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1 Natriuretic peptides in the control of lipid metabolism
2 and insulin sensitivity

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32 Abbreviations

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

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57 **1. Abstract**

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

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85 **2. Introduction**

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

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

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

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

126 **3. Secretion and processing of NP**

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

134 the central nervous system^{30,31}, is mainly viewed as a peptide regulating vascular blood
135 pressure³² and bone growth³³, although a minor role in metabolic regulation has been
136 suggested^{34,35}. In mammals, dendroaspis natriuretic peptide (DNP) (of which the synthesis
137 and secretion sites have not been identified) exerts renal actions via its specific receptor³⁶ but
138 because it has not been well studied with respect to metabolic effects in humans, DNP will not
139 be further discussed in the current review.

140 Currently, ANP and BNP are the main metabolically active NPs described in literature and
141 will therefore be discussed in more detail in the present review. These peptides have a 17-
142 amino acid ring structure in common, formed by an intramolecular disulfide linkage, of which
143 the sequence is highly preserved within the biologically active form of these peptides³⁷.
144 Structural differences between NP family members are due to specific amino- and carboxy-
145 terminal extensions³⁸. At rest, ANP (normal concentration range 5-50 pg/mL) is mainly
146 produced and secreted by the (right) atrial myocardium as a prehormone³⁹. The
147 intracellular granules of the atrial myocardium contain proANP which is cleaved by corin
148 (type II transmembrane serine protease expressed primarily in the heart) during secretion,
149 resulting in a 28-amino acid, bioactive α ANP peptide⁴⁰, with a very short plasma half-life of
150 about 2-4 min⁴¹, and inactive fragments (N-terminal ANP and mid-regional-proANP) which
151 have a longer plasma half-life (about 40-50 min)^{41,42}. BNP is mainly produced and secreted
152 by the ventricular myocardium as preproBNP⁴³. To become biologically active, preproBNP is
153 cleaved to proBNP and subsequently, like for ANP, a cardiac protease, corin or furin, is
154 responsible for the conversion to the 32-amino acid BNP (plasma concentration range 0-65
155 pg/mL) which is secreted in the circulation having a plasma half-life of about 15-25 min⁴⁴,
156 and the inactive N-terminal fragment proBNP⁴⁵. The latter inactive fragment has a plasma
157 half-life of about 60-120 min and a plasma concentration in the range of 7-220 pmol/L in
158 healthy individuals⁴¹. While the structure of BNP varies distinctly among species, ANP is

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

161 **4. Determinants of NP secretion**

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

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

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

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

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

201 **5. NP receptors and signaling**

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

207 thereby playing a role in long bone growth⁶⁸. Ligand binding to a NPRA homodimer results
208 in the internalization of the bound ligand-receptor complex and the activation of cytosolic
209 GC, the catalytic effector of the receptor, subsequently causing hydrolysis of GTP into cyclic
210 GMP (cGMP). This second messenger is able to activate various biological responses via
211 cGMP-dependent protein kinases, cGMP-gated ion channels or other effector proteins⁶⁹. A
212 large proportion of the ligand-bound receptor undergo lysosomal degradation, in which about
213 75% of the internalized ANP is processed in the lysosomes and 25% is released as intact
214 molecules through a recycling pathway. A small amount of internalized receptors is recycled
215 back to the plasma membrane or released into the cell exterior⁷⁰. The NP receptor C (NPRC)
216 is the third subtype, having an extracellular domain that is partly homologous to those of
217 NPRA receptors and thus has the ability to bind NPs, with the highest affinity to ANP and
218 lowest to BNP⁷¹. However, NPRC lacks GC activity and instead its main function is to
219 facilitate scavenging of its ligands, internalization of ligand-receptor complexes and recycling
220 of NPRC, together with lysosomal degradation of its ligands^{72,73}. Besides via lysosomal
221 clearance, NPs can be degraded intracellularly by endopeptidases including neutral
222 endopeptidase (NEP)⁷⁴, which is also produced in adipocytes⁷⁵. In addition, insulin-
223 degrading enzyme (IDE) enzymatically cleaves NP^{45,76} and dipeptidyl peptidase-4 (DPP4 or
224 CD26) cleaves the N-terminal peptide of NPs thereby lowering biological activity⁷⁷. Another
225 route to clear circulating NP is via secretion into body fluids like urine (via glomerular
226 filtration) and bile⁴⁵.

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

232 the cardiovascular system and blood monocytes^{73,80,81}. Interestingly, NPRA and NPRC
233 display diurnal regulations (in antiphase of one other) in the rodent white AT⁸², not in the
234 heart muscle⁸³, which together with the circadian regulated plasma NPs^{84,85}, may be a
235 characteristic for energy homeostasis during the day. Furthermore, the local tissue specific
236 and systemic effects of NP are thought to depend on the ratio between NPRA and NPRC^{86,87}.
237 Collectively, NPs mediate their effects via NP receptors, of which three subtypes have been
238 described. The diverse effects of NPs, systemically as well as the local tissue effects, are
239 determined by NP receptor expression profiles and their ligand-affinity.

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

241 Evidence from several epidemiological studies demonstrated an inverse association between
242 systemic NPs levels (both ANP and BNP) and body weight (mostly expressed by body mass
243 index (BMI))^{21,23,88-92}. The inverse relationship between NP levels and BMI was also found in
244 the presence of left ventricular hypertrophy⁹³. However, these studies lack detailed body
245 composition analyses thereby not being able to differentiate between fat accumulation or fluid
246 retention as a cause of the increased BMI. In contrast, other smaller cohorts showed that there
247 is no or even a positive relationship between circulating NPs levels and BMI^{94,95}.
248 Furthermore, NPs may affect AT distribution^{96,97}. Variations in regional and particularly
249 visceral adiposity were strongly related to circulating N-terminal-pro-BNP. The relationship
250 of NPs with subcutaneous adiposity was less strong⁹⁷. This relation could be partly
251 moderated by the hyperinsulinemic state that is frequently observed in visceral adiposity, as
252 high insulin levels have been shown to suppress NPs secretion and activity^{98,99}. The Dallas
253 Heart Study recently showed that both BNP and N-terminal-proBNP are inversely related to
254 visceral and liver fat, while being positively associated with gluteofemoral body fat,
255 independent of insulin sensitivity¹⁰⁰.

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

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

281 blood pressure, antihypertensive treatment, age and sex) or renal function (cystatin C)²⁵. In
282 the Atherosclerosis Risk in Communities Study (a community-based population study), it was
283 shown that having low N-terminal-proBNP levels (lowest quartile, <31pg/mL) was associated
284 with higher risk of incident diabetes over a 12 year follow-up period in subjects without
285 T2DM at baseline. These results were consistent across race, gender and BMI categories²⁶,
286 and were independent of age^{111,112}. Of interest, statistical adjustment for BMI did not
287 abrogate the association between low NP levels and diabetes onset¹¹³. These results are in
288 line with the Framingham Heart Study and the Malmö Diet and Cancer Study, which showed
289 that lower N-terminal-proBNP levels were associated with higher incidence of IR in lean as
290 well as in obese subjects in a cross-sectional study²³. Additionally, prospective cohort data
291 from the Women's Health Study showed that subjects with N-terminal-proBNP levels near
292 the upper limit of the normal range (>117 pg/mL) have a significantly lower risk of
293 developing diabetes¹¹⁴.

294 Thus, there is consistent evidence that increased NP concentrations are protective against IR
295 and T2DM¹¹⁴⁻¹¹⁶. In line, lower blood glucose concentrations were transiently observed upon
296 systemic BNP infusion during intravenous glucose tolerance testing in young, healthy lean
297 men with normal glucose tolerance¹¹⁷. Moreover, in a random subset of a general middle-
298 aged population (age >45 years) a genetic variant of the ANP gene (single nucleotide
299 polymorphism rs5068) was associated with higher N-terminal-proANP levels and a beneficial
300 cardiometabolic profile (*i.e.* reduced systemic blood pressure, BMI, waist circumference and a
301 lower risk of metabolic syndrome) compared to the A/A carriers⁸⁹. This ANP gene-
302 polymorphism was accompanied with a lower incidence of T2DM after a 14-year follow-up
303¹¹⁸. Similarly, a genetic polymorphism in the promotor region of the BNP gene (T-381C
304 polymorphism) is associated with higher plasma BNP levels and lower risk of T2DM in

305 several population samples including individuals with normoglycemia, impaired glucose
306 tolerance and T2DM ¹¹⁹.

