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Natriuretic peptides in the control of lipid metabolism and insulin sensitivity

Kenneth Verboven[1,2], Dominique Hansen[2,3], Johan W.E. Jocken[1] and Ellen E. Blaak[1]

[1] Department of Human Biology, NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University Medical Center+, Maastricht, The Netherlands

[2] REVAL - Rehabilitation Research Center, BIOMED - Biomedical Research Institute, Faculty of Medicine and Life Sciences, Hasselt University, Hasselt, Belgium

[3] Heart Centre Hasselt, Jessa Hospital, Hasselt, Belgium

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Corresponding author:

Prof. dr. Ellen E. Blaak

Department of Human Biology, NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University Medical Center +

PO Box 616, 6200 MD, Maastricht, The Netherlands

Phone: +31 433 881 503

Email: e.blaak@maastrichtuniversity.nl

Abbreviations

Insulin resistance, IR; type 2 diabetes mellitus, T2DM; fatty acid, FA; adipose tissue, AT; natriuretic peptides, NPs; atrial natriuretic peptide, ANP; B-type natriuretic peptide, BNP; C-type natriuretic peptide, CNP; dendroaspis natriuretic peptide, DNP; glucagon-like peptide 1, GLP1; guanylyl cyclase, GC; natriuretic peptide receptor A, MAPK; mitogen-activated protein kinase, NPRA; natriuretic peptide receptor B, NPRB; cyclic GMP, cGMP; natriuretic peptide receptor C, NPRC; neutral endopeptidase, NEP; insulin-degrading enzyme, IDE; dipeptidyl peptidase-4, DPP4; body mass index, BMI; phosphatidylinositol 3-kinase, PI3K; protein kinase G, PKG; perilipin-1, PLIN-1; hormone sensitive lipase, HSL; adipose triglyceride lipase, ATGL; protein kinase A, PKA; cyclic AMP, cAMP; human ANP, hANP; phosphodiesterase 3B, PDE3B; tumor necrosis factor alpha, TNF-α; brown adipose tissue, BAT; uncoupling protein 1, UCP-1; peroxisome proliferator-activated receptor gamma coactivator 1-alpha, PGC-1α; peroxisome proliferator activated receptor, PPAR; non-alcoholic fatty liver, NAFL; lipopolysaccharides, LPS.

1. Abstract

Natriuretic peptides have long been known for their cardiovascular function. However, a growing body of evidence emphasizes the role of natriuretic peptides in human substrate and energy metabolism, thereby connecting the heart with several insulin sensitive organs like adipose tissue, skeletal muscle and liver. Obesity may be associated with an impaired regulation of the natriuretic peptide system, also indicated as a natriuretic handicap. Evidence points towards a contribution of this natriuretic handicap to the development of obesity, type 2 diabetes mellitus and cardiometabolic complications, although the causal relationship is not fully understood. Nevertheless, targeting the natriuretic peptide pathway may improve metabolic health in obesity and type 2 diabetes mellitus. This review will focus on current literature regarding the metabolic roles of natriuretic peptides with emphasis on lipid metabolism and insulin sensitivity. Furthermore, it will be discussed how exercise and lifestyle intervention may modulate the natriuretic peptide-related metabolic effects.

2. Introduction

Obesity is one of the major health problems of the twenty-first century as it is closely associated with the development of chronic metabolic diseases, including cardiovascular disease, insulin resistance (IR) and type 2 diabetes mellitus (T2DM) [1-3]. Different insulin sensitive organs tightly orchestrate energy and substrate metabolism in the human body. Therefore, alterations in these organs may contribute to the development of disturbances in fatty acid (FA) metabolism, ultimately leading to impaired glucose metabolism, IR and cardiometabolic disease. Next to the liver, skeletal muscle, the gastrointestinal tract and the pancreas, the adipose tissue (AT) is an important central organ in the inter-organ crosstalk in human energy and substrate metabolism. The AT is the primary site for long-term energy storage, mainly as triglycerides. However, a chronic excessive energy intake, like in obesity, results in enlargement (hypertrophy) of existing adipocytes. Since adipocytes have a limited expansion capacity, hypertrophy will lead to a reduced buffering capacity and dysfunctional AT may develop [4,5]. This AT dysfunction is further characterized by an altered lipid storage capacity and adipokine release, immune cell infiltration and low-grade inflammation, plays an important role in the development and/or progression of IR by promoting ectopic lipid storage and low-grade inflammation [6-10]. Physical activity intervention, whether or not combined with diet, may reduce the progression towards T2DM [11,12], possibly due to modulation of AT, liver and/or skeletal muscle FA metabolism [10].

Only recently, research proposed natriuretic peptides (NPs) as important endocrine hormones implicated in the regulation of whole-body energy and substrate metabolism [13-16]. Atrial natriuretic peptide (ANP), B-type natriuretic peptide (BNP), both secreted by the heart, and C-type natriuretic peptide (CNP), mainly expressed by vascular endothelial cells and to a lower extent in cardiomyocytes [17], are the three NP family members that may play a role in human substrate and energy metabolism. Besides their cardiovascular and renal effects

(described extensively by Volpe *et al.* [18]), NPs play a role in different metabolic processes including lipid mobilization in human white AT [13,15], energy dissipation in brown AT, browning of white AT [19] and fat oxidation in human skeletal muscle [20], possibly influencing whole-body FA metabolism, glucose homeostasis and insulin sensitivity. In addition to their wide range of metabolic effects in human insulin sensitive tissues, plasma NP levels were also reported to be negatively associated with cardiometabolic diseases [21-26] and have been even suggested to have a predictive value in the development of new onset T2DM [25]. However, a better understanding of the interaction between NPs and tissue energy, FA and glucose metabolism is necessary to obtain a better insight in the role of NPs in the development of IR, T2DM and cardiometabolic complications.

In this review, we will discuss the current knowledge regarding the role of NPs in the control of human lipid metabolism and insulin sensitivity. At first, NP secretion, clearance and signaling is discussed. Subsequently, the role of NPs in the etiology of obesity and T2DM is discussed, as well as their effects on AT, skeletal muscle and liver metabolism. Moreover, the benefits of lifestyle and/or exercise interventions with respect to NPs in the control of insulin sensitivity are considered.

