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Habilitation

The Role of Natriuretic Peptides in the Regulation of Energy Metabolism, Lipid- and Glucose Homeostasis

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We think for ourselves,

that is why we are scientists.

Gerald I. Shulman

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Abbreviations

AMP = adenosine monophosphate

AMPK = AMP activated protein kinase

ANP = atrial natriuretic peptide

AR = adrenergic receptors

ATGL = adipose triglyceride lipase

BNP = brain natriuretic peptide

cAMP = cyclic adenosine monophosphate

cGKI = cGMP-dependent protein kinase I

cGMP = cyclic guanosine monophosphate

CNP = C-type natriuretic peptide

DNP = dendroaspis natriuretic peptide

FFA = free fatty acids

Kcal = kilocalories

HSL = hormone sensitive lipase

mRNA = messenger ribonucleic acid

NP = natriuretic peptides

NPR-A = natriuretic peptide receptor A

NPR-B = natriuretic peptide receptor B

NPR-C = natriuretic peptide receptor C

OXPHOS = oxidative phosphorylation

PDE-3A = phosphodiesterase 3A

PDE-5 = phosphodiesterase 5

PGC-1α = peroxisome proliferator activated receptor-γ coativator-1α

1. Introduction and Aims

Metabolic research may have started with a fundamental question: why is metabolic regulation necessary? Metazoans depend on the transformation of chemical into biochemical energy. Typically, chemical energy is supplied in a discontinuous manner, while life *per se* leads to a constant depletion of energy. The capacity to bridge the gaps between energy demand and availability is, hence, a vital function of metabolic regulation. The aim of this habilitation is to characterize the physiological role of novel endocrine players in the regulation of metabolism.

1.1 Regulation of Energy Expenditure

Mammalian energy expenditure is the result of 3 components: first, basal metabolic rate accounts for about 60% of whole energy expenditure and is mainly determined by the amount of skeletal muscles in mammals. Second, thermogenesis, which is induced by the digestion of food, and third, physical activity, which accounts for up to 30% of total energy expenditure (1-4). Physical activity is the most variable component, ranging from less than one hundred kcal per day in sedentary adults to thousands of kcal per day in endurance athletes (5). Apart from physical activity, regulation of energy expenditure results from a complex crosstalk between higher regions within the central nervous system (3), the liver (6; 7), skeletal muscle (4), white and brown adipose tissue (8; 9), pancreas (10) and the gut (11). The autonomous nervous system, whose origins have been mapped to central nervous system nuclei in the hypothalamus, midbrain, and brain stem signals via first-order, leptin- and melanocortin-responsive neurons using distinct ganglia to synapse into target organs. Postganglionic neurons release either norepinephrine (sympathetic) or acetylcholine (parasympathetic) from terminals, stimulating adrenergic receptors. Adrenergic regulation has been postulated to be the most important regulator of energy expenditure in response to dietary excess (3). Furthermore, specific hormones are involved in the regulation of energy expenditure. Thyroid hormone was first identified to be responsible for approximately 30% of basal thermogenesis (12). More recently, adipokines such as adiponectin and leptin were found to be involved in the regulation of energy expenditure (13). Finally, energetic substrates, such as specific lipids, are ligands for distinct nuclear receptors that control the regulation of energy expenditure (14).

1.2 Regulation of Lipid Metabolism

Lipids in the gestalt of triglycerides emerged as the preferred storage nutrient to buffer against fluctuations in energy demand and availability. The ubiquitous selection of triglycerides for this role is attributable to two physicochemical properties: triglycerides provide twice as much caloric density than carbohydrates or proteins and they are insoluble in water, so they can accumulate to high levels with no adverse osmotic or colloidal effects on cells. Triglycerides are built from three fatty acids assembled on one glycerol backbone. Diet is the premier source of fatty acids and they are condensed into chylomicrones after absorption in the gut and hydrolyzed by endothelial lipoprotein lipase, so as to release fatty acids for the uptake in peripheral tissues. The fatty acids flux in the circulation equals 100 g of fat per day of which 20% are extracted by the liver (6). There, fatty acids can be either oxidized in the mitochondrial matrix to generate energy and ketone bodies, re-esterified and stored in lipid droplets, or coupled to apolipoproteins and secreted as a constituent of very-low-density lipoproteins (15; 16).

Circulating excess fatty acids are stockpiled in adipose tissue. During fasting, plasma levels of insulin decrease and levels of glucagon and epinephrine increase. Epinephrine and glucagon stimulate lipid mobilization, which is catalyzed by adipocyte triglyceride lipase (ATGL), followed by hormone sensitive lipase (HSL) (17; 18). HSL activity is induced by cAMP dependent protein kinase A (PKA), a kinase which is effectively suppressed by insulin through phosphodiesterase 3A (PDE-3A). Lipolysis is generally regarded to be mainly under the control of catecholaminergic receptor activation and insulin (16).

1.3 Natriuretic Peptides

Rats injected with extracts prepared from cardiac atria featured massive diuresis and natriuresis together with blood pressure reduction (19). The active molecule mediating the response was shown to be atrial natriuretic peptide (ANP). Later, three additional natriuretic peptide family members have been identified, namely brain natriuretic peptide (BNP), C-type natriuretic peptide (CNP) and dendroaspis natriuretic peptide (DNP) (20). The different genes encoding for ANP, BNP and CNP have subsequently been identified in humans, while the gene for DNP has not been found in humans yet (20; 21).

