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European National Society Cardiovascular Journals: 
Background, Rationale and Mission Statement of the "Editors' Club"
(Task Force of the European Society of Cardiology)

FERNANDO ALFONSO,1 GIUSEPPE AMBROSIO, 2 FAUSTO J. PINTO, 3 ERNST E. VAN DER WALL 4

From Editors-in-Chief of 1Revista Española de Cardiología, published by the Spanish Society of Cardiology, 2(Former Editor) Giornale Italiano di Cardiologia, published by the Italian Federation of Cardiology, 3Revista Portuguesa de Cardiologia, published by the Portuguese Society of Cardiology, 4Netherlands Heart Journal, published by the Netherlands Society of Cardiology and Chairman of the Task Force.


Cardiovascular scientific production in Europe is growing both in quantity and in quality. Promoting high-quality research is a major goal of the European Society of Cardiology (ESC).1-3 The ESC has two highly respected official general journals, namely the European Heart Journal and Cardiovascular Research, devoted to clinical and basic research respectively.1-3 The ESC also publishes several subspeciality official journals covering the full spectrum of cardiovascular diseases and related techniques. Most European countries, however, also have their own cardiovascular journals. National Society Cardiovascular Journals (NSCJ) are time-honoured and classically disseminate high-quality scientific research mainly originating from each particular European country. They also play a major role in education and harmonisation of clinical practice. Most NSCJ are published in local languages but many...
of them also incorporate English editions. Altogether, NSCJ provide a highly effective means to disseminate cardiovascular research produced in Europe. Scientific knowledge, however, has no barriers and many of these journals have gained an undisputed international profile. Some NSCJ, however, are just emerging and would benefit from networking support. It became clear that enhancing collaboration among NSCJ Editors would facilitate advancement in knowledge and further diffusion of scientific and educative contents.

Developing a "Constitution Document" and "Mission Statement" was considered desirable to set the basis of future collaboration among NSCJ Editors. We assumed this responsibility in recognising the crucial role of NSCJ in Europe. Our target was to produce and issue a core document with fundamental principles upon which all NSCJ Editors would agree. Common goals will be identified and agreed-on measures will be pursued. The constitution document presented herein was therefore developed to formalise the NSCJ Editors' Club Task Force.

National Society Cardiovascular Journals: Background and Basic Data

All Editors-in-Chief of the official cardiovascular journals of the ESC National Societies are de facto members of the Editors' Club. On April 2007, during the "spring days" at the Heart House in Nice, the ESC Board formally approved the initiative and the Editors' Club Task Force was officially launched. The organisation of the Task Force consists of a Nucleus of NSCJ Editors and remains within the membership division of the ESC, coordinated by the ESC vice-president. Further involvement of the ESC publishing department will be also considered as required.

The initial steps of the Editors' Club Task Force moved in the direction to gain further insights on who we are and where we are now. Accordingly, several proactive measures were taken:

1. Upon request of this Task Force, the portal on the ESC web page for the NSCJ was modified to increase its visibility. Currently, this site may be reached, not only from the area corresponding to members and National Societies, but also directly from the scientific area of the ESC. It is clear that NSCJ significantly contribute to the enormous scientific input provided by the ESC as a whole and appropriate recognition to this fact should be granted.

2. Electronic communication brings the scientific community closer together. Therefore, direct links to NSCJ have been updated and implemented. This would further stimulate exchange of scientific research amongst European authors, researchers and readers. Submission of high quality original research articles should be encouraged by NSCJ Editors, establishing efficient networking tools connecting all European journals.

3. As a final preliminary step, the Task Force strived to obtain detailed editorial and organisational data from all corresponding journals. Accordingly, feedback was directly requested from the NSCJ Editors and Presidents of the National Societies. A comprehensive structured questionnaire (23 items), was devised. Corporate mailing and subsequent collection of all editorial data were guaranteed with the help of the ESC membership department. Consistency checks were performed and, when required, data confirmation was directly obtained from the corresponding national Editor. Fully detailed results of this survey are currently freely available from the ESC web page (metafile of national journals). This posted material will be updated annually.