3. Secretion and processing of NP

The discovery of the endocrine properties of the heart by deBold in 1981, as shown by a potent natriuretic and diuretic effect of atrial myocardial extracts in rats [27], led to the reexamination of the function of the earlier discovered atrial myocardium granules [28]. The dual nature of atrial cardiomyocytes (i.e. secretory-contractile function) became obvious and research led to the identification of ANP [29] and later the other NP-hormone family members BNP, which is found at highest levels in cardiac ventricles, CNP, which is mainly expressed in and produced by endothelial cells [17]. CNP, previously thought to act as a neuropeptide in

the central nervous system [30,31], is mainly viewed as a peptide regulating vascular blood pressure [32] and bone growth [33], although a minor role in metabolic regulation has been suggested [34,35]. In mammals, dendroaspis natriuretic peptide (DNP) (of which the synthesis and secretion sites have not been identified) exerts renal actions via its specific receptor [36] but because it has not been well studied with respect to metabolic effects in humans, DNP will not be further discussed in the current review.

Currently, ANP and BNP are the main metabolically active NPs described in literature and will therefore be discussed in more detail in the present review. These peptides have a 17-amino acid ring structure in common, formed by an intramolecular disulfide linkage, of which the sequence is highly preserved within the biologically active form of these peptides [37]. Structural differences between NP family members are due to specific amino- and carboxy-terminal extensions [38]. At rest, ANP (normal concentration range 5-50 pg/mL) is mainly produced and secreted by the (right) atrial myocardium as a preprohormone [39]. The intracellular granules of the atrial myocardium contain proANP which is cleaved by corin (type II transmembrane serine protease expressed primarily in the heart) during secretion, resulting in a 28-amino acid, bioactive αANP peptide [40], with a very short plasma half-life of about 2-4 min [41], and inactive fragments (N-terminal ANP and mid-regional-proANP) which have a longer plasma half-life (about 40-50 min) [41,42]. BNP is mainly produced and secreted by the ventricular myocardium as preproBNP [43]. To become biologically active, preproBNP is cleaved to proBNP and subsequently, like for ANP, a cardiac protease, corin or furin, is responsible for the conversion to the 32-amino acid BNP (plasma concentration range 0-65 pg/mL) which is secreted in the circulation having a plasma half-life of about 15-25 min [44], and the inactive N-terminal fragment proBNP [45]. The latter inactive fragment has a plasma half-life of about 60-120 min and a plasma concentration in the range of 7-220 pmol/L in healthy individuals [41]. While the structure of BNP varies distinctly among species, ANP is

strongly homologous between human and rodent [45]. The post-translational processing, cleavage and degradation sites of NPs were recently reviewed by Volpe *et al.*[18].

4. Determinants of NP secretion

Mechanical stretch of cardiomyocytes is the most important trigger for NP release in the circulation [45]. Atrial wall stretching causes an increase in ANP gene transcription and increased release of stored granules [46]. Ventricular wall stress, in case of volume or pressure overload, is mainly responsible for BNP transcription and secretion [38]. In addition, hypoxic conditions or myocardial ischemia are potent inducers of ANP secretion due to the presence of hypoxia-responsive elements in the promotor region of the ANP gene [47].

Another potent stimulus for NP secretion is cold exposure. Animals and humans exposed to cold display a greater expression of ANP (mRNA) and BNP (mRNA and protein) in the heart, combined with higher plasma levels, in conjunction with an elevated sympathetic nervous system activation in the heart [19]. Cold-induced elevation of NPs levels might result from increased blood pressure in response to skin vasoconstriction and altered central blood volumes, thereby augmenting cardiac filling pressure and thus NPs' secretion [48,49].

Besides mechanical stretch and cold exposure, endocrine regulation of NP secretion is present as well. Sex steroids, thyroid hormones, glucocorticoids, endothelin-1, angiotensin II and inflammatory cytokines (tumor necrosis factor-α, interleukin-1 and -6) all are able to modulate NP secretion [38,45,50]. Inflammatory cytokines stimulate BNP transcription and translation *in vitro* in murine cardiomyocyte cultures [51] and *in vivo* secretion into the plasma in human transplant patients specifically [52]. In this regard, it was shown that the glucagon-like peptide 1 (GLP1) receptor agonist liraglutide was able to induce a significant increase in ANP secretion in mice due to the presence of GLP1 receptors on right atrial cardiomyocytes [53]. However, the existence of a GLP-1-ANP axis could not be confirmed in men or patients with

T2DM [54-57]. ANP and BNP plasma levels also increase with age, possibly due to an age-related reduction in coronary blood flow reserve and thus increased myocardial ischemia [58-60]. Modulation by sex steroids may result in sex dependent regulation of NP levels [60-62]. An effect of sex hormones during adolescence was already observed in pubertal versus post-pubertal adolescents, where NP concentrations are lower in post-pubertal boys compared with pubertal boys [63]. Estrogens might have a stimulatory effect on the production and secretion of ANP and BNP by the cardiomyocyte, whereas androgens may have an inhibitory effect [61]. In part, the increased NP levels in women might have clinical implications for sex-related difference in relative risk of developing metabolic and cardiovascular disease. Furthermore, during adolescence NPs levels seem to increase progressively in girls [58,59], probably the result of an interaction between the increased estrogen concentration and ANP transcription and secretion or via the regulation of the NPs receptor expression [64]. Plasma CNP levels alternatively decrease during adolescence until the age of fifty, whereupon they tend to increase. CNP concentrations are higher in men than in women as testosterone and growth hormone are able to induce CNP [65].

Finally, metabolically compromised conditions like obesity, insulin resistance and T2DM may be characterized by altered systemic NP concentrations, which will be discussed more extensively later in this review.

5. NP receptors and signaling

To exert their main biological effects NPs bind to NP receptors, of which three subtypes have been described (reviewed recently by Kuhn) [66]. ANP and BNP bind with a high affinity to a membrane-bound receptor, containing a transmembrane segment, with specific guanylyl cyclase (GC) activity called NP receptor A (NPRA). CNP is mainly bound to NP receptor B (NPRB) [67], similar in structure and function to NPRA and mainly expressed by chondrocytes,

thereby playing a role in long bone growth [68]. Ligand binding to a NPRA homodimer results in the internalization of the bound ligand-receptor complex and the activation of cytosolic GC, the catalytic effector of the receptor, subsequently causing hydrolysis of GTP into cyclic GMP (cGMP). This second messenger is able to activate various biological responses via cGMP-dependent protein kinases, cGMP-gated ion channels or other effector proteins [69]. A large proportion of the ligand-bound receptor undergo lysosomal degradation, in which about 75% of the internalized ANP is processed in the lysosomes and 25% is released as intact molecules through a recycling pathway. A small amount of internalized receptors is recycled back to the plasma membrane or released into the cell exterior [70]. The NP receptor C (NPRC) is the third subtype, having an extracellular domain that is partly homologous to those of NPRA receptors and thus has the ability to bind NPs, with the highest affinity to ANP and lowest to BNP [71]. However, NPRC lacks GC activity and instead its main function is to facilitate scavenging of its ligands, internalization of ligand-receptor complexes and recycling of NPRC, together with lysosomal degradation of its ligands [72,73]. Besides via lysosomal clearance, NPs can be degraded intracellularly by endopeptidases including neutral endopeptidase (NEP) [74], which is also produced in adipocytes [75]. In addition, insulin-degrading enzyme (IDE) enzymatically cleaves NP [45,76] and dipeptidyl peptidase-4 (DPP4 or CD26) cleaves the N-terminal peptide of NPs thereby lowering biological activity [77]. Another route to clear circulating NP is via secretion into body fluids like urine (via glomerular filtration) and bile [45].

