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Published in:
EBioMedicine

DOI:
10.1016/j.ebiom.2017.02.026

Publication date:
2017

Document Version
Publisher's PDF, also known as Version of record

EBioMedicine 17 (2017) 88–94

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

Research Paper

Effect of Pancreatic Hormones on pro-Atrial Natriuretic Peptide in Humans

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ARTICLE INFO

Article history:
Received 27 November 2016
Received in revised form 24 February 2017
Accepted 27 February 2017
Available online 1 March 2017

Keywords:
ProANP
Insulin resistance
Metabolism
Hyperglycemia
Prediabetes

ABSTRACT

Plasma concentrations of pro-Atrial natriuretic peptide, proANP, are decreased in obesity and diabetes. Decreased proANP concentrations have also been noted after meal intake, and recently, a glucose-mediated regulation of ANP gene expression was reported. Hence, we evaluated the effects of insulin, glucagon and glucose on plasma proANP in a series of observational and experimental studies.

Six healthy men underwent seven days of bed rest. Before and after the bed rest, hyperinsulinemic euglycemic clamps with serial plasma measurements of proANP were performed. Moreover, plasma proANP was quantified in 65 individuals with normal or impaired glucose regulation. Finally, the effects of infusion-induced hyperglucagonemia were examined in ten healthy men.

Bed rest decreased insulin sensitivity and plasma proANP. The decrease in proANP was not associated with insulin sensitivity and the peptide concentrations remained constant during euglycemic hyperinsulinemia and hyperglycemic hyperglucagonemia. Impaired glucose regulation was not associated with decreased proANP concentrations.

Bed rest per se induces a marked decrease in plasma proANP concentrations whereas insulin resistance and impaired glucose regulation was not associated with lower proANP concentrations. Neither acute hyperinsulinemia nor hyperglucagonemia seems to affect plasma proANP. Our findings thus suggest that decreased plasma proANP concentrations occur late in the development of insulin resistance.

1. Introduction

Atrial natriuretic peptide (ANP) is a member of the natriuretic peptide family comprising also B-type and C-type natriuretic peptides. Together with B-type natriuretic peptide, ANP is expressed mainly in the cardiac atria and secreted to circulation upon cardiac strain; accordingly, increased circulating concentrations are seen in heart failure settings (Mukoyama et al., 1991). In the last decade, however, these cardiac hormones have also been implicated in metabolic dysfunction, where decreased circulating concentrations have been reported in obesity, insulin resistance, and diabetes (Jujić et al., 2016; Then et al., 2013; Wang et al., 2007).

Recently, a glucose-dependent transcriptional regulation of cardiac ANP expression mediated by micro-ribonucleic acid-425 was reported in stem cell-derived cardiomyocytes (Arora et al., 2016). Based on this observation, hyperglycemia may be the mechanism underlying decreased plasma concentrations of ANP and its precursor, proANP, observed in obesity and type 2 diabetes. In support of this, ANP secretion decreases after a meal (Arora et al., 2016; Goetze, 2013), but whether glucose per se, or in conjunction with post-prandial hormones (Jujić et al., 2016), regulates the cardiac ANP expression, remains unclear. Importantly, cardiomyocytes express receptors for both glucagon and insulin and increased plasma concentrations of both hormones are well-known features of obesity and type 2 diabetes (Brubaker and Drucker, 2002; Gammeltoft and Van Obberghen, 1986).

Hence, the objective of the present study was to examine the cardiac ANP response to insulin, glucagon and glucose. Specifically, we investigated the effects of increased plasma concentrations of insulin and glucagon in human infusion experiments, where plasma glucose was either

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http://dx.doi.org/10.1016/j.ebiom.2017.02.026

clamped or increased. Moreover, we measured circulating peptide concentrations in middle-aged individuals with normal and impaired glucose regulation. As physical inactivity is associated with not only decreased muscle mass but also reduced glucose extraction in skeletal muscle (Biensø et al., 2012), we also examined the ANP response to short-term reduced insulin sensitivity by subjecting healthy humans to a strict bed rest regimen. The mature ANP hormone is however considered unstable under most storage conditions. Hence, we quantified plasma concentrations of the midregional part of N-terminal proANP, a peptide that is co-secreted into the circulation in equimolar amounts with mature ANP and thus considered a robust marker of ANP secretion (Davidson et al., 1995; Hall et al., 1995; Nelesen et al., 1995).

2. Materials and Methods

2.1. Research Design

The study included a series of three studies for which the design, study populations and inclusion and exclusion criteria have been described previously (Biensø et al., 2012; Færch et al., 2008; Guerra et al., 2014; Hansen et al., 2015; Kiilerich et al., 2011; Ringholm et al., 2011). The three studies were all performed according to the Declaration of Helsinki and were approved by the regional ethical committee (KA98155g, H-A-2008-0024 and H-1-2012-129). Prior to the experiments, all participants gave written, informed consent to participate.

2.2. Bed Rest-induced Insulin Resistance

The study was performed at the Copenhagen University Hospital, Rigshospitalet, Denmark, and the Department of Nutrition, Exercise and Sports, University of Copenhagen, Denmark, and included six healthy males with a mean (SD) age of 24.7 (4.4) years, weight of 68.9 (4.8) kg, height of 180.3 (4.8) cm, and body mass index (BMI) of 21.2 (4.4) kg/m^2. As previously described (Biensø et al., 2012; Guerra et al., 2014; Kiilerich et al., 2011; Ringholm et al., 2011), the participants were subjected to seven days of bed rest including transportation by wheel chair at all times and were served regular food and water ad libitum. The day before the bed rest was commenced and on day 6 of the bed rest, each subject underwent a standard oral glucose tolerance test (OGTT). At 0, 30, 60, and 120 min after glucose intake, blood was sampled and plasma concentrations of insulin and glucose were quantified. At baseline and at day 7, a 3 h hyperinsulinemic euglycemic clamp (HIEC) was performed by infusing insulin (Actrapid; Novo Nordisk, Bagsvaerd, Denmark) at a constant rate of 50 mU/m^2/min for 3 h (Biensø et al., 2012). Arterial blood glucose concentrations were measured every 5–10 min throughout the procedure with a glucose analyzer (Radiometer ABL 725 series Acid-Base Analyser) and the glucose infusion rate (GIR) was adjusted to maintain euglycemia. The average GIR from 90 to 120 min from the start of the insulin infusion was used as a measure of insulin sensitivity (the *M*-value). Moreover, the modified Matsuda index was calculated from the OGTT as follows: 10^4/(fasting plasma glucose (FPG) × fasting plasma insulin × mean plasma glucose during the OGTT × mean plasma insulin during the OGTT) (Matsuda and DeFronzo, 1999). Femoral venous blood samples were collected into tubes containing EDTA before and after the bed rest period as well as 30 min prior to initiation of the clamp, at clamp initiation (t = 0) and 30, 60, 120, 150, and 180 min into the clamp. Blood samples were centrifuged immediately and stored at −20 °C until analysis.

2.3. Prevalent Impaired Glucose Regulation

Participants were recruited from the Inter99 study which is a population-based non-pharmacological intervention study to prevent ischemic heart disease and type 2 diabetes (clinical trial reg. no. NCT00289237, clinicaltrials.gov) (Jørgensen et al., 2003). After a 5-year follow-up examination, a sample of 120 participants with either normal glucose tolerance (NGT; defined as FPG < 6.1 mmol/l and 2 h plasma glucose < 7.8 mmol/l), isolated impaired fasting glycemia (IFG; defined as 6·1 mmol/l ≤ FGP < 7.0 mmol/l but 2 h plasma glucose < 7.8 mmol/l), or isolated impaired glucose tolerance (IGT; defined as FPG < 6.1 mmol/l but a 7.8 ≤ 2 h plasma glucose <11.1 mmol/l) were subjected to another OGTT at the Steno Diabetes Center, Gentofte, Denmark, and, if glucose tolerance status was confirmed, they were invited to participate also in the present part of the study (Færch et al., 2008). A total of 66 men and women (20 with NGT, 18 with IFG, and 28 with IGT) agreed to participate and underwent sampling of blood from the femoral vein followed by a 2-h HIEC. Details have been provided elsewhere (Færch et al., 2008), but in brief, the HIEC was started with a 9 min stepwise decline in insulin infusion (Actrapid; Novo Nordisk, Bagsvaerd, Denmark) after which insulin was infused at a constant rate of 40 mU/m^2/min for 2 h. Blood glucose concentrations were measured every 5 min throughout the clamp with a glucose analyzer (One Touch Profile glucose meter, Lifescan, Milpitas, CA, USA) and the glucose infusion rate was adjusted to maintain blood glucose concentration of 5 mmol/l. The *M*-value was calculated from the average GIR during the last 30 min of the clamp. Blood samples for proANP quantification were collected at baseline into tubes containing sodium fluoride and immediately placed on ice. After centrifugation, the samples were stored at −80 °C until analysis.