Main results of the survey are as follows. A total of 40 National Societies responded to the structured questionnaire including a total of 34 journals. Eight National Societies have no official journal, the 3 Baltic countries share the same Journal and 3 National Societies have more than 1 journal. The oldest cardiovascular journal in Europe is
Archives des Maladies du Coeur et des Vaisseaux founded in 1908. Overall, 11 journals have more than 30 years of existence, 2 are older than 20 years and 12 have been published for more than a decade. In addition to NSCJ in local languages, 12 journals are also available in English (full text) and 27 journals systematically include English abstracts. Thirty-three journals include original papers whereas 1 exclusively consists of review papers or state of the art articles. Thirteen journals are published monthly. The journals print run varies from 1000 to 9000 copies (mean 3135 copies). A system of "peer review" is selected to evaluate manuscripts by 31 journals and 23 journals adhere to the requirements of the International Committee of Medical Journals Editors. Twenty nine journals are indexed (Index Medicus), 18 appear in PubMed (MEDLINE) and 5 have obtained an impact factor in 2006. In addition to the print edition, 26 journals have an electronic edition, and 13 have also implemented an electronic system for manuscript submission. A dedicated web page is offered by 25 journals whereas 26 publications are directly accessible via the web page of the corresponding national society.

General Editorial Considerations

Both, technical and ethical considerations should be addressed. Promoting editorial quality standards is of paramount importance to increase the attractiveness of our publications in the globalised and highly competing field of academic cardiovascular medicine. In this regard the Task Force believes that every effort should be made to follow the uniform recommendations initially issued by the International Committee of Medical Journal Editors (ICMJE) nearly 30 years ago. These recommendations have been recently updated (6th edition) and the emphasis has shifted from the original technical requirements (focused on unifying technical and formal aspects of manuscript preparation), to general principles of editorial ethics and global policies that should govern biomedical publishing.

Technical requirements are indeed important to guarantee clarity, precision and to facilitate dissemination of medical studies. In turn, implementation and strict compliance with these requirements eventually raises the overall quality of research. In this regard, the suggestions provided by the CONSORT (CONsolidated Standards Of Reporting Randomised Trials) group should be followed to improve presentation of randomised clinical trials. These studies should comply with special requirements, including a check list and flow diagram. We should keep in mind that cardiology is one of the medical disciplines where performance of randomised trials has more clearly fructified and the concept of evidence-based medicine is widely embraced.

Currently, online editions represent the most efficient means for disseminating the information that journals publish. Visits to electronic editions are ever-increasing and full article downloads grow exponentially. Therefore, electronic connectivity should be facilitated so that online journal editions are made more visible to readers and, if possible, freely available. In this regard, a provocative novel index, known as the "web impact factor", has been proposed and the field of webometrics is just emerging.

On the other hand, ethical considerations directly affect the credibility of the scientific content. Therefore, they should ensure transparency, trust and honesty in the scientific process involved in performance and publication of research. The final purpose is to protect the process of scientific exchange. It should be acknowledged that a sizable bulk of corporative research has recently moved from academic and university centres to close agreements between sponsors and private contract research organisations. Accordingly, explicitly disclosing the role of the sponsor in designing, conducting, analysing, interpreting and writing the trial is becoming increasingly relevant. Other concepts such as Editorial Freedom and Editorial Independence have been recently emphasised by the ICMJE, WAME
Authority and autonomy are critical to ensure appropriate editorial decisions. In this regard, NSCJ Editors should jealously safeguard the editorial independence of their respective national journals.

The peer review process – despite its limitations – has been enshrined at the highest level and it is now currently identified as an essential part of the editorial scientific process. Therefore, standards for peer review excellence should be developed. This requires both fairness in judgement and expertise in the field. Editors are responsible for monitoring and ensuring fairness, timeliness, and thoroughness in this process.

Other issues such as conflicts of interest (for authors, reviewers and editors) and requirements for authorship are also intended to protect the credibility of the scientific information. Disclosure of potential conflicts of interest should be enforced. Disclosure on data accessibility and accepting a full responsibility for accurate data presentation and interpretation are key considerations. Confidentiality and agreed-on embargos should be maintained. Publication bias (selective reporting of positive findings and lack of publication of studies with negative results) should be prevented by NSCJ Editors. The whole publication process is based on the credibility, trust, authenticity and scientific honesty. To further preserve scientific credibility, NSCJ Editors should harmonise their policies regarding scientific misconduct and scientific fraud.

The HEART Group (Heart Editors Action Round Table) of cardiovascular editors issued a consensus document focused on redundant publication. The impact factor (Journal Citation Reports) represents a widely accepted means to evaluate the scientific prestige of journals. However, flaws in the impact factor calculation should be acknowledged and research or scholarly merits should not be rewarded based on the impact factor of the journal in which articles are eventually published. Padding the impact factor should be discouraged. However, NSCJ Editors should develop common policies to stimulate diffusion of European studies exclusively based on scientific quality and clinical relevance criteria. This would overcome current citation biases, particularly against non-English biomedical journals. Joint support of European research by increasing recognition of European scientific and editorial quality is considered, therefore, highly advisable.