ANP is primarily expressed and stored in granules in cardiac atria. The peptide is present at lower concentrations in the ventricles and kidneys. BNP was first isolated from porcine brain

(22). Yet, subsequent studies showed substantially higher BNP concentrations in ventricular cardiomyocytes. Biological responses to natriuretic peptides are mediated through interaction with specific natriuretic peptide receptors (NPR) named NPR-A, NPR-B, and NPR-C. All NPR are seven transmembrane receptors. NPR-A and NPR-B receptors possess guanylyl cyclase activity. Increased cyclic guanosine monophosphate (cGMP) production mediates the specific biological responses to NPR-A and NPR-B stimulation (20). With chronic exposure to their ligands, guanylyl cyclase activities of NPR-A and NPR-B decrease due to dephosphorylation (23). The phenomenon is commonly referred to as homologous desensitization. NPR-A and/or NPR-B induced intracellular signaling is reduced through different mechanisms by angiotenin II (24), vasopressin (25), lysophophatic acid (26), spingosine-1-phosphate, platelet derived growth factor, basic fibroblast growth factor, and endothelin (20). NPR-A and NPR-B are expressed in various tissues including heart, kidney, brain, lung, adipose tissue, vascular smooth muscle, and adrenal glands (27; 28). NPR-A shows a strong affinity for ANP and BNP, whereas NPR-B is more specific for CNP (29).

NPR-C lacks intrinsic intracellular second messenger activity. It has been suggested that NPR-C may inhibit adenylyl cyclase and activate phospholipase C (30). However, the most important role of NPR-C is to control local concentrations of natriuretic peptides through constitutive receptor-mediated internalization and degradation (31; 32). In addition, natriuretic peptides are cleared from the circulation by enzymatic degradation through plasma membrane bound neutral endopeptidases such as neprilysin (20; 33).

1.4 Natriuretic peptides and Cardiovascular Regulation

The main stimulus for ANP release is atrial stretch (34). Myocardial stretch causes BNP release. Therefore, ANP and BNP are secreted in physiological conditions associated with increased venous return including physical exercise (35; 36), water immersion (37), and head down tilt (38). ANP and BNP concentrations are also raised in disorders with increased intravascular volume, such as congestive heart failure (39; 40). Once released, ANP and BNP are distributed to their target tissues via the blood stream, thus acting in an endocrine manner. In numerous studies, ANP and BNP have been shown to regulate arterial blood pressure, glomerular filtration-rate, and renal sodium- and fluid reabsorption. Furthermore, ANP and BNP alter transvascular volume balance by changing endothelium permeability and they inhibit the renin-angiotensin-aldosterone-system (20). Natriuretic peptide measurements have

been validated as prognostic biomarkers for heart failure and myocardial infarction (41).

1.5 Metabolic Effects of Natriuretic Peptides

Natriuretic peptides are generally regarded as cardiovascular hormones so far. But why then is NPR-A expressed in white adipose tissue? As pointed out, an important physiological function of adipose tissue is to store excess energy as triglycerides and to release free fatty acids and glycerol from triglycerides to meet the metabolic demands of the organism. Recent studies suggested that natriuretic peptides affect lipolysis. The investigators tested the lipolytic response to natriuretic peptides in isolated human adipocytes. The non-selective beta-adrenoreceptor agonist isoproterenol served as positive control intervention (42). ANP elicited a maximal lipolytic response that was comparable in magnitude to the isoproterenol response. However, ANP was more potent than isoproterenol. The lipid mobilizing effect of DNP was similar to that of ANP (43).BNP induced 64% of the maximal lipolytic response of Isoproterenol while the lipid mobilizing effect of CNP was negligible (43).

Radioligand binding assays using [^{123}I]-ANP as the ligand showed high affine binding sites on human adipocytes (42). Maximal stimulation of lipolysis with ANP increased intracellular cGMP concentrations 187 fold. In contrast, cAMP concentrations remained unchanged. In acellular systems, cGMP inhibits PDE-3B (44). However, pharmacological PDE-3B inhibition or PDE-3B stimulation through insulin did not affect ANP induced lipolysis in isolated adipocytes (16).

White adipocytes from human subjects, macaques, rats, mice, hamster, rabbits and dogs were tested for their susceptibility to ANP mediated lipolysis (45). Remarkably, ANP had no effect on lipolysis in mouse, rat, hamster, rabbit, and dog adipocytes, but it induced a profound lipolytic response in human and in macaque adipocytes. The difference between primates and non-primates may be related to species differences in adipose NPR expression. In binding assays and real time PCR studies, the NPR-A/NPR-C ratio is about a 100 fold higher in human than in rat fat cells (45). Rat adipocyte-membranes possess a high density of NPR-C, the “clearance-receptors”, while primate fat cell membranes are densely packed with NPR-A, the “effector-receptors”. Thus, the lipolytic effect of ANP seems to be a primate specificity.

1.6 Aims

The leitmotiv of the habilitation is to characterize metabolic effects of Natriuretic Peptides and to delineate the potential physiological and pathophysiological role in human metabolic regulation. Aims are addressed using biochemical methods, cell-culture experiments as well as comprehensive metabolic and cardiovascular clinical research methods in human subjects.