The main effector receptor for ANP and BNP, NPRA, is highly expressed throughout the cardiovascular system (vascular smooth muscle and endothelial cells with only a limited expression in the heart), in kidney and adrenal gland, as well as in different metabolic organs like skeletal muscle, pancreas, liver, brain, gut and AT [37,78,79]. Expression of the scavenging NPRC, the most widely expressed NPs receptor, is mainly present in the AT, kidneys, lungs,

the cardiovascular system and blood monocytes [73,80,81]. Interestingly, NPRA and NPRC display diurnal regulations (in antiphase of one other) in the rodent white AT [82], not in the heart muscle [83], which together with the circadian regulated plasma NPs [84,85], may be a characteristic for energy homeostasis during the day. Furthermore, the local tissue specific and systemic effects of NP are thought to depend on the ratio between NPRA and NPRC [86,87]. Collectively, NPs mediate their effects via NP receptors, of which three subtypes have been described. The diverse effects of NPs, systemically as well as the local tissue effects, are determined by NP receptor expression profiles and their ligand-affinity.

6. Systemic NP deficiency in obesity, insulin resistance and type 2 diabetes mellitus

Evidence from several epidemiological studies demonstrated an inverse association between systemic NPs levels (both ANP and BNP) and body weight (mostly expressed by body mass index (BMI)) [21,23,88-92]. The inverse relationship between NP levels and BMI was also found in the presence of left ventricular hypertrophy [93]. However, these studies lack detailed body composition analyses thereby not being able to differentiate between fat accumulation or fluid retention as a cause of the increased BMI. In contrast, other smaller cohorts showed that there is no or even a positive relationship between circulating NPs levels and BMI [94,95]. Furthermore, NPs may affect AT distribution [96,97]. Variations in regional and particularly visceral adiposity were strongly related to circulating N-terminal-pro-BNP. The relationship of NPs with subcutaneous adiposity was less strong [97]. This relation could be partly moderated by the hyperinsulinemic state that is frequently observed in visceral adiposity, as high insulin levels have been shown to suppress NPs secretion and activity [98,99]. The Dallas Heart Study recently showed that both BNP and N-terminal-proBNP are inversely related to visceral and liver fat, while being positively associated with gluteofemoral body fat, independent of insulin sensitivity [100].

Of interest, it is important to take into account obesity comorbidities including the presence of cardiac burden in considering these results [101]. The decrease in systemic NPs levels may be accompanied by higher blood aldosterone concentrations, as a consequence of an impaired NPs-mediated renin-angiotensin-aldosteron system inhibition [102,103], thereby leading to obesity-related hypertension [22] or an increased incidence of all-cause mortality [91]. The substantial role of NPs deficiency in the pathogenesis of obesity-related hypertension was recently corroborated in healthy obese men, showing a negative association between serum mid-regional-proANP and mean 24-hour systolic ambulatory blood pressure [104]. Moreover, research indicated that NPs deficiency might enhance cardiovascular risk [18]. Although not all mechanisms involved in obesity-related hypertension are well understood [105], NPs might partially link obesity and metabolic syndrome to hypertension [106].

As obesity is often associated with IR or T2DM, a link between the NPs system, obesity and T2DM seems plausible. Recent research indicated that NPs deficiency might increase the risk of T2DM onset [107]. Indeed, two recent prospective cohort studies showed evidence supporting this hypothesis [25,26]. Results of the Malmö Diet and Cancer Study showed mid-regional-proANP plasma levels to be inversely associated with new-onset diabetes development (*i.e*, diabetes incidence) and an impaired glucose metabolism over the 16-year follow-up period of the study, which was also true for N-terminal-proBNP plasma levels, although not statistically significant [25]. In this regard, mid-regional-proANP is believed to be a better predictor of T2DM incidence compared to N-terminal-proBNP [25], the latter being more sensitive to mild forms of left ventricular dysfunction [108] which is relatively frequent (even subclinically) in the obese state [109]. Circulating ANP (measured as mid-regional-proANP) within the normal high range (~212-372 pg/mL) was associated with lower risk of IR during a 16-year follow-up period in a middle-aged cohort [110]. This association was independent of diabetes risk factors (including waist circumference, plasma levels of triglycerides, HDL-cholesterol, systolic

blood pressure, antihypertensive treatment, age and sex) or renal function (cystatin C) [25]. In the Atherosclerosis Risk in Communities Study (a community-based population study), it was shown that having low N-terminal-proBNP levels (lowest quartile, <31pg/mL) was associated with higher risk of incident diabetes over a 12 year follow-up period in subjects without T2DM at baseline. These results were consistent across race, gender and BMI categories [26], and were independent of age [111,112]. Of interest, statistical adjustment for BMI did not abrogate the association between low NP levels and diabetes onset [113]. These results are in line with the Framingham Heart Study and the Malmö Diet and Cancer Study, which showed that lower N-terminal-proBNP levels were associated with higher incidence of IR in lean as well as in obese subjects in a cross-sectional study [23]. Additionally, prospective cohort data from the Women's Health Study showed that subjects with N-terminal-proBNP levels near the upper limit of the normal range (>117 pg/mL) have a significantly lower risk of developing diabetes [114].

