothermia in women	Increased quantity Participates in mechanism of weight loss

GLP-1 = glucagon-like peptide-1; NP = natriuretic peptide; NPR-A = natriuretic peptide receptor A; NPR-C = natriuretic peptide receptor C.

expenditure mediated by BAT. Activation of cGMP-dependent protein kinase through NPR-A on adipocytes not only stimulates lipolysis but also activates p38 MAPK, leading to increased expression of UCP-1 and PGC-1α, markers consistent with activation of BAT thermogenesis.[18] The cGMP-mediated browning effect of NPs may underlie the ability of sildenafil (a phosphodiesterase-5 inhibitor) to cause browning.[59] The parallel pathways by which sympathetic nerves and NPs convergence on p38 MAPK suggest cooperativity in these systems to activate the machinery required for adaptive thermogenesis. In this regard, cardiac expression and circulating levels of ANP and BNP are both increased after exposure to cold temperature.[39,60] Cold exposure increases the expression of NPR-A and reduces the expression of NPR-C in both BAT and WAT, creating an environment favoring natriuretic peptide induction of beige fat cellular formation.[18] Selective β_3-adrenergic receptor activation in adipose tissues of mice produces a similar shift in the ratio of NP receptors, implicating that sympathetic nerves play a role in this response.[60]

Cold exposure induces activation of adrenergic receptors and upregulation and translocation of glucose transporters (GLUTs), with GLUT1 and GLUT4 facilitating the uptake of glucose into the cell to be used for triglyceride formation and/or be "burned" to generate heat.[61-63] In adult humans exposed to cold, glucose uptake increases 12-fold in BAT and is correlated with an increase in whole body energy expenditure.[64] Cold-induced thermogenesis decreases body weight by inducing transformation of white/storage adipocytes into beige/burning adipocytes.[65,66] The capacity to increase clearance of glucose in beige adipocytes after exposure to cold is also associated with improved insulin sensitivity and glucose homeostasis.[67,68] A great deal of interest is now focused on ways to increase the mass of beige cells to capitalize on their thermogenic potential as a method to increase total body energy expenditure and reduce body fat mass through enhanced lipolysis, thereby providing a novel approach to combat obesity and associated metabolic disorders (Figure 3).[69]

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NATRIURETIC PEPTIDES AND HYPOXIA

Exposure to hypoxia in humans and rodent models leads to increased circulating levels of NPs.[70,71] The vasodilating properties of NPs serves to limit hypoxia-induced pulmonary vasoconstriction and attenuate hypoxia-induced pulmonary hypertension and vascular remodeling through direct transcriptional regulation of hypoxia-inducible factor (HIF)-induced expression of vascular endothelial growth factor (Table 2). Stabilization of HIF-1 under conditions of hypoxia leads to increased ANP and BNP gene expression, suggesting that HIF interacts with the promotor region of these genes to induce increased expression of the NPs.[71,74]

Altitude (hypobaric hypoxia) also activates factors known to cause browning of adipose tissue. Although not well studied in humans, animals taken to high altitude undergo significant browning potentially due to cold exposure, increased levels of NPs, and activation of sympathetic nerve activity. Interestingly, animals such as the plateau pika (*Ochotona curzoniae*), also known as the black-lipped pika, mostly undergo browning of visceral fat, whereas most studies in humans demonstrate that recruitment of brown fat occurs in subcutaneous fat.[75] One can speculate that browning in subcutaneous fat would be much less efficient at defending core temperature because these animals have short appendages leading to only small volumes of blood at any one time exposed to the environment. In contrast, in humans, expansion of subcutaneous BAT in the cervical-axillary region and inguinal fossa is more strategically located in defending core body temperature because much greater volumes of blood in the arms and legs are exposed to cold. It is likely that NPs conspire with sympathetic nerves to facilitate the browning of adipose tissue in this setting. Exposure of mice to cold increases HIF gene expression in BAT, and these effects may be relevant to the browning of WAT as HIF activation shifts metabolism toward glycolysis and increased lactate production.[76,77] Lactate accumulation may induce browning of WAT with expression of functional UCP-1. Last, vascular endothelial growth factor is another HIF-induced protein whose expression in BAT is upregulated on exposure to cold.[78] Vascular endothelial growth factor plays a critical role in expanding the thermogenic capacity of BAT by promoting angiogenesis and increasing the density of blood vessels, allowing for heat dissipation.

