ABSTRACTS

Abstracts for Oral Presentation:

OP1.

LONG-TERM FENOFIBRATE THERAPY INCREASES FIBROBLAST GROWTH FACTOR 21 AND RETINOL-BINDING PROTEIN 4 IN SUBJECTS WITH TYPE 2 DIABETES

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Introduction: Fenofibrate is a peroxisome proliferator-activated receptor (PPAR)-α agonist which showed beneficial effects on total cardiovascular risk in patients with type 2 diabetes in the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study. This study aimed to investigate the long-term effect of fenofibrate therapy on three novel biomarkers of cardiovascular risk, namely adipocyte-fatty acid binding protein (A-FABP), fibroblast growth factor 21 (FGF21), and retinol-binding protein 4 (RBP4), which are all downstream targets of PPAR-α or PPAR-γ, in patients with type 2 diabetes.
Methods: A total of 216 patients (108 in the fenofibrate group and 108 in the placebo group) were randomly selected from the FIELD study cohort. A-FABP, FGF21, and RBP4 levels were measured in serum samples at both baseline and the fifth year of the study.
Results: Relative to the placebo group, the changes of serum FGF21 and RBP4 levels were 85% (P<0.001) and 10% (P=0.032) higher in the fenofibrate group respectively over five years. Fenofibrate treatment had no detectable effect on serum A-FABP level (P>0.05). The effect of fenofibrate treatment on serum FGF21, but not RBP4, remained significant after adjusting for fenofibrate-induced changes in glycosylated hemoglobin, total cholesterol, triglycerides, apolipoprotein A-II, fibrinogen, plasma creatinine, and homocysteine (P=0.002).
Conclusions: Long-term fenofibrate treatment could increase serum FGF21 levels over five years in patients with type 2 diabetes. Further studies are needed to investigate the potential role of FGF21 in the fenofibrate-mediated reduction of cardiovascular risk.

OP2.

PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR DELTA (PPARδ) MEDIATES THE VASCULAR BENEFITS OF METFORMIN IN DIET-INDUCED OBESE MICE

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Objectives: Metformin, an anti-diabetic drug, is known to activate AMP-activated protein kinase (AMPK); whilst AMPK and *PPARδ* have been shown to form a transcriptional complex and synergistically induce gene expression. The present study investigated whether *PPARδ* is a critical mediator for metformin in ameliorating endothelial dysfunction in diet-induced obese (DIO) mice.
Methods: Aortae from C57BL/6J mice were cultured with tunicamycin [endoplasmic reticulum (ER) stress inducer], metformin, or GSK0660 (*PPARδ* antagonist). Male *PPARδ* wild-type and knockout mice were fed with high-fat diet for 3 months to induce obesity, followed by oral administration with metformin for 7 days. Vascular reactivity and protein expression levels were determined by wire myograph and Western blotting, respectively. Fluorescence imaging measured the levels of reactive oxygen species (ROS) and nitric oxide (NO) under confocal microscopy.
Results: Tunicamycin impaired endothelium-dependent relaxations (EDR) in response to acetylcholine, and increased the levels of ROS and ER stress markers, such as phosphorylated eIF2α, ATF6 and ATF3 in mouse aortae. These harmful effects of tunicamycin were reversed by co-treatment with metformin while such benefits of metformin were abolished by GSK0660. Chronic metformin treatment alleviated EDR and reduced the levels of ROS and above-described ER stress markers in DIO *PPARδ* wild-type but not in *PPARδ* knockout mice. Tunicamycin-induced reduction of NO production in endothelial cells was also reversed by metformin.
Conclusions: The present study provides novel evidence that *PPARδ* plays a critical role in the vascular benefits of chronic metformin treatment in restoring the impaired endothelial function and curtailing ER and oxidative stress in obese mice. Dual medication of metformin together with *PPARδ* agonist could be a more effective venue against diabetic vasculopathy (Supported by GRF grants).

ABSTRACTS

Abstracts for Oral Presentation:

OP3.

PR INTERVAL PROLONGATION STRONGLY PREDICTS NEW-ONSET MYOCARDIAL INFARCTION, ISCHEMIC STROKE, HEART FAILURE AND CARDIOVASCULAR DEATH IN HIGH-RISK CARDIOVASCULAR PATIENTS: PATHOPHYSIOLOGICAL INSIGHTS OF ADVERSE VASCULAR FUNCTION

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Objectives: To investigate whether PR prolongation predicts adverse cardiovascular (CV) events in coronary patients or risk equivalent, and the underlying pathophysiological mechanisms in terms of vascular phenotypes. Methods: We prospectively followed up 597 high-risk CV outpatients (mean age 66±11 years; male 67%; coronary disease 55%, stroke 22%, diabetes 52%) for new-onset ischemic stroke, myocardial infarction (MI), congestive heart failure (CHF), and CV death. Vascular phenotypes were assessed by high-resolution ultrasound for mean carotid Intima-Media Thickness (IMT), and Vascular Profiling System (VP-2000; subgroup n=338) for Pulse Wave Velocity (PWV). Impaired left ventricular ejection fraction (LVEF) from transthoracic echocardiography (subgroup n=194) was defined as <35%. PR interval was determined from 12-lead electrocardiogram.

Results: PR prolongation >200 ms was present in 79 patients (13%) at baseline. PR prolongation >200 ms was associated with significantly higher mean carotid IMT (1.05±0.37 mm vs 0.94±0.28 mm, P=0.010, Figure 1), higher PWV (1144±142 cms^{-1} vs 1091±143 cms^{-1}, P=0.024), and impaired LVEF<35% (16% vs 5%, P=0.027). After mean follow-up of 63±11 months, increased PR interval significantly predicted new-onset ischemic stroke (P=0.006), CHF (P=0.040), CV death (P<0.001), and combined CV endpoints (P<0.001) at cut-off >200 ms, and new-onset MI (C-statistic 0.70, P=0.001) at >162 ms (P=0.008). Adjusting for potential confounders using multivariable cox regression, PR prolongation independently predicted increased risk of new-onset ischemic stroke (HR 5.05, 95%CI: 1.34 to 19.10, P=0.017), CV death (HR 16.40, 95%CI: 3.99 to 67.46, P<0.001, Figure 2), combined CV endpoints (HR 2.33, 95%CI: 1.26 to 4.32, P=0.007) at cut-off >200ms, and increased new-onset MI (HR 8.05, 95%CI: 1.66 to 39.06, P=0.010) at cut-off >162 ms.
Conclusions: PR prolongation >200ms strongly predicts new-onset ischemic stroke, MI, and CV death, and combined CV endpoint including CHF in high-risk CV patients. Increased risk of MI was observed at PR interval >162 ms. Whether adverse vascular function in PR prolongation may mechanistically represent mediating pathways prior to clinical events warrants further investigations.

Table 1. Prediction of New-Onset CV Events by PR Interval

New-Onset CV Events	Total	PR Interval	PR Interval	P-value	ROC C-Statistic	
n (%)	(n=597)	<200 ms (n=518)	>200 ms (n=79)		Estimate	P-value
MI	26 (4%)	20 (4%)	6 (8%)	0.130	0.70	0.001*
Ischemic stroke	21 (4%)	14 (3%)	7 (9%)	0.006*	0.50	0.99
HF	37 (6%)	28 (5%)	9 (11%)	0.040*	0.60	0.042*
CV death	27 (5%)	17 (3%)	10 (13%)	<0.001*	0.63	0.020*
Combined endpoints	87 (19%)	65 (13%)	22 (28%)	<0.001*	0.61	0.001*

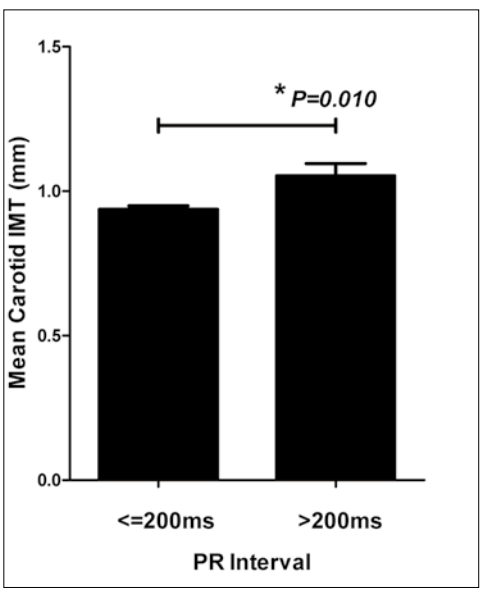

Figure 1. *PR interval and carotid IMT .*

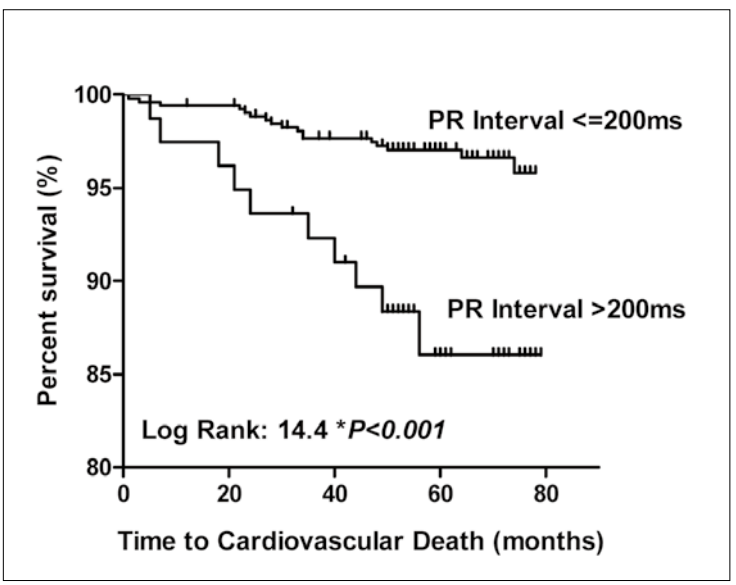

Figure 2. *Kaplan-Meier curves for CV death.*

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Abstracts for Oral Presentation:

OP4.

CIGARETTE SMOKE-INDUCED ANGIOGENESIS THROUGH AMPK ACTIVATION IN RAT HEART

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Objectives: Vascular endothelial growth factor (VEGF) is recognized as an angiogenic predictor. It has been reported that there is a link between VEGF release and adenosine monophosphate (AMP)-activated kinase (AMPK) activation in many cells (Fisslthaler B & Fleming I, Circ Res 2009; 105:114). This study aims to investigate the effects of cigarette smoke (CS) on the levels of VEGF, anti-inflammatory mediators, IL-10 and adiponectin, and AMPK pathway in heart of CS-exposed rat.
Methods: Male Sprague-Dawley rats (aged 6-7 weeks) were randomly divided into sham air (SA) group (n=8) and CS group (n=8) respectively. CS group was exposed to 4% CS for 1h each day for 56 days in ventilated smoking chambers, while the SA group was exposed to fresh air. Animals were sacrificed 24 h after the last exposure and heart tissue was collected. Heart homogenates were prepared for the determination of IL-10 and adiponectin and VEGF by ELISA and activation of AMPK by Western blot analysis.
Results: CS exposure caused significant decrease in the levels of cardiac IL-10 (348.9±37.22 pg/mg protein and 259.5±16.73 pg/mg protein for SA and CS group respectively, $p<0.05$) and cardiac adiponectin (375.1±14.77 ng/mg protein and 270.9±31.67 ng/mg protein for SA and CS group respectively, $p<0.05$). On the contrary, we found a significant elevation of cardiac VEGF level (545.1±43.66 pg/mg protein and 722.6± 28.60 pg/mg protein for SA and CS group respectively, $p<0.01$) after CS exposure. Western blot analysis showed a significant increase in the phosphorylation of AMPK (Thr172).
Conclusion: Our data demonstrated that a pro-inflammatory state existed in the heart of CS-exposed rat, leading to elevation of VEGF production. The AMPK pathway might play a role in CS-induced angiogenesis.