Thus, there is consistent evidence that increased NP concentrations are protective against IR and T2DM [114-116]. In line, lower blood glucose concentrations were transiently observed upon systemic BNP infusion during intravenous glucose tolerance testing in young, healthy lean men with normal glucose tolerance [117]. Moreover, in a random subset of a general middle-aged population (age >45 years) a genetic variant of the ANP gene (single nucleotide polymorphism rs5068) was associated with higher N-terminal-proANP levels and a beneficial cardiometabolic profile (*i.e.* reduced systemic blood pressure, BMI, waist circumference and a lower risk of metabolic syndrome) compared to the A/A carriers [89]. This ANP gene-polymorphism was accompanied with a lower incidence of T2DM after a 14-year follow-up [118]. Similarly, a genetic polymorphism in the promotor region of the BNP gene (T-381C polymorphism) is associated with higher plasma BNP levels and lower risk of T2DM in

several population samples including individuals with normoglycemia, impaired glucose tolerance and T2DM [119].

Altogether, the presence of a NPs deficiency in metabolic disease is generally accepted, as acknowledged by large (but challenged by some smaller) cohorts, but the cause remains incompletely understood. Nonetheless, it is imperative to understand the etiology of this anomaly to further establish the clinical relevance of using mid-regional-ANP and/or N-terminal-proBNP as biomarkers for diabetes prediction [15].

7. Underlying mechanism for systemic NP deficiency in obesity

Several potential explanations for the observed systemic NPs deficiency in human obesity, and more general human metabolic disease, have been proposed, apart from common variants of the human ANP and BNP genes that affect circulating NP concentrations [114,116,120]. One explanation could be that the NP deficiency may be due to an increased NP degradation in human AT of obese [78,71,86] and obese hypertensive individuals [22], which is mainly fulfilled by NPRC-mediated lysosomal breakdown as mentioned before [74,121]. In addition, hyperinsulinemia increased NPRC expression *in vitro* in 3T3-L1 adipocytes [86], human adipocytes [86,122] and in human subcutaneous AT of healthy, moderately obese individuals with normal glucose tolerance during hyperinsulinemic-euglycemic and hyperinsulinemic-hyperglycemic clamps [81], mainly through the phosphatidylinositol 3-kinase (PI3K) pathway [86]. Moreover, previous work of Sarzani *et al.* [96] with a genetic NPRC variant shows that a reduced NPs clearance (or resulting increased systemic levels) might be associated with a reduced content of upper body fat and a lower risk of developing abdominal obesity. Together, these data suggest that the AT may be responsible for the increased NP clearance and lowering systemic NP availability in obese insulin resistant conditions [81,123].

However, results from the Dallas Heart Study showed that the association between BMI and circulating NP levels is explained by the amount of lean mass, and not AT mass, indicating that lean tissue could also be important for plasma NP regulation [88]. Indeed, upregulation of NPRC in human skeletal muscle tissue, next to down-regulation of the NPRA expression in the AT and skeletal muscle of obese and/or obese diabetic humans and mice has been found [124,125]. Thus, besides AT, also skeletal muscle may contribute to the NP deficiency observed in T2DM in the long-term, certainly considering that muscle mass accounts for up to 40% of total body weight. Therefore, even a moderate increase in skeletal muscle NPRC expression could markedly reduce NPs plasma levels [124].

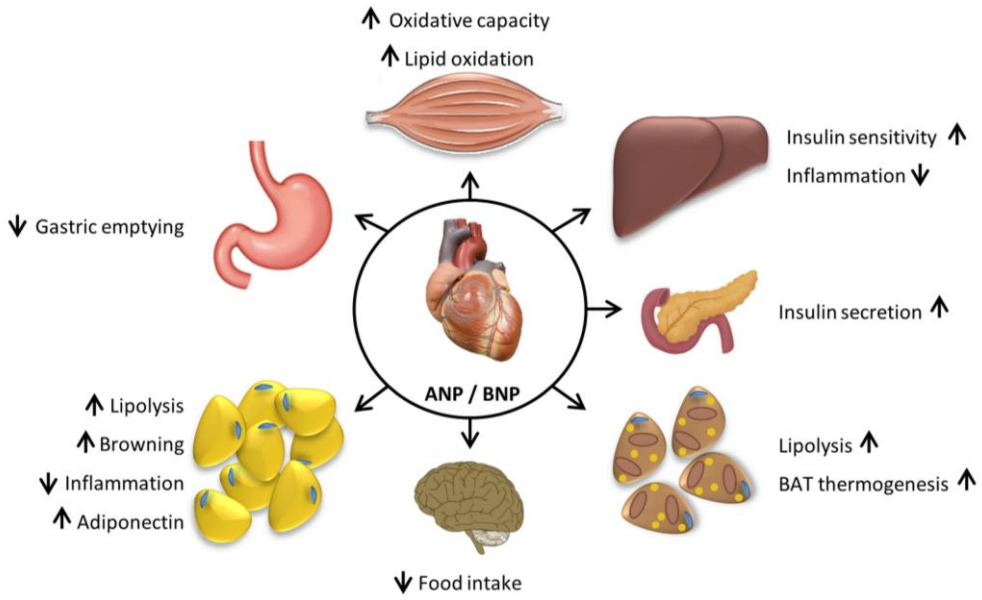
Additionally, it has been shown that NPRC mRNA expression is down-regulated *in vitro* following starvation in human differentiated adipocytes [122] and *in vivo* in rat white and brown AT [126] while the opposite was true under high fat feeding in wild-type mice skeletal muscle, white and brown AT [127]. Like NPRC, NEP expression is increased in the plasma (protein) and AT (mRNA) of obese subjects, although there is no direct evidence for an increased NEP activity in human obesity [128]. Together, these findings propose an altered NPs receptor ratio in obese insulin resistant or T2DM individuals mainly due to an increased expression of adipose NPRC and NEP, possibly leading to elevated NP clearance.

On the other hand, a decreased cardiac ANP and BNP release in metabolic disease has also been proposed. Not only circulating NPs levels but also the side products of NPs release (N-terminal-proANP and N-terminal-proBNP) are reduced in obesity. These proteins are structurally distinct and are biologically inactive compounds, which makes NPRC mediated clearance of these components rather unlikely [88,129]. Of interest, NPs levels in the aortic root and the coronary sinus were observed to be negatively correlated with BMI [130]. These findings suggest that besides an increased clearance, a reduced cardiac NPs release might potentially contribute to the systemic NPs deficiency in metabolic diseases as well. This hypothesis was

353 further acknowledged by the notion that cardiac ANP and BNP mRNA expressions were
354 reduced in obese Zucker fatty rats and *db/db* mice [131,132]. Putative impairments in cardiac NPs
355 secretion in human metabolically compromised conditions needs to be explored further.