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

312 **7. Underlying mechanism for systemic NP deficiency in obesity**

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

328 However, results from the Dallas Heart Study showed that the association between BMI and
329 circulating NP levels is explained by the amount of lean mass, and not AT mass, indicating
330 that lean tissue could also be important for plasma NP regulation ⁸⁸. Indeed, upregulation of
331 NPRC in human skeletal muscle tissue, next to down-regulation of the NPRC expression in
332 the AT and skeletal muscle of obese and/or obese diabetic humans and mice has been found
333 ^{124,125}. Thus, besides AT, also skeletal muscle may contribute to the NP deficiency observed in
334 T2DM in the long-term, certainly considering that muscle mass accounts for up to 40% of
335 total body weight. Therefore, even a moderate increase in skeletal muscle NPRC expression
336 could markedly reduce NPs plasma levels ¹²⁴.

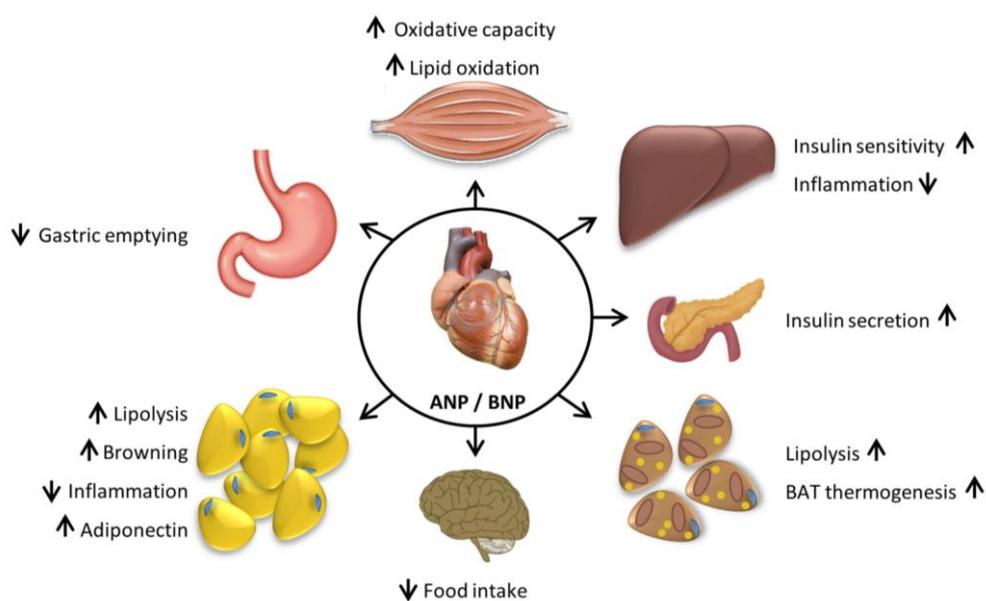
337 Additionally, it has been shown that NPRC mRNA expression is down-regulated *in vitro*
338 following starvation in human differentiated adipocytes ¹²² and *in vivo* in rat white and brown
339 AT ¹²⁶ while the opposite was true under high fat feeding in wild-type mice skeletal muscle,
340 white and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

345 On the other hand, a decreased cardiac ANP and BNP release in metabolic disease has also
346 been proposed. Not only circulating NPs levels but also the side products of NPs release (N-
347 terminal-proANP and N-terminal-proBNP) are reduced in obesity. These proteins are
348 structurally distinct and are biologically inactive compounds, which makes NPRC mediated
349 clearance of these components rather unlikely ^{88,129}. Of interest, NPs levels in the aortic root
350 and the coronary sinus were observed to be negatively correlated with BMI ¹³⁰. These findings
351 suggest that besides an increased clearance, a reduced cardiac NPs release might potentially
352 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 actions¹⁸ resulting in the use of NPs
358 (or their fragments) as cardiovascular biomarkers in the clinic¹³³. 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 diseases¹³⁴. 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.