In response to cold, activity of BAT mostly relies on lipid metabolism to generate fatty acids that directly activate UCP1. In unstimulated BAT, the source of fatty acids is mostly the stored triglyceride within BAT itself, and, in part, de novo synthesis from glucose cleared from the circulation by the upregulation of GLUTs.[42] Cold exposure leads to an increase in lipoprotein lipase activity, allowing enhanced fatty acid synthesis leading to increased uptake and internalization of plasma triglycerides. Norepinephrine increases the amount of lipoprotein lipase gene expression through cAMP.[79,80] Activation of BAT after exposure to cold has been shown to correct hyperlipidemia and improve the deleterious effects of fatty acid on insulin resistance in an animal model of diet-induced obesity and insulin resistance.[81-83]

In addition to lipolysis and delivery of fatty acids, NPs contribute to fuel availability through increasing food intake. Natriuretic peptides decrease circulating leptin levels by inhibiting its release from adipocytes.[84] Leptin is an adipokine that is generated and released selectively from adipocytes. Leptin binds to its receptors in the hypothalamus and regulates food intake and energy expenditure. Conversely, reductions in circulating leptin levels, which are seen after weight loss, are associated with increased food intake. Cold exposure reduces leptin levels and causes increased food intake, providing an exogenous source of fuel for continued maintenance of nonshivering thermogenesis.[85,86]

SEXUAL DIMORPHISM AND NPs

Women have greater quantities of BAT as measured by PET-computed tomography imaging studies.[45] Women have a higher metabolic rate per kilogram adipose tissue than do men because women have more BAT and higher expression of genes involved in mitochondrial function including UCP-1.[45,46] Estrogens, through activation of estrogen receptor α, increase brain-derived neurotropic factor in the hypothalamus, which induces browning through neural modulation of WAT.[87,88] Circulating ANP and BNP concentrations are higher in women than in men, providing

an additional factor as to why women have more BAT than do men[89-91] (Table 2). In girls during adolescence, the NPs increase progressively and reach 2-fold higher levels in women with normal menstrual cycles than in men at the same age.[89] After menopause, women have levels of NPs similar to those in men, which correlates to reductions in BAT in women after menopause. Furthermore, estrogen administration increases circulating ANP levels in postmenopausal women, and estradiol enhances NP activity by reducing NPR-C and increasing NPR-A expression levels.[89,91] Higher levels of NPs and BAT may serve a protective role in premenopausal women for maintenance of body temperature because premenopausal women have a higher surface area-to-volume ratio than do men and therefore are more prone to hypothermia.

During pregnancy, the basal metabolic rate progressively increases, particularly in the third trimester; however, the increase in metabolic rate is thought to be disproportionate to the increase in body adiposity, suggesting that the accumulated adipose tissue has enhanced metabolic activity.[92] Perhaps elevated levels of estrogens during pregnancy contribute to increasing the metabolic rate by enhancing the browning of adipose tissues mediated by ANP and BNP, which progressively rise during the course of pregnancy and reach a plateau during the last trimester.[93]

Finally, NP levels are elevated in infants during the first several weeks after birth, and at the same time the newborn has accumulated large amounts of BAT.[94,95] It is interesting to speculate whether estrogens influence placental production of ANP and whether this accounts for higher levels of NPs in the developing infant contributing to the accumulation of BAT.[96,97]

OBESITY

Obesity is associated with increased fat deposition in the classical WAT depots such as the visceral and subcutaneous depot. After expansion of WAT in obesity, obese humans characteristically have reductions in BAT activity, which facilitates further weight gain. In mice and humans, nutrient overload associated with caloric excess and obesity induces catecholamine resistance in adipose tissue, resulting in suppressed lipolysis and fatty acid oxidation with the net effect of enhancing fat accumulation.[98] Catecholamine resistance in adipose tissue is thought to occur by down-regulating β-adrenergic receptor expression and interfering with downstream β-adrenergic receptor signaling pathways, such as protein kinase A activity.[99,100] The development of catecholamine resistance also limits the activation of BAT and the browning of adipose tissue given the central role of β-adrenergic stimulation in the activation and recruitment of BAT.

There is an inverse relationship between BMI and BAT. The energy-burning feature of BAT and the evidence of an inverse correlation between BAT metabolism and BMI have given rise to the hypothesis that obesity may be linked to BAT malfunction. One potential contributor to this malfunction is resistance to the effects of NPs. Leptin levels are increased in obese subjects, but there is end-organ resistance at the level of the hypothalamus. In addition, obesity is characterized by insulin resistance. This combined resistance would be additional factors limiting browning, as leptin and insulin have been shown to induce browning through a synergistic effect in the arcuate nucleus of the hypothalamus, specifically at the level of the pro-opiomelanocortin neuron.[101]

Obesity is characterized by high levels of circulating fatty acids, which contribute to insulin resistance.[102] In addition, high levels of fatty acids have been shown to exert adverse effects on cardiac function. As mentioned previously, low levels of NPs and increased expression of the NPR-C clearance receptor on adipocytes in obesity can be viewed as an appropriate response in this setting because natriuretic peptide—mediated lipolysis would be minimized.[19,21,103] However, low levels are also detrimental to body weight regulation, as thermogenesis and energy expenditure would be reduced (Table 2).