356 **8. NPs and their role in inter-organ crosstalk**

357 NPs are long known for their cardiovascular and renal actions [18] resulting in the use of NPs
358 (or their fragments) as cardiovascular biomarkers in the clinic [133]. Over the last two decades,
359 pioneering studies by the group of Lafontan *et al.* suggested a protective role for the heart in
360 metabolic diseases [134]. The inter-organ crosstalk effectuated by ANP and BNP integrates
361 effects on AT function, skeletal muscle, liver, gut, central nervous system and pancreas, as
362 indicated in Figure 1.



363

364 **Figure 1 –** Overview of the multiple metabolic actions of natriuretic peptides in the control of
365 lipid metabolism and insulin sensitivity.

366

367

8.1. NP and adipose tissue function

As the human white AT is one of the main sites of NP receptor expression, investigating the effects of NPs on AT function seems obvious, in particular in the obese state. Next, the effects on AT lipolysis, brown AT metabolism (Table 1) and the role of NP in AT lipid metabolism in obesity and T2DM will be discussed in more detail.

8.1.1. NP and adipose tissue lipolysis

The potency of inducing a lipolytic effect by NPs was first described in isolated human adipocytes, with ANP being the most potent activator of lipolysis as compared to BNP and CNP, the latter only showing a minor maximal lipolytic response (17% as compared to maximal stimulation with isoproterenol) [134]. In addition, these lipolytic properties seem to be primate-specific, which may be due to the differential NPRC clearance receptor expression profiles in other mammalian adipocytes, especially in rodents, making ANP-mediated lipolysis less likely [135]. The signaling pathway relies on cGMP-dependent activation of protein kinase G (PKG), thereby promoting phosphorylation of perilipin-1 (PLIN-1) and hormone sensitive lipase (HSL) to trigger triglyceride hydrolysis [134,136,137], in which adipose triglyceride lipase (ATGL) might be involved as well [122], the latter probably via a different signaling pathway (*i.e.* AMP-activated kinase) [138] as compared with HSL activation (*i.e.* protein kinase A (PKA) and PKG) [139]. NPs-induced lipolysis is completely independent from the catecholamine-induced (cyclic AMP (cAMP)/PKA mediated) lipolysis, as they rely on different pathways [140,141]. However, an additive lipolytic effect occurs when human adipocytes are stimulated with ANP and a beta-adrenergic agonist (*e.g.* isoproterenol) simultaneously [142]. Infusion studies of human ANP (hANP), either intravenous or *in situ* through microdialysis in the subcutaneous AT, showed promotion of lipid mobilization in healthy subjects, also in the presence of local beta-adrenergic blockade [140]. Infusing

intravenous hANP (doses from 6.25-25 ng*kg^{-1}min^{-1}), corresponding to the physiological range observed during moderate exercise, stimulates whole-body lipid mobilization and oxidation (in a dose dependent way), even in the postprandial state [137,141,143]. Furthermore, exercise-induced increases in systemic ANP concentrations (which may vary depending on the exercise/subjects' characteristics) lead to an increase in lipid mobilization, at least in lean healthy subjects [144]. In human obesity, lipolytic catecholamine-resistance is most commonly observed in the subcutaneous AT in the obese insulin resistant state [145-147]. Additionally, an impaired ANP-mediated lipolysis has been reported *in vitro* and *in situ* in human subcutaneous AT of patients with obesity and/or type 2 diabetes and overweight men compared to non-obese counterparts [125,148]. Of interest, ANP-mediated lipid mobilization was reported to be higher in subcutaneous compared to visceral adipocytes of lean individuals [148], a difference that was not present in individuals with obesity [148,149]. The blunted ANP-mediated lipolysis in the subcutaneous AT of obese insulin resistant individuals may be in part due to an up-regulation of NPRC and a down-regulation of NPRA mRNA and protein in human subcutaneous adipocytes [125,148].

An interaction between the NPs' system and the anti-lipolytic hormone insulin was first suggested by Endre *et al.* [150], who showed that hyperinsulinemic euglycemic clamping caused a decrease in serum ANP in normotensive and hypertensive men. This finding was confirmed in obese men [81] but not in young lean individuals [151]. Insulin inhibits the catecholamine-induced lipolysis via activation of phosphodiesterase 3B (PDE3B), but does not have a direct anti-lipolytic effect on the ANP-mediated lipolytic pathway [152]. However, insulin might attenuate ANP-mediated lipolysis by inducing NPRC expression, as described earlier in this review. Of interest, the presence of low-glucose conditions together with insulin stimulation abolished NPRC expression to basal levels, indicating the existence of a "nutritional signaling" in NPRC regulation [122]. The relative ratio of NPRA to NPRC mRNA levels in

subcutaneous AT was decreased depending on glucometabolic status since patients with T2DM had the lowest ratio compared to subjects with normal glucose tolerance or impaired glucose metabolism [148,153].. Acute increases in systemic blood glucose decreased circulating N-terminal-proANP in lean, overweight and obese subjects, a mechanism mediated through glucose-induced miR-425 expression [154], a negative regulator of NPRA [155]. Insulin thus seems to be a key hormone in the connection between glucose/lipid metabolism and NPs' metabolic activities.

Together, these findings indicate that augmenting ANP-mediated lipolysis, possibly by improving insulin sensitivity, might be a target to improve lipid turnover in the obese insulin resistant and/or T2DM state.

8.1.2. NP and adipokines

Another way to link NPs to AT function is the ability of NPs to alter expression and secretion of adiponectin, an adipokine with insulin sensitizing properties, both *in vitro* [156] and *in vivo* [157] in healthy subjects. Moreover, adiponectin is positively associated with NPs [100,111,158,159]. Other insulin desensitizing mediators frequently linked to NPs include tumor necrosis factor-α (TNF-α) or interleukin-6. The secretion of these pro-inflammatory factors was reduced by treating human AT pieces with physiological ANP concentrations *in vitro*, possibly through a direct effect on both adipocytes and macrophages [160]. In this regard, reducing pro-inflammatory cytokines and increasing adiponectin secretion from AT could indirectly ameliorate the insulin sensitizing effects by NPs (Table 1).

