The interaction between circadian rhythms of endothelial function: resting versus recruitable endothelial function

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Purpose: We set out to investigate the circadian rhythm of endothelial function using two methods that assess endothelial recruitability and resting function.

Background: Previous studies have shown the existence of a circadian rhythm in endothelium-dependent vasomotor function, with a reduction in Flow-Mediated Dilation (FMD) in the early morning hours and progressive increase in the afternoon and evening. These changes have been previously taken as evidence of endothelial dysfunction, and have been proposed to be one of the mechanisms explaining the higher incidence of cardiovascular events in the morning hours. One limitation, however, is that FMD assay reflects endothelial responsiveness to specific stimuli, but provides no information on resting endothelial function.

Methods and results: We studied 10 young healthy male volunteers (mean age, 28.9 ± 3.7 years). Radial artery endothelium-dependent flow-mediated vasodilation (FMD) and Low-flow-Mediated Constriction (L-FMC) were measured at 8 AM, 2 PM and 8 PM. Compared with 2 PM and 8 PM, FMD decreased markedly in the early morning (8 AM: 2.9 ± 3.3%; 2PM: 6.2 ± 2.7%; 8PM: 6.0 ± 3.8%; P<0.02 for the comparison between 8AM and 2PM). In contrast, L-FMC was maximal at 8AM, decreased significantly at 2PM, and returned to higher values at 8PM (8AM: -5.1 ± 1.2%; 2PM: -2.7 ± 1.9%; 8PM: -6.6 ± 2.1%; P<0.02 for the comparison between 8AM and 2PM), such that the composite endpoint function (sum of FMD+L-FMC) did not differ between 8AM and 2PM and was maximal at 8 PM (P=0.03 by ANOVA). Vascular sympathetic tone was assessed using laser Doppler flowmetry and Fourier transform analysis. Sympathetic tone was maximal in the early morning and lowest in the evening (P<0.05).

Conclusions: FMD is blunted in the early morning in healthy subjects. In contrast, L-FMC is maximal at 8PM, and higher at 8AM and 8PM. These data emphasize the concept that circadian rhythms of endothelial function are complex and cannot be reduced to the interpretation of one single parameter.

Bone morphogenetic protein receptor activation plays a crucial role in endothelial dysfunction and osteogenic differentiation in mice with chronic kidney disease

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Background: Endothelial dysfunction and vascular calcification are the major cardiovascular complications in patients with chronic kidney disease (CKD). Recently, we showed that endothelial dysfunction is caused by reduced endothelial nitric oxide synthase (eNOS) phosphorylation in CKD. Bone morphogenetic protein (BMP) receptor signaling is a key pathway of bone metabolism through Smad1/5/8 phosphorylation. The aim of this study was to examine the role of BMP receptor signaling in endothelial dysfunction and vascular osteogenic differentiation in CKD.

Methods and results: We created a CKD model by performing 5/6 nephrectomy (Sham AL) and by reducing protein intake to 10% of normal diet (BWM). Under these conditions, plasma creatinine levels were markedly increased in BWM. In the early morning (8AM), while the eNOS expression was reduced (sham AL 0.45 ± 0.1; BWM 0.74 ± 0.1; P<0.05) and the eNOS activity was lower (sham AL 1.0 ± 0.3; BWM 0.5 ± 0.1 vs RYGB 0.74 ± 0.1). Moreover, PDK4, which is downstream of GLP-1r activation and inhibits JNK signaling, was upregulated in RYGB compared to the AL and BWM sham rats. Accordingly, JNK phosphorylation was blunted only in the aorta of RYGB rats. Plasma fasting levels of GLP-1 were higher after RYGB compared to sham AL and BWM. sham rats. Moreover, plasma bile acids, which stimulate GLP-1 secretion from L cells in the lower intestine, were also increased in RYGB compared to both sham AL and BWM. In conclusion, GLP-1 may be a crucial mediator of the endothelial function improvement observed immediately after RYGB, in addition to weight loss.

Endothelial function and vascular remodelling

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Increased plasma Glucagon Like Peptide-1 improves endothelial dysfunction immediately after Roux-en-Y gastric bypass prior to body weight loss inhibiting the e-Jun N-Terminal Protein Kinase Signaling

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Purpose: Roux-en-Y Gastric bypass (RYGB) reduces weight and cardiovascular risk in obese patients. The mechanism of RYGB cardiovascular protection (CV) seems to be partly weight-independent, but still unclear. Glucagon Like Peptide-1 (GLP-1) exerts endothelial protective actions, through endothelial-NOS-Synthase (eNOS) activation. Thus, we investigated the role of GLP-1 in obesity-induced endothelial dysfunction in rats after RYGB, prior to significant weight loss.

Methods: After 7 weeks of high fat/high-cholesterol diet, obes Wistar male rats underwent RYGB and were compared to sham-operated rats fed Ad Lib (AL) or body weight-matched (BWM) to rats receiving RYGB. Thoracic aortic rings were perfused and subjected to isometric tension recording, cumulative relaxation responses to 5-HT was performed to peptide GLP-1 (7–36) amide (10-12-6-10-6MO/L) after submaximal contraction with norepinephrine (10-6MO/L) and repeated after preincubation with full GLP-1 receptor antagonist exendin (9-36) (10-7 mol/L) and eNOS-inhibitor (L-NAME, 10-4MO/L). Western blotting of aortic lysates using GLP-1 receptor (GLP-1r), eNOS, Protein Kinase A (PKA) and c-Jun N-Termal Protein Kinase (JNK) antibodies was performed to address the role of GLP-1 in gluco- lipidemic function. GLP-1 and bile acids plasma fasting levels were measured.

Results: 8 days post-surgery body weight difference among the 3 groups was yet not significant. GLP-1-induced vasorelaxation was impaired in sham AL and BWM compared with RYGB rats (max relaxation: sham AL: 17±3.1% and 15±2.8% vs 36±4.8%, respectively, n=6-8, p<0.05). Exendin (9-36) and L-NMIE NAME inhibited GLP-1-induced vasodilation, suggesting GLP-1 receptor and eNOS activation. Interestingly, GLP-1 protein expression was decreased in aortic lysates from sham AL and BWM compared to RYGBPersons (0.44±0.1 vs 0.49±0.1 vs 0.86±0.2 relative units, p<0.05) while eNOS expression was reduced (sham AL 0.45±0.1 and BWM 0.34±0.1 vs 0.74±0.1). Moreover, PDK4, which is downstream of GLR activation and inhibits JNK signaling, was upregulated in RYGB compared to the AL and BWM sham rats. Accordingly, JNK phosphorylation was blunted only in the aorta of RYGB rats. Plasma fasting levels of GLP-1 were higher after RYGB compared to sham AL and BWM. sham rats. Moreover, plasma bile acids, which stimulate GLP-1 secretion from L cells in the lower intestine, were also increased in RYGB compared to both sham AL and BWM.

Conclusion: Our study suggests that GLP-1 may be a crucial mediator of the endothelial function improvement observed immediately after RYGB, in addition to weight loss.

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Does cellular sex matter? Dimorphic transcriptional differences between female and male endothelial cells

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Purpose: Significant sex differences exist in cardiovascular diseases. A critical impact of gonadal hormones on pathogenesis, disease progression and outcome is presumed. However, it is largely unknown whether sexually dimorphic gene expression also plays a role, and whether cells themselves show intrinsic sex differences.

Methods: We performed whole genome expression analyses in Human Umbilical Vein Endothelial Cells (HUVEC) from 20 male and 20 female donors and compared levels of gene transcription between the sexes. Furthermore, to investigate whether there is a sex-specific response to stress, we subjected male and female HUVEC to laminar shear stress of 6 dyn/cm² for 24 hours and analyzed gene expression.

Results: Genes indicative for greater immune responsiveness were stronger expressed in female compared to male HUVEC. There was a significant enrichment of 77 immune-related genes in female HUVEC. Increased transcriptional levels in female cells were verified for 20 genes by real-time RT-PCR. After shear stress, 6.7% of all mRNAs were regulated. Female HUVEC showed a much stronger pronounced transcriptional response to shear than did their male counterparts.

Conclusions: Our results suggest that sexual dimorphism in endothelial cells...
may contribute to explain gender differences in endothelial function or atherosclerosis. In addition, these data indicate caution in interpretation of cell culture experiments and point to the importance for differentiation between male and female cells when doing in vitro experiments.

MECHANISMS OF HEART FAILURE DETERIORATION

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Natriuretic peptide/cyclic guanosine monophosphate pathway exerts compensatory roles against endothelin system in the pulmonary circulation of left heart disease

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Purpose: Imbalance between vasoactive substances is implicated in pulmonary hypertension. In the Left Heart Disease (LHD), elevated Pulmonary Artery (PA) pressure due to passively-transmitted post-capillary Pulmonary Venous (PV) congestion has progressed with disproportionate pre-capillary vasoconstriction and remodeling. We aimed to assess detailed relationships among these substances and hemodynamic variables.

Methods: Right heart catheterization was performed for heart failure patients (n=49, age 74) and blood samples were obtained from PA and wedged PA catheter. Both PA and PV (median 2.8 versus 0.9 pg/mL, respectively), yielding trans-pulmonary ET-1 release (median 3.5 ng/min) as a function of mPAP (R 0.57). Mean PAWP 15 mmHg was observed in 16 patients and 10 patients showed the reactive PVR increase with TPG≥12 mmHg. Linear correlations of ET-1 levels in both PA and PV with mPAP (R 0.52, 0.44, respectively) and the marked increase of ET-1 in PA over PA (median 2.8 versus 0.9 pg/mL, respectively), yielding trans-pulmonary ET-1 release (median 3.5 mg/min) as a function of mPAP (R 0.37), were significantly observed. Similarly, increased CGMP levels in relation to mPAP were observed. Mean calculated CGMP release (median 3.4 remol/min) and the ET-1 release correlated with the CGMP release (R 0.38). In contrast, increased BNP levels especially in PA (median 276 pg/mL) elicited trans-pulmonary BNP uptake (median 101 ng/min), reflecting a target activity for the pulmonary circulation. A negative relationship (R -0.35) between mPAP and %BNP uptake was observed. Although plasma CyPA levels were not different in PA and PV (619 versus 662 ng/mL, respectively) and CyPA in PV did not correlate with either mPAP or PVR, CyPA levels were increased in patients with elevated uric acid, log-C reactive protein, and hemoglobin.

Conclusion: CyPA may not reflect pulmonary but systemic inflammatory status. vascular changes through endogenous BNP elevated in LHD operates in the pulmonary circulation of left heart disease.

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Left ventricular contraction pattern in asymptomatic patients with moderate to severe aortic regurgitation

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Background: The aim of the study was to compare systolic left ventricular (LV) contraction pattern in patients with moderate to severe aortic regurgitation (AR) with healthy controls (C) by real-time three-dimensional echocardiography (RT3DE).

Methods: Twenty patients and 15 age matched controls were compared (35±10 years, AR 38±3 years, respectively, p=NS). All were examined with transthoracic three-dimensional speckle tracking. LV end-diastolic volume (EDV), LV mass index (LVMi) and global tissue deformation parameters (strain and strain rate) were measured in the three orthogonal axes (long axis, circumferential axis and radial axis). Results: Heat rate was similar in the AR and the C group (62±11 vs 59±8 bpm, respectively, p=NS). Athlete blood pressure was higher in AR patients (71±17 vs 50±5 mmHg, p<0.002). In the two groups (AR vs C) LVEDV was (118±43 and 110±23 mL/m2), LV mass (119±29 and 110±19 g/m2), LVMi (119±29 and 110±19 g/m2), and LVEF (66±2% and 68±2%) were similar. The mean stroke index (SI) was similar in both groups (AR vs C; 4.1±0.7 vs 4.0±0.6 L/min/m2, p=NS).