During times of abundant food supply, there was a biological need to enhance the storage capacity of adipocytes to facilitate survival upon starvation. The availability of foods with high caloric content in industrialized societies, together with reduced physical activity, has resulted in an obesity pandemic. Adipocytes possess remarkable adaptive plasticity to maintain metabolic homeostasis in conditions of excess nutrients, fasting, or cold exposure. Identifying critical regulators of adaptive

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responses to metabolic stress in adipocytes may lead to interventional strategies to promote more metabolically active adipose tissue and recruitment of beige adipoctyes and improve metabolic health in obesity.

In morbidly obese subjects who undergo weight loss after adjustable gastric banding surgery, cold-activated BAT mass increases as detected by fluorodeoxyglucose-PET scanning.[104-107] The precise mechanism by which this occurs is not known. Brown adipose tissue holds therapeutic potential for ameliorating obesity and the metabolic syndrome via increasing energy expenditure. Weight loss in severely obese subjects and in subjects with type 2 diabetes substantially increases BAT activity, suggesting that weight loss per se may contribute by restoring sensitivity to adrenergic stimulation in WAT as well as sensitivity to insulin and leptin in the hypothalamus, and here we posit that additional benefits of weight loss may be mediated through increased activity of the NPs to induce browning of adipose tissues. Specifically, weight loss after Roux-en-Y gastric bypass in rodents has been demonstrated to induce adipose tissue browning, which occurs independent of weight loss per se.[108] After this procedure, circulating levels of NPs are increased and there is an alteration in the NPR ratio favoring an elevation in NPR-A/B and a reduction in the expression of NPR-C consistent with elevations in UCP-1 and NP-induced browning of WAT.[108] Gastric bypass is also known to increase circulating levels of GLP-1, and in this regard GLP-1 is known to stimulate the release of NPs.[5,104] Increased NPs along with improved sensitivity to adrenergic nerves in adipose tissue would provide a favorable environment for browning to occur. In addition, improvements in insulin and leptin sensitivity centrally would facilitate the potential for browning of adipose tissues and would contribute to improvements in insulin sensitivity and metabolism seen after this procedure.

CONCLUSION

Although most clinicians are aware of the renal effects and natriuretic properties of NPs, there is much less awareness of how these peptides regulate energy homeostasis. With the recent approval and proven efficacy of a combination drug containing sacubitril and valsartan that enhances the effectiveness of NPs for the treatment of patients with heart failure, it is now important and timely to revisit the myriad functions of this class of peptides. In this comprehensive review, we have introduced novel ideas and provide a synthesis of literature with respect to NPs. We specifically focused on the functions of these peptides in the regulation of body weight and energy homeostasis and discussed the role of NPs in transforming adipose tissue into a more highly active endocrine organ through "beiging/browning." In addition, the role of these peptides in nonshivering thermogenesis and how they are affected in obesity supports their potential critical function in energy homeostasis. In our review, we provided an insight into the role of NPs in a cardiac-adipose tissue loop, a new feedback loop that is gaining appreciation and attention. Last, we discussed the sexual dimorphism that exists in the metabolism of these compounds. In summary, this review addressed new therapeutic actions of NPs at a point when the Food and Drug Administration has recently approved a drug that augments these peptides. We discussed a potential therapeutic use of these peptides by providing a pharmacological approach to increasing energy expenditure and transitioning WAT into metabolically active BAT. With the appreciation that adult humans possess active BAT, fighting obesity by increasing adipose tissue energy expenditure induced by NPs and their receptors has emerged as a promising strategy and has the potential to reduce manifestations of end organ injury.

Abbreviations and Acronyms: **ANP** = atrial natriuretic peptide; **ATP** = adenosine triphosphate; **BAT** = brown adipose tissue; **BMI** = body mass index; **BNP** = brain natriuretic peptide; **cGMP** = cyclic guanosine monophosphate; **CNP** = C-type natriuretic peptide; **FFA** = free fatty acid; **GLUT** = glucose transporter; **HIF** = hypoxia-inducible factor; **NP** = natriuretic peptide; **NPR** = natriuretic peptide receptor; **PGC-1α** = peroxisome proliferator-activated receptor-gamma coactivator; **PET** = positron emission tomographic; **UCP-1** = uncoupling protein 1; **WAT** = white adipose tissue