8.1.3. NP and brown adipose tissue metabolism

Next to white AT, the presence and the importance of human brown AT (BAT) for human metabolic diseases receives increasing attention [161-164], although the quantitative importance of BAT in human energy and substrate metabolism remains uncertain. BAT is a thermogenic tissue having the ability to dissipate energy in the form of heat, thereby maintaining body temperature. Substrates including glucose and free fatty acids, delivered by white AT lipolysis, are necessary for heat dissipation, a process that is mediated by mitochondrial inner membrane uncoupling protein 1 (UCP-1) [165]. In addition, UCP-1 may have a regulatory function in whole-body energy homeostasis [166]. However, most of these data are derived from rodent studies and because adult human BAT may have a differential gene expression profile as either rodent BAT or beige fat [167], it physiological function in humans still needs to be determined in more detail. Support for a role of NPs in non-shivering thermogenesis was recently provided by showing that cold exposure acutely increases cardiac BNP secretion and NPRA/NPRC ratio in white AT in mice[19]. Mechanistic experiments indeed showed that ANP (and BNP) might activate mitochondrial biogenesis and uncoupling in human and mouse white adipocytes, via p38 MAPK/ATF2 signaling [19]. Chronic BNP treatment of *db/+* and *db/db* mice further confirmed these findings, showing increased UCP-1 expression and browning of the white fat pads [131]. ANP treatment also enhanced mitochondrial function in human adipocytes [168]. Taken together, *in vitro* studies have shown that the NP system is able to induce a thermogenic process in the AT and to induce brown AT activation. Since cold exposure is able to increase both NPs secretion and brown AT activation, the role of NPs in white AT "browning" might be of interest in the human *in vivo* situation, particularly in human metabolic disease. Nevertheless, until today, the role of NPs in human brown AT remains elusive.

8.2. NP and skeletal muscle metabolism

The mobilization of free fatty acids from AT depots by NPs provides substrates for energy production by oxidative tissues [137,140]. However, enhancement of AT and muscle lipid oxidation has been shown to be susceptible for NPs as well. Birkenfeld *et al.* [148] observed an acute increase in whole-body lipid oxidation (predominantly resulting from increased muscle lipid oxidation) following intravenous ANP infusion. Additionally ANP infusion leads to higher energy expenditure in the postprandial state [143]. The oxidative effect of ANP, as well as BNP, was later confirmed *in vitro* in human muscle cells. Transgenic *in vivo* experiments in mice showed increased skeletal muscle mitochondrial biogenesis, respiration and lipid oxidation upon chronic overexpression of BNP or cGMP-dependent protein kinase, thereby protecting for high fat diet induced obesity and glucose intolerance [127] . A physiological role of NPs in the regulation of skeletal muscle oxidative capacity in human primary myotubes was established by showing that ANP, BNP and cGMP analogs induce peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) expression, mitochondrial oxidation and lipid (palmitate) oxidation *in vitro* [20]. In addition, PGC-1α expression was associated with NPRA expression in skeletal muscle of healthy human subjects [20]. This proposes that NPs affect mitochondrial respiration and lipid oxidation in skeletal muscle through a cGMP dependent pathway, which was shown to be mediated by the induction of transcription and protein expression of PGC-1α and several OXPHOS complexes (complex I and complex IV), accompanied by an unchanged peroxisome proliferator activated receptor (PPAR) δ expression and mitochondrial DNA content [20].

Moreover, in skeletal muscle of obese and glucose intolerant humans and mice an altered NPRA/NPRC protein ratio was recently reported [124]. In *db/db* and *db/+* mice, this was accompanied with a diminished phosphorylation and activation of p38 MAPK, a downstream effector of the NPs receptor signaling pathway. However, chronic treatment of mice with

obesity-induced glucose intolerance and T2DM with BNP showed a reduced total diacylglycerol content in skeletal muscle, which was accompanied with higher oxidative capacity and PGC-1α gene expression [124]. This observation was further confirmed in human primary myotubes, showing that increased NP mediated lipid oxidation was accompanied by reduced *de novo* ceramide production [124] (Table 1).

Yet, recent data indicate a conceivable interaction between the NPs system and an exercise induced myokine called musclin a protein homologous to members of the NPs family [169] which results in the amelioration of the NPs' effectiveness [170]. Its physiologically relevant interaction was shown *in vitro* and *in vivo* and indicates the NPRA-mediated increase in skeletal muscle mitochondrial biogenesis to be potentiated by a musclin-NPRC interaction during exercise in mice [170]. Musclin is significantly upregulated in skeletal muscle of obese IR mice [169] and its gene expression is known to be increased upon palmitate treatment in C2C12 myotubes [171] and high fat diet in rats [172]. Furthermore, as musclin was proposed to exert its effects on glucose uptake in skeletal muscle via PPAR-γ [173], this suggests a possible role for musclin in substrate metabolism which needs to be explored in humans in the future. These studies indicate the importance of NPs signaling in skeletal muscle lipid oxidative capacity, which is imperative for long-term maintenance of insulin sensitivity in obesity and T2DM.

8.3. NP and liver function

Hepatic IR is an additional hallmark frequently observed in the metabolic syndrome and develops in case of an imbalance between fatty acid supply and utilization of fatty acids by hepatocytes, leading to ectopic lipid accumulation and whole-body IR [174]. In obesity the presence of non-alcoholic fatty liver (NAFL) is frequently observed [175], which may further lead to non-alcoholic steatohepatitis, liver cirrhosis or even liver carcinoma [176]. Recent studies show inverse relationships between NPs, in particular N-terminal-proBNP, and liver fat

content in individuals without diabetes or self-reported liver disease [177], as well as between NPs and liver function as indicated by aminotransferases enzymes in individuals without cardiovascular disease [178]. Additionally, NPs could ameliorate hepatic function as the presence of NPs receptors was shown in the human liver [179]. More precisely, these receptors were found on Kupffer-cells, resulting in a hepatoprotective effect of ANP by reducing Kupffer-cell-derived oxidant stress [180] and inhibiting lipopolysaccharides (LPS)-induced release of pro-inflammatory TNF-α via a cGMP-mediated signaling [181]. ANP or its analogs inhibited hepatic glycolysis and stimulated gluconeogenesis and cGMP production in perfused livers of fed rats [182]. Besides, ANP also induced hepatic lipid oxidation in healthy lean individuals, thereby reducing lipid spill-over and ectopic lipid deposition [143]. Consequently, lower liver TAG content was observed in BNP- or cGKI-transgenic mice on a high fat diet [127]. These findings were later confirmed in a cGKI knock out model, indicated by the presence of interleukin-6 mediated liver inflammation, fasting hyperglycemia and reduced insulin signaling [183]. These data together suggest a direct role of NPs in liver lipid catabolism (Table 1) next to indirect effects via AT mass reduction [127].