Specifically, my aims are:

1) to determine the role of ANP in human lipolysis *in vivo;*
2) to find mechanisms mediating the lipolytic effect of ANP;
3) to test whether or not ANP affects lipid oxidation and energy expenditure in humans;
4) to show metabolic consequences of chronic ANP activation;
5) to characterize the interaction between ANP and specific adipokines;
6) to determine the physiological role and molecular mechanisms of ANP and BNP-activated oxidative metabolism in human skeletal muscle cells.

2. Own Work

2.1 **Birkenfeld AL,** Boschmann M, Moro C, Heusser C, Adams F, Franke G, Schroeder C, Berlan M, Luft FC, Lafontan M, Jordan J: Lipid Mobilization with Physiological Atrial Natriuretic Peptide Concentrations in Humans**.** ***J Clin Endocrinol Metab 2005***; 90:3622-628.(46)

ANP in pharmacological concentrations stimulates lipid mobilization in extracted human adipoctes. Whether or not the response is important in humans *in vivo* was unknown. Therefore, the aim was to determine the hemodynamic and metabolic response to physiologically relevant ANP concentrations in fourteen healthy normal-weight men (30 ± 1.2 yr). Probands received an intravenous infusion of human ANP (h-ANP) at rates of 6.25, 12.5, and 25 ng/kg·min and local changes in blood flow and glucose and lipid metabolism of abdominal subcutaneous adipose tissue and femoral skeletal muscle was studied by microdialysis. Overall changes in energy expenditure and substrate oxidation rates were monitored by indirect calorimetry. ANP infusion resulted in an increase in serum free fatty acids and glycerol concentrations (correlation with ANP $r^2 = 0.86$ and $r^2 = 0.76$, for NEFA and glycerol, respectively). In adipose tissue, glycerol increased from 53 ± 6 μmol/liter to 87 ± 13 μmol/liter ($P < 0.001$). In femoral skeletal muscle, glycerol concentrations did not change, whereas lactate-to-pyruvate ratio decreased from 91 ± 23 to 32 ± 4 ($P < 0.001$). Indirect calorimetry indicated an increase in lipid oxidation ($P < 0.05$) concomitantly with a decrease in carbohydrate oxidation ($P < 0.01$), without changes in overall energy expenditure. Our data show that ANP briskly stimulates lipid mobilization and oxidation at plasma concentrations that are encountered in conditions such as heart failure. We conclude that natriuretic-peptide induced metabolic effects could be relevant in conditions with increased natriuretic peptide availability such as physical activity and in pathophysiological conditions such as heart failure. Drugs that interfere with the natriuretic peptide system should be evaluated for potential metabolic side effects.

2.2 Birkenfeld AL, Boschmann M, Moro C, Adams F, Heusser C, Tank J, Diedrich A, Schroeder C, Franke G, Berlan M, Luft FC, Lafontan M, Jordan J: Beta-Adrenergic and Atrial Natriuretic Peptide Interactions on Human Cardiovascular and Metabolic Regulation. ***J Clin Endocrinol Metab 2006***; 91:5069-5075.(47)

We have previously shown that ANP modifies lipid and carbohydrate metabolism in humans. However, the blood pressure lowering effects of ANP might lead to reflex-mediated β-adrenergic receptor stimulation, which might explain some of the metabolic effects induced by ANP. Therefore, we tested the hypothesis that ANP-induced changes in glucose and lipid metabolism, in particular adipose tissue lipolysis, are secondary to β-adrenergic receptor stimulation. Patients included 10 healthy young male subjects (body mass index 24 ± 1 kg/m^2), in which incremental ANP doses (6.25, 12.5, and 25 ng/kg·min) with and without propranolol (0.20 mg/kg in divided doses followed by 0.033 mg/kg·h infusion) were infused intravenously. Metabolism was monitored through venous blood sampling, intramuscular and subcutaneous microdialysis and indirect calorimetry. Cardiovascular changes were monitored by continuous electrocardiogram and beat-by-beat blood pressure recordings. Venous free fatty acid, glycerol, glucose, and insulin and microdialysate glucose, glycerol, lactate, and pyruvate were measured. ANP increased heart rate dose dependently. β-Adrenergic receptor blockade abolished the response. ANP elicited a dose-dependent increase in serum free fatty acid and glycerol concentrations. The response was not suppressed with propranolol. Venous glucose and insulin concentrations increased with ANP, both without and with propranolol. ANP induced lipid mobilization in sc adipose tissue. In skeletal muscle, microdialysate lactate increased, whereas the lactate to pyruvate ratio decreased, both with and without propranolol. Higher ANP doses increased lipid oxidation, whereas energy expenditure remained unchanged. Propranolol tended to attenuate the increase in lipid oxidation. Selected cardiovascular ANP effects are at least partly mediated by β-adrenergic receptor stimulation. ANP-induced changes in lipid mobilization and glycolysis are mediated by another mechanism, presumably stimulation of natriuretic peptide receptors, whereas substrate oxidation might be modulated through adrenergic mechanisms.