OP5.

cAMP/PKA-ACTIVATED CFTR IS REQUIRED FOR ACIDOSIS-INDUCED RELEASE OF ATP FROM SKELETAL MYOCYTES

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ATP is an extracellular signaling molecule involved in the regulation of skeletal muscle blood flow.

Our previous study showed that depression of pH using lactic acid treatment stimulated the efflux of ATP from skeletal myocytes through a mechanism that involved the cystic fibrosis transmembrane conductance regulator (CFTR). The present study was undertaken to further explore the signal transduction mechanism linking the decrease in pH to the ATP release from muscle.

Accumulation of ATP in the medium surrounding the myocytes was measured using a luminescence assay. Incubation of the myocytes in lactic acid (10 mM) for 3 hours increased the extracellular ATP from 0.67±0.08 to 1.15±0.11 nM (n=36; $P<0.001$). The specific inhibitor of CFTR, CFTRinh-172, inhibited the lactic-acid-induced increase in extracellular ATP. The CFTR expression was significantly increased with 3 hours incubation with lactic acid. Lactic acid treatment also increased the intracellular cAMP from 3.2±0.3 to 7.1±1.0 nM, and the Protein Kinase A (PKA) activity from 30.6±4.5 to 37.0±5.4 pmol/ml, whereas the lactic-acid-induced increase in extracellular ATP was inhibited by the PKA inhibitor, KT5720, but enhanced by the phosphodiesterase inhibitor IBMX, suggesting that the PKA/cAMP pathway was involved in CFTR activation. Furthermore, an in vivo phosphorylation study using 32P-ATP showed that CFTR phosphorylation was increased by forskolin alone or foskolin with dibutyryl-cAMP and IBMX, further supporting a role for the cAMP/PKA pathway in CFTR activation in muscle. Amiloride, an inhibitor of the Na/H exchanger (NHE), prevented the lactic-acid-induced increases in intracellular cAMP and extracellular ATP; inhibitors of the Na/Ca exchanger (NCX), SN-6 and KB-R7943, also inhibited the lactic-acid-induced accumulation of ATP in the medium surrounding the cultured myocytes.

Based on these data, we propose that depression of the pH increases the efflux of ATP through a mechanism that involves CFTR activation through the cAMP/PKA pathway, and that NHE and NCX may be involved.

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Abstracts for Oral Presentation:

OP6.

HIGH FERRITIN AND LOW TRANSFERRIN SATURATION ARE ASSOCIATED WITH PRE-DIABETES AMONG ADULTS

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Background and Aims: Iron overload is known to cause diabetes. However, the underlying mechanism is poorly understood. We therefore studied the association of different markers of iron metabolism, namely ferritin, erythrocyte protoporphyrin and transferrin saturation (TSAT, as defined by a percentage of transferrin that is saturated with iron) with pre-diabetes (preDM) in US adults without chronic kidney disease, anemia, and iron deficiency.
Methods: Data on 2,575 participants of the National Health and Nutrition Examination Survey (NHANES) 1999-2002 who were free of diabetes, chronic kidney disease, iron deficiency, and anemia were analyzed. Data on 3,876 participants of the NHANES III (1988-1994) were used as replication. Homeostasis model assessment of insulin resistance (HOMA-IR), blood glycosylated hemoglobin level (A1C), fasting glucose, insulin, and preDM (defined as a fasting plasma glucose 100-125 mg/dl or an A1C value 5.7-6.4%) were measured as the outcomes.
Results: Logistic regression analyses indicated independent associations of high ferritin (P_{trend}=0.028) and low TSAT (Ptrend=0.029) with preDM after adjusting for sociodemographics, physical activity (active/sedementary), metabolic and inflammatory markers (triglycerides, total cholesterol, HDL cholesterol, mean arterial pressure, CRP, white cell count, and albumin), and liver enzymes (GGT, ALP, AST, and ALT). The NHANES III data showed similar associations. Combining the results showed a more significant association for high ferritin (P_{meta}=0.016) and low TSAT (P_{meta}=0.002). Moreover, TSAT was associated with A1C, fasting glucose, insulin, and HOMA-IR (P_{meta} ≤0.001).
Conclusions: Higher ferritin and lower TSAT are associated with higher risk of preDM in a general population without confounding diseases. Further research is needed to examine the underlying mechanism of these two indices, especially TSAT, in the pathophysiology of preDM.

OP7.

FUNCTIONAL ROLE OF TRPC5 CHANNELS IN AORTIC BARORECEPTOR

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Aortic baroreceptor is the mechanosensor to detect blood pressure in aortic arch. Upon changes in arterial blood pressure, the baroreceptor nerve terminal on the aortic arch adventitia will be activated, resulting in action potentials that propagate to the cardiovascular control centre in the brain. However, the molecular identity of the baroreceptor mechanosensors is not well understood.

TRP channels are a superfamily of non-selective cation channels that can be divided into seven subfamilies: TRPA, TRPC, TRPM, TRPML, TRPN, TRPP, and TRPV. Many TRP isoforms have been reported to be sensors for diverse source of external and/or internal stimuli. Recently, one of the isoforms, TRPC5, has been reported to be hypo-osmolarity and pressure sensitive.

In the present study, the expression of TRPC5 channels in the aortic baroreceptor nerve terminal, which is located on the aortic arch, along the nerve fiber (aortic depressor nerve) and in the ganglion region (nodose ganglion) was demonstrated by immunohistochemistry. RT-PCR and immunoblot studies confirmed the expression of TRPC5 channels in the aortic baroreceptor. In Ca^{2+} imaging studies of cultured aortic baroreceptor neurons, a TRPC5 potentiator daidzein was able to potentiate the hypotonicity-induced $[Ca^{2+}]i$ response while a TRPC5 blocking antibodies T5E3 inhibited the response. Electrophysiological studies showed that hydrostatic pressure could activate the whole-cell current in cultured baroreceptor neurons and the current displayed a double rectifying I-V relationship, which is typical of TRPC5. Daidzein treatment also potentiated the pressure-induced action potential firing in isolated aortic baroreceptor neurons, which could be blocked by a TRPC blocker 2-APB. Furthermore, *trpc5* knockout mice manifested a significant reduction in aortic depression nerve activity upon blood pressure elevation when compared with wild-type mice.

Taken together, our study provides the evidence that TRPC5 is involved in pressure sensing of aortic baroreceptor neuron and is participated in the aortic baroreceptor function.

ABSTRACTS

Abstracts for Oral Presentation:

OP8.

ANGIOTENSINOGEN (M235T) GENE AND BLOOD PRESSURE RESPONSE TO ACE INHIBITOR (ENALAPRIL) IN PATIENTS WITH ESSENTIAL HYPERTENSION

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Objective: We determined the association of angiotensinogen (M235T) gene polymorphism with essential hypertension and the relationship between polymorphism in the angiotensinogen (M235T) gene and blood pressure response to ACE inhibitor (Enalapril) in patients with essential hypertension.
Methods: 250 patients with essential hypertension and 250 controls from Delhi and surrounding areas were recruited for the investigation. Blood pressure was recorded before and after 6 weeks of treatment with ACE inhibitors, Enalapril. Genotyping were carried out by polymerase chain reaction and Restriction fragment length polymorphism technique.
Results: Statistically significant association of T allele was observed with essential hypertension [x^2=14.67, p=0.00013, odds ratio=1.76 (1.3-2.32) at 95% CI]. The decrease in systolic and diastolic blood pressure after six weeks of treatment of the patients carrying TT genotype were greater than the groups carrying MT and MM genotypes.
Conclusions: The angiotensinogen (M235T) gene polymorphism is significantly associated with essential hypertension. Patients carrying TT genotype had higher blood pressure lowering response when treated with ACE inhibitor, Enalapril than those carrying MM and MT genotypes suggesting that the T allele may be a possible genetic marker for essential hypertension.