**Rationale for the Editors' Club**

European NSCJ are heterogeneous and, above all, are published in different languages. This highlights that cooperation among NSCJ Editors is crucial to avoid "Tower of Babel" phenomena precluding efficient dissemination of scientific information across Europe. Even relatively humble journals should not be condemned to ostracism but rather considered highly successful providing they have a broad dissemination and are deeply appreciated by their readers. We should break boundaries and set free scientific knowledge from any constrictions generated by language, logistic, bureaucratic or economic barriers. Cross-links between European Journals are highly advisable. Cross-references should be stimulated but only when based on strict criteria of scientific quality. A minimal list of important issues should be developed with principles that all NSCJ Editors could agree upon. Common goals, priorities and challenges should be readily identifiable. Finally, proactive global decisions should be made in order to capture a wider audience.
All the above described editorial recommendations, however, leave enough room for specific editorial policies that shape the particular interest of every specific journal. Room for diversity should be jealously maintained as the focus and scope of different national journals actually differ. Nevertheless, advancement in knowledge is founded in the exchange of novel information by investigators, and NSCJ Editors have full responsibility for stimulating cooperation among European researches.

Here, we would like to present three typical examples where these collaborative efforts could be applicable:

1. Novel recommendations suggesting to register all clinical trials prior to definitive publication should be discussed on the light of currently available administrative national laws and recent European directives (EudraCT). Proposals for a uniform European "Repository" of clinical trials fulfilling not only administrative and regulatory issues but also editorial requirements (including free public access) should be considered. This will allow early recognition of undue trial design changes or methodological flaws. Eventually, most NSCJ Editors could joint uniform recommendations and common editorial policies and platforms might be devised at a European level.

2. Collaboration among NSCJ Editors is essential to further disseminate and promote clinical application of ESC clinical practice guidelines. After endorsement by National Societies, translation of these guidelines into national local languages should facilitate their implementation into clinical practice. Foot notes, incorporating comments of local experts, are pivotal in this regard. Publication of these guidelines in NSCJ should follow the general rules for "secondary publication", after primary publication in the European Heart Journal has been granted. Nevertheless, time matters, and this detailed and rigorous editorial process (typically affecting uniquely long documents) should be expedited to streamline the translation process and to monitor its accuracy. Implementation of an "early translation process" would be desirable. A full collaboration between NSCJ Editors and the ESC committee of practice guidelines is, therefore, of paramount importance. The circle of knowledge will be closed when the corresponding feedback is ensured by dissemination of selected national activity registries unraveling local practices in patient care. This will help to elucidate success, viability and implementation of different ESC initiatives at the national level. Hopefully, this bidirectional exchange in knowledge will promote widespread implementation of these recommendations and harmonisation of cardiovascular practices across Europe. Eventually, uniform and consistent clinical practices should translate into improvements in patient care.

3. Boosting dissemination of official ESC late breaking clinical trials, by readily translating their abstracts into local languages and publishing the main results of these important studies, while paying maximal attention to preserve accuracy and scientific integrity, remains a challenge. This final proposal will require, once more, a close coordination between ESC scientific bodies, ESC publishing department and NSCJ Editors.

Mission Statement

1. To increase collaboration among NSCJ Editors. The main purpose of this Task Force is to foster interaction among NSCJ Editors. Selected editorial topics will be discussed and addressed using a systematic and comprehensive approach. Standing and "ad hoc" committees will be created. Common editorial policies should be developed. As needed, editorials, uniform requirements, and consensus documents will be issued. Regular meetings (annual ESC Congress and others) will be scheduled and a formal agenda will be proposed.

2. To promote editorial excellence. A major objective of the Task Force is to devise means to improve
the scientific standards of NSCJ. Scientific content, quality requirements, credibility, and editorial and research ethics will be promoted. 5-8

3. To improve diffusion of scientific knowledge. Coordination of editorial initiatives among NSCJ and also official ESC journals will further facilitate diffusion of editorial and scientific content. To develop common strategies to increase awareness of the high quality scientific research generated in Europe which, in turn, would positively affect bibliometric indicators. Recognition and diffusion of European cardiovascular research, ESC clinical practice guidelines and other scientific or education initiatives should be promoted. Distribution of common academic material, core curriculum, and additional teaching tools should be also facilitated. Fostering of electronic editions should be encouraged to increase diffusion and NSCJ visibility.

4. To share technical editorial information, experiences, initiatives, publishing resources and technical tools among NSCJ Editors. To address common issues regarding free access to scientific content. To foresee common strategies to advance into the dynamic field of standardised platforms for manuscript submission. To adopt common policies aimed to increase efficiency in the publication process. To promote parallel electronic and English-editions in an increasing number of NSCJ and, eventually, sharing copy-editing resources. To develop joint efforts to more efficiently tackle the problem of finite editorial resources, and finally, to ensure economic viability of NSCJ.