2.3 **Birkenfeld AL,** Budziarek P, Boschmann M, Moro C, Adams F, Franke G, Berlan M, Marques MA, Sweep FC, Luft FC, Lafontan M, Jordan J. Atrial Natriuretic Peptide Induces Postprandial Lipid Oxidation in Man. ***Diabetes 2008***; 57:3199-204.(48)

ANP has recently been shown to promote human adipose tissue lipolysis through cGMP-mediated hormone-sensitive lipase activation, independently of the sympathetic nervous system. We hypothesized that ANP increases postprandial free fatty acid (FFA) availability and energy expenditure while decreasing arterial blood pressure. We infused human ANP (25 ng . kg^{-1}) . min($^{-1}$)) in 12 men (age 32 +/- 0.8 years, BMI 23.3 +/- 0.4 kg/m^2) before, during, and 2 h after ingestion of a standardized high-fat test meal in a randomized, double-blind, cross-over fashion. Cardiovascular changes were monitored by continuous electrocardiogram and beat-by-beat blood pressure recordings. Metabolism was monitored through venous blood sampling, intramuscular and subcutaneous abdominal adipose tissue microdialysis, and indirect calorimetry. ANP infusion decreased mean arterial blood pressure by 4 mmHg during the postprandial phase ($P < 0.01$ vs. placebo). At the same time, ANP induced lipolysis systemically ($P < 0.05$ vs. placebo) and locally in subcutaneous abdominal adipose tissue ($P < 0.0001$ vs. placebo), leading to a 50% increase in venous glycerol ($P < 0.01$) and FFA ($P < 0.05$) concentrations compared with placebo. The increase in FFA availability with ANP was paralleled by a 15% increase in lipid oxidation rates ($P < 0.05$ vs. placebo), driving a substantial increase in postprandial energy expenditure ($P < 0.05$ vs. placebo). Our data identify the ANP system as a novel pathway regulating postprandial lipid oxidation, energy expenditure, and concomitantly arterial blood pressure. The findings could have therapeutic implications.

2.4 **Birkenfeld AL**, Adams F, Schroeder C, Engeli S, Jordan J. Metabolic actions could confound advantageous effects of combined angiotensin II-receptor and neprilysin inhibition. ***Hypertension 2011***, 57: e4-5.(49)

We recently identified the ANP system as a novel pathway regulating lipolysis, postprandial lipid oxidation and energy expenditure. The effect may be relevant in conditions such as heart failure, with chronically elevated natriuretic peptide levels. However, this is only possible if the metabolic effects do not desensitize through chronically elevated natriuretic peptide concentrations. We tested the hypothesis that the *ex vivo* lipolytic response to atrial natriuretic peptide is attenuated in isolated adipocytes from patients with severely impaired left ventricular function in part through changes in the expression of natriuretic peptide receptors. We studied patients with left ventricular ejection fraction of 27±4% (6 men, 1 postmenopausal woman; 60±2 years of age; waist-to-hip ratio 0.97±0.02; body mass index 29.4±0.5 kg/m^2) and control subjects with left ventricular ejection fraction of 64±4% (7 men, 1 postmenopausal woman; 60±4 years of age; waist-to-hip ratio 1.04±0.02; body mass index 30.5±1.0 kg/m^2). Groups were also matched for co-morbidities and medication. After an overnight fast, we obtained periumbilical subcutaneous adipose tissue needle biopsies and lipolytic activity was assessed by calculation of the relative increase of glycerol concentration in medium of ANP-treated adipocytes compared with untreated controls. Moreover, natriuretic peptide receptor-A and -C mRNA expression were determined. The surprising finding of our study is that the adipose tissue natriuretic peptide system does not desensitize in heart failure patients, as evidenced by a preserved lipolytic response to atrial natriuretic peptide. Whether preserved lipolytic responses predispose to cardiac cachexia or sustain metabolism in the failing heart remains to be shown. However, the preserved lipolytic response in patients with impaired left ventricular function is likely relevant because atrial and brain natriuretic peptide concentrations are chronically elevated.

2.5 **Birkenfeld AL,** Boschmann M, Engeli S, Moro C, Arafat A, Luft FC, Jordan J. Atrial natriuretic peptide and adiponectin interactions in man. ***PLoS One 2012***; 7:e43238 (50)

Several aspects of the metabolic effects of ANP closely resemble the metabolic profile of the adipocyte secreted hormone adiponectin. Observations *in vitro* and in heart failure patients suggest that ANP promotes adiponectin transcription and release. We tested the hypothesis that ANP acutely raises adiponectin levels in 12 healthy men. We infused ANP intravenously while collecting venous blood and adipose tissue microdialysates at baseline and at the end of ANP-infusion**.** We obtained blood samples at identical time-points without ANP infusion in 7 age and BMI matched men. With infusion, venous ANP concentrations increased ~10 fold. Systemic and adipose tissue glycerol concentrations increased 70% and 80%, respectively ($P<0.01$). ANP infusion increased total adiponectin 14±5% and high molecular-weight (HMW)-adiponectin 13±5% ($P<0.05$). Adiponectin did not change in the control group ($P<0.05$ vs. infusion). ANP-induced changes in HMW adiponectin and adipose tissue lipolysis were directly correlated with each other, possibly suggesting a common mechanism. Our data show that ANP acutely increases systemic total and HMW-adiponectin concentrations in healthy subjects. Our study could have implications for the physiological regulation of adiponectin and for disease states associated with altered natriuretic peptide availability.

