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Genetic Factors Influencing B-type Natriuretic Peptide-Mediated Production of Cyclic Guanosine Monophosphate and Blood Pressure Effects in Heart Failure Patients

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Abstract

Natriuretic peptides (NPs) represent a critical pathway in heart failure (HF). We explored genetic determinants of pharmacodynamic effects of B-type NP (BNP) and changes in plasma cyclic guanosine monophosphate (cGMP) and blood pressure (BP). HF patients ($n=135$) received recombinant human BNP (nesiritide) at standard doses, and plasma cGMP levels were measured at baseline and during infusion. We tested the association of 119 single nucleotide polymorphisms (SNPs) in 4 candidate genes (NPR1, NPR2, NPR3, and membrane metallo-endopeptidase (MME)) with the change in cGMP and BP. Gene-based testing for association of genetic variation with endpoints was significant only for MME. Upon individual SNP testing, two loci in MME were associated with cGMP; another (rs16824656) showed association with BP change. In summary, the pharmacodynamic effects of BNP vary substantially in HF patients and are associated with genetic variation in MME. MME genetic variation may be an important determinant of NP-mediated effects in humans.

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Conflict of Interest The authors declare that they have no competing interests.

Compliance with Ethical Standards

Ethical Approval All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 [5].

Informed Consent Informed consent was obtained from all individual participants included in the study.

Keywords

Natriuretic peptide; Heart failure; Drug metabolism; Pharmacogenetics; Genetic polymorphisms

Introduction

There have been numerous advances in the treatment of heart failure (HF) over the last two decades, but despite this, it remains a critical public health problem with a high prevalence, substantial morbidity and mortality, and enormous associated costs [1]. The natriuretic peptide (NP) system, particularly B-type NP (BNP), has emerged as a critical factor in HF. It is helpful in establishing diagnosis and prognosis, plays an important role in the pathophysiology, and has even led to novel therapeutic agents [2–5]. However, there is still an incomplete understanding of the impact and inner workings of the NP system, particularly the marked variability in NP system function across the broad spectrum of HF patients [6]. For example, it is clear that BNP levels, rates of BNP elimination, and clinical response to extrinsic NP (e.g., nesiritide, carperitide) vary substantially among HF patients [4, 7, 8]. Part of this variability is genetic in origin; BNP levels are heritable [9], variation in NP pathway genes is associated with differences in BNP production [10, 11] and cardiovascular phenotypes [12–15], and our group recently showed that BNP clearance rate appears to be affected by genetic variation [7]. An important related question is whether the pharmacodynamic impact of BNP varies with genetic background. This would have obvious implications in terms of understanding the HF disease process, as well as potentially influencing the effectiveness of interventions targeting the NP system.

From a molecular standpoint, there are primarily two effector receptors and two clearance mechanisms previously identified for NPs; NP receptor (NPR) A (gene name *NPR1*), B (*NPR2*), and C (*NPR3*) and membrane metalloendopeptidase (aka neutral endopeptidase [NEP]), encoded by the gene *MME* (Fig. 1) [16–18]. NPRA and NPRB are transmembrane guanylate cyclases that generate cyclic guanosine monophosphate (cGMP), the key second messenger, which is thought to ultimately mediate most physiologic effects, after NP binds to the receptor. MME enzymatically degrades NP (and other peptides) to predominantly inactive forms. NPRC is a non-catalytic NPR, which shares homology with the other NPRs in the extracellular and transmembrane portions, but lacks the intracellular guanylate cyclase domain. It is thought that its primary role is in peptide internalization and clearance although effector roles have also been proposed. With these as our target genes, the goal of the present study was to systematically assess whether genetic variation in these candidates impacts the physiologic effects of BNP. To do this, we studied HF patients infused with exogenous BNP (nesiritide) and quantified the response by measuring the change in plasma cGMP levels (primary end point) as well as in blood pressure (BP).

Methods

Patients and Sample Collection

The primary study, Pharmacogenetics of BNP (PGBNP), was an investigator-initiated National Institutes of Health-sponsored study centered at Henry Ford Hospital in Detroit,

MI. Patients were enrolled at two sites: Henry Ford Hospital (88 subjects) and Butterworth Hospital (13 subjects) in Grand Rapids, MI. The study was approved by the Henry Ford Hospital and Butterworth Hospital institutional review boards, and written informed consent was obtained from all patients. A total of 101 patients with a history of HF who were receiving nesiritide either as part of clinical care (hospitalized HF patients) or only for the purposes of the study (ambulatory chronic HF patients) were included. Subjects with baseline systolic BP (SBP) less than 110 mmHg or with end-stage renal disease were excluded. Patients received intravenous nesiritide at standard doses: 2 mcg/kg bolus (not required for participation—i.e., the bolus could be held at the discretion of the physician) followed by 0.01 mcg/kg/min continuous infusion. Patients were monitored for 1 h prior to bolus and an additional 2 h following initiation of nesiritide. Patients were monitored for at least 1 h after discontinuation (if the drug was stopped). Blood samples were drawn at baseline and at 2 h of continuous infusion. BP was monitored every 15 min throughout the study. Six patients were not included in the final analysis because either they did not receive the study drug (excluded during baseline phase due to vital signs) or samples could not be collected, leaving 95 subjects for analysis. All patient samples were centrifuged, aliquoted, and frozen within 30 min and were stored at -70°C until batch testing could be performed.

In order to increase our sample size and power, we also pooled data from the Assessment of Biomarkers and Cardiorenal Syndrome Heart Failure Trial (ABCHF) study [19], the design and primary results of which have been previously published [20]. Briefly, this was an investigator-initiated clinical trial which randomized patients with exacerbated HF to receive nesiritide or nitroglycerin and collected blood samples, BP, and other data at baseline through 48 h of infusion. Patients were also consented for genetic analyses at the time of study. Patient samples and data from 43 subjects in the nesiritide arm were examined; after missing data or samples were excluded, 40 subjects had analyzable data and were included in the final analysis for the present work. Sample handling and processing for cGMP and genotyping was performed the same as in the PGBNP. BP measurements were taken at baseline and hourly through the study period. Analyses were performed on the baseline measure, and mean BP at 1 and 3 h, to be as consistent as possible with the PGBNP study. Blood samples for cGMP were collected at baseline and 24 h.

Cyclic Guanosine Monophosphate Quantification

All cGMP assays were performed in the Cardiovascular Research Laboratory at Henry Ford Hospital. Commercially available kits from Arbor Assays (Ann Arbor, MI) were used according to standard processes. Proteins in stored plasma samples were precipitated by adding 95 % alcohol, and the resulting lyophilized supernatant was acetylated; cGMP was assayed as described in the assay kit. Values of cGMP were expressed as picomoles per milliliter. Among the total cohort of 135 patients, a cGMP level (either baseline or infusion) could not be obtained in 9 subjects (2 from PGBNP and 7 from ABCHF), so that paired sample results were available on a total of 126 individuals.

Genotyping

DNA samples were genotyped using a custom Illumina Goldengate[®] array that contained candidate-gene coverage relevant to HF including focused attention on the genes of interest

to the NP pathway. Single nucleotide polymorphisms (SNPs) were chosen for the array by attempting to include all coding variants and also utilizing HAPMAP to select optimal noncoding (“tag”) variants to capture blocks with minor allele frequency (>0.1 prevalence) in whites or African-Americans within the gene regions of interest. After processing requirements for the Goldengate technology and quality control of genotyping, 119 SNPs in the 4 candidate genes of interest, *NPR1* (8), *NPR2* (18), *NPR3* (52), and *MME* (41), were available for analysis. Genotyping calls were made using GenomeStudio software automatic algorithms (Illumina, San Diego, CA), and then individual SNPs were reviewed manually. Sample call rates were greater than 90 %, and none of the SNPs analyzed deviated significantly from Hardy-Weinberg equilibrium.

Statistical Analysis

The primary analysis was a SNP-wise test of association with change in cGMP levels (cGMP) for all SNPs genotyped using linear regression, adjusted for self-identified race, hospitalization status (inpatient vs. ambulatory), and bolus use. We estimated the association between genotype and outcome for each SNP within each cohort separately (PGBNP or ABCHF), and significant heterogeneity was observed using Cochran’s *Q* test [21]. We then combined the estimates using the random effect model for each SNP as shown below:

$$y_i = \mu + \theta_i + \varepsilon_i$$

where y_i is the observed effect size for each cohort i , μ is the overall mean, and θ_i is the random effect for the cohort which is normally distributed with mean 0 and variance τ^2 representing between-cohort variance. $\varepsilon_i \sim N(0, s_i^2)$ in which s_i^2 represents within-cohort variability. τ^2 was estimated using the DerSimonian-Laird method [22]. We similarly examined genotype associations with the secondary end points of (1) change in SBP and (2) change in diastolic BP (DBP). Since BP was measured every 15 min throughout the study, we used the average SBP over the infusion period minus the average SBP over the baseline period as the change in SBP (i.e., average SBP during infusion–average SBP during baseline=change in SBP). Similarly, the average DBPs over the same two periods were used to calculate the difference in DBP due to nesiritide infusion. In order to try to assess which specific genes may be key contributors to pharmacodynamic outcomes (as opposed to attempting to identify the individual SNP), we also performed a gene-wise analysis in which the p values of all SNPs across each gene were combined using Simes’ procedure into a single p value [23].

Analyses were performed using PLINK, R, and SAS (SAS Institute, NC). Since the nature of the study was exploratory with inherent challenges regarding statistical power for genetic effects, we considered p values less than 0.05 significant, without formal adjustment for multiple comparisons. For clarity, we also present the false discovery rate (Hochberg method [24]) for each finding of potential interest and considered 0.1 to be significant [25]. We calculated power using proc power in SAS (two sample means). Inputting the achieved sample size of 135, and taking the standard deviation of cGMP change from a previous published study [21], and group proportions of 4.26:1 (reflecting a minor allele frequency [MAF] of 0.1) and 1:1 (reflecting MAF 0.3), our estimated power to detect a 50 % relative

difference in cGMP is 89–99 % for MAF 0.1 to 0.3, respectively. The study was not designed or sized to achieve standard power in assessing changes in BP. Using the standard deviation measured in SBP in a previous study [22] and our cohort size, we again estimated power to detect a 50% change using PROC POWER; this was between 38 and 56 % for MAF of 0.1 to 0.3, respectively.

Results

The overall study included 135 HF patients with a mean age of 65 (± 15) years; 35 % were female and 60 % were non-white. The baseline characteristics of each of the 2 cohorts (95 subjects from the PGBNP study and 40 from the ABC study) are shown in Table 1. In total, 64 subjects were hospitalized for HF at the time of study entry and received nesiritide by clinical indication, with the remaining 71 subjects being patients with a history of stable chronic HF that received nesiritide for study purposes only.

Infusion of nesiritide was associated with a significant increase in plasma cGMP and decreases in both SBP and DBP. The mean change in cGMP from baseline to steady-state infusion was 7.25 ± 8.23 pmol/ml ($p < 0.0001$); the distribution is depicted in Fig. 2. SBP declined on average 7.3 mmHg ($p < 0.0001$) while DBP declined 3.5 mmHg ($p = 0.0001$). As expected, there was no observed impact on heart rate ($\uparrow 0.94$ beats per minute (bpm), $p = 0.18$). There was also no significant difference in the sodium excretion rate ($p = 0.26$). Interestingly, cGMP was poorly correlated with changes in BP. For example, the Pearson correlation coefficient for cGMP with SBP was -0.045 ($p = 0.61$).

The changes in cGMP and BP were also examined according to age, gender, race, body mass index, creatinine (continuous variables dichotomized at the median), and use of a bolus. These results are summarized in Table 2, and characteristics with significant association to endpoints are depicted in supplemental Figure 1. Non-white race was associated with cGMP ($p = 0.038$), such that non-white patients showed a greater increase in cGMP compared to white patients. In contrast, change in SBP and DBP in response to infused nesiritide was less in non-whites compared to their white counterparts (5 vs. 10 mmHg reduction; $p = 0.019$). Inclusion of a bolus dose appeared to have no effect on cGMP (7.23 vs. 7.25, $p = 0.99$), but it did affect BP and was associated with an additional 9-mmHg reduction in SBP ($p = 0.0003$).

Genetic Associations of Pharmacodynamic Response to Infused BNP

Examination of 119 SNPs in the 4 candidate genes and the association with the pharmacodynamic phenotypes of interest was undertaken. Linkage maps of each gene with an associated heat map of phenotypic associations are depicted in Fig. 3. Gene-wise tests of association were performed by using Simes' procedure to calculate a single p value for overall variation in each candidate gene. Only *MME* appeared to be of interest, showing a trend for cGMP ($p = 0.09$) and significant associations with SBP and DBP ($p = 0.004$ and 0.003 , respectively), while all the other candidates were not significant.

In terms of individual SNPs, there were two SNPs in *MME* that had statistically significant associations with cGMP: rs9829347 ($p = 0.0037$) and rs9864287 ($p = 0.0069$). These two

SNPs are in high linkage disequilibrium ($D'=0.97$); they appear to both represent the same linkage block, as can be seen in Fig. 3, and are thus unlikely to be two independent findings. The full linear regression analysis is shown in Table 3 (Forest plot in supplemental Figure 2), and boxplots of each endpoint for each SNP are depicted in Fig. 4. The stronger association was with rs9829347, which carried a 2.5-pmol/ml difference in cGMP per G allele (1.38-fold difference). However, this association did not meet multiple comparison adjustment significance (false discovery rate (FDR)=0.39). This genotype was also associated with changes in SBP ($p=0.017$) and DBP ($p=0.0039$) but not in the anticipated direction relative to cGMP (i.e., they were not inversely correlated). Similarly, rs9864287 was associated with DBP ($p=0.0096$) but not SBP. There was one other locus in *MME* which had a more borderline association with cGMP: rs1126073 ($p=0.029$, FDR 0.93).

While change in cGMP was our primary endpoint, we also explored changes in BP with BNP infusion. There were a number of variants showing significant associations with change in BP (Supplemental Table), and most of these, particularly the most statistically significant SNPs, were within *MME*. Four variants in *MME* showed significant associations to SBP with BNP infusion. The strongest was rs16824656 ($p=0.00015$, FDR=0.017). The same SNP was also related to DBP ($p=0.00011$, FDR=0.012). Interestingly, this SNP had no significant association to cGMP (bottom of Table 3, $p=0.74$), perhaps due to disparate cGMP trends in the two cohorts (right panel of Fig. 4). Three other SNPs had more borderline associations with SBP (rs9829347, rs701109, rs3773878) with p values ranging from 0.017 to 0.029 and all with FDR=0.77. There were no significant variants in the other genes for SBP. Diastolic BP change showed 15 variants with significant associations (Supplemental Table 1), from *MME*, *NPR2*, and *NPR3*. Interestingly, the strongest and the only one with significance by FDR was rs16824656, same as noted above for SBP.

Discussion

The aim of the present study was to better understand the variability in the pharmacodynamic response to BNP, taking advantage of the existence of human recombinant BNP as a pharmacologic agent (nesiritide) to assess the acute response in humans with HF. These data demonstrate substantial variability in the second messenger response, which is related to self-identified race as well as specific genetic variants, particularly in *MME*. We did not detect a corresponding change in BP associated with the genotypes associated with cGMP, but we were underpowered for those end points and did find additional variants associated with BP alone. To our knowledge, this study is one of the largest of its kind, interrogating the impact of genetic variation on NP system physiology in humans. This is an important question as the NP system is a key player in the development, diagnosis, prognosis, and treatment of HF.

Overall, *MME* appears to be the best candidate gene for modulating the response to NPs of those examined in this study. It was the only gene of the four examined that was significant in gene-wise testing and had several strong associations with cGMP and BP. Although the individual variants identified as important to the pharmacodynamic effect were not the same as from recently published pharmacokinetic [7] and gene expression [26] analyses, there is significant linkage disequilibrium (LD) in *MME* that could partially explain this

inconsistency (supplemental Figure 2). Indeed, some of the loci in the middle LD block of the gene, which were associated with expression in our previous study (rs1816558, rs10513469, rs1436630, rs1025192, rs3773895), show linkage disequilibrium with the 3' LD block containing the SNPs associated with pharmacodynamic endpoints (cGMP and BP); specifically, rs1025192, which was the strongest SNP associate of gene expression, shows LD with both rs9829347 and rs9864287 ($D'=0.54$ and 0.64 , respectively). Similarly, the linkage block near the 5' end, which had several SNP associations to BNP pharmacokinetics in the previous study (rs989692, rs6798179, rs9834487), does show some longer range LD with the 3' block of interest in the current study. Thus, while no specific variant was functionally validated, the totality of the data suggests that genetic variation in *MME* is likely an important determinant of NP system function and the response to pharmacologic interventions directed at it. Overall, the idea that *MME* is critical to BNP response in HF is consistent with the PARADIGM-HF trial [27] results, which showed that inhibiting *MME* (aka NEP) improved HF outcomes. It is intriguing to consider whether genetic variation may influence response to NEP-inhibitor therapy as well.

Limitations

Our study has several limitations that should be noted. The study sample size provided adequate power for cGMP but was underpowered for BP. Nonetheless, identification of a significant correlation between cGMP and genetic variation in *MME* can provide a foundation for future studies with larger sample sizes to assess the impact of genetic variation on BP or other clinical end points, such as symptoms or outcomes. While we have examined what were felt to be the key characteristics in describing our cohorts, it is possible that there were unaccounted differences in the two cohorts that made up this study and this somehow could have impacted the results. We attempted to mitigate this concern by accounting for study site in our models, but residual confounding of unmeasured or untested variables cannot be definitively excluded. Another potential limitation is the nonrandomized nature of the study, raising the possibility that other factors could have contributed to the changes in cGMP. It is unlikely, however, that other simultaneous changes drove our findings given the short time frame of the study and that BNP is a key driver of cGMP. Finally, this was a candidate gene study, and the sample size does not provide adequate power to take an unbiased approach. Thus, we could be overlooking other important genetic contributors to NP response.

Conclusions

Cyclic GMP, the second messenger of NPs, is increased in plasma following BNP infusion, but the magnitude of change varies substantially across HF patients and is associated with both self-identified race and specific genetic variants in *MME*. Genetic variation in *MME* likely impacts NP system physiology and could have implications for the risk of HF development, disease progression in HF patients, and targeting of therapies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

BP	Blood pressure
BPM	Beats per minute
BNP	B-type natriuretic peptide
cGMP	Cyclic guanosine monophosphate
DBP	Diastolic blood pressure
GMP	Guanosine monophosphate
HF	Heart failure
MME	Membrane metallo-endopeptidase
NEP	Neutral endopeptidase
NP	Natriuretic peptide
NPR	Natriuretic peptide receptor
NPRC	Natriuretic peptide receptor C
PGBNP	Pharmacogenetics of N-type natriuretic peptide
SBP	Systolic blood pressure
SNP	Single nucleotide polymorphism

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Fig. 1. Schematic of BNP processing. *NPRC* natriuretic peptide receptor C, *MME* membrane metallo-endopeptidase, *BNP* B-type natriuretic peptide

Histograms and Boxplots of Change in cGMP by Cohort

Fig. 2. Histograms and boxplots of change in cGMP by cohort. *cGMP* cyclic guanosine monophosphate

Fig. 3. Linkage disequilibrium plot of each candidate gene and heat map of p values for genetic variants' association with cGMP, SBP, and DBP. *NPR* natriuretic peptide receptor, *MME* membrane metallo-endopeptidase, *cGMP* cyclic guanosine monophosphate, *SBP* systolic blood pressure, *DBP* diastolic blood pressure. The directionality of the beta coefficients (positive or negative) refers to the correlation to a specific allele and is given so that the reader can tell which direction the three endpoints are changing relative to each other (i.e., to

understand whether the changes for cGMP vs. BP are moving in the same direction or opposite directions)

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Fig. 4. Boxplots of $cGMP$, SBP , and DBP changes for significant variants. Genotype frequencies are shown on the plots below each genotype. $cGMP$ cyclic guanosine monophosphate, SBP systolic blood pressure, DBP diastolic blood pressure

Table 1

Baseline Characteristics

Characteristics	PGBP (cohort 1, N=95)	ABCHF (cohort 2, N=40)	<i>p</i>
Age (years), mean±SD	63 ± 16	69 ± 14	0.036
Female gender, <i>N</i> (%)	29 (31 %)	18 (45 %)	0.11
Non-white race, <i>N</i> (%)	47 (49 %)	34 (85 %)	0.0001
Ejection fraction 50 %, <i>N</i> (%)	25 (26 %)	10 (25 %)	0.696
Mean ejection fraction (Among those <50%), <i>N</i> (%)	26% ± 11	25% ± 10	0.87
Creatinine (mg/dL), mean±SD	1.38 ± 0.54	1.27 ± 0.43	0.87
Body mass index (kg/m ²), mean ± SD	32.6 ± 7.9	30.06 ± 11.7	0.175
Inpatient status, <i>N</i> (%)	25 (26%)	40 (100%)	<0.0001
Bolus used, <i>N</i> (%)	82 (86%)	24 (60%)	<0.0001

Statistical tests are *t* test or chi square as appropriate.

ABCHF Assessment of Biomarkers and Cardiorenal Syndrome Heart Failure Trial, PGBNP Pharmacogenetics of BNP, *SD* standard deviation

Table 2

Mean change in outcome measures by subgroups

Characteristic	cGMP (pmol/ml)	<i>p</i>	SBP (mmHg)	<i>p</i>	DBP (mmHg)	<i>p</i>
Overall	7.25 (±8.2)		-7.31 (±12.5)		-3.5 (±9.2)	
Female	6.73	0.62	-5.01	0.12	-4.55	0.071
Male	7.51		-8.54		-1.55	
Non-white	8.39	0.038	-5.29	0.021	-2.28	0.043
White	5.54		-10.3		-5.35	
EF low	7.21	0.93	-6.89	0.43	-3.04	0.31
EF high	7.35		-8.86		-4.92	
Age low	8.61	0.11	-7.86	0.65	-4.34	0.35
Age high	6.18		-6.87		-2.84	
BMI low	6.45	0.19	-6.01	0.165	-2.65	0.22
BMI high	8.30		-9.04		-4.64	
Creatinine low	7.85	0.45	-7.15	0.88	-3.61	0.89
Creatinine high	6.71		-7.48		-3.41	
Bolus	7.25	0.99	-1.02	0.0003	0.44	0.0028
No bolus	7.23		-9.68		-4.9	
In-hospital	5.7	0.09	-3.83	0.0017	-1.09	0.0035
Ambulatory	8.46		-10.5		-5.75	

All *p* values in table from *t* tests*cGMP* change in cyclic guanosine monophosphate, *DBP* change in diastolic blood pressure, *SBP* change in systolic blood pressure, *BMI* body mass index, *EF* ejection fraction

Table 3

Results of full linear regression models for cGMP

SNP	Factor	PGENP			ABCHF			Meta-analysis		
		Coef.	95% CI	p	Coef.	95% CI	p	Coef.	95% CI	p
rs9864287	Genotype	2.22	0.52, 3.92	0.012	3.14	-3.74, 10.01	0.379	2.27	0.62, 3.92	0.007
	Race	3.92	1.36, 6.49	0.004	9.18	-4.74, 23.1	0.206			
	Bolus	2.82	-0.9, 6.54	0.142	-6.64	-15.15, 1.86	0.136			
	Inpatient	-2.51	-5.54, 0.52	0.108						
rs9829347	Genotype	2.53	0.8, 4.27	0.005	1.98	-5.39, 9.35	0.602	2.5	0.81, 4.20	0.004
	Race	3.98	1.41, 6.55	0.003	7.58	-5.98, 21.14	0.282			
	Bolus	3.08	-0.62, 6.79	0.107	-6.74	-15.44, 1.96	0.139			
	Inpatient	-2.66	-5.67, 0.35	0.087						
rs16824656	Genotype	-1.52	-3.96, 0.92	0.226	5.46	-1.69, 12.61	0.145	1.13	-5.52, 7.77	0.739
	Race	2.45	0.13, 4.78	0.043	5.02	-6.64, 16.68	0.405			
	Bolus	2.33	-1.31, 5.98	0.214	-4.60	-13.17, 3.98	0.302			
	Inpatient	-2.80	-5.79, 0.20	0.071						