Conclusions: RT3DE identifies non-homogenous remodeling in patients with chronic moderate to severe aortic regurgitation. Isolated reduction of longitudinal strain and strain rate was demonstrated, and was strongly correlated to the volume loaded left ventricle.

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Radial function correlates with heart failure symptoms in hypertrophic cardiomyopathy with normal ejection fraction

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Purpose: To assess 2D and 3D left ventricular (LV) myocardial deformation pattern in HCM and its relation with clinical status.

Methods: In 32 HCM pts and 32 age- and gender-matched controls, we analyzed peak global 2DLS and 3D strain (longitudinal, 3DLS; circumferential, 3DCS; radial, 3DRS; area, 3DAS), LV ejection fraction (LVEF), mass and outflow tract area (LVOTA) were measured by 3D echo. LV filling pressures were estimated by E/e' ratio, and maximal provokable LVOT gradient was recorded. Symptomatic status was defined by NYHA class II (IV).

Results: Although LVEF was similar in pts and controls (64±6% vs 62±4%, respectively), the systolic deformation was significantly impacted in pts (p<0.01) and was lost for 3DCS, which was only marginally lower (p=0.07). In HCM pts, all strain parameters were more closely correlated with LV end-systolic volume (r=0.55 to 0.67), LVEF (r=0.82 to 0.86) and mass (r=0.33 to 0.56) than in controls. Symptomatic pts (n=11) had more impaired 3DAS, 3DRS, and 3DSCS, but also had more LVOT obstruction and concentric remodelling, and higher E/e' (Table). Neither 2DLS or 3DSCS were significantly related to symptomatic status. AIROC curve analysis, 3DAS, 3DRS and 3DAS had a similar accuracy to identify symptomatic pts (AUCs 0.72-0.73).

Table 1

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Noninvasive and mainstream end-tidal carbon dioxide monitoring of venous cannula is a unique and useful technique for diagnosing pulmonary hypertension in patients with heart failure

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Purpose: End-tidal carbon dioxide (ETC02) monitoring is commonly used for intubated patients receiving mechanical ventilation in the fields of anesthesiology and critical care medicine. Noninvasive and mainstream ETC02 monitoring has been long-awaited so as to expand its use to other clinical settings. The partial pressure of ETC02 (PETCO2) depends on the pulmonary ventilation-perfusion (V/Q) relationship and cell metabolism, and in normal subjects, PECTO2 values of around 40 mmHg reflect normal alveolar PCO2. Heart failure (HF) is one of the most common pathological conditions to develop ETC02 monitoring and recent PETC02 has been reported to be a predictor of prognosis in patients with HF. In this study, we aimed to clarify the association between hemodynamic parameters and PETC02 in patients with HF.

Methods: Twenty-four consecutive patients who received both right heart catheterization (RHC) and ETC02 monitoring were enrolled for analysis. We measured the mixed pulmonary capillary wedge pressure (PCWP), pulmonary arterial pressure (PAP), and right atrial pressure. Cardiac output was calculated using two approaches, thermodilution and the Fick method. The cardiac index, pulmonary vascular resistance index (PVR) and trans-pulmonary gradient (TPG) were also calculated. Mixed venous oxygen saturation (SvO2) was obtained by analyzing pulmonary arterial blood gas. PETCO2 has been reported to be a predictor of prognosis in patients with HF.

Conclusions: In HCM with normal LVEF, a significant longitudinal dysfunction exists, irrespective of symptomatic status. Although in HCM symptom development is multifactorial, in our population it was also related to further impairment in radial function.

Conclusion: RT3DE identifies non-homogenous remodeling in patients with chronic moderate to severe aortic regurgitation. Isolated reduction of longitudinal strain and strain rate was demonstrated, and was strongly correlated to the volume loaded left ventricle.