# ABSTRACTS

Abstracts for Oral Communications:

## OC1.

### LOSS-OF-FUNCTION IN TOLL-LIKE RECEPTOR 4 RECEPTORS NITRIC OXIDE-MEDIATED RELAXATIONS IN LEPR$^{db/db}$ MICE

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The present study analyzes the role of toll-like receptor 4 (TLR4), a target for saturated fatty acids and lipopolysaccharides, in modulating endothelial function in the mouse aorta. A type-2 diabetes model with double knockout (DKO) of leptin receptors (Lepr) and TLR4, was obtained by crossing Lepr$^{db/+}$ and TLR4$^{-/-}$ mice. Mice were sacrificed and rings [with and without endothelium] of their aorta were studied for measurement of isometric tension in a Mulvany-Halpern myograph.

Acetylcholine-induced endothelium-dependent relaxations were potentiated in DKO mice compared with Lepr$^{db/db}$ control mice. The eNOS dimer to monomer ratio, as well as the basal and acetylcholine-stimulated eNOS phosphorylation levels were higher in preparations from DKO mice. This difference in relaxations and eNOS activity [dimer to monomer ratio and phosphorylation levels] between Lepr$^{db/db}$ and DKO mice was abolished by apocynin [non-specific NADPH oxidase inhibitor]. Lucigenin-enhanced chemiluminescence assay suggested a lower basal endothelialproduction of superoxide anions by NADPH oxidase in DKO mice preparations. Quantitative PCR results revealed that NADPH oxidase isoform 4 and isoform 1 was downregulated in DKO mice arteries. Administration of lipopolysaccharide inhibited acetylcholine-evoked relaxations in wild type mice but not in TLR4$^{-/-}$ mice. Western blotting analysis revealed that lipopolysaccharide decreased eNOS dimer to monomer ratio and phosphorylation levels, which was accompanied by phosphorylation of glycogen synthase kinase 3β and increased expression of β-catenin and NADPH oxidase isoform 4 in wild type mice aortae and cultured endothelial cells (HUVEC EA.Hy926). The attenuated relaxations to acetylcholine and the changes in eNOS activity induced by lipopolysaccharide were inhibited by apocynin, LY294002 (PI3K inhibitor) and enhanced by LiCl [non specific inhibitor of glycogen synthase kinase 3β].

In conclusion, TLR4 activation decreases eNOS-mediated relaxations by enhancing oxidative stress produced by NADPH oxidase and resulting in the stimulation of the glycogen synthase kinase 3β/β catenin cascade. TLR4 inhibition or down-regulation may be a potential target for the treatment of vascular dysfunction.

## OC2.

### DEFICIENCY OF EP4 RECEPTOR ON BONE MARROW-DERIVED CELLS BOOSTED INFLAMMATION AND ABDOMINAL AORTIC ANEURYSM FORMATION INDUCED BY ANGIOTENSIN II IN HYPERLIPIDEMIC MICE

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**Objective:** Chronic inflammation during abdominal aortic aneurysm (AAA) formation contributes to remodeling and eventual weakening of the vessel wall. Prostaglandin $E_2$ ($PGE_2$), through activation of its receptor EP4, can mute inflammation. Whether EP4 participates directly in the pathogenesis of aneurysm remains unknown. We tested the hypothesis that a lack of EP4 receptor on bone marrow-derived cells would increase local inflammation and enhance the formation of AAA *in vivo*.
**Methods and Results:** Hypercholesterolemic low-density lipoprotein receptor knockout (LDLR$^{-/-}$) mice transplanted with either EP4$^{+/+}$ [EP4$^{+/+}$/LDLR$^{-/-}$] or EP4$^{-/-}$ [EP$^{-/-}$/LDLR$^{-/-}$] bone marrow received infusions of angiotensin II to induce AAA. Deficiency of EP4 on bone marrow-derived cells increased the incidence and severity of AAA, increased monocyte chemoattractant protein-1 (MCP-1), and enhanced infiltration of macrophages and T cells into the AAA lesions. Lack of EP4 also augmented elastin fragmentation, increased the number of cells bearing markers of apoptosis, and decreased smooth-muscle cell accumulation within the AAA lesions.

**Conclusions:** Deficiency of EP4 receptor boosted inflammation and AAA formation induced by angiotensin II in hyperlipidemic mice. This study affirms the pathophysiologic importance of $PGE_2$ signaling through EP4 as an endogenous anti-inflammatory path involved in AAA formation.