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368 **8.1. NP and adipose tissue function**

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

373 **8.1.1. NP and adipose tissue lipolysis**

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

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

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

417 subcutaneous AT was decreased depending on glucometabolic status since patients with
418 T2DM had the lowest ratio compared to subjects with normal glucose tolerance or impaired
419 glucose metabolism^{148,153}. Acute increases in systemic blood glucose decreased circulating
420 N-terminal-proANP in lean, overweight and obese subjects, a mechanism mediated through
421 glucose-induced miR-425 expression¹⁵⁴, a negative regulator of NPRA¹⁵⁵. Insulin thus seems
422 to be a key hormone in the connection between glucose/lipid metabolism and NPs' metabolic
423 activities.

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

427 **8.1.2. NP and adipokines**

428 Another way to link NPs to AT function is the ability of NPs to alter expression and secretion
429 of adiponectin, an adipokine with insulin sensitizing properties, both *in vitro*¹⁵⁶ and *in vivo*¹⁵⁷
430 in healthy subjects. Moreover, adiponectin is positively associated with NPs^{100,111,158,159}.
431 Other insulin desensitizing mediators frequently linked to NPs include tumor necrosis factor-
432 α (TNF- α) or interleukin-6. The secretion of these pro-inflammatory factors was reduced by
433 treating human AT pieces with physiological ANP concentrations *in vitro*, possibly through a
434 direct effect on both adipocytes and macrophages¹⁶⁰. In this regard, reducing pro-
435 inflammatory cytokines and increasing adiponectin secretion from AT could indirectly
436 ameliorate the insulin sensitizing effects by NPs (Table 1).

437

438

439

440 **8.1.3. NP and brown adipose tissue metabolism**

441 Next to white AT, the presence and the importance of human brown AT (BAT) for human
442 metabolic diseases receives increasing attention ¹⁶¹⁻¹⁶⁴, although the quantitative importance
443 of BAT in human energy and substrate metabolism remains uncertain. BAT is a thermogenic
444 tissue having the ability to dissipate energy in the form of heat, thereby maintaining body
445 temperature. Substrates including glucose and free fatty acids, delivered by white AT
446 lipolysis, are necessary for heat dissipation, a process that is mediated by mitochondrial inner
447 membrane uncoupling protein 1 (UCP-1) ¹⁶⁵. In addition, UCP-1 may have a regulatory
448 function in whole-body energy homeostasis ¹⁶⁶. However, most of these data are derived from
449 rodent studies and because adult human BAT may have a differential gene expression profile
450 as either rodent BAT or beige fat ¹⁶⁷, its physiological function in humans still needs to be
451 determined in more detail. Support for a role of NPs in non-shivering thermogenesis was
452 recently provided by showing that cold exposure acutely increases cardiac BNP secretion and
453 NPRA/NPRC ratio in white AT in mice¹⁹. Mechanistic experiments indeed showed that ANP
454 (and BNP) might activate mitochondrial biogenesis and uncoupling in human and mouse
455 white adipocytes, via p38 MAPK/ATF2 signaling ¹⁹. Chronic BNP treatment of *db/+* and
456 *db/db* mice further confirmed these findings, showing increased UCP-1 expression and
457 browning of the white fat pads ¹³¹. ANP treatment also enhanced mitochondrial function in
458 human adipocytes ¹⁶⁸. Taken together, *in vitro* studies have shown that the NP system is able
459 to induce a thermogenic process in the AT and to induce brown AT activation. Since cold
460 exposure is able to increase both NPs secretion and brown AT activation, the role of NPs in
461 white AT “browning” might be of interest in the human *in vivo* situation, particularly in
462 human metabolic disease. Nevertheless, until today, the role of NPs in human brown AT
463 remains elusive.

464

465 **8.2. NP and skeletal muscle metabolism**

466 The mobilization of free fatty acids from AT depots by NPs provides substrates for energy
467 production by oxidative tissues^{137,140}. However, enhancement of AT and muscle lipid
468 oxidation has been shown to be susceptible for NPs as well. Birkenfeld *et al.*¹⁴⁸ observed an
469 acute increase in whole-body lipid oxidation (predominantly resulting from increased muscle
470 lipid oxidation) following intravenous ANP infusion. Additionally ANP infusion leads to
471 higher energy expenditure in the postprandial state¹⁴³. The oxidative effect of ANP, as well as
472 BNP, was later confirmed *in vitro* in human muscle cells. Transgenic *in vivo* experiments in
473 mice showed increased skeletal muscle mitochondrial biogenesis, respiration and lipid
474 oxidation upon chronic overexpression of BNP or cGMP-dependent protein kinase, thereby
475 protecting for high fat diet induced obesity and glucose intolerance¹²⁷. A physiological role
476 of NPs in the regulation of skeletal muscle oxidative capacity in human primary myotubes
477 was established by showing that ANP, BNP and cGMP analogs induce peroxisome
478 proliferator-activated receptor gamma coactivator 1-alpha (PGC-1 α) expression,
479 mitochondrial oxidation and lipid (palmitate) oxidation *in vitro*²⁰. In addition, PGC-1 α
480 expression was associated with NPRA expression in skeletal muscle of healthy human
481 subjects²⁰. This proposes that NPs affect mitochondrial respiration and lipid oxidation in
482 skeletal muscle through a cGMP dependent pathway, which was shown to be mediated by the
483 induction of transcription and protein expression of PGC-1 α and several OXPHOS complexes
484 (complex I and complex IV), accompanied by an unchanged peroxisome proliferator activated
485 receptor (PPAR) δ expression and mitochondrial DNA content²⁰.

486 Moreover, in skeletal muscle of obese and glucose intolerant humans and mice an altered
487 NPRA/NPRC protein ratio was recently reported¹²⁴. In *db/db* and *db/+* mice, this was
488 accompanied with a diminished phosphorylation and activation of p38 MAPK, a downstream
489 effector of the NPs receptor signaling pathway. However, chronic treatment of mice with

490 obesity-induced glucose intolerance and T2DM with BNP showed a reduced total
491 diacylglycerol content in skeletal muscle, which was accompanied with higher oxidative
492 capacity and PGC-1 α gene expression¹²⁴. This observation was further confirmed in human
493 primary myotubes, showing that increased NP mediated lipid oxidation was accompanied by
494 reduced *de novo* ceramide production¹²⁴ (Table 1).

495 Yet, recent data indicate a conceivable interaction between the NPs system and an exercise
496 induced myokine called musclin a protein homologous to members of the NPs family¹⁶⁹
497 which results in the amelioration of the NPs' effectiveness¹⁷⁰. Its physiologically relevant
498 interaction was shown *in vitro* and *in vivo* and indicates the NPRA-mediated increase in
499 skeletal muscle mitochondrial biogenesis to be potentiated by a musclin-NPRC interaction
500 during exercise in mice¹⁷⁰. Musclin is significantly upregulated in skeletal muscle of obese IR
501 mice¹⁶⁹ and its gene expression is known to be increased upon palmitate treatment in C2C12
502 myotubes¹⁷¹ and high fat diet in rats¹⁷². Furthermore, as musclin was proposed to exert its
503 effects on glucose uptake in skeletal muscle via PPAR- γ ¹⁷³, this suggests a possible role for
504 musclin in substrate metabolism which needs to be explored in humans in the future. These
505 studies indicate the importance of NPs signaling in skeletal muscle lipid oxidative capacity,
506 which is imperative for long-term maintenance of insulin sensitivity in obesity and T2DM.

507 **8.3. NP and liver function**

508 Hepatic IR is an additional hallmark frequently observed in the metabolic syndrome and
509 develops in case of an imbalance between fatty acid supply and utilization of fatty acids by
510 hepatocytes, leading to ectopic lipid accumulation and whole-body IR¹⁷⁴. In obesity the
511 presence of non-alcoholic fatty liver (NAFL) is frequently observed¹⁷⁵, which may further
512 lead to non-alcoholic steatohepatitis, liver cirrhosis or even liver carcinoma¹⁷⁶. Recent studies
513 show inverse relationships between NPs, in particular N-terminal-proBNP, and liver fat

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

529 **9. NP and control of insulin sensitivity**

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

536

537

538 **9.1. NP and insulin sensitivity**

539 A role of NPs and their signaling pathway(s) in the control of insulin sensitivity was
540 corroborated by BNP- and cGK-I-transgenic animals, which were protected against the
541 development of diet-induced IR and glucose intolerance¹²⁷, and chronic BNP infusion studies
542 in obese diabetic *db/db* mice^{124,131}, which improved insulin sensitivity and glucose tolerance
543 and was accompanied by a reduced ectopic lipid accumulation^{124,127,131}. On the contrary,
544 genetic knock-down of the NPs signaling cascade impaired fasting glycemia in mice, possibly
545 reflecting an attenuated insulin-mediated regulation of hepatic gluconeogenesis¹⁸³. Little is
546 known about the mechanisms responsible for the improvements in the NP-induced metabolic
547 effects, but these may relate to NP secretion as well as NPs receptor expression and post-
548 receptor signaling. Indeed, in humans, whole-body insulin sensitivity was recently shown to
549 strongly correlate with NPRA in subcutaneous AT¹⁵³ and skeletal muscle¹²⁴. In line, AT
550^{148,153} and skeletal muscle¹²⁴ NPRC expression was negatively associated with whole-body
551 insulin sensitivity. Additionally, NPs degradation by NEP⁷⁴ may contribute to the
552 development of IR (as was shown in obese Zucker rats)^{184,185}. Indeed NEP expression in
553 plasma and adipocytes was positively associated with obesity and cardiometabolic risk in the
554 presence of IR¹²⁸.

555 **9.2. NP and insulin secretion**

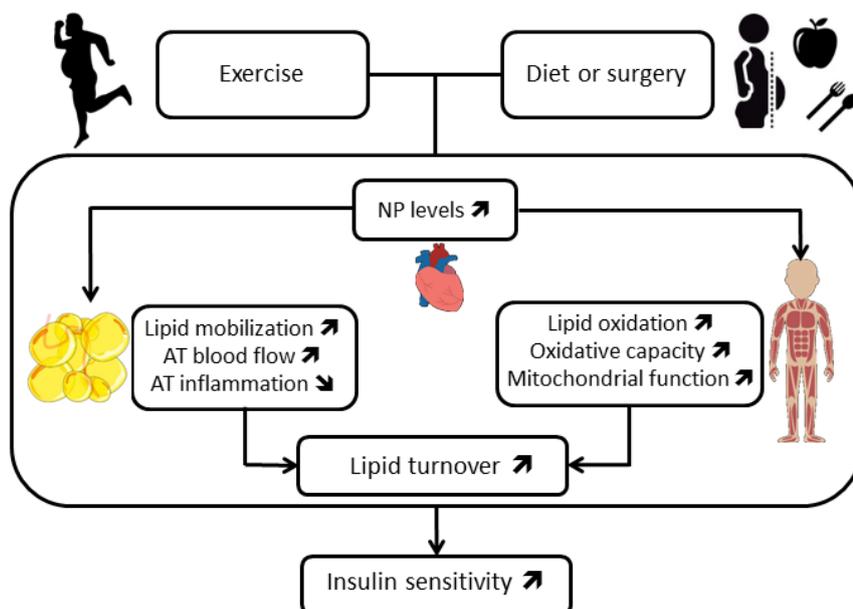
556 In addition to changes in insulin sensitivity, NPs may also affect pancreatic insulin secretion.
557 Increased insulin levels were observed during ANP infusion in healthy subjects^{143,186}, while
558 others showed no alterations during physiological infusion¹⁸⁷. This effect of NPs on insulin
559 concentration could be (partly) mediated by an increased secretion, since NPRA receptors
560 were shown to be present on pancreatic α and β cells¹⁸⁸. Furthermore, β cell insulin content,
561 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.

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



596 **Figure 2** – Lifestyle or surgical intervention effects on natriuretic peptide levels and
 597 associated metabolic effects in humans.
 598

599

600

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 activity²⁰⁵, rather than caloric restriction²⁰⁰, augments ANP-mediated increases in AT blood flow¹⁹² 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 exercise²⁰⁷. Moreover, exercise training enhances mitochondrial function at the level of the skeletal muscle and ultimately insulin sensitivity²⁰⁸, at least partly due to an increased NPRA expression and signaling²⁰⁸, the latter also resulting from caloric restriction-induced weight loss¹²⁴ in obese subjects²⁰. 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 T2DM¹⁵³. Liraglutide-induced weight loss in obese individuals with T2DM was correlated with change in NPs levels, although the mechanism responsible remained elusive²⁰⁹.

Diet-induced¹³⁵ and gastric bypass-mediated weight loss¹²⁵ 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 in exercise 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

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

666 **12. Conclusions**

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

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

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