2.4. Hormone Infusions

Ten healthy males were enlisted in the study which involved four different infusion protocols each separated by at least a 14-day interval: 1) 1-h intravenous glucagon infusion (GlucaGen, Novo Nordisk Scandinavia, Copenhagen, Denmark; 6 ng/kg/min); 2) 2-h and 10 min somatostatin-analogue infusion (Octreotide, Hospira Nordic AB, Stockholm, Sweden; 100 ng/kg/min) concurrent with 1-h glucagon infusion (6 ng/kg/min); 3) 2-h and 10 min somatostatin-analogue infusion (100 ng/kg/min), and 4) 1-h saline infusion (at the same rate as the one used for the infusion of glucagon alone) (Hansen et al., 2015).

During the isolated somatostatin-analogue infusion, four additional males were recruited to replace study participants who were not available at the time. The mean (SD) age, weight, height, and BMI of the study subjects in infusions 1, 2, and 4 was: 22.9 (0.4) years, 75.4 (2.1) kg, 183.8 (2.6) cm, and 22.3 (0.5) kg/m^2, whereas study subjects in trial 3 had a mean (SD) age, weight, height, and BMI of 23.0 (0.5) years, 72.9 (1.9) kg, 180.8 (1.7) cm, and 22.3 (0.5) kg/m^2. Blood samples were collected in tubes coated with EDTA at the onset (t = 0) and 30, 60, 90, and 120 min into the infusions and placed on ice. The blood samples were centrifuged and plasma was stored at −80 °C until analysis.

2.5. Plasma proANP Measurement

ProANP concentrations in plasma samples were measured at the Department of Clinical Biochemistry, Rigshospitalet. ProANP was quantified as proANP$_{53–90}$ corresponding to the mid-regional part of proANP by use of an automated assay on the KRYPTOR Plus system (ThermoFisher Scientific, Hennigsdorf, Germany). According to the manufacturer, the detection limit of the assay is 2.1 pmol/l and the limit of quantitation is 4.5 pmol/l. The intra-assay coefficient of variation in the range between 20 pmol/l and 1000 pmol/l is <2.5%.

2.6. Statistical Analyses

Statistical analyses were performed using SAS statistical software version 9.4 (SAS institute, Cary, North Carolina, USA). When appropriate, logarithmic transformations were applied to achieve normality and homogeneity of the residuals. A p-value < 0.05 was considered significant. The present study includes a series of studies of previously published cohorts. Hence, no sample size estimation was performed to determine the sample size needed to detect a significant difference in

plasma proANP. Instead, plasma samples from all participant in the respective cohorts were included.

In the bed rest study, the respective areas under the plasma insulin and glucose curves during the OGTT, the *M*-value, the Matsuda index and the plasma concentrations of proANP were compared before and after bed rest by use of either paired t-tests or Wilcoxons signed rank tests. Moreover, Spearman's rank order correlations were used to assess the associations between plasma proANP and the areas under the insulin and glucose curves, the *M*-value and the Matsuda index, respectively. In extension, the associations between the fold change in plasma proANP (calculated as: (proANP concentration after bed rest - proANP concentration before bedrest)/proANP concentration before bed rest) and the *M*-value and Matsuda index after bed rest were assessed by use of Spearman's rank order correlations. To assess the proANP response to clamp-induced hyperinsulinemia before and after bed rest, plasma proANP concentrations were modeled as linear functions of time of blood sampling during the two HIECs. To account for correlation among measurements of the same individual, a shared random effect of each individual was included in the linear mixed model. Moreover, random individual intercepts and slope coefficients were included to allow for differences before and after bed rest.

For the population-based study of impaired glucose regulation, the baseline characteristics, including plasma proANP concentrations, among the three glucose tolerance groups were compared by use of one-way ANOVAs with Tukey's post hoc tests or by use of the nonparametric Kruskal-Wallis test followed by pairwise comparisons using the Mann–Whitney *U* test. Gender distribution among the groups was tested by use of a Chi square test. Least squares multiple linear regression analyses were used to assess the associations between proANP concentrations as the dependent variable and the *M*-value and the presence or absence of impaired glucose regulation (defined by either IGT or IFG) as the explanatory variables. The two explanatory variables were entered separately and the respective models were adjusted for age and sex. Finally, Pearson's correlations were used to assess the associations between plasma proANP and BMI, systolic and diastolic blood pressures, respectively.

For the hormone infusion study, repeated measures ANOVAs with compound symmetry covariance structure were used to describe the changes in plasma proANP concentrations during the four infusion experiments. Time of blood sampling during the infusions was entered as a repeated factor in the four respective statistical models. Post hoc t-tests with Tukey–Kramer correction of the p-values were performed if a significant overall effect of time was found in the respective models.

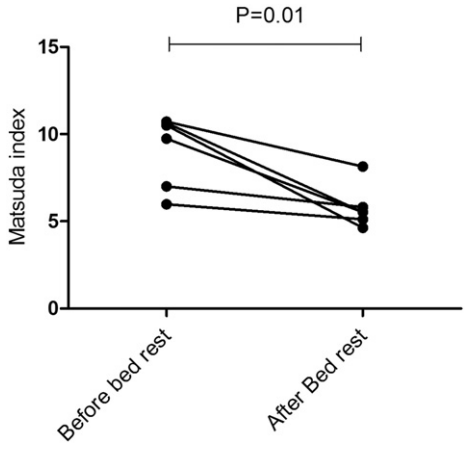

Fig. 1. The Matsuda indices before and after bed rest. In six healthy young men, oral glucose tolerance tests (OGTTs) were performed immediately before bed rest and at day 6 into the bed rest. The modified Matsuda index was calculated from fasting glucose and insulin and mean glucose and insulin during the OGTTs. The modified Matsuda index was compared before and at day 6 of bed rest by use of a Wilcoxon signed rank test ($p = 0.01$).

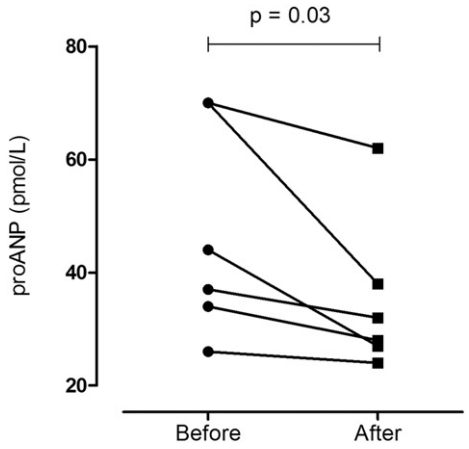

Fig. 2. Plasma concentrations of pro-atrial natriuretic peptide before and after bed rest. Peptide plasma concentrations were quantified before and after seven days of complete bed rest in healthy young men ($n = 6$) and was compared by use of Wilcoxon signed rank test ($p = 0.03$).

3. Results

3.1. Plasma proANP During Bed Rest-induced Insulin Resistance

After bed rest, insulin resistance was confirmed by increased plasma insulin concentrations during oral glucose challenge (mean (SD) of the areas under the insulin curves: 23,950.0 ± 8207.1 pmol/l versus 33,465.0 ± 9908.4 pmol/l; $p = 0.03$) whereas plasma glucose concentrations remained unaffected (mean of the areas under the glucose curves 841.5 ± 101.1 mmol/l versus 882.5 ± 139.0 mmol/l; $p = 0.6$). Accordingly, the Matsuda index was significantly decreased by 45% after bed rest (from 10.1 to 5.5 arbitrary units; $p = 0.01$; Fig. 1). Compared to before bed rest, the median (IQR) *M*-value decreased after bed rest (37.5 (32.1–46.7) μmol/min/kg body weight versus 27.0 (20.2–38.6)) μmol/min/kg body weight) but this difference did not reach statistical significance ($p = 0.1$). Plasma proANP concentrations decreased from 40.1 (32.0–70.0) pmol/l before bed rest to 30.0 (26.3–44.0) pmol/l after bed rest ($p = 0.03$, Fig. 2) but peptide concentrations were not correlated to the *M*-value ($p = 0.6$) or the Matsuda index ($p = 0.8$). Likewise when analyzed as fold change, plasma proANP concentrations were not correlated to either the *M*-value or the Matsuda index after bed rest (both $p = 0.9$). The change in plasma proANP concentrations during the HIEC after bed rest was not statistically

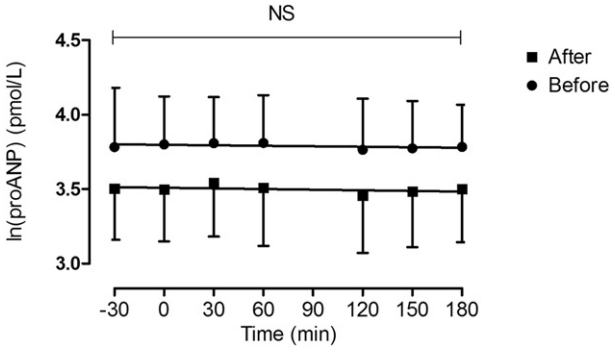

Fig. 3. Plasma concentrations of proANP during acute hyperinsulinemia before and after bed rest. Plasma concentrations of proANP were modeled as linear functions of time of blood sampling during the two clamps. The slopes of the fitted lines representing the mean proANP concentrations during hyperinsulinemic euglycemic clamps before and after bed rest, respectively, were not statistically significantly different. Thus assuming equal slopes, the estimated resultant slope of proANP concentrations over time during hyperinsulinemia was not statistically significantly different from 0 ($n = 6$). Data have been log base e transformed and are shown as mean ± sd. ProANP = proatrial natriuretic peptide.

Table 1
Descriptive data of the individuals with normal or impaired glucose tolerance.

	NGT (n = 20)	IFG (n = 18)	IGT (n = 27)	p-value
Gender (M/F)	11/9	16/2	15/12	0.04
Age (years)	49.8 ± 10.6	53.9 ± 7.6	53.9 ± 8.3	0.2
BMI (kg/m^2)	25.6 ± 3.3	27.8 ± 3.6	28.1 ± 3.5*	0.04
Diastolic BP (mmHg)[a]	76.6 ± 12.3	85.1 ± 11.4	82.5 ± 7.0	0.05
Systolic BP (mmHg)[a]	126.7 ± 17.5	136.9 ± 22.8	136.8 ± 17.3	0.2
Fasting plasma glucose (mmol/l)	5.5 ± 0.4	6.2 ± 0.3#	5.7 ± 0.4	<0.001
Fasting serum insulin (pmol/l)	28.5 (22.5–38.5)	39.5 (29.0–55.5)	39.0 (27.0–66.0)	0.2
M-value ($\times 10^{-3}$ mmol/min/kg body weight)	49.3 ± 12.2	45.0 ± 20.3	35.4 ± 15.2*	0.01
HOMA-IR	1.3 (1.0–2.0)	1.7 (1.4–2.5)	1.8 (1.2–2.7)	0.5
Pro-atrial natriuretic peptide (pmol/l)	46.3 ± 18.8	40.5 ± 20.4	47.7 ± 22.4	0.5

Overall group-wise differences were analyzed by use of one-way ANOVA or Kruskal-Wallis tests followed by pairwise comparison of groups. NGT, normal glucose tolerance, IFG, isolated impaired fasting glucose, IGT, isolated impaired glucose tolerance, HOMA-IR, Homeostatic model assessment of insulin resistance calculated as fasting insulin (mIU/L) × fasting glucose (mmol/L) divided by 22.5.
Data are presented as mean ± SD or, in case of non-normal distribution, median and interquartile ranges.
[a] Due to missing observations for systolic and diastolic blood pressure (BP) measurements, the sample size is reduced to n = 19, n = 17 and n = 21 for NGT, IFG and IGT groups, respectively.
* $p < 0.05$ vs. the NGT group.
# $p < 0.05$ vs. the NGT and the IGT groups.

significantly different from the change in plasma proANP concentrations during the HIEC before bed rest ($p = 0.9$). Hence assuming equal change in proANP, no effect of hyperinsulinemia was found ($p = 0.4$). Nonetheless, mean plasma proANP in the HIEC after bed rest was significantly decreased compared to mean plasma proANP in the HIEC before bed rest ($p = 0.002$; Fig. 3), corroborating the net effect of bed rest.

3.2. Plasma proANP in Impaired Glucose Regulation

The circulating proANP concentration was not quantified in one individual with IGT due to inadequate amount of plasma. A total of 65 individuals were thus included in the study (Table 1). The distribution of gender differed among groups ($p = 0.04$) and individuals with IGT displayed higher BMI and lower insulin sensitivity than individuals with NGT ($p = 0.04$ and 0.01, respectively). Plasma proANP concentrations did not differ among the three groups ($p = 0.5$, Table 1). Likewise, proANP concentrations were not associated with either presence of impaired glucose tolerance ($p = 0.3$) or the M-value ($p = 0.7$). Plasma proANP was not correlated to either BMI ($p = 0.7$) or systolic or diastolic blood pressures ($p = 0.8$ and $p = 0.9$, respectively).

3.3. Plasma proANP During Hormonal Infusions

Overall, infusion of glucagon alone significantly affected plasma proANP concentrations ($p = 0.008$) and on post hoc testing, the peptide concentrations decreased from 48.2 ± 16.5 pmol/l at baseline to 44.1 ± 13.1 pmol/l 90 min into the infusion ($p = 0.03$). Neither infusions of glucagon in the presence of a somatostatin-analogue nor infusions of a somatostatin-analogue alone affected the circulating proANP concentrations ($p = 0.07$ and $p = 0.1$, respectively). Saline infusions were per se associated with an overall change in circulating proANP ($p = 0.004$) comprising a decrease in peptide concentrations at 60, 90, and 120 min into the infusion compared to onset of the infusion ($p = 0.02$; $p = 0.004$ and $p = 0.02$, respectively) (Fig. 4).

4. Discussion

In the present study, we found that seven days of bed rest induced a marked decrease in proANP concentrations in plasma from healthy young men. Moreover, bed rest was associated with decreased insulin sensitivity, but a significant association between the magnitude of the induced insulin resistance and plasma proANP concentrations was not found. Likewise, presence of impaired glucose regulation among middle-aged men and women was not associated with low plasma proANP concentrations. Finally, neither hyperinsulinemia nor isolated hyperglucagonemia acutely affected proANP concentrations in healthy lean individuals.

The effects of (supine and head-down) bed rest on ANP concentrations have been examined before. In the circulation, however, ANP has a very short half-life and may mainly represent hemodynamic changes including altered renal elimination. Therefore, the overall decrease in ANP concentrations noted in these early studies was ascribed the cardiovascular changes observed during bed rest (Gauquelin et al., 1996, 1995; Maillet et al., 1994). Bed rest, however, also has profound effects on the metabolic homeostasis and the induction of insulin resistance, including decreased activity of key proteins involved in glucose transport, has been well described (Biensø et al., 2012; Stuart et al., 1988). Hence, in the present study we investigated the bed rest-induced proANP response, as proANP is a much more stable marker of cardiac ANP expression and without the same elimination mechanism in circulation. Decreased insulin sensitivity including glucose-mediated hyperinsulinemia was successfully induced and plasma proANP was significantly decreased after bed rest. Nonetheless, we were not able to detect a significant association between indices of insulin sensitivity and plasma proANP or between the decrease in proANP and the magnitude of the induced insulin resistance in the population in question. The bed rest-induced decrease in plasma proANP concentrations may thus reflect other physiological processes than decreased insulin sensitivity such as plasma volume reduction and cardiac atrophy due to reduced myocardial load in the supine position (Perhonen et al., 2001).

To further elucidate the link between circulating markers of ANP expression and insulin resistance, we quantified circulating proANP concentrations in middle-aged men and women with prevalent impaired glucose regulation. We found that individuals with impaired glucose tolerance had similar proANP levels as individuals with normal glucose tolerance. In line with this, proANP concentrations did not differ between individuals with isolated impaired fasting glycemia and individuals with isolated impaired glucose tolerance, and the concentrations were not associated with insulin sensitivity. These findings contrast previous cross-sectional population-based studies reporting lower circulating proANP concentrations in obesity, insulin resistance, and diabetes (Then et al., 2013; Wang et al., 2007). Head-on comparisons with these studies are hampered by the broad clinical spectrum of the syndromes of insulin resistance ranging from obesity over type 2 diabetes to extreme insulin resistance. In the present study, the deteriorated insulin sensitivity induced by bed rest was characterized mainly by an increased insulin response to an oral glucose challenge, whereas the prevalent cases of insulin resistance represented normal to prediabetic states of hyperglycemia and not type 2 diabetes (Færch et al., 2008).

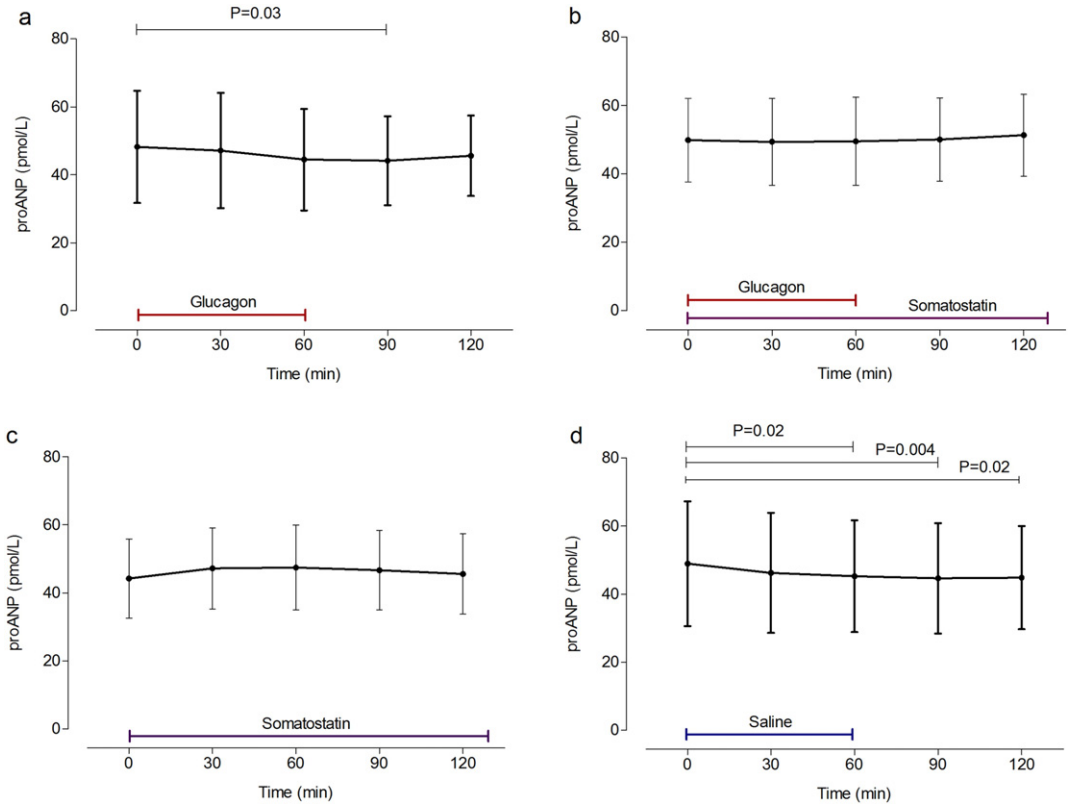

Fig. 4. Effect of hyperglucagonemia on plasma concentrations of pro-atrial natriuretic peptide. In ten healthy men, the infusion of glucagon alone resulted in significantly decreased plasma proANP concentrations at 90 min into the infusion compared to the onset (t = 0 min) (A). In the presence of a somatostatin-analogue, however, infusions of glucagon did not alter plasma proANP concentrations (B). Likewise, plasma proANP was unaltered during the infusion of a somatostatin-analogue alone (C). Saline infusions per se altered proANP concentrations overall, comprising a decrease at 60, 90, and 120 min into the infusion compared to the onset of infusions (D). Data are shown as mean ± sd and was analyzed by use of repeated measures ANOVA.

These differences notwithstanding, the individuals in the present study were non-obese and with early low-grade insulin resistance, whereas the previous population-based studies included significantly more individuals and a wider range of insulin resistance including overt type 2 diabetes. Taken together, it is therefore conceivable that decreased cardiac ANP secretion may represent a later event in disease progression, where the early stages are not affected or perhaps compensated.

In the present study we also investigated plasma pro-ANP in acute hyperinsulinemic settings but found that peptide concentrations were unaffected by acute euglycemic hyperinsulinemia before and after bed rest. Early studies investigated circulating concentrations of the mature ANP hormone iunder hyperinsulinemic conditions but reached divergent conclusions in individuals with normal glucose regulation (Clark et al., 1993; Gans et al., 1996; Miller et al., 1993; Nannipieri et al., 2002; Ohno et al., 2001; Trevisan et al., 1990) as well as in individuals with type 2 diabetes Several factors may account for these discordant findings, including different methodology and demographics of the included study subjects, the lack of stability of mature ANP and the high variability among assays quantifying ANP (Clerico et al., 2000). One previous study however quantified circulating proANP concentrations in obese men and reported decreased concentrations of proANP after euglycemic and hyperglycemic hyperinsulinemia, respectively (Pivovarova et al., 2012). In contrast to this latter study, the population investigated in the present study were young individuals with a BMI in the low range of what is classified as normal weight. Differential natriuretic peptide clearance in obese and normal weight adults mediated by an increased expression of *NPRC*, the gene encoding the natriuretic peptide clearance receptor, has been indicated in previous studies (Kovacova et al., 2016) and it is thus possible that also a differential secretion of ANP exists between obese individuals and their normal weight counterparts. Even though the lack of a statistically significant effect does not equal the lack of true biological effect (particularly in small populations such as ours), the changes in plasma proANP concentrations during the two HIECs were extremely small, thus supporting a lack of effect of euglycemic hyperinsulinemia in the present population. Our findings thus discourage the notion of ANP expression being regulated directly or indirectly by plasma insulin in lean individuals but this notion cannot necessarily be extended to an overweight and obese population.

Apart from hyperinsulinemia, early stages of insulin resistance are characterized by hyperglycemia as well as a defective suppression of glucagon secretion (Ahrén, 2009; Færch et al., 2016). Therefore, we also investigated the proANP response to manipulations of insulin, glucose, and glucagon in healthy young men. During isolated hyperglucagonemia, i.e., increased plasma glucagon in the presence of somatostatin-induced suppression of other hormones, proANP concentrations remained unaffected, indicating that glucagon per se is not involved in the regulation of cardiac ANP secretion. Surprisingly, a modest decrease in plasma proANP was observed during infusions of glucagon and saline per se. Seeing that the infusion of glucagon alone was accompanied by a glucagon-induced release of glucose (peaking at 6·8 mmol/l), which in turn stimulated pronounced insulin release (Hansen et al., 2015), it cannot be rejected that the combination of hyperglucagonemia and hyperinsulinemia may downregulate cardiac ANP expression. Of note, however, the magnitude of the observed decrease in plasma proANP was very modest and may thus not be biologically significant. Therefore, further studies are needed before this notion is convincingly supported. Although our experimental setup was not aimed at investigating the effects of blood glucose, our data do not support the notion of glucose

per se being involved in the regulation of ANP secretion, as has recently been suggested (Arora et al., 2016). First, marked hyperglycemia (peaking at 12.5 mmol/l), as was observed in isolated hyperglucagonemia due to blockage of endogenous insulin release, did not affect plasma proANP, whereas proANP concentrations decreased during the infusions of saline and glucagon where normoglycemic conditions were present. Second, the middle-aged individuals with a prediabetic state of hyperglycemia, represented by either increased blood glucose in the fasting state or, after an oral glucose challenge, did not exhibit significantly decreased plasma proANP.

Of note, the lack of detectably decreased cardiac ANP expression in low-grade insulin resistance, acute hyperinsulinemia and acute hyperglucagonemia found in the present study does not necessarily equal preserved ANP signaling to target organs. Several lines of evidence have pointed at defective ANP signaling being an early event in the progression of metabolic dysfunction (Coué et al., 2015; Kovacova et al., 2016; Pivovarova et al., 2012). Specifically, the ratio of the gene expression levels of the natriuretic peptide receptors type A and C is decreased already at early stages of a dysregulated glucose metabolism (Kovacova et al., 2016). As the natriuretic peptide receptors type A is considered primary responsible for ANP signaling whereas the natriuretic peptide receptors type C is mainly a clearance receptor, this change conveys a reduced biological activity of ANP. We did however not quantify the expression levels of the genes encoding the peripheral receptors in the present study and decreased ANP bioavailability may thus have been present.

Moreover, we do acknowledge the fact that proANP may not fully report on acute changes due to the prolonged elimination phase. However, as plasma half-life of approximately 60 min has been reported for proANP (Murthy et al., 1988) we do expect significant changes in proANP secretion to be reflected during infusion experiments of 2–3 h' duration as were performed in the present study.

In conclusion, short term bed rest induced a marked decrease in plasma proANP concentrations. According to our findings, this decrease reflected other aspects of bed rest than the induced insulin resistance. In line with this, individuals with impaired glucose regulation had similar plasma proANP levels as age-matched normoglycemic controls. Finally, neither acute euglycemic hyperinsulinemia nor hyperglycemic hyperglucagonemia per se were associated with changes in circulating proANP in lean individuals. Altogether, our findings suggest that cardiac proANP – today used as a clinical marker of cardiac dysfunction – is not affected by acute hyperinsulinemia, hyperglucagonemia, or hyperglycemia in non-diabetic lean individuals.

Funding

JPG received an unrestricted research grant from the Novo Nordisk Foundation (Hellerup, Denmark) which has been used for postdoc salary for NEZ. Otherwise, no additional funding was received for the present study. None of the authors (including the corresponding author and the co-authors) has disclosures to report, whether being from government sources or industry.

Author Contributions

Study design: NEZ, DT, KF, PP, JSH, PR, JPG.
Data collection: DT, KF, PP, JSH, JPG.
Data analysis: NEZ.
Literature search: NEZ, DT.
Figures: NEZ.
Data interpretation: NEZ, DT, KF, PP, JSH, PR, JPG.
Manuscript writing, original draft: NEZ, DT, JPG.
Manuscript writing, review and editing: NEZ, DT, KF, PP, JSH, PR, JPG.

Conflicts of Interest

None of the authors have any personal or financial conflicts of interest in relation to the data presented in the present study.

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