9. NP and control of insulin sensitivity

A role of NPs in the regulation of insulin sensitivity is plausible since several studies indicated an inverse association between NP deficiency and IR in human cohorts [21,24,92,113]. Moreover, the broad range of metabolic effects in insulin sensitive tissues makes these peptides putative targets for lifestyle and exercise interventions in metabolic diseases However, unraveling the mechanistic link via which NPs signaling can modulate insulin sensitivity remains incompletely understood

9.1. NP and insulin sensitivity

A role of NPs and their signaling pathway(s) in the control of insulin sensitivity was corroborated by BNP- and cGK-I-transgenic animals, which were protected against the development of diet-induced IR and glucose intolerance [127], and chronic BNP infusion studies in obese diabetic *db/db* mice [124,131], which improved insulin sensitivity and glucose tolerance and was accompanied by a reduced ectopic lipid accumulation [124,127,131]. On the contrary, genetic knock-down of the NPs signaling cascade impaired fasting glycemia in mice, possibly reflecting an attenuated insulin-mediated regulation of hepatic gluconeogenesis [183]. Little is known about the mechanisms responsible for the improvements in the NP-induced metabolic effects, but these may relate to NP secretion as well as NPs receptor expression and post-receptor signaling. Indeed, in humans, whole-body insulin sensitivity was recently shown to strongly correlate with NPRA in subcutaneous AT [153] and skeletal muscle [124]. In line, AT [148,153] and skeletal muscle [124] NPRC expression was negatively associated with whole-body insulin sensitivity. Additionally, NPs degradation by NEP [74] may contribute to the development of IR (as was shown in obese Zucker rats) [184,185]. Indeed NEP expression in plasma and adipocytes was positively associated with obesity and cardiometabolic risk in the presence of IR [128].

9.2. NP and insulin secretion

In addition to changes in insulin sensitivity, NPs may also affect pancreatic insulin secretion. Increased insulin levels were observed during ANP infusion in healthy subjects [143,186], while others showed no alterations during physiological infusion [187]. This effect of NPs on insulin concentration could be (partly) mediated by an increased secretion, since NPRA receptors were shown to be present on pancreatic α and β cells [188]. Furthermore, β cell insulin content, fasting glycemia as well as islet size and β cell mass were shown to be attenuated in the

562 NPRA knock out state [188], the latter being confirmed in isolated rat pancreatic islands [189]
563 (Table 1).

564 **10. Impact of exercise/lifestyle intervention on NP-related metabolic effects**

565 The use of exercise as a non-pharmacological strategy to combat NP deficiency is appropriate
566 as several studies have shown that exercise increases ANP levels, at least in healthy subjects
567 (Figure 2) [144,190-192]. In particular, ANP secretion is enhanced by increasing venous return and
568 cardiac filling pressure (*i.e.* cardiac output) [193-195], while only slightly increasing [193] or even
569 not altering circulatory BNP levels [194] in healthy volunteers, possibly indicating opposing
570 regulation in cardiac atria and ventricles. One study reported plasma BNP levels to be
571 positively associated with physical activity levels, although this remains controversial [196,197].
572 Recently, 7 days of bed rest induced a decrease in plasma proANP in young healthy males,
573 which was also accompanied with a decreased insulin sensitivity [151]. Exercise in elderly,
574 healthy subjects caused significantly increased proANP and N-terminal-proBNP levels [198]. Of
575 interest, data about exercise effects on NPs in metabolic conditions are scarce. Tanaka *et al.*
576 [199] showed that NP secretion (ANP and BNP) is more sensitive to sympathetic activity in
577 normotensive subjects compared to hypertensive patients, where increased NPs levels may
578 represent cardiac stress. In overweight and obese patients mid-regional-proANP
579 concentrations increased upon incremental exhaustive exercise both before and after diet
580 intervention, with no difference in exercise response between these conditions [200]. Moreover,
581 NPs were also evaluated in relation to resistance training [201,202]. Resistance training induces a
582 significant increase in N-terminal-proBNP, which might be partly due to myocardial damage
583 [201]. However, N-terminal-proBNP concentrations did not change in elderly following
584 resistance training [202]. The latter two studies indicate that the effects of strength training on
585 NPs concentrations are still controversial and thus need more investigation, especially in
586 metabolically compromised conditions.

With respect to CNP, exercise preconditioning (that is, an enhancement of the tolerance of an ischemic heart to subsequent ischemic insult by a single bout of interval exercise) promotes the secretion of CNP in rodents, thereby elevating CNP levels in the myocardium and protecting against high-intensity exercise-induced myocardial injury [203]. Indeed, exercise training (more specifically walking or walking plus resistance exercise training) increased plasma CNP levels, which may be one of the mechanisms through which exercise intervention may reverse endothelial-dependent dysfunction in middle-aged individuals with impaired glucose tolerance [204]. This supports the use of (combined) exercise training to prevent T2DM.

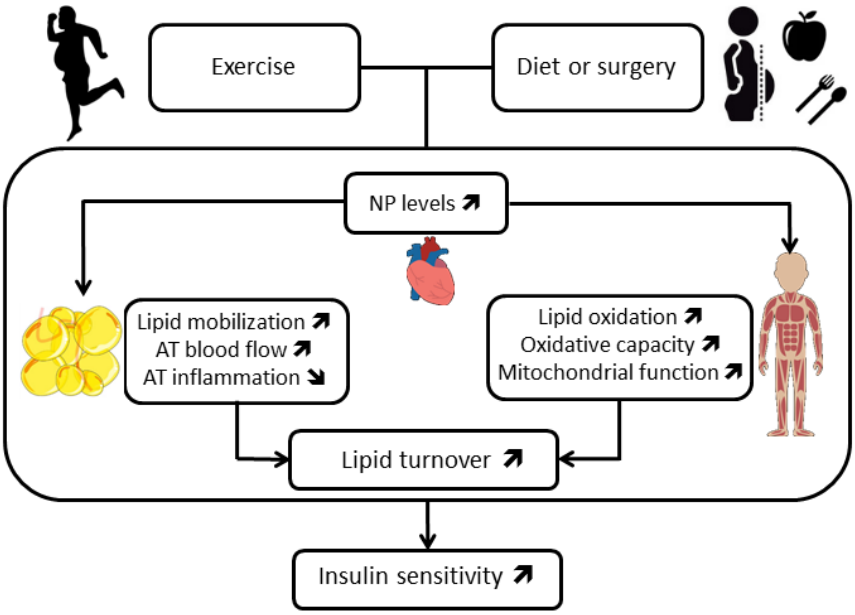


Figure 2 – Lifestyle or surgical intervention effects on natriuretic peptide levels and associated metabolic effects in humans.

