

Serum lipids and cardiac function correlate with nitrotyrosine and MMP activity in CAD patients

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Abstract

Aims: Peroxynitrite - matrix metalloproteinase (MMP) signaling has been shown to contribute to myocardial ischemia/reperfusion injury and heart failure and to be influenced by hyperlipidemia in preclinical models. Therefore, here we investigated the correlation between the markers of peroxynitrite – MMP signaling and hyperlipidemia in patients with significant coronary stenosis. **Methods:** Five minutes before percutaneous coronary intervention (PCI), arterial blood samples were collected from 36 consecutive coronary artery disease (CAD) patients selected for elective PCI. **Results:** Serum nitrotyrosine positively correlated with MMP-9 activity ($r=0.54$, $p=0.01$) but not with MMP-2 activity. Nitrotyrosine positively correlated with total ($r=0.58$; $p<0.01$) and LDL cholesterol ($r=0.55$; $p<0.01$), serum triglyceride ($r=0.47$; $p<0.05$), and creatinine ($r=0.42$; $p<0.05$), and negatively correlated with HDL cholesterol ($r=-0.46$; $p<0.05$) and with left ventricular ejection fraction (LVEF; $r=-0.55$; $p<0.05$), respectively. MMP-2 activity correlated positively with total ($r=0.55$; $p<0.05$) and LDL cholesterol ($r=0.45$; $p<0.05$). In statin-treated patients, a significantly reduced serum nitrotyrosine was found as compared to statin naives, however, MMP activities and serum cholesterol levels were not different. MMP-9 activity correlated with urea nitrogen ($r=0.42$; $p<0.05$) and LVEF ($r=-0.73$; $p<0.01$). Serum creatinine correlated negatively with LVEF ($r=-0.49$, $p<0.05$). **Conclusions:** This is the first demonstration that (i) serum nitrotyrosine correlates with MMP-9 activity, (ii) lipid parameters correlate with nitrotyrosine and MMP-2 activity, (iii) myocardial function correlates with creatinine, nitrotyrosine, and MMP-9 activity, and (iv) creatinine correlates with nitrotyrosine and urea nitrogen with MMP-9 activity in CAD patients. Studying the biomarkers of peroxynitrite – MMP pathway in large prospective trials may reveal their diagnostic avails.

Keywords: cardiac function, coronary artery disease, lipids, matrix metalloproteinase, nitro-oxidative stress, nitrotyrosine

Introduction

Matrix metalloproteinases (MMPs) are major targets of drug and diagnostic development in several pathologies including cardiovascular diseases [1,2]. The most abundant MMPs in the human myocardium are MMP-2 and MMP-9, which have been shown to play a crucial role in myocardial ischemia/reperfusion injury in several animal models [3]. Furthermore, it has been shown that serum MMP-2 predicts infarct size and ventricular dysfunction in ST-elevation myocardial infarction patients [4]. The activation of MMP-2 and MMP-9 may occur not only by proteolysis but also due to nitro-oxidative stress via posttranslational modification of pro-MMPs and the formation of transcriptional complexes [5,6].

Nitro-oxidative stress plays a pivotal role in the pathomechanism of several cardiovascular diseases including ischemic heart disease [7]. The main effector of nitro-oxidative stress is peroxynitrite, which may activate several enzymes including MMPs via S-nitrosylation/S-glutathiolation of their tyrosine and/or cysteine residues [7,8]. Furthermore, peroxynitrite nitrates free tyrosine residues to form nitrotyrosine, which is a widely used marker for peroxynitrite generation [9].

The contribution of increased nitrotyrosine formation to the development of atherosclerosis and thus to coronary artery disease (CAD) has been described in patients with hypercholesterolemia-combined CAD [10]. Hyperlipidemia is well-characterized risk factor for cardiovascular diseases at least in part due to its nitro-oxidative stress enhancing effect [11,12] and deterioration of cardioprotective mechanisms [13,14]. Nevertheless, the correlation between serum nitrotyrosine and lipid levels is not consistent in the literature. Moreover, correlations between serum nitrotyrosine and MMP-2 and MMP-9 activities are still unknown in CAD patients.

Therefore, we have conducted a pilot clinical study to determine the correlation between serum nitrotyrosine and MMP-2 and MMP-9 activities in single-vessel CAD patients subjected to elective percutaneous coronary intervention. Furthermore, we have investigated the correlation of serum lipids (total-, LDL-, HDL cholesterol; triglyceride) with serum nitrotyrosine as well as with serum MMP-2 and MMP-9 activities, respectively. To investigate whether serum nitrotyrosine or MMP-2 and MMP-9 activities may correlate with cardiac function, we determined left ventricular ejection fractions (LVEF). Finally, we have examined if there is a difference in serum MMP-2 and MMP-9 activities in statin naïve and statin-treated CAD patients.

Materials and Methods

Patients and blood sampling

The study was approved by the Ethics Committee of the University of Szeged. A written informed consent was obtained from all patients enrolled in the study. The investigations were carried out in consecutive patients with single-vessel coronary disease elected for percutaneous coronary intervention (PCI). Blood samples were collected from patients five minutes before the PCI.

Inclusion criteria were as follows:

- (1) Class II or III stable angina pectoris by the Canadian Cardiovascular Society grading system;
- (2) Single-vessel coronary artery disease defined as $\geq 70\%$ diameter stenosis by visual assessment of the coronary angiogram.

Exclusion criteria were as follows:

- (1) angina pectoris or other signs of myocardial ischemia within one week before intervention;
- (2) Lown 3–4 ventricular arrhythmia before the intervention;
- (3) severe left ventricular dysfunction (<35% LVEF or New York Heart Association functional circulatory stage III-IV);
- (4) treatment with ATP-sensitive potassium channel inhibitors;
- (5) history of myocardial infarction or baseline ST-segment abnormalities;
- (6) serum electrolytes out of the physiologic range;
- (7) serum creatinine level >150 $\mu\text{mol/L}$;
- (8) hypothyreosis
- (9) angiographically visible collateral vessels interfering with the treated coronary artery.