## ABSTRACTS

Abstracts for Oral Communications:

## OC3.

### IDENTIFICATION OF CHANNELS ON SKELETAL MYOBLASTS THAT MAY CONTRIBUTE TO ACIDOSIS-INDUCED ATP RELEASE

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Low pH stimulated ATP efflux from skeletal myoblasts, and inhibition of Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) abolished the acidosis-induced ATP release. CFTR is a chloride channel that has been reported to mediate ATP efflux from some cell types. Several other chloride channels or stretch-activated channels are also reported to conduct ATP. In this study, I aim to identify channels on skeletal myoblasts that are firstly opened at low pH, and secondly contribute to ATP release.

RTPCR was used to determine which of the channels reported to conduct ATP in other cell types were present on skeletal myoblasts. The chloride channels CFTR, Clcn-2, Clcn-3, Clcn-7, $Ca^{2+}$ activated chloride channel and volume-sensitive outwardly-rectifying channels, and the stretch-activated channels, Connexin 36, Connexin 43 and Pannexin 3, were expressed on the cells.

Whole-cell patch clamp was performed on skeletal myoblasts using potassium-free solutions: the pipette (intracellular) solution contained 110 mM Cs-Aspartate, 20 mM CsCl, 5 mM $Na_2$-Phophocreatine, 1.0 mM $MgCl_2 \cdot 6H_2O$, 10 mM HEPES, 5 mM $Cs_2$-EGTA, 0.1 mM GMP and 5 mM $Mg_2$-ATP, pH adjusted to 7.2 with CsOH, while the bath (extracellular) solution contained 140 mM NaCl, 5 mM CsCl, 1.0 mM $MgCl_2$, 5.0 mM HEPES and 1 mM $CaCl_2$, pH adjusted to 7.3-7.4 with NaOH. The reversal potential of the whole-cell current was -28.3±1.34 mV (n=6), which suggests that the current recorded was the chloride current. The current increased when the medium pH was reduced from 7.4 to 6.8. A specific inhibitor of CFTR, $CFTR_{inh}$-172, had no effect on the current at pH 7.4, but reduced it at pH 6.8; application of forskolin (10 μM), which elevates intracellular cAMP, produced a similar increase in whole-cell current to that produced by low pH.

These data suggest that the CFTR $Cl^-$ channels were opened at low pH. Further experiments are in hand to determine which of the other channels contribute to acidosis-induced ATP release.

## OC4.

### DIPEPTIDYL-PEPTIDASE 4 INHIBITOR IMPROVES ENDOTHELIAL FUNCTION OF SPONTANEOUSLY HYPERTENSIVE RATS THROUGH ACTIVATION OF GLP-1/GLP-1 RECEPTOR/AMPK/NO CASCADE

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**Objectives** Sitagliptin, a highly selective dipeptidyl peptidase 4 (DPP-4) inhibitor, is a new anti-diabetic drug through inhibiting inactivation and degradation of glucagon like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide. This study investigated whether sitagliptin could protect endothelial function in spontaneously hypertensive rats (SHRs).
**Methods** SHRs and normotensive WKYs were treated orally with sitagliptin ($10\ mg.kg^{-1}.d^{-1}$) or vehicle for 2 weeks. Renal blood flow was measured by magnetic resonance imaging. Intrarenal arteries were suspended in myograph for force measurement and levels of marker proteins were assayed by Western blotting. Nitric oxide (NO) production was determined by confocal microscopy in primary culture of SHR aortic endothelial cells.
**Results** Relaxation to GLP-1 receptor agonist exendin-4 was impaired in SHR arteries, which was restored by sitagliptin. The improved relaxation and sitagliptin- or exendin-4-stimulated rises in $[NO]_i$ was reversed by GLP-1 receptor antagonist exendin 9-39, AMPK inhibitor compound C and NOS inhibitor L-NAME. Overexpression of AMPKα2 further increased while expression of dominant negative AMPK inhibited phosphorylation of AMPKα at $Thr^{172}$ and eNOS at $Ser^{1177}$ in response to sitagliptin and exendin-4 in endothelial cells. 12h incubation with sitagliptin and exendin-4 augmented endothelium-dependent relaxation (EDR) in SHR arteries, which was antagonized by exendin 9-39 and compound C. In addition, two-week administration of sitagliptin improved EDR, increased phosphoryation of AMPKa and eNOS, up-regulated GLP-1 and GLP-1 receptor in SHR arteries, and restored renal blood flow in SHR.
**Conclusions** Sitagliptin improves endothelial function in hypertensive rats by restoring NO bioavailability via AMPK/eNOS activation. Stimulating GLP-1/GLP-1 receptor signaling pathway could be another therapeutic option in controlling hypertension-related vascular events.

# ABSTRACTS

Abstracts for Oral Communications:

## OC5.

### INTERMITTENT HYPOXIA INDUCES PARADOXICAL CARDIO-PROTECTIVE EFFECTS IN AN ANIMAL MODEL VIA HEME OXYGENASE-1 UPREGULATION

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**Background:** Obstructive sleep apnea (OSA), with intermittent hypoxia during sleep, is increasingly recognized as an independent risk factor of cardiovascular diseases (CVD). Oxidative stress and inflammation are pathogenic mechanisms in CVD, but majority of studies have focused on the systemic status. Heme-oxygenase-1 (HO-1) is a stress inducible protein and catalyzes rate-limiting step of degradation of cellular heme into free iron, carbon monoxide (CO) and biliverdin/bilirubin, all of which may exert anti-oxidative and anti-inflammatory effects. The aim of this study was to use an animal model to evaluate oxidative stress and inflammation in response to intermittent hypoxia (IH) with or without diet-induced hyperlipidemia, with special reference to any difference in the systemic and cardiac response, and the mechanistic pathways involved.
**Methods:** Male Sprague-Dawley rats were divided into four groups: regular chow diet or high fat high cholesterol (HFHC) diet plus intermittent air (IA) or IH treatment, and rats were sacrificed at 2 or 4 weeks. Serum and cardiac levels of oxidative and pro-inflammatory markers were assayed with ELISA and semi-quantitative PCR, the expression of HO-1 and activation of signaling pathways in the heart were analyzed by Western blot.
**Results:** IH and HFHC diet alone or together caused time-dependent elevation in serum malondialdehyde (MDA) and CINC-1 and reduction in serum adiponectin levels. In contrast, elevation of cardiac adiponectin level and suppression of the cardiac levels of oxidative and pro-inflammatory markers were seen, accompanied by upregulation of expression of cardiac HO-1 at 4 weeks when cardiac activation of ERK and Akt signaling pathways were also observed.
**Conclusions**: Oxidative stress and inflammation resulted from IH and hyperlipidemia may serve as a potential mechanism underlying OSA-related CVD. However, the upregulation of HO-1 expression may exert a local protective function in the heart via activation of ERK and Akt signaling pathway from systemic oxidative and inflammatory insults.
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## OC6.

### BETA1 SUBUNIT-DEPENDENT MODULATION OF BK CHANNEL BY MEMBRANE CHOLESTEROL

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**Background**: The large conductance $Ca^{2+}$-activated K+ (BK, or slo) channels are ubiquitously expressed in different tissues without (brain, liver, etc) or with (smooth muscle and heart) regulatory beta-subunit, and play an important role in regulating various physiological processes such as cell excitability, hormone secretion, vascular activity, etc. Recent studies have shown that membrane cholesterol is a major regulator of several potassium channels including Kir and Kv1.5 channels. However, the regulation of BK channels by cholesterol is not fully understood.
**Methods**: Whole cell BK current and BK single channel current were recorded in whole-cell patch clamp mode and cell-attached single channel recording, respectively, in HEK 293 cells stably expressing Maxi-K with beta1-subunit. Western Blotting was performed to detect the protein expression of BK channel.
**Results**: We found that whole-cell BK current was significantly suppressed with cholesterol enrichment by cholesterol saturated methyl-beta-cyclodextrin (MβCD), whereas cholesterol depletion by MβCD had no effect on the current amplitude. Low-density lipoprotein (LDL), a class of lipoprotein particles carrying cholesterol around the body, also largely decreases BK current. Single channel recording showed that cholesterol enrichment significantly reduced the open probability of BK channel, suggesting that cholesterol increase likely decreases the membrane channel number. In the opposite, in the cells stably expressing BK channel without beta1-subunit, cholesterol-saturated-MβCD has no significant effect on the current amplitude of BK channels. Western Blotting data shows that BK channel α subunit expression was reduced by cholesterol-saturated-MβCD or LDL while the β1 subunits expression did not alter. However, both á and b1 subunits expression of BK channel in cultured HCASMCs (Human Coronary Artery Smooth Muscle Cells) was suppressed by cholesterol-saturated-MβCD or LDL.
**Conclusion**: Our results demonstrate the important evidence that BK channels exhibit beta1-subunit-dependent responses to cholesterol. The enriched-cholesterol and LDL reduce the activity of BK channels co-expressed with α and β1 subunit, which may at least in part accounts for the occurrence of hypertension in patients with high plasma cholesterol level, since both of α and β1 subunit transcripts are abundant in vascular smooth muscle.

## ABSTRACTS

Abstracts for Oral Communications:

## OC7.

### LIPOCALIN-2 DEFICIENCY PROTECTS HEARTS AGAINST ISCHEMIA/REPERFUSION INJURY

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Lipocalin-2 is a pro-inflammatory adipokine causally involved in the development of obesity-associated metabolic and vascular diseases. Clinical evidences suggest that there are close associations between circulating levels of lipocalin-2 and cardiac dysfunction. However, little is known about the detailed roles of lipocalin-2 in regulating pathophysiological functions of the heart. The present study has used a Langendorff system to evaluate the heart function of mice lacking lipocalin-2. The results demonstrate that in response to a 20-min global ischemia injury and during the 60-min reperfusion, hearts from lipocalin-2 knockout mice (Lcn2-KO) show a better recovery and improved myocardial contractile functions, compared to those littermates with normal lipocalin-2 expressions. These phenomena can be observed in mice under both standard chow and high fat feeding conditions. Deficiency of lipocalin-2 significantly reduced heart infarct size and lactate dehydrogenase release. These protective functions are partly attributed to the enhancement of mitochondrial respiratory chain activities in Lcn2-KO mice. Furthermore, lipocalin-2 treatment is able to block the recovery of heart function during ischemia/reperfusion injury and acutely damage the mitochondrial functions. In summary, the results support a potential role of lipocalin-2 in the pathogenesis of obesity-related cardiac disorders.

## OC8.

### UP-REGULATION OF HEME OXYGENASE-1 IMPROVES ENDOTHELIAL FUNCTIONS BY IMPAIRING ENDOTHELIUM-DEPENDENT CONTRACTIONS AND ENHANCING ENDOTHELIUM-DEPENDENT HYPERPOLARIZATIONS

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**Objective:** Heme oxygenase (HO) attenuates the production of reactive oxygen species (ROS) through its ability to degrade heme and produce carbon monoxide (CO), biliverdin/ bilirubin, and to release free iron. Up-regulation of HO lowers blood pressure in animals. However, the underlying mechanism is still unknown. The present study was designed to investigate whether or not up-regulation of HO by the pharmacological agent hemin improves endothelial function in arteries of the spontaneous hypertensive rat (SHR).
**Methods:** 36 weeks old SHR were divided into two groups. One group received an intraperitoneal injection of hemin (50 $mg \bullet kg^{-1}$, 24 hours before sacrifice) while; the control group was injected with normal saline. Rings of aorta and mesenteric arteries were suspended in organ chambers for isometric tension recording. The intracellular reactive oxygen species (ROS) concentration was measured by confocal microscopy. The release of prostanoids was measured by enzyme immunoassay. Expression of HO-1, cyclooxygenase (COX) and prostaglandin $I_2$ synthase in the aorta was measured by Western blotting.
**Results:** HO-1 protein expression was significantly higher in the hemin treatment group than in controls, implying that HO-1 is induced by hemin. This up-regulation of HO-1 resulted in an impairment of both acetylcholine- and A23187-induced endothelium-dependent contractions. A lower expression level of COX-1, a lower intracellular ROS production and a lower release of prostaglandin $F_{1\alpha}$ (the major stable metabolite of prostacyclin) were observed in the hemin treatment group as compared to the controls. The sensitivity of thromboxane-prostanoid (TP) receptors to prostacyclin and iloprost (prostacyclin analog) were significantly attenuated in hemin-treated rats. Moreover, hemin treatment potentiated acetylcholine-evoked relaxations in mesenteric arteries in the presence of L-NAME and indomethacin, implying a facilitation of endothelium-dependent hyperpolarizations. These enhanced EDHF response were abolished by ouabain ($Na^+$-$K^+$-ATPase blocker) and barium chloride (Kir channel blocker), indicating an involvement of $Na^+$-$K^+$-ATPase and Kir channel.
**Conclusions:** Up-regulation of HO-1 improves endothelial function by attenuating endothelium-dependent contractions as well as potentiating endothelium-dependent hyperpolarizations. The impairment of endothelium-dependent contractions can be explained by an impairement of the expression of COX-1 resulting in the attenuation of the production of ROS and vasoconstrictor prostaglandins combined with a reduced TP receptor sensitivity. The enhancement of endothelium-dependent hyperpolarizations is related to $Na^+$-$K^+$-ATPase and Kir channel.