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REFERENCES

1. Lang RE, Thölken H, Ganten D, Luft FC, Ruskoaho H, Unger T. Atrial natriuretic factor is a circulating hormone

stimulated by volume loading. *Nature*. 1985;314(6008):264-266.
2. Sagnella GA, Markandu ND, Shore AC, MacGregor GA. Effects of changes in dietary sodium intake and saline infusion on immunoreactive atrial natriuretic peptide in human plasma. *Lancet*. 1985;2(8466):1208-1211.
3. Qu XW, Rozenfeld RA, Huang W, Crawford SE, Gonzalez-Crussi F, Hsueh W. Interaction of platelet-activating factor, spleen and atrial natriuretic peptide in plasma volume regulation during endotoxaemia in rats. *J Physiol*. 1998;512(Pt 1):227-234.
4. Tojo K, Sato S, Tokudome G, et al. Stimulation by corticotropin-releasing factor of atrial natriuretic peptide and brain natriuretic peptide secretions from cultured neonatal rat cardiomyocytes. *Biochem Biophys Res Commun*. 1996;225(2):340-346.
5. Kim M, Platt MJ, Shibasaki T, et al. GLP-1 receptor activation and Epac2 link atrial natriuretic peptide secretion to control of blood pressure. *Nat Med*. 2013;19(5):567-575.
6. Sudoh T, Kangawa K, Minamino N, Matsuo H. A new natriuretic peptide in porcine brain. *Nature*. 1988;332(6159):78-81.
7. Maack T, Camargo MJ, Kleinert HD, Laragh JH, Atlas SA. Atrial natriuretic factor: structure and functional properties. *Kidney Int*. 1985;27(4):607-615.
8. Schlueter N, de Sterke A, Willmes DM, Spranger J, Jordan J, Birkenfeld AL. Metabolic actions of natriuretic peptides and therapeutic potential in the metabolic syndrome. *Pharmacol Ther*. 2014;144(1):12-27.
9. Pham I, Sediame S, Maistre G, et al. Renal and vascular effects of C-type and atrial natriuretic peptides in humans. *Am J Physiol*. 1997;273(4, Pt 2):R1457-R1464.
10. Lisy O, Lainchbury JG, Leskinen H, Burnett JC Jr. Therapeutic actions of a new synthetic vasoactive and natriuretic peptide, dendroaspis natriuretic peptide, in experimental severe congestive heart failure. *Hypertension*. 2001;37(4):1089-1094.
11. Meyer M, Richter R, Brunkhorst R, et al. Urodilatin is involved in sodium homeostasis and exerts sodium-state-dependent natriuretic and diuretic effects. *Am J Physiol*. 1996;271(3, Pt 2):F489-F497.
12. Nagase M, Katafuchi T, Hirose S, Fujita T. Tissue distribution and localization of natriuretic peptide receptor subtypes in stroke prone spontaneously hypertensive rats. *J Hypertens*. 1997;15(11):1235-1243.
13. Suga S, Nakao K, Hosoda K, et al. Receptor selectivity of natriuretic peptide family, atrial natriuretic peptide, brain natriuretic peptide, and C-type natriuretic peptide. *Endocrinology*. 1992;130(1):229-239.
14. Kenny AJ, Bourne A, Ingram J. Hydrolysis of human and pig brain natriuretic peptides, urodilatin, C-type natriuretic peptide and some C-receptor ligands by endopeptidase-24.11. *Biochem J*. 1993;291(Pt 1):83-88.
15. Choi YH, Park S, Hockman S, et al. Alterations in regulation of energy homeostasis in cyclic nucleotide phosphodiesterase 3B-null mice. *J Clin Invest*. 2006;116(12):3240-3251.
16. Sengenes C, Bouloumie A, Hauner H, et al. Involvement of a cGMP-dependent pathway in the natriuretic peptide-mediated hormone-sensitive lipase phosphorylation in human adipocytes. *J Biol Chem*. 2003;278(49):48617-48626.
17. Moro C, Galitzky J, Sengenes C, Crampes F, Lafontan M, Berlan M. Functional and pharmacological characterization of the natriuretic peptide-dependent lipolytic pathway in human fat cells. *J Pharmacol Exp Ther*. 2004;308(3):984-992.
18. Bordicchia M, Liu D, Amri EZ, et al. Cardiac natriuretic peptides act via p38 MAPK to induce the brown fat thermogenic program in mouse and human adipocytes. *J Clin Invest*. 2012;122(3):1022-1036.
19. Wang TJ, Larson MG, Levy D, et al. Impact of obesity on plasma natriuretic peptide levels. *Circulation*. 2004;109(5):594-600.
20. Daniels LB, Clopton P, Bhalla V, et al. How obesity affects the cut-points for B-type natriuretic peptide in the diagnosis of acute heart failure: results from the Breathing Not Properly Multinational Study. *Am Heart J*. 2006;151(5):999-1005.
21. Dessì-Fulgheri P, Sarzani R, Tamburrini P, et al. Plasma atrial natriuretic peptide and natriuretic peptide receptor gene expression in adipose tissue of normotensive and hypertensive obese patients. *J Hypertens*. 1997;15(12, Pt 2):1695-1699.
22. Nakatsuji H, Maeda N, Hibuse T, et al. Reciprocal regulation of natriuretic peptide receptors by insulin in adipose cells. *Biochem Biophys Res Commun*. 2010;392(1):100-105.
23. Standeven KF, Hess K, Carter AM, et al. Neprilysin, obesity and the metabolic syndrome. *Int J Obes (Lond)*. 2011;35(8):1031-1040.
24. McMurray JJ, Packer M, Desai AS, et al; PARADIGM-HF Investigators and Committees. Angiotensin-neprilysin inhibition versus enalapril in heart failure. *N Engl J Med*. 2014;371(11):993-1004.
25. Fitzgibbons TP, Czech MP. Epicardial and perivascular adipose tissues and their influence on cardiovascular disease: basic mechanisms and clinical associations. *J Am Heart Assoc*. 2014;3(2):e000582.
26. Rosei CA, Withers SB, Belcaid L, De Ciuceis C, Rizzoni D, Heagerty AM. Blockade of the renin-angiotensin system in small arteries and anticontractile function of perivascular adipose tissue. *J Hypertens*. 2015;33(5):1039-1045.
27. Withers SB, Bussey CE, Saxton SN, Melrose HM, Watkins AE, Heagerty AM. Mechanisms of adiponectin-associated perivascular function in vascular disease. *Arterioscler Thromb Vasc Biol*. 2014;34(8):1637-1642.
28. Birkenfeld AL, Boschmann M, Engeli S, et al. Atrial natriuretic peptide and adiponectin interactions in man. *PLoS One*. 2012;7(8):e43238.
29. Lopaschuk GD, Ussher JR, Folmes CD, Jaswal JS, Stanley WC. Myocardial fatty acid metabolism in health and disease. *Physiol Rev*. 2010;90(1):207-258.
30. Ohba H, Takada H, Musha H, et al. Effects of prolonged strenuous exercise on plasma levels of atrial natriuretic peptide and brain natriuretic peptide in healthy men. *Am Heart J*. 2001;141(5):751-758.
31. Birkenfeld AL, Budziarek P, Boschmann M, et al. Atrial natriuretic peptide induces postprandial lipid oxidation in humans. *Diabetes*. 2008;57(12):3199-3204.
32. Engeli S, Birkenfeld AL, Badin PM, et al. Natriuretic peptides enhance the oxidative capacity of human skeletal muscle. *J Clin Invest*. 2012;122(12):4675-4679.
33. Tuunanen H, Engblom E, Naum A, et al. Free fatty acid depletion acutely decreases cardiac work and efficiency in cardiomyopathic heart failure. *Circulation*. 2006;114(20):2130-2137.
34. Jaswal JS, Keung W, Wang W, Ussher JR, Lopaschuk GD. Targeting fatty acid and carbohydrate oxidation—a novel therapeutic intervention in the ischemic and failing heart. *Biochim Biophys Acta*. 2011;1813(7):1333-1350.
35. Blondin DP, Tingelstad HC, Mantha OL, Gosselin C, Haman F. Maintaining thermogenesis in cold exposed humans: relying on multiple metabolic pathways. *Compr Physiol*. 2014;4(4):1383-1402.
36. Templeman NM, Beaudry JL, Le Moine CM, McClelland GB. Chronic hypoxia- and cold-induced changes in cardiac enzyme and gene expression in CD-1 mice. *Biochim Biophys Acta*. 2010;1800(12):1248-1255.
37. Cheng Y, Hauton D. Cold acclimation induces physiological cardiac hypertrophy and increases assimilation of triacylglycerol metabolism through lipoprotein lipase. *Biochim Biophys Acta*. 2008;1781(10):618-626.
38. Hassi J, Rintamaki H, Ruskoaho H, Leppaluoto J, Vuolteenaho O. Plasma levels of endothelin-1 and atrial natriuretic peptide in men during a 2-hour stay in a cold room. *Acta Physiol Scand*. 1991;142(4):481-485.

39. Yuan K, Jin X, Park WH, Kim JH, Park BH, Kim SH. Modification of atrial natriuretic peptide system in cold-induced hypertensive rats. *Regul Pept*. 2009;154(1-3):112-120.
40. Palmer BF, Clegg DJ. The sexual dimorphism of obesity. *Mol Cell Endocrinol*. 2015;402:113-119.
41. Lee P, Swarbrick MM, Ho KK. Brown adipose tissue in adult humans: a metabolic renaissance. *Endocr Rev*. 2013;34(3):413-438.
42. Cannon B, Nedergaard J. Brown adipose tissue: function and physiological significance. *Physiol Rev*. 2004;84(1):277-359.
43. Enerback S, Jacobsson A, Simpson EM, et al. Mice lacking mitochondrial uncoupling protein are cold-sensitive but not obese. *Nature*. 1997;387(6628):90-94.
44. Seale P, Bjork B, Yang W, et al. PRDM16 controls a brown fat/skeletal muscle switch. *Nature*. 2008;454(7207):961-967.
45. Cypess AM, Lehman S, Williams G, et al. Identification and importance of brown adipose tissue in adult humans. *N Engl J Med*. 2009;360(15):1509-1517.
46. van Marken Lichtenbelt WD, Vanhommerig JW, Smulders NM, et al. Cold-activated brown adipose tissue in healthy men. *N Engl J Med*. 2009;360(15):1500-1508.
47. Virtanen KA, Lidell ME, Orava J, et al. Functional brown adipose tissue in healthy adults. *N Engl J Med*. 2009;360(15):1518-1525.
48. Tam CS, Lecoultre V, Ravussin E. Brown adipose tissue: mechanisms and potential therapeutic targets. *Circulation*. 2012;125(22):2782-2791.
49. Hu HH, Perkins TG, Chia JM, Gilsanz V. Characterization of human brown adipose tissue by chemical-shift water-fat MRI. *AJR Am J Roentgenol*. 2013;200(1):177-183.
50. Sacks H, Symonds ME. Anatomical locations of human brown adipose tissue: functional relevance and implications in obesity and type 2 diabetes. *Diabetes*. 2013;62(6):1783-1790.
51. Symonds ME, Henderson K, Elvidge L, et al. Thermal imaging to assess age-related changes of skin temperature within the supraclavicular region co-locating with brown adipose tissue in healthy children. *J Pediatr*. 2012;161(5):892-898.
52. Harms M, Seale P. Brown and beige fat: development, function and therapeutic potential. *Nat Med*. 2013;19(10):1252-1263.
53. Bartelt A, Heeren J. Adipose tissue browning and metabolic health. *Nat Rev Endocrinol*. 2014;10(1):24-36.
54. Rosenwald M, Perdikari A, Rülicke T, Wolfrum C. Bi-directional interconversion of brite and white adipocytes. *Nat Cell Biol*. 2013;15(6):659-667.
55. Sanchez-Gurmaches J, Guertin DA. Adipocyte lineages: tracing back the origins of fat. *Biochim Biophys Acta*. 2014;1842(3):340-351.
56. Cao W, Medvedev AV, Daniel KW, Collins S. Beta-adrenergic activation of p38 MAP kinase in adipocytes: cAMP induction of the uncoupling protein 1 (UCP1) gene requires p38 MAP kinase. *J Biol Chem*. 2001;276(29):27077-27082.
57. Cao W, Daniel KW, Robidoux J, et al. p38 mitogen-activated protein kinase is the central regulator of cyclic AMP-dependent transcription of the brown fat uncoupling protein 1 gene. *Mol Cell Biol*. 2004;24(7):3057-3067.
58. Puigserver P, Wu Z, Park CW, Graves R, Wright M, Spiegelman BM. A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. *Cell*. 1998;92(6):829-839.
59. Mitschke MM, Hoffmann LS, Gnad T, et al. Increased cGMP promotes healthy expansion and browning of white adipose tissue. *FASEB J*. 2013;27(4):1621-1630.
60. Collins SA. heart-adipose tissue connection in the regulation of energy metabolism. *Nat Rev Endocrinol*. 2014;10(3):157-163.
61. Shimizu Y, Nikami H, Tsukazaki K, et al. Increased expression of glucose transporter GLUT-4 in brown adipose tissue of fasted rats after cold exposure. *Am J Physiol*. 1993;264(6, Pt 1):E890-E895.
62. Olsen JM, Sato M, Dallner OS, et al. Glucose uptake in brown fat cells is dependent on mTOR complex 2-promoted GLUT1 translocation. *J Cell Biol*. 2014;207(3):365-374.
63. Marette A, Bukowiecki LJ. Noradrenaline stimulates glucose transport in rat brown adipocytes by activating thermogenesis: evidence that fatty acid activation of mitochondrial respiration enhances glucose transport. *Biochem J*. 1991;277(Pt 1):119-124.
64. Orava J, Nuutila P, Lidell ME, et al. Different metabolic responses of human brown adipose tissue to activation by cold and insulin. *Cell Metab*. 2011;14(2):272-279.
65. Yoneshiro T, Aita S, Matsushita M, et al. Recruited brown adipose tissue as an antiobesity agent in humans. *J Clin Invest*. 2013;123(8):3404-3408.
66. Chondronikola M, Volpi E, Borsheim E, et al. Brown adipose tissue improves whole-body glucose homeostasis and insulin sensitivity in humans. *Diabetes*. 2014;63(12):4089-4099.
67. Matsushita M, Yoneshiro T, Aita S, Kameya T, Sugie H, Saito M. Impact of brown adipose tissue on body fatness and glucose metabolism in healthy humans. *Int J Obes (Lond)*. 2014;38(6):812-817.
68. Blondin DP, Labbe SM, Tingelstad HC, et al. Increased brown adipose tissue oxidative capacity in cold-acclimated humans. *J Clin Endocrinol Metab*. 2014;99(3):E438-E446.
69. Poher AL, Altirriba J, Veyrat-Durebex C, Rohner-Jeanrenaud F. Brown adipose tissue activity as a target for the treatment of obesity/insulin resistance. *Front Physiol*. 2015;6:4.
70. Chen YF. Atrial natriuretic peptide in hypoxia. *Peptides*. 2005;26:1068-1077.
71. Casals G, Ros J, Sionis A, Davidson MM, Morales-Ruiz M, Jiménez W. Hypoxia induces B-type natriuretic peptide release in cell lines derived from human cardiomyocytes. *Am J Physiol Heart Circ Physiol*. 2009;297(2):H550-H555.
72. Trayhurn P, Alomar SY. Oxygen deprivation and the cellular response to hypoxia in adipocytes—perspectives on white and brown adipose tissues in obesity. *Front Endocrinol (Lausanne)*. 2015;6:19.
73. Martinez D, Fiori CZ, Baronio D, et al. Brown adipose tissue: is it affected by intermittent hypoxia? *Lipids Health Dis*. 2010;9:121.
74. Palmer BF, Clegg DJ. Ascent to altitude as a weight loss method: the good and bad of hypoxia inducible factor activation. *Obesity (Silver Spring)*. 2014;22(2):311-317.
75. Bai Z, Wuren T, Liu S, et al. Intermittent cold exposure results in visceral adipose tissue "browning" in the plateau pika (*Ochotona curzoniae*). *Comp Biochem Physiol A Mol Integr Physiol*. 2015;184:171-178.
76. Nikami H, Nedergaard J, Fredriksson JM. Norepinephrine but not hypoxia stimulates HIF-1alpha gene expression in brown adipocytes. *Biochem Biophys Res Commun*. 2005;337(1):121-126.
77. Carriere A, Jeanson Y, Berger-Muller S, et al. Browning of white adipose cells by intermediate metabolites: an adaptive mechanism to alleviate redox pressure. *Diabetes*. 2014;63(10):3253-3265.
78. Xue Y, Petrovic N, Cao R, et al. Hypoxia-independent angiogenesis in adipose tissues during cold acclimation. *Cell Metab*. 2009;9(1):99-109.
79. Carneheim C, Nedergaard J, Cannon B. Beta-adrenergic stimulation of lipoprotein lipase in rat brown adipose tissue during acclimation to cold. *Am J Physiol*. 1984;246(4, Pt 1):E327-E333.
80. Carneheim C, Nedergaard J, Cannon B. Cold-induced beta-adrenergic recruitment of lipoprotein lipase in brown fat is due to increased transcription. *Am J Physiol*. 1988;254(2, Pt 1):E155-E161.
81. Berbee JF, Boon MR, Khedoe PP, et al. Brown fat activation reduces hypercholesterolaemia and protects from atherosclerosis development. *Nat Commun*. 2015;6:6356.

82. Bartelt A, Bruns OT, Reimer R, et al. Brown adipose tissue activity controls triglyceride clearance. *Nat Med*. 2011;17(2):200-205.
83. Berkseth KE, Schur E, Schwartz MW. A role for natriuretic peptides in the central control of energy balance? *Diabetes*. 2013;62(5):1379-1381.
84. Fain JN, Kanu A, Bahouth SW, Cowan GS, Lloyd Hiler M. Inhibition of leptin release by atrial natriuretic peptide (ANP) in human adipocytes. *Biochem Pharmacol*. 2003;65(11):1883-1888.
85. Peinó R, Piñeiro V, Gualillo O. Cold exposure inhibits leptin secretion in vitro by a direct and non-specific action on adipose tissue. *Eur J Endocrinol*. 2000;142(2):195-199.
86. Bing C, Frankish HM, Pickavance L, et al. Hyperphagia in cold-exposed rats is accompanied by decreased plasma leptin but unchanged hypothalamic NPY. *Am J Physiol*. 1998;274(1, Pt 2):R62-R68.
87. Solum DT, Handa RJ. Estrogen regulates the development of brain-derived neurotrophic factor mRNA and protein in the rat hippocampus. *J Neurosci*. 2002;22(7):2650-2659.
88. Cao L, Choi EY, Liu X, et al. White to brown fat phenotypic switch induced by genetic and environmental activation of a hypothalamic-adipocyte axis. *Cell Metab*. 2011;14(3):324-338.
89. Maffei S, Del Ry S, Prontera C, Clerico A. Increase in circulating levels of cardiac natriuretic peptides after hormone replacement therapy in postmenopausal women. *Clin Sci (Lond)*. 2001;101(5):447-453.
90. Belo NO, Sairam MR, Dos Reis AM. Impairment of the natriuretic peptide system in follitropin receptor knockout mice and reversal by estradiol: implications for obesity-associated hypertension in menopause. *Endocrinology*. 2008;149(3):1399-1406.
91. Mahmoodzadeh S, Pham TH, Kuehne A, et al. 17β-Estradiol-induced interaction of ERalpha with NPPA regulates gene expression in cardiomyocytes. *Cardiovasc Res*. 2012;96(3):411-421.
92. Lof M, Olausson H, Bostrom K, Janerot-Sjoberg B, Sohlstrom A, Forsum E. Changes in basal metabolic rate during pregnancy in relation to changes in body weight and composition, cardiac output, insulin-like growth factor I, and thyroid hormones and in relation to fetal growth. *Am J Clin Nutr*. 2005;81(3):678-685.
93. Yoshimura T, Yoshimura M, Yasue H, et al. Plasma concentration of atrial natriuretic peptide and brain natriuretic peptide during normal human pregnancy and the postpartum period. *J Endocrinol*. 1994;140(3):393-397.
94. Koch A, Singer H. Normal values of B type natriuretic peptide in infants, children, and adolescents. *Heart*. 2003;89(8):875-878.
95. Mir TS, Laux R, Hellwege HH, et al. Plasma concentrations of aminoterminal pro atrial natriuretic peptide and aminoterminal pro brain natriuretic peptide in healthy neonates: marked and rapid increase after birth. *Pediatrics*. 2003;112(4):896-899.
96. Graham CH, Watson JD, Blumenfeld AJ, Pang SC. Expression of atrial natriuretic peptide by third-trimester placental cytotrophoblasts in women. *Biol Reprod*. 1996;54(4):834-840.
97. Huang W, Lee D, Yang Z, et al. Evidence for atrial natriuretic peptide-(5-28) production by rat placental cytotrophoblasts. *Endocrinology*. 1992;131(2):919-924.
98. Guilherme A, Virbasius JV, Puri V, Czech MP. Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. *Nat Rev Mol Cell Biol*. 2008;9(5):367-377.
99. Guo T, Marmol P, Moliner A, et al. Adipocyte ALK7 links nutrient overload to catecholamine resistance in obesity. *Elife*. 2014;3:e03245.
100. Song Y, Altarejos J, Goodarzi MO, et al. CRTC3 links catecholamine signalling to energy balance. *Nature*. 2010;468(7326):933-939.
101. Dodd GT, Decherf S, Loh K, et al. Leptin and insulin act on POMC neurons to promote the browning of white fat. *Cell*. 2015;160(1-2):88-104.
102. Karpe F, Dickmann JR, Frayn KN. Fatty acids, obesity, and insulin resistance: time for a reevaluation. *Diabetes*. 2011;60(10):2441-2449.
103. Krauser DG, Lloyd-Jones DM, Chae CU, et al. Effect of body mass index on natriuretic peptide levels in patients with acute congestive heart failure: a ProBNP Investigation of Dyspnea in the Emergency Department (PRIDE) substudy. *Am Heart J*. 2005;149(4):744-750.
104. Salehi M, Prigeon RL, D'Alessio DA. Gastric bypass surgery enhances glucagon-like peptide 1-stimulated postprandial insulin secretion in humans. *Diabetes*. 2011;60(9):2308-2314.
105. Chen-Tournoux A, Khan AM, Baggish AL, et al. Effect of weight loss after weight loss surgery on plasma N-terminal pro-B-type natriuretic peptide levels. *Am J Cardiol*. 2010;106(10):1450-1455.
106. Marney AM, Brown NJ, Tamboli R, Abumrad N. Changes in B-type natriuretic peptide and BMI following Roux-en-Y gastric bypass surgery. *Diabetes Care*. 2014;37(4):e70-e71.
107. Vijgen GH, Bouvy ND, Teule GJ, et al. Increase in brown adipose tissue activity after weight loss in morbidly obese subjects. *J Clin Endocrinol Metab*. 2012;97(7):E1229-E1233.
108. Neinast MD, Frank AP, Zechner JF, et al. Activation of natriuretic peptides and the sympathetic nervous system following Roux-en-Y gastric bypass is associated with gonadal adipose tissues browning. *Mol Metab*. 2015;4(5):427-436.