According to inclusion and exclusion criteria, 36 patients were enrolled into the present study.

PCI was performed using the standard femoral approach in all patients after premedication with 100 mg of aspirin and 75 mg of clopidogrel once a day for two days prior to PCI. Serum levels of total-, LDL-, HDL cholesterol, triglyceride, creatinine and urea nitrogen were quantitated by enzymatic methods on a Roche Hitachi Modular P800 automated analysers (Roche Diagnostics, Indianapolis, IN). Additional blood samples were collected from patients 5 minutes before the percutaneous coronary intervention from femoral artery. Blood samples were centrifuged at 1000g for 15 min, at 4°C then serum samples were collected into polypropylene microcentrifuge tubes and stored at -20°C until the performance of nitrotyrosine and MMP-2, MMP-9 zymographic measurements.

Biochemical analyses

To measure circulatory MMP-2 and MMP-9 activities, gelatin zymography was performed from serum samples (for a representative zymogram see Fig. 1). Gelatinolytic activities of MMPs were examined as previously described [15]. Briefly, 8% polyacrylamide gels were copolymerized with gelatin (2 mg/ml, type A from porcine skin, Sigma-Aldrich), and 40 µg of protein per lane were loaded. An internal standard (American Type Culture Collection, Manassas, VA) was loaded into each gel to normalize activities between gels. After electrophoresis (150 V, 1.5 h), gels were washed with 2.5% Triton X-100 for 3 x 15 min and incubated for 20 h at 37°C in incubation buffer (50 mM Tris·HCl, 150 mM NaCl, 5 mM CaCl₂, and 0.05% NaN₃, pH 7.4). Gels were then stained with 0.05% Coomassie brilliant blue (G-250, Sigma-Aldrich) in a mixture of methanol-acetic acid-water [2.5:1:6.5 (vol/vol)] and destained in aqueous 4% methanol-8% acetic acid (vol/vol). For positive controls gelatinase zymography standard containing human MMP-2 and -9 (Cat. No.: CC73, Chemicon Europe Ltd., Southampton, UK) and MMP-2 standard (Cat. No.: CC071, Chemicon Europe Ltd.) were used. For negative control, lanes containing serum samples were cut off after renaturation of the gel and were separately incubated for 20 h at 37°C in development buffer in the presence of the calcium chelator EGTA (ethylene glycol-bis(2-aminoethylether)-N,N,N',N'-tetraacetic acid; 10 mM). Gelatinolytic activities were detected as transparent bands against the dark-blue background. Band intensities were quantified, expressed as the ratio to the internal standard, and presented in arbitrary units.

To estimate systemic peroxynitrite formation, we have measured free nitrotyrosine by ELISA in serum samples as previously described [16,17]. Briefly, serum samples were deproteinized by addition of 4 volumes of ice-cold ethanol. After centrifugation, supernatants were separated and evaporated under nitrogen

flow, then they were dissolved in phosphate buffer. Deproteinised sera were incubated overnight with anti-nitrotyrosine rabbit IgG and nitrotyrosine acetylcholinesterase tracer in precoated (mouse anti-rabbit IgG) microplates followed by development with Ellman's reagent. Serum nitrotyrosine levels were expressed in ng/mL.

Measurement of cardiac function

Echocardiographic examinations were performed prior to PCI on a GE Vivid 3 (GE Healthcare, Milwaukee, WI) cardiac ultrasound and data was digitally recorded. Images were acquired using a 3.5 MHz transducer. Images and cines were obtained in standard apical and parasternal two- and four-chamber views. To assess LVEF, calculations were performed according to recommendations for chamber quantification in consensus. LVEF was calculated using the modified Simpson's method.

Statistical analysis

Data are expressed as mean \pm S.E.M. Univariate correlations between lipid parameters and MMP activities as well as nitrotyrosine were analyzed using the Pearson correlation coefficient. **Because of the relatively small sample sizes in the current pilot study, bootstrapped confidence intervals were calculated, using 1000 samples with replacement, in order to make robust estimates of the correlation coefficients as described elsewhere [18,19].** Linear regression analysis was used to estimate the prediction of the selected variables. The effects of statin treatment on variables were analyzed by unpaired two-tailed Student's t-test.

Results

Baseline characteristics of the 36 coronary artery disease patients are summarized in Table 1.

Correlation between serum nitrotyrosine and MMP-2 or MMP-9 activities

We have determined whether serum nitrotyrosine correlates with MMP-2 and MMP-9 activities in CAD patients. MMP-9 activity correlated positively with nitrotyrosine (Fig. 2/A; $r=0.535$, $p=0.010$), however, there was no significant correlation between serum nitrotyrosine and MMP-2 activity (Fig. 2/B).

Correlation between serum lipids and nitrotyrosine or MMPs activity

In CAD patients, serum total cholesterol (Fig. 3/A), LDL cholesterol (Fig. 3/B) and triglyceride (Fig. 3/C) levels correlated positively ($r=0.582$, $p=0.003$; $r=0.552$, $p=0.008$; $r=0.471$, $p=0.023$, respectively), while HDL cholesterol negatively (Fig. 3/D; $r=-0.455$, $p=0.033$) with serum nitrotyrosine. Serum MMP-2 activity correlated positively with serum total cholesterol (Fig. 3/E) and LDL cholesterol (Fig. 3/F) levels ($r=0.550$, $p=0.015$ and $r=0.448$, $p=0.028$, respectively), however, neither serum triglyceride (Fig. 3/H) nor HDL cholesterol (Fig. 3/G) showed a significant correlation with MMP-2 activity. None of the serum lipid parameters correlated with MMP-9 activity in CAD patients (Table 2).

Correlation between myocardial function and serum nitrotyrosine or MMPs activity

Serum nitrotyrosine level (Fig. 4/A) and MMP-9 activity (Fig. 4/C) correlated negatively with LVEF ($r=-0.548$, $p=0.010$ and $r=-0.732$, $p=0.0002$, respectively),

however, MMP-2 activity did not correlate with LVEF (Fig. 4/B) in CAD patients. We did not find any correlation between serum lipid parameters and LVEF (data not shown).

Serum nitrotyrosine or MMPs activity in statin-treated patients

Fifteen out of the 36 CAD patients involved in the present study were treated with different types of statins. In statin-treated patients, a significantly reduced serum nitrotyrosine level (Fig. 5/A) was found (from 23.5 ± 4.5 ng/ml to 13.6 ± 5.1 ng/ml; $p < 0.05$) as compared to statin naive patients. MMP-2 and MMP-9 activities did not show any difference between statin-treated and statin naive patients (Fig. 5/B-C).

Correlation between renal function and serum nitrotyrosine or MMPs activity

In addition, we tested the correlation of renal function parameters with markers of peroxynitrite – MMP pathway and also with LVEF. Serum creatinine correlated positively with nitrotyrosine ($r = 0.422$, $p = 0.036$; Fig. 6/A) but not with MMP-2 and MMP-9 activities (Table 3). Urea nitrogen level correlated positively with MMP-9 activity ($r = 0.423$, $p = 0.035$; Fig. 6/B) but not with nitrotyrosine or MMP-2 activity (Table 3). Serum creatinine correlated negatively with LVEF ($r = -0.503$, $p = 0.009$; Fig. 6/C), however, urea nitrogen did not correlate with LVEF (Table 3).

Discussion

In the present pilot study, we have demonstrated for the first time in the literature that (i) serum nitrotyrosine correlates with MMP-9 activity, (ii) serum lipid parameters correlate with nitrotyrosine level and MMP-2 activity, (iii) myocardial function correlates with nitrotyrosine, MMP-9 activity as well as with creatinine, and

(iv) creatinine correlates with nitrotyrosine and urea nitrogen correlates with MMP-9 activity in CAD patients.

Serum nitrotyrosine correlates with MMP activity

Here we found a positive correlation of serum nitrotyrosine with MMP-9 activity but not with MMP-2 activity. This suggests that peroxynitrite activates MMPs in humans as well. Previously, a positive correlation between nitrotyrosine content and MMP-9 expression was found in instable atherosclerotic plaques derived from ischemic heart disease patients, which supports our present results [20]. However, the reason why we have not found a significant correlation between peroxynitrite and MMP-2 activity is not known. A plausible explanation is that while MMP-9 is an abundant enzyme in different cells and tissues including macrophages and leukocytes, MMP-2 is an intracellular enzyme mainly in contractile tissues that can be activated and released e.g. due to acute myocardial ischemia/reperfusion and infarction [8,21-24], however, our CAD patient population did not have severe ischemia at the time of tissue sampling, therefore, MMP-2 activation and release can be less sensitively detected in the serum.

Serum lipids correlate with nitrotyrosine and MMPs activity

Hyperlipidemia is a well-characterized risk factor for cardiovascular diseases and it has been also demonstrated that it contributes to elevated nitro-oxidative stress in humans [10,25]. We have previously shown in rats and in apoB-100 transgenic mice that hypercholesterolemia increases nitro-oxidative stress, which leads to myocardial functional deterioration [26,27]. Here, we have also examined the correlation pattern of serum lipids with nitrotyrosine in CAD patients and shown

that nitrotyrosine correlated positively with triglyceride, total- and LDL cholesterol levels, and negatively with HDL cholesterol. In a previous study [28], an increased serum nitrotyrosine level was reported in hypercholesterolemic patients, wherein only LDL cholesterol showed significant positive correlation with nitrotyrosine. In CAD patients, a minor correlation was found between only serum protein-bound nitrotyrosine and triglyceride [10]. A similar correlation was shown by others in patients with metabolic syndrome [29]. In our present study, we have measured serum free nitrotyrosine, which seems to be a more sensitive marker for systemic peroxynitrite generation.

Here we have found that serum MMP-2 activity correlated positively with total- and LDL cholesterol levels. However, neither HDL cholesterol, nor triglyceride levels correlated with MMP-2 activity. According to our best knowledge, MMP-2 activity has been investigated by only one previous study in CAD patients [30], in which a negative correlation between serum MMP-2 activity and HDL cholesterol was found, however, other correlations were not assessed. In another study [31], plasma MMP-2 protein level was shown to correlate negatively with HDL cholesterol in CAD patients. Noji et al. [32] measured plasma MMP-2 in CAD patients several days before coronary intervention, and interestingly found a positive correlation between MMP-2 protein level and HDL cholesterol, however, they did not assess MMP-2 activity. In our present study, serum MMP-9 activity did not show correlation with any of the serum lipids. A similar finding was published previously in hypercholesterolemic patients [33]. Two independent studies in patients with CAD and acute coronary syndrome showed that MMP-9 protein level positively correlated with LDL cholesterol [31,32]. Furthermore, correlation of plasma MMP-9 protein

content with triglyceride level has been also shown [31]. However, in these studies, the authors did not measure MMP-9 activities, only MMP-9 protein immunoreactivity.

Myocardial function correlates with serum nitrotyrosine and MMPs activity

Here we have demonstrated first time in the literature in CAD patients that cardiac function characterized by LVEF showed a significant negative correlation with serum nitrotyrosine. This may suggest that the nitro-oxidative stress is detrimental to cardiac function in humans, which is in accordance with preclinical studies. Furthermore, we have found here that LVEF correlated negatively with MMP-9 activity, however, there was no correlation with MMP-2 activity. This is partly in accordance with previous findings that both MMP-2 and MMP-9 protein levels correlate with LVEF or left ventricular end-diastolic volume index in cardiac patients with acute myocardial infarction or heart failure [34-36]. We have also examined here the correlation of serum cholesterol and triglyceride levels with LVEF, however, we did not observe any significant correlation among these data. In contrast, Wang et al. [37] described a positive correlation of LVEF with serum HDL cholesterol and a negative correlation with serum triglyceride in angina patients.

Decreased serum nitrotyrosine in statin-treated patients

Most CAD patients are treated with various statins. Statins are well-characterized inhibitors of endogenous cholesterol synthesis and it has been also described that they attenuate nitro-oxidative stress [38] as assessed by plasma nitrotyrosine level in CAD patients [10]. In accordance, we have found here a reduced serum nitrotyrosine level in statin-treated CAD patients compared to statin naives.

Here we have found that MMP-2 and MMP-9 activities were not significantly different in statin-treated CAD patients compared to statin naives, although a tendency of decrease was found. In contrast, It has been shown in 48 CAD patients undergone coronary artery bypass surgery that protein levels and activities of MMP-2 and MMP-9 measured both in plasma and in pericardial fluid were reduced after 2-month pravastatin therapy as compared to untreated patients [39]. Other authors have also reported a reduction in plasma MMP-2 and/or MMP-9 protein levels after statin treatment of cardiac patients, however, in these studies, only MMP protein levels were measured [34-36,40].

Renal function correlates with serum nitrotyrosine and MMPs activity

It has been previously described that renal dysfunction may develop and contribute to mortality in patients with myocardial infarction [41]. It is also well known that ischemic coronary disease is a leading cause of death in uremic patients [42]. Although uremic patients and patients with severe left ventricular dysfunction were not enrolled into the present study, we have shown a significant negative correlation between serum creatinine level and LVEF for the first time in the literature in CAD patients. Moreover, we have found a positive correlation between serum creatinine and nitrotyrosine as well as between urea nitrogen and MMP-9 activity in CAD patients. These results suggest that both increased nitrotyrosine level and MMP-9 activity may have diagnostic values for the development of an incipient renal dysfunction after myocardial infarction even when the patients have creatinine and/or urea nitrogen levels near or within the normal range.

Limitations

The present manuscript provides data from a pilot study, therefore, the number of the enrolled patients is relatively low. However, bootstrap analysis of the correlation coefficients were performed, which is a widely accepted statistical method of making robust estimates of the coefficients in case of small sample sizes. The correlations we found were relatively weak, but statistically significant. This may show that there might be several other players in the interactions between the parameters we correlated in the present study. Indeed, MMP activity is determined by several confounding factors, such as level of the endogenous tissue inhibitors of MMPs (TIMPs), Zn^{2+} availability, phosphorylation status of MMPs, redox state etc. E.g. TIMPs may dissociate from MMPs during electrophoresis, therefore, TIMP free or TIMP bound MMPs in the serum cannot be assessed by zymography. Nevertheless, our results show that serum MMP-2 and -9 activities as measured by zymography are valuable diagnostic markers.

Conclusions

This is the first demonstration that serum (i) nitrotyrosine correlates with MMP-9 activity, (ii) lipid parameters correlate with nitrotyrosine level and MMP-2 activity, (iii) myocardial function correlates with nitrotyrosine, MMP-9 activity as well as with creatinine, and (iv) creatinine correlates with nitrotyrosine and urea nitrogen correlates with MMP-9 activity in CAD patients. These findings suggest that nitrotyrosine and MMP-2 and MMP-9 activities may be of diagnostic values in cardiac and renal function of CAD patients and attenuation of nitro-oxidative stress and MMP activities may provide therapeutic benefits in this patient population. Thus,

to study markers of peroxynitrite – MMP pathway in large prospective studies may reveal their diagnostic avails.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Legends

Figure 1

Representative gelatin zymogram from serum samples of CAD patients.

Figure 2

Correlation of serum nitrotyrosine content with MMP-2 and MMP-9 activities. Serum MMP-9 activity correlated positively with serum nitrotyrosine (A). Serum MMP-2 activity (B) did not correlate with serum nitrotyrosine. Continuous line: linear regression of data; dotted line: 95% confidence interval. **r: Pearson correlation coefficient, p: significance, bCI: bootstrapped confidence interval of correlation coefficient**

Figure 3

Correlation of serum nitrotyrosine and MMP-2 activities with serum lipids. Serum nitrotyrosine level correlated positively with total- (A) and LDL cholesterol (B) as well as with triglyceride (C) and correlated negatively with HDL cholesterol (D). Serum MMP-2 activity correlated positively with serum total- (E) and LDL cholesterol levels (F). Neither serum triglyceride (G) nor HDL cholesterol (H) showed a significant correlation with serum MMP-2 activity. Continuous line: linear regression of data; dotted line: 95% confidence interval. **r: Pearson correlation coefficient, p: significance, bCI: bootstrapped confidence interval of correlation coefficient**

Figure 4

Correlation of left ventricular ejection fraction (LVEF) with serum nitrotyrosine, and serum MMP-2 and MMP-9 activities. Serum nitrotyrosine (A) and MMP-9 (C) activity correlated negatively with LVEF. Serum MMP-2 activity did not correlate with LVEF

(B). Continuous line: linear regression of data; dotted line: 95% confidence interval. **r**: Pearson correlation coefficient, **p**: significance, **bCI**: bootstrapped confidence interval of correlation coefficient

Figure 5

Serum nitrotyrosine levels (A) and serum MMP-2 (B) and MMP-9 (C) activities in statin-treated and statin naive CAD patients. Serum nitrotyrosine was significantly reduced in statin-treated CAD patients as compared to statin naive patients (* $p < 0.05$, data are shown as mean \pm S.E.M.). MMP-2 and MMP-9 activities were not changed in statin-treated CAD patients as compared to statin naive ones ($p = 0.409$ and $p = 0.564$, respectively).

Figure 6

Correlation of renal function with serum nitrotyrosine, MMP activities and myocardial function. Serum nitrotyrosine correlated positively with serum creatinine (A). Serum MMP-9 activity correlated positively with urea nitrogen level (B). Continuous line: linear regression of data; dotted line: 95% confidence interval. **r**: Pearson correlation coefficient, **p**: significance, **bCI**: bootstrapped confidence interval of correlation coefficient

Table 1 Baseline characteristics of the 36 coronary artery disease patients

Variables	Mean±SD or Occurance (number of individuals)
<i>Biographic and anamnestic variables</i>	
Age (years)	67.4 ± 10.5
Gender, m/f	19/17
Type 1 diabetes	0/36
Type 2 diabetes	5/36
Statin treatment	15/36
ACE inhibitor treatment	31/36
ARB treatment	1/36
Nitrate treatment	18/36
<i>Hemodynamic variables</i>	
Systolic blood pressure (mmHg)	141.7 ± 11.1
Diastolic blood pressure (mmHg)	89.8 ± 21.4
Heart rate (beats/min)	70.4 ± 12.1
LVEF (%)	56.0 ± 10.5
Coronary stenosis (%)	63.5 ± 8.0
Occurrence of hypertension	29/36
<i>Serum variables</i>	
Total cholesterol (mmol/L)	4.6 ± 0.9
LDL cholesterol (mmol/L)	2.5 ± 0.8
HDL cholesterol (mmol/L)	1.3 ± 0.4
Triglycerides (mmol/L)	1.8 ± 0.8
3-nitrotyrosine (ng/mL)	17.4 ± 11.3
Urea nitrogen (mmol/l)	5.9 ± 2.4
Creatinine (μmol/l)	86.3 ± 27.5

Table 2 Correlations between serum MMP-9 activity and lipid parameters

<i>Variables</i>	<i>Pearson correlation coefficient</i>	<i>Bootstrapped confidence intervals of correlation coefficient</i>	<i>p</i>
MMP-9 / total cholesterol	0.171	-0.27-0.49	0.436
MMP-9 / LDL cholesterol	0.020	-0.38-0.30	0.939
MMP-9 / HDL cholesterol	0.050	-0.31-0.59	0.840
MMP-9 / triglyceride	0.024	-0.34-0.40	0.913

Table 3 Correlations between serum nitrotyrosine level, MMPs activities, or LVEF and renal function parameters

<i>Variables</i>	<i>Pearson correlation coefficient</i>	<i>Bootstrapped confidence intervals of correlation coefficient</i>	<i>p</i>
MMP-2 / creatinine	0.125	-0.20-0.45	0.504
MMP-9 / creatinine	0.118	-0.30-0.48	0.550
NTyr / BUN	0.105	-0.22-0.39	0.602
MMP-2 / BUN	0.071	-0.20-0.42	0.700
LVEF / BUN	-0.250	-0.57-0.00	0.217

Negative
control

Serum #1

Serum #2

Serum #3

Serum #4

Serum #5

Serum #6

Serum #7

MMP-2
standard

Zymography
standard

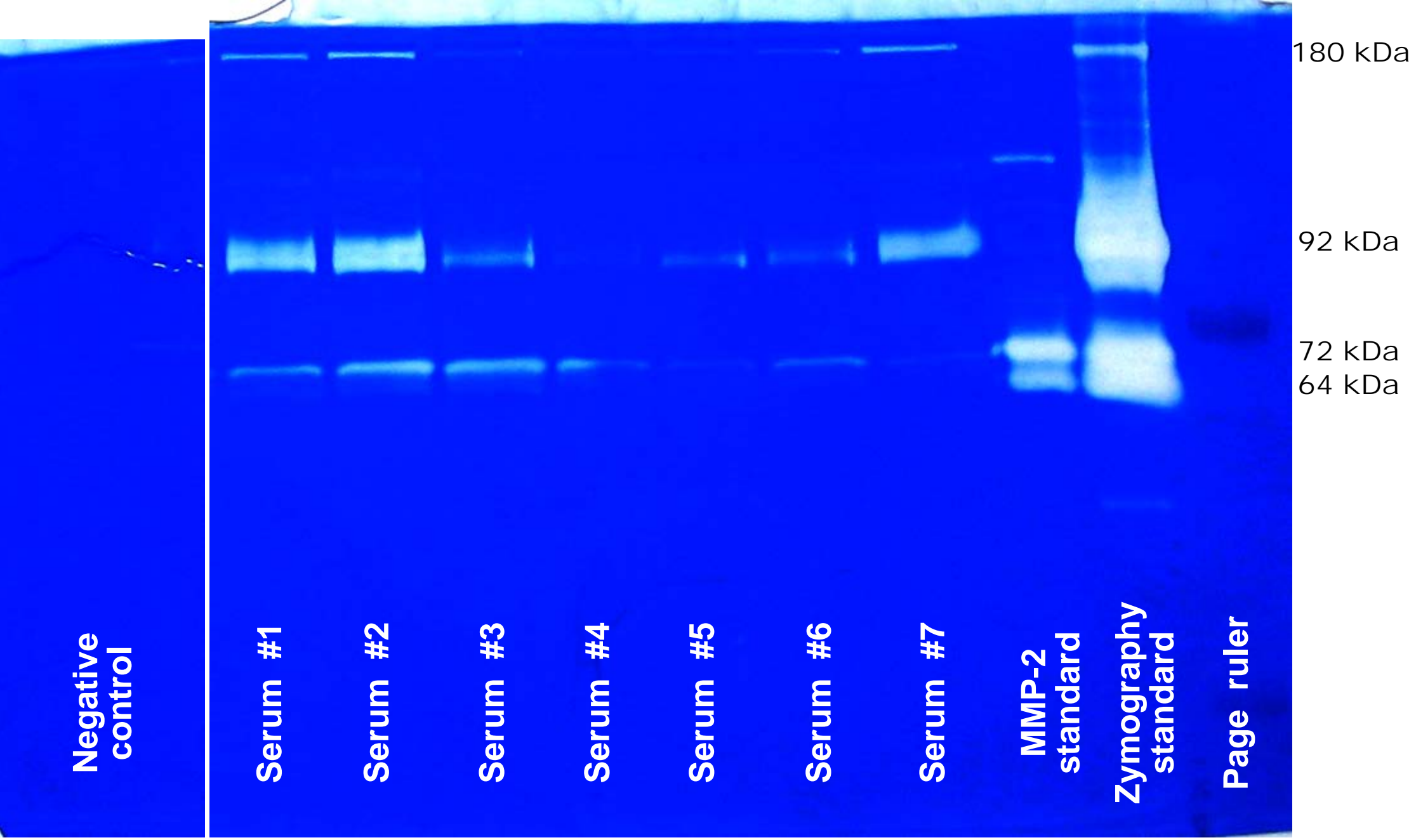
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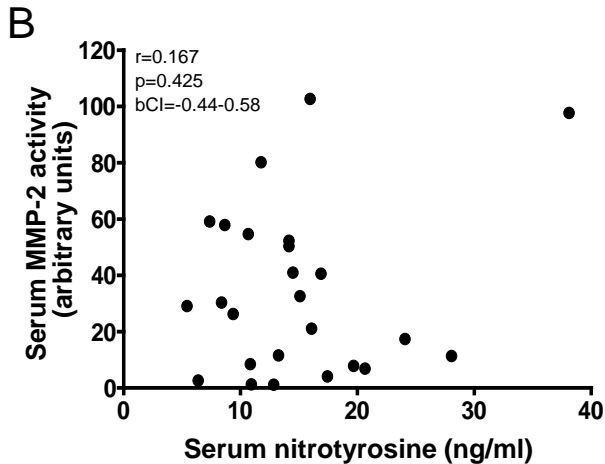
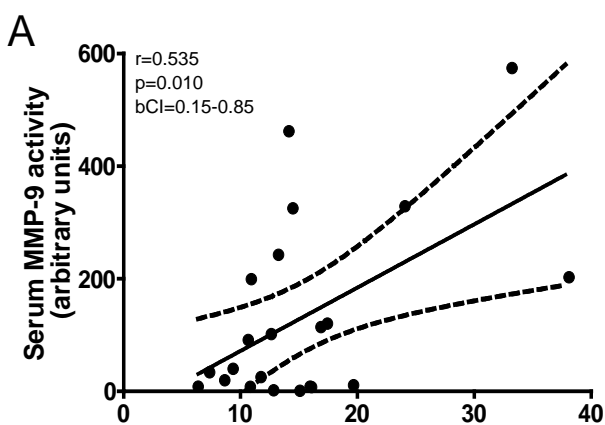
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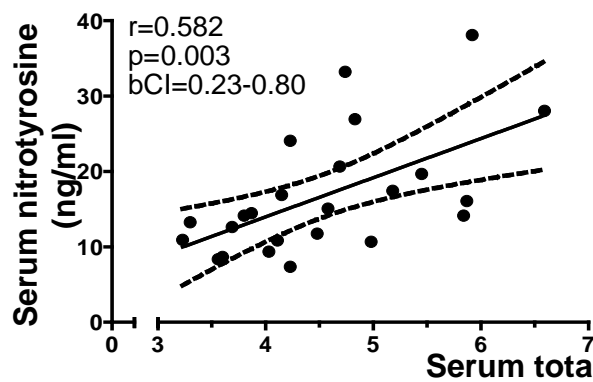
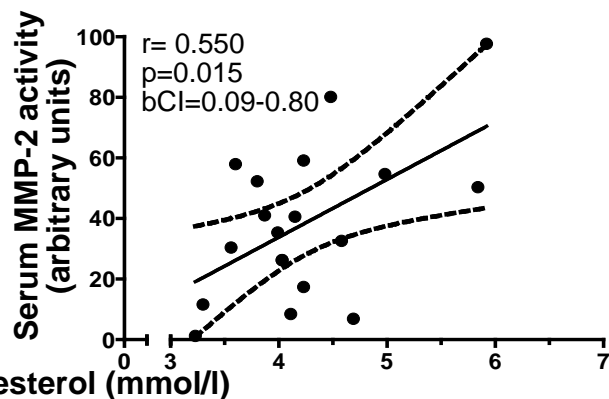
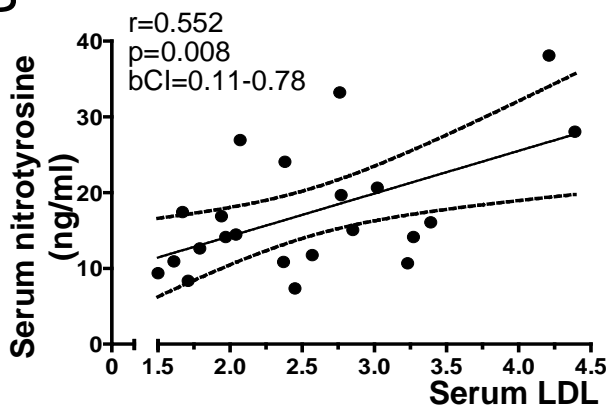
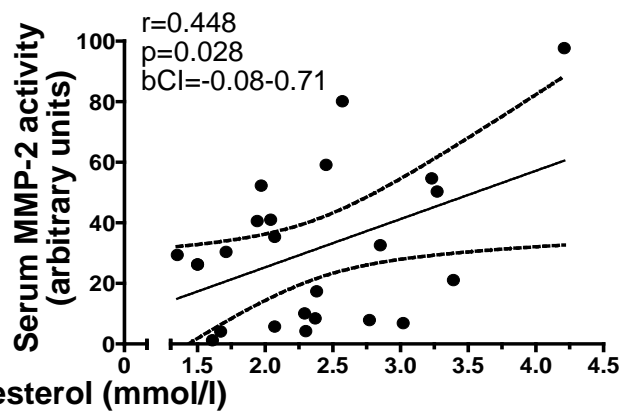
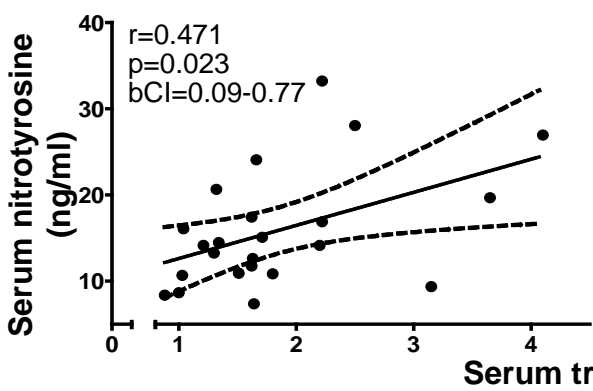
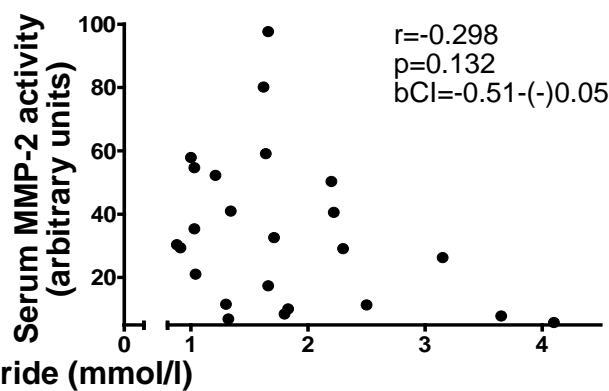
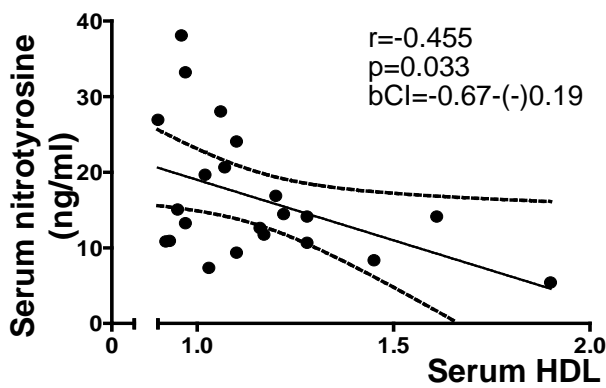
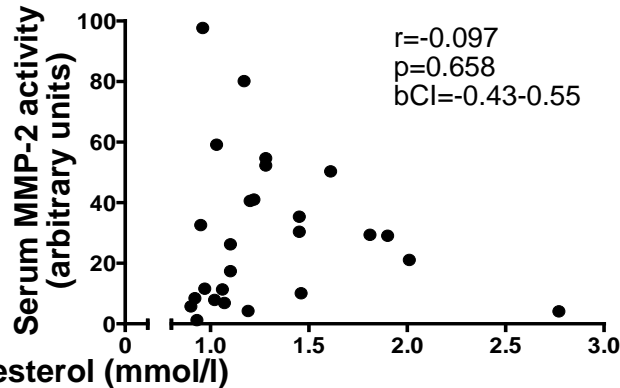
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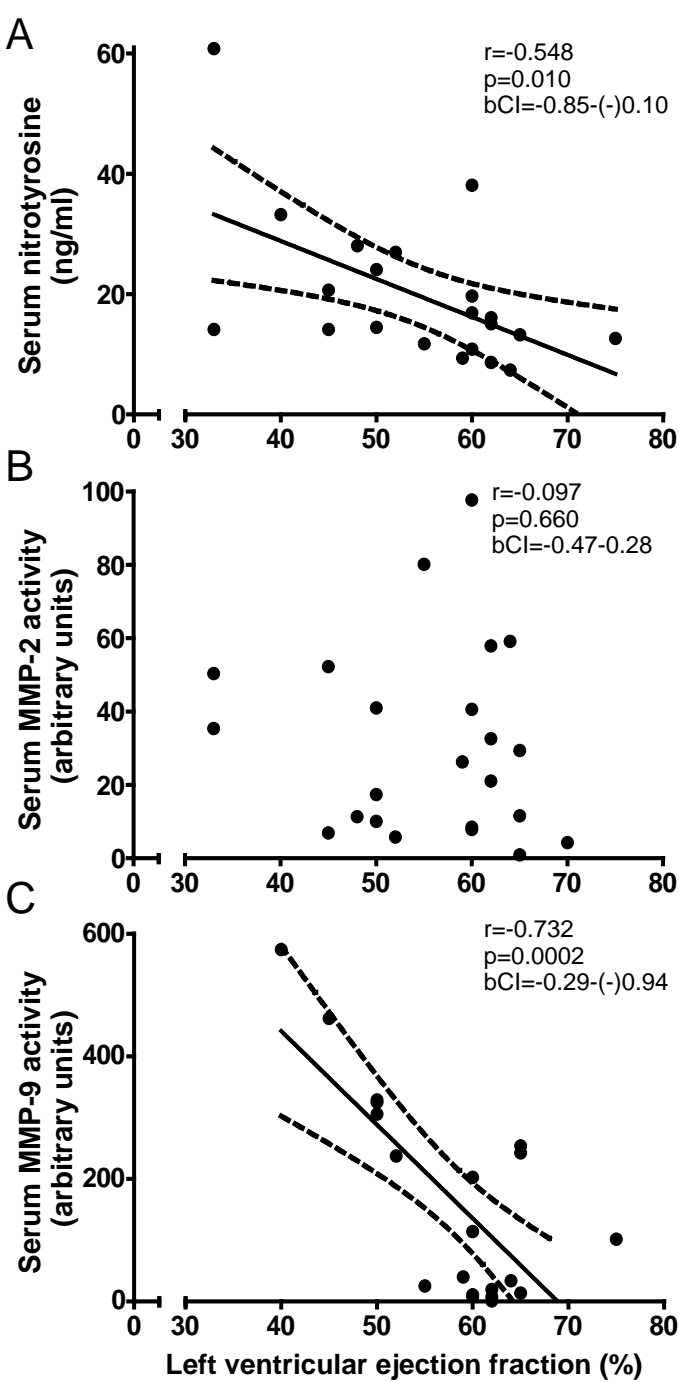
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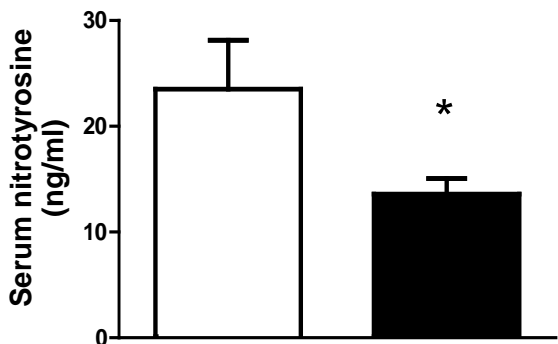
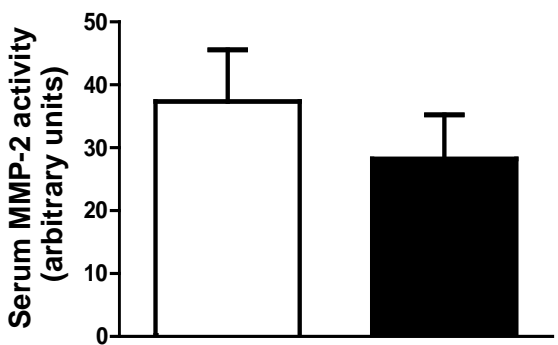
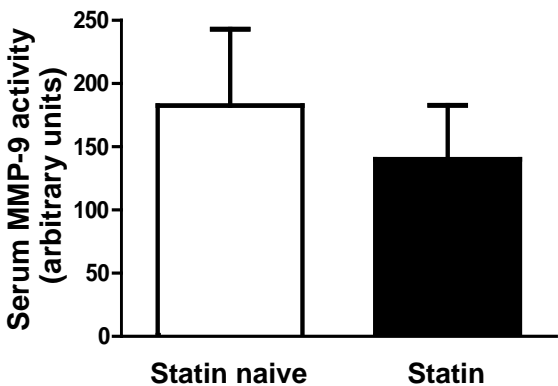
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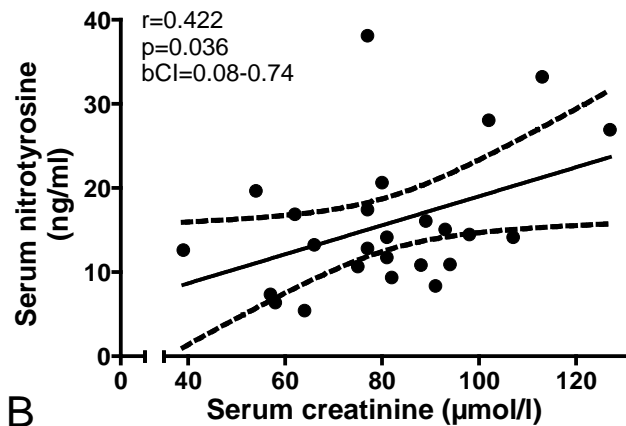


A**E****B****F****C****G****D****H**

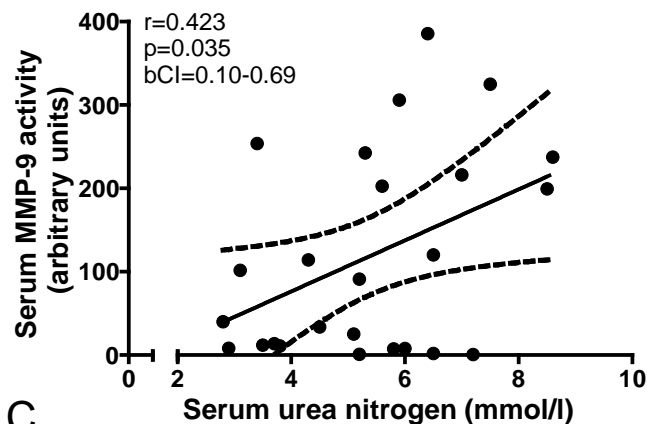


A**B****C**

A



B



C