2.6 Engeli S, **Birkenfeld AL**, Badin PM, Bourlier V, Louche K, Viguerie N, Thalamas C, Montastier E, Larrouy D, Harant I, de Glisezinski I, Lieske S, Reinke J, Beckmann B, Langin D, Jordan J and Moro C. Natriuretic peptides enhance the oxidative capacity of human skeletal muscle. ***J Clin Invest 2012*** epub ahead of print (51)

Cardiac natriuretic peptides are major activators of human fat cell lipolysis and have recently been shown to control thermogenesis. Here, we investigated the physiological role of NP on human skeletal muscle oxidative metabolism. NP receptor type A (NPR-A) gene expression is positively correlated to mRNA levels of peroxisome proliferator activated receptor-γ coativator-1α (PGC-1α) and several oxidative phosphorylation (OXPHOS) genes in human skeletal muscle. NPR-A, PGC-1α and OXPHOS gene expression is co-ordinately up-regulated in response to aerobic exercise training in human skeletal muscle. Human primary myotubes express functional NPR-A and downstream signalling components. In human myotubes, NP induce PGC-1α and mitochondrial OXPHOS gene expression in a cGMP-dependent manner. Moreover, NP increase OXPHOS protein expression, fat oxidation and maximal respiration independently of significant changes in mitochondrial proliferation and mass. NP recapitulate *in vitro* the effect of exercise training on muscle fat oxidative capacity *in vivo*. Collectively, these data show that activation of NP signalling in human skeletal muscle enhances mitochondrial oxidative metabolism and fat oxidation. We propose that NP could contribute to exercise training-induced improvement in skeletal muscle fat oxidative capacity in humans.

3. Discussion

3.1 The Effect of Natriuretic Peptides on Lipid Mobilization

Based on *in vitro* studies, I tested the hypothesis that ANP stimulates lipolysis in physiologically relevant concentrations in humans. To address the issue, we infused incremental intravenous ANP doses in healthy lean men.(46) ANP stimulated lipolysis in concentrations that are observed during physical exercise or in heart failure patients. During intravenous ANP administration, lipolysis is stimulated mainly in subcutaneous adipose tissue, while lipid mobilization in skeletal muscle does not change.(46) Together the data suggest that physiological or pathological changes in ANP concentrations could affect lipid mobilization in human subjects.

In the fasted state, intravenous ANP infusion does not change resting energy expenditure. However, lipid oxidation rates decreased slightly at low intravenous ANP infusion rates. Lipid oxidation increased when ANP concentration was raised to a high physiological range. In contrast, glucose oxidation rates increased with low ANP infusion rates, and decreased with higher ANP infusion rates.(46) A similar pattern of substrate use is observed during prolonged physical training: initially, carbohydrate oxidation predominates but ith prolonged exercise, lipid oxidation is increased.

The previous observations do not prove that ANP mediated lipolysis in human subjects is mediated through NPR. Unfortunately, specific blockers for these receptors are not available for clinical studies. However, alternative mechanisms have been excluded in previous studies. Among others,insulin is a potent lipolysis inhibitor. Insulin concentrations increase with intravenous ANP infusion.(47) Furthermore, ANP mediated glycerol release from subcutaneous adipose tissue was not attenuated during euglycemic hyperinsulinemic clamp testing.(52) Therefore,ANP mediated lipolysis *in vivo* cannot be explained by insulin mediated responses.

Systemic norepinephrine concentrations increased with intravenous ANP infusion.(46; 53) The response may be explained by ANP induced vasodilatation and volume depletion leading to the/an activation of the baroreflex mediated sympathetic nervous system. Indeed, ANP infusion elicits an increase in heart rate that can be abolished with beta-adrenoreceptor blockade.(47) On the other hand, ANP might also have a direct sympathoinhibitory

effect.(53). Neither local (54) nor systemic (47) near-complete beta-adrenergic receptor blockade with propranolol reduced ANP mediated lipolysis. The observations support the idea that beta-adrenergic receptor agonists and ANP induce lipolysis through distinct receptor and post receptor mechanisms in human subjects.

Meanwhile, the molecular mechanisms mediating the ANP response in human adipocytes have been worked up in more detail (Figure 1). ANP binding to NPR-A stimulates cyclase activity and increases intracellular cGMP concentrations.(55) Gene expression analysis showed cGMP-dependent protein kinase I (cGKI) mRNA expression in human adipocytes. ANP induced lipolysis in human preadipocytes was markedly attenuated with cGKI inhibition.(55) The activation of cGKI by cGMP promotes HSL phosphorylation.(56) ANP-induced lipolysis is associated with increased phosphorylation of critical HSL serine residues. cGMP-dependent protein kinase II, cAMP-dependent protein kinases, and MAP-kinases ERK and p38 do not contribute to ANP induced lipolysis.(55) Taken together, the activation of NPR-A by ANP increases guanylyl cyclase activity, which then catalyses the synthesis of cGMP. cGMP activates cGKI, which phosphorylates HSL. Phosphorylated HSL breaks triglycerides into glycerol and free fatty acids.

Figure 1:

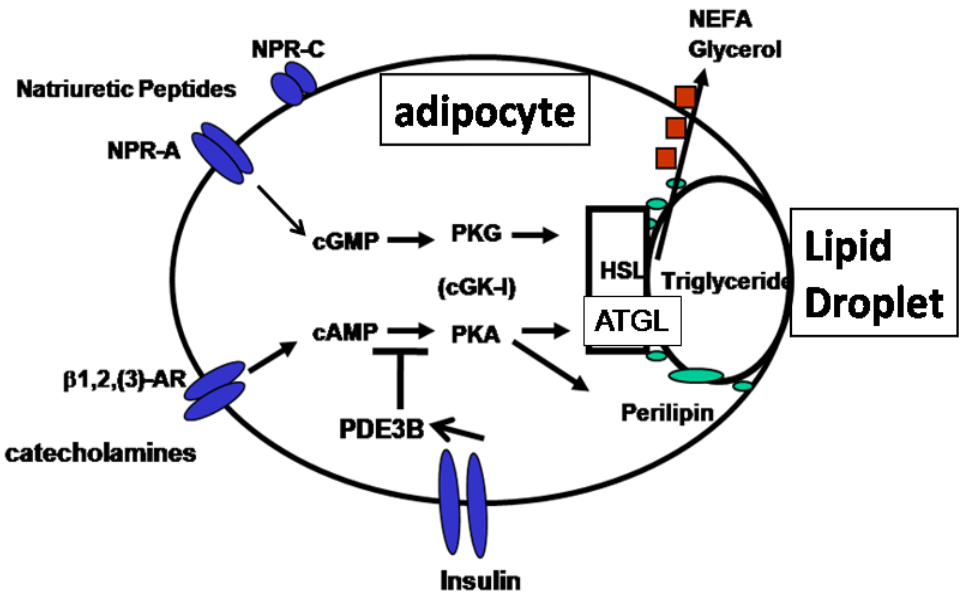

Figure 1: Intracellular pathways of ANP- and catecholamine induced lipolysis transduction pathways. Catecholamines signal via activation of adrenergic receptors (AR), and natriuretic peptides via type A receptor (NPR-A) activation. Protein kinases (PKA and PKG [cGK-I]) are involved in target protein phosphorylation. HSL phosphorylation promotes its translocation from the cytosol to the surface of the lipid droplet. Perilipin phosphorylation leads to the physical alteration of the droplet surface that facilitates the action of HSL and the initiation of lipolysis. Docking of adipocyte lipid binding protein to HSL favors the outflow of fatty acids released by the hydrolysis of triglycerides. Monoglyceride lipase catalyzes monoacylglycerol hydrolysis.

3.2 Activation of Oxidative Metabolism through Natriuretic Peptides

My work consistently shows that ANP infusion induces lipid oxidation in the fasted and postabsorptive state (48). The activation of lipid oxidation requires the induction of mitochondrial oxidative metabolism. Plasma ketone concentrations, which reflect hepatic lipid oxidation, increase? sharply in the postprandial phase with ANP infusion (48). The switch towards lipid oxidation is paralleled by an increase in postabsorptive energy expenditure. Circulating free carnitine concentrations have been reported to decrease with ANP infusion (57). Carnitine is a critical factor for fatty acid intramitochondrial transport by carnitine palmityl transferase I and thus, beta-oxidation (58). Together, these data clearly suggest that the ANP-induced lipid oxidation is driven by an activation of

oxidative phosphorylation. But how can natriuretic peptides induce oxidative metabolism?
We addressed the issue in cultured human myotubes - the tissue with the highest capacity to increase oxidative phosphorylation under physiological conditions. ANP and BNP dose-dependently increased intracellular cGMP concentrations and induced the transcription and protein concentration of peroxisome proliferator activated receptor (PPAR)-g coactivator 1α (PGC-1α), the master regulator of mitochondrial biogenesis and metabolism (51; 59). Similar results were obtained with cGMP analogues and the selective PDE-5 inhibitor sildenafil in human myotubes indicating that the induction of mitochondrial master-regulators involves the cGMP pathway. Chronic ANP treatment of human myotubes enhanced palmitate oxidation and improved oxidative phosphorylation efficiency (higher P/O ratio) (51). This data is in line with animal studies showing that overexpression of BNP also activates mitochondrial biogenesis and the oxidative capacity in skeletal muscle of mice (60). These data point towards a major physiological role of NP in the regulation of the skeletal muscle oxidative capacity in humans. Clinically, the effect might be further enhanced through the ability of ANP to induce adiponectin secretion, as shown in my work (50).