10.1. Lifestyle interventions and NP-mediated metabolic effects

Mechanisms responsible for the exercise-/exercise training-induced normalization of the NP deficiency in metabolic disease might include multiple paths, which are currently incompletely understood (Figure 2). Regular physical activity [205], rather than caloric restriction [200], augments ANP-mediated increases in AT blood flow [192] and AT functional NPRA receptor expression thereby presumably recovering white AT ANP responsiveness [192, 206], together resulting in an improved AT lipid mobilization process in the overweight/obese state. As an alternative, water-based exercise has been shown to be not advantageous over moderate intensity land-based exercise with respect to lipid mobilization or fat oxidation in humans despite higher systemic ANP levels during exercise [207]. Moreover, exercise training enhances mitochondrial function at the level of the skeletal muscle and ultimately insulin sensitivity [208], at least partly due to an increased NPRA expression and signaling [208], the latter also resulting from caloric restriction-induced weight loss [124] in obese subjects [20]. Of interest, pharmacologically improved insulin sensitivity (by the anti-diabetic drug pioglitazone) was accompanied by an increased NPRA/NPRC ratio in subcutaneous AT of obese individuals with T2DM [153]. Liraglutide-induced weight loss in obese individuals with T2DM was correlated with change in NPs levels, although the mechanism responsible remained elusive [209].

Diet-induced [135] and gastric bypass-mediated weight loss [125] confirmed the reversibility of the reduced maximal ANP responsiveness in the subcutaneous AT of obese women, postulating that the observed impairments in NPs-mediated metabolic effects are secondary to the obese state. With respect to caloric restriction, fasting was shown to restore NPs signaling by reducing NPRC expression in the AT [126,127]. However, weight loss studies, either involving caloric restriction or gastric bypass surgery, indicated increased systemic NPs levels (*i.e.* NT-proBNP) [210-215], which was not confirmed in all studies [200,216,217]. Comparing these

626 interventions, it was recently shown that amount of weight loss is associated with the increase
627 in systemic NPs concentrations [218]. Besides changing the NPs' signaling pathway and as
628 many studies also observe changes in the inactive fragments (NT-proANP and NT-proBNP),
629 which are not cleared by NPRC, these findings suggest not only changes in signaling but also
630 adjustments in cardiac production and release following weight loss. In this regard,
631 improvements in other comorbidities which could affect the NPs system following this type of
632 interventions should be taken into account as well.

633 Together, these data indicate that the NPs signaling pathway may be a suitable target to
634 improve insulin sensitivity inexercise interventions, weight loss interventions or a
635 combination between both (Figure 2). Their modulatory effects with respect to NPs related
636 improvements in insulin sensitivity need further investigation in human metabolic disease.
637 However, the present clinical studies do not show a causal relationship but they do indicate
638 the presence of a strong association between the NPs system and insulin sensitivity
639 management, which needs further focus in upcoming human non-pharmacological
640 intervention studies.

641 11. Therapeutic opportunities for ANP in metabolic diseases

642 The natriuretic deficiency present in obesity, the metabolic syndrome and T2DM is described
643 by reduced plasma levels of NPs together with impaired tissue responses in AT and skeletal
644 muscle tissue. Normalizing systemic NPs levels or tissue responses may therefore be
645 imperative in the prevention of metabolic disturbances in the obese state. With respect to
646 systemic NPs levels, pharmacological treatment with the anti-diabetic drug liraglutide was
647 unable to acutely increase cardiac ANP secretion [54,55,57]. However, upon chronic treatment in
648 obese individuals with T2DM systemic NPs levels increased, thereby being associated with
649 the amount of induced weight loss [209]. This might indicate that, based on the pleiotropic

effects of NPs in metabolic tissues, several molecular targets of the NP system may be targeted. Strategies aimed to chronically inhibit NPs degradation might be a way to sustain appropriate systemic NPs levels and thus NP signaling in metabolic tissues. Inhibiting NPRC-, NEP- or IDE-mediated NPs breakdown could be suitable options in this respect as recently reviewed [219]. Secondly, NPs delivery may be envisioned, a therapeutic option in which adequate delivery is crucial to obtain clinical efficacy [219]. In addition to the use of recombinant ANP (carperitide) and BNP (nesiritide) in acute heart failure treatment [220-222], the therapeutic potential of these compounds in metabolic diseases may be tested. Furthermore, selective NPRC antagonists or NPs analogs resisting NPRC-mediated clearance might be suitable as well. Finding novel peptides or optimized delivery methods will be a new frontier in the development of therapeutics for metabolic diseases in future research. In addition, to gain knowledge about causality and to identify potential determinants and mechanisms determining tissue-specific effects, more mechanistic studies in whole-body and tissue-specific knockout models of NPs or their signaling pathway are crucial. These insights may be implemented in the optimization of non-pharmacologic treatment strategies of metabolic diseases.

12. Conclusions

The potential role of NPs as an important metabolic target affecting insulin sensitivity in metabolically compromised conditions has been put forward over the last decade. Preclinical and clinical research indicated the presence of a NP deficiency in obesity and T2DM, which is a well-accepted anomaly that could result from inadequate cardiac NPs production and secretion, as well as an increased degree of peripheral degradation. Of interest, several impairments in NPs receptor and post-receptor signaling have been observed in peripheral tissues like the AT or the skeletal muscle tissue of patients with metabolic disease, including overweight, obesity, IR and T2DM. Despite several animal and human data suggest a causal

link between these NPs (signaling) deficiencies and the development of T2DM, the exact molecular mechanism remains incompletely understood. Unraveling the molecular background of these anomalies could therefore further highlight the emerging role of NPs in metabolic diseases. From a clinical point of view, it remains important to investigate therapeutic options to restore this NP deficiency. Next to implementing pharmacological intervention strategies, lifestyle interventions (including physical activity and diet) are of interest in the treatment of metabolic disease. Indeed, previous studies indicated diet-induced weight loss and physical exercise training to sensitize NPs signaling in AT and skeletal muscle tissue, two main metabolic organs with a role in obesity-related IR and T2DM, but also to improve cardiac NPs secretion. However, selecting the appropriate intervention with respect to reducing cardiovascular and metabolic risk factors is of special interest to further improve cardiometabolic health and reduce the development and progression of obesity-related risk factors. Therefore, long-term human intervention studies are needed to clarify the role of NPs in the control of body weight and insulin sensitivity, including investigation of the underlying molecular machinery.

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