5. To provide an operative framework and dataset that will enable future joint ventures and comprehensive European publishing initiatives. To further stimulate collaboration between NSCJ Editors and the ESC scientific bodies and publishing department. In this way, promotion of spotlight, theme or monographic issues, covering burning cardiovascular topics, might be nicely coordinated.

6. Public relations. To provide a common voice when issues concerning NSCJ arise. To serve as a liaison in the relations with governmental bodies, professional or scientific organisations, industry, the media and the public.

7. To foster collaboration between National Societies and the ESC. To close the gap between ESC official journals and NSCJ. To promote European incentives to stimulate publication of quality research.

Final Remarks

All the information presented in the present document set the basis to support this exciting editorial initiative. NSCJ Editors should be committed to progressively adapt their local policies, including instructions to authors, to follow general editorial recommendations. 5-8,32,33 The main challenge of the Editors Club will be to foster consensus and agreements upon strategic priorities among NSCJ. The breadth and quality of articles should be improved and strategic actions should be aimed to foster inclusion of most NSCJ in well respected international bibliographic databases and electronic search systems. Joint efforts should aim to broaden distribution and dissemination of these journals and to consolidate their prestige and recognition by the international scientific community. The main goals of this pioneering effort are, therefore, already quite clear: to increase collaboration among NSCJ Editors, enhance editorial standards, improve quality requirements, preserve publication ethics, guarantee scientific credibility and expand dissemination of scientific knowledge.

Commitment of NSCJ Editors to achieve these objectives is crucial and this Editors' Club emerging forum should provide a unique opportunity to foster global editorial policies. Overtime, the results and implications of these ambitious editorial initiatives should be critically evaluated.
Acknowledgements

The continuous help of Anne Mascarelli (ESC) deserves special recognition.

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### Appendix.

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* not official National Society journal, but major cardiology journal in Austria
** common journal for the Baltic countries
Heart Rate Variability in Children with Congenital Heart Disease Before and After Open Heart Surgery

MURAT ÖZEREN,1 OLGU HALLIOĞLU,2 KHATUNA MAKHAROBLIDZE,2 HANDAN ANKARALI3

From 1Department of Cardiovascular Surgery; 2Department of Pediatric Cardiology, Mersin University Medical Faculty, Mersin, Turkey; 3Department of Biostatistics, Zonguldak Karaelmas University, Zonguldak, Turkey

ÖZEREN ET AL.: Heart Rate Variability in Children with Congenital Heart Disease Before and After Open Heart Surgery. Background: Spectrum analysis of heart rate variability (HRV) is a noninvasive procedure that provides information on sympathetic and parasympathetic controls. Reduced HRV may indicate cardiac autonomic dysfunction and susceptibility to hemodynamic instability during anesthesia, after myocardial infarction or cardiac operations. Aim: This study was designed to investigate the effects of cardiopulmonary bypass on HRV variability in children with congenital heart disease and if HRV is turning to the normal in postoperative period and when, as well as the duration of the process. Methods: HRV data were obtained from 29 pediatric patients with congenital heart disease, who underwent elective cardiac surgery. Electrocardiographic data were collected with PC-based ECG acquisition system (PC-ECG 1200). ECG results were obtained by assessing 200 heart beats that recorded in supine position. Clinical data including age, type of cardiac lesion, type of surgical procedure, cardiopulmonary bypass (CPB) time, cross clamp time were recorded. Results: 16 male and 13 female patients mean aged 8.08±3.8 (1-15), 27 had an acyanotic heart disease, 2 of them had a cyanotic heart disease. Standard deviation of all normal RR intervals (SDNN) (p=0.048) and HRV triangular index (p=0.017) were significantly lower in the postoperative first month than preoperatively. There were no significant preoperative differences in other time or frequency domain measures of HRV between the preoperative recordings and postoperative for the first month. SDNN, low and high frequency were found significantly low when compared between the postoperative first and third month, although HF was decreased in the first postoperative month, but did not reach statistical significance. Conclusion: Our findings showed that decreased HRV is a nonspecific marker of cardiovascular stress just after the cardiac operations, reflecting an alteration in autonomic nervous system input to the heart and turning to the normal in the third month.