3.3. Physiological Significance

To address the physiological relevance of NP induced metabolic changes, we tested the relevance of the relationship between the activating cGMP coupled NP receptor, NPR-A, and expression and oxidative metabolism in human skeletal muscle. We were able to show that NPR-A positively correlates with the expression of PGC-1α and important mitochondrial OXPHOS genes. Therefore, the next logical step was to investigate the potential link between NP signalling and skeletal muscle mitochondrial oxidative metabolism during a physical exercise training program. The exercise training program improved oxygen uptake and the? resting metabolic rate. This physiological adaptation could partly involve mitochondrial uncoupling in skeletal muscle (61). Gene set enrichment analysis of skeletal muscle microarray data revealed pathways related to mitochondrial metabolism and oxidative phosphorylation as top ranking biological functions in response to exercise training. Exercise training increased the expression of the skeletal muscle OXPHOS proteins without evidence for an increase in mitochondrial density. These data are consistent with previous findings and suggest that exercise training can improve mitochondrial function *per se (62)*. In addition, we show a concomitant up-regulation of NPR-A and total PGC-1α transcripts in human skeletal muscle after an 8 week aerobic exercise training program in obese man. These data suggest a

physiological link between NP/NPR-A signalling and mitochondrial oxidative capacity in human skeletal muscle. It is tempting to speculate that increased NP signalling in skeletal muscle may contribute, at least in part, to exercise training-induced improvement in fat oxidative capacity. Indeed, physical exercise is a strong physiological stimulus for cardiac ANP release (35).

3.4 Pathophysiological Significance: Heart Failure, Obesity and Insulin Resistance

Endogenous plasma NP levels are also elevated in a number of pathological conditions in humans and experimental animals. ANP and BNP are increased in congestive heart failure and ischemic heart disease, chronic renal failure, the syndrome of inappropriate antidiuretic hormone secretion, sepsis, liver cirrhosis, and pulmonary hypertension.(20) Very high NP concentrations occur in congestive heart failure, because the congestion of blood increases atrial and ventricular stretch. In the setting of congestive heart failure, mRNA expression levels of ANP and BNP in cardiomyocytes are markedly increased and plasma ANP concentrations are elevated up to 20 fold.(63) Our work shows that the lipolytic effect of ANP does not desensitize in patients with chronically elevated ANP concentrations due to severe heart failure.(49) Thus, ANP mediated changes in lipid mobilization may be relevant in congestive heart failure. The failing heart is particularly dependent on fatty acid oxidation for the maintenance of its work capacity.(64) It is possible that increased lipid mobilization through natriuretic peptides sustains substrate supply in heart failure patients – a hypothesis that needs to be tested. However, an increased ANP mediated lipid mobilization may predispose to cardiac cachexia, which is a serious complication of heart failure and carries a poor prognosis (65). Cardiac cachexia is associated with a reduction in the total body fat mass, lean tissue mass and bone mineral density.(66) BNP concentrations are inversely related to the BMI of heart failure patients.(67) In another study, median BNP concentrations where 484 pg/ml in cachectic heart failure patients and 151 pg/ml in non-cachectic heart failure patients.(68)

Reduced circulating natriuretic peptide concentrations are independently associated with obesity, insulin resistance and type 2 diabetes (69; 70). NP clearance receptor, NPR-C, increases in subcutaneous adipose tissues from obese hypertensive (71), and insulin resistant subject, presumably through the regulation by insulin (72). Upregulation of the NPR-C clearance receptor may decrease natriuretic peptide availability, both systemically and in adipose tissue. In the event of what??, obese subjects do not respond with adequate ANP

release with volume loading.(73) Moreover, BMI and circulating ANP and BNP concentrations are inversely correlated.(69) Variation in the promoter region of the NPR-C gene seems to be associated with reduced NPR-C expression in human adipose tissue. (74) Individuals with the variation had lower BMI and waist circumference and a lower prevalence of overweight and obesity than individuals without the variation. The observation is strengthened by the fact that NPR-C knockout mice are exceptionally thin. In this animal model, normal body fat deposits are absent on necropsy.(31) It is possible that reduced natriuretic peptide availability contributes to obesity. The mechanism could also provide a pathophysiological link between obesity and arterial hypertension.(75). It is reassuring that impaired ANP responses in obesity can be recovered. Five kg weight loss in obese women increased the sensitivity to the ANP mediated lipolysis. (76) In rats, weight reduction decreased adipose NPR-C expression, which might increase ANP availability due to diminished clearance.(77)

Figure 2

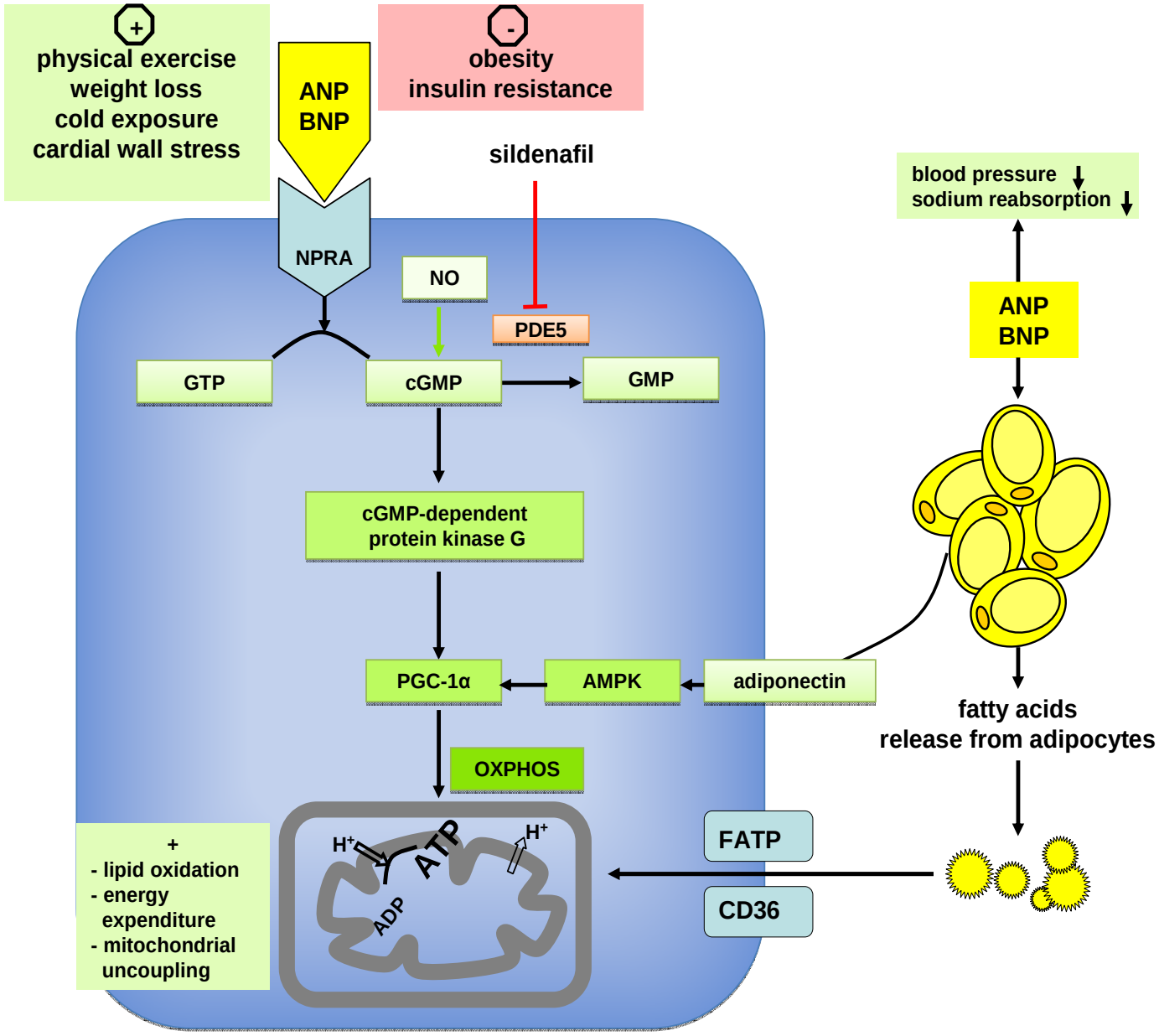

Figure 2: Natriuretic Peptide mediated metabolic interactions.

Natriuretic peptides are secreted by the heart. Natriuretic peptide concentrations increase due to cardiac wall stress, weight loss, exercise, and exposure to cold and are reduced by obesity and insulin resistance. The target receptor of circulating natriuretic peptides is natriuretic peptide receptor A (NPR-A), a membrane-bound guanylyl cyclase. The binding of natriuretic peptides to NPR-A on adipocytes leads to the production of intracellular second messenger cyclic guanosine monophosphate (cGMP). The cGMP activates cGMP-dependent protein

kinas. PKG-mediated phosphorylation triggers a cascade that includes enhanced lipolysis and activation of PGC-1α, which is a master regulator of mitochondrial biogenesis and beta-oxidation. Atrial natriuretic peptides induce adiponectin secretion, which is known to induce AMPK - a master regulator of PGC-1α and cellular energy status.

4. Summary and Conclusions

In summary, my data show that in addition to their well-established blood pressure lowering effects, Natriuretic Peptides are a novel class of hormones that act as potent regulators of energy metabolism. Natriuretic Peptides activate adipose tissue lipolysis using an alternative signalling pathway compared to catecholamines providing the body with fatty acids as an energy source. At the same time, Natriuretic Peptides induce the molecular and cellular program that is needed for the generation of biochemical energy from mitochondrial fatty acid oxidation through a cGMP dependent pathway (51; 78). Moreover, our data suggest that Natriuretic Peptides mediate the beneficial effects of physical exercise in human skeletal muscle at least in part. Reduced levels of Natriuretic Peptides might contribute to the development of obesity and type 2 diabetes.

These data raise the tantalizing possibility that modulating the levels or function of Natriuretic Peptides could lead to some of the health-promoting effects of physical exercise, without requiring rigorous physical activity. Potential Natriuretic Peptide agonists that are “capable of lowering blood pressure, preventing sodium retention, and reducing fat accumulation seem almost ideally suited for the fight against cardiometabolic disease”.(79)

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Declaration

Eidesstattliche Versicherung

gemäß Habilitationsordnung der Medizinischen Fakultät Charité Berlin.

Hiermit erkläre ich, dass

- weder früher noch gleichzeitig ein Habilitationsverfahren durchgeführt oder angemeldet wurde,
- die vorgelegte Habilitationsschrift ohne fremde Hilfe verfasst, die beschriebenen Ergebnisse selbst gewonnen wurden, sowie die verwendeten Hilfsmittel, die Zusammenarbeit mit anderen Wissenschaftlerinnen oder Wissenschaftlern und technischen Hilfskräften sowie die verwendete Literatur Vollständig in der Habilitationsschrift angegeben wurden,
- mir die geltende Habilitationsordnung bekannt ist.

Berlin, 23.03.2013 Dr. med. Andreas Birkenfeld