Cardiopulmonary bypass, heart rate variability, spectral analysis

摘要
背景：心率變異性的光譜分析（HRV），是一種能獲得交感和副交感控制資訊的非創傷性方法。麻醉過程中、心梗後及心臟手術後心率變異性下降，可能說明心臟自主功能障礙和血液流變學不穩定性敏感性。目的：本研究所心肺分流術（體外轉流）對先天性心臟病患兒心率變異性影響，以及術後過程及術期HRV是否恢復正常。方法：採集29例選擇性心臟手術後的先天性心臟病患兒心率變異性數據。心電圖採用PC-ECG 1200，採集200次臥位心搏。記錄其他臨床指標包括年齡、心臟病變類型、手術方式、體外轉流時間（CPB）時間和十字鉗閉時間。結果：16例男性和13例女性平均年齡8.08±3.8（1-15）。27例患有非發绀性心臟病，其餘2例患有發绀性心臟病。術後一月的正常RR間期的標準差（SDNN）（p=0.048）及心率變異性三角指數（p=0.017）均術前顯著降低。術前和術後一月的其他時間和心率變異頻率域無顯著差異，比較術後一月及三月SDNN、低頻、高頻顯著降

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HRV IN CONGENITAL HEART DISEASE

Introduction

Heart rate variability (HRV), which reflects autonomic nervous system activity, is useful for assessing autonomic control under various physiologic and clinical conditions. Spectrum analysis of HRV is a noninvasive procedure that provides quantitative information on sympathetic and parasympathetic controls. Reduced HRV may indicate cardiac autonomic dysfunction and susceptibility to hemodynamic instability during anesthesia, after myocardial infarction or cardiac operations.

Reduced HRV has been observed in patients before and after operations of the congenital heart disease. Previous studies have mainly demonstrated HRV changes between preoperative and postoperative values. Little is known about course of HRV after the open heart operations for congenital heart disease. Consequently, the aim of this study was to investigate the effects of cardiopulmonary bypass on HRV variability in children with congenital heart disease and if HRV is turning to the normal in postoperative period, as well as the duration of the process.

Patients and Methods

HRV data were obtained from 29 consecutive pediatric patients with congenital heart disease, who underwent elective cardiac surgery. Informed consent from the patient’s parents was taken before surgery to participate in the study. Exclusion criteria included: a) Patients with arrhythmia or pacemaker; b) Weight less than 6 kg; c) Preoperative instable clinical conditions; d) Preoperative cardiovascular medications.

Clinical data including age, type of cardiac lesion, preoperative medications, type of surgical procedure, cardiopulmonary bypass (CPB) time, cross clamp time were recorded for all patients. Postoperative data included duration of mechanical ventilation, inotropic support, pleural drainage, and hospitalization period.

The anesthesia and surgical management of all patients were performed same manner. Anesthesia was induced and maintained with ketamine hydrochloride (Ketalar®; EWL Eczacıbaşı Warner Lambert Istanbul-Turkey) and Fentanyl (Fentanyl Citrate®; Abbott Laboratories, USA). After surgery, all patients were admitted to the intensive care unit (ICU) and ventilated mechanically. Patients received routine postoperative care, including administration of analgesics as needed for pain relief, and dopamine for cardiac support if necessary. When cardiopulmonary function was stable, the patients were transferred to the ward. Patients receiving cardiovascular medications (beta-blockers, digoxin, and calcium antagonists) in postoperative follow-up were excluded from the study. Measurements were obtained on the preoperative day and in postoperative months 1 and 3. All HRV measurements were taken in the afternoon (2:00-6:00 PM) to avoid influences of night/day differences.

Heart Rate Variability

Electrocardiographic data were collected with PC-based ECG acquisition system (PC-ECG 1200; NORAV Medical Ltd. Yorkeam, Iceland). ECG results were obtained by assessing 200 heart beats that recorded in supine position. HRV parameters were assessed with careful attention given to the rhythm in order to be sure that patient was in sinus rhythm, and all of the marked QRS complexes were controlled. Mistakenly marked artifacts were corrected manually. For ECG recordings in which more than 10% had artifacts, the process was repeated.

A short period analysis of HRV was performed
for both the frequency and time domain parameters by using PC-based ECG acquisition system (PC-ECG 1200) that allows automatic measurements.

**Heart Rate Variability Analysis**

HRV measurement is based on the sequence of RR intervals. SDNN is the standard deviation of all normal RR intervals (those measured between consecutive sinus beats). HRV indices described above are measures of variability in RR interval. Among time domain parameters; SDNN and HRV triangular index were measured separately.

HRV may similarly be broken into the frequency components that compose the overall variability. Frequency domain analysis is performed by taking a series of numbers along the axis and computing the Fourier transform. Akselrod et al showed that low frequency (LF) band (0.04-0.15 Hz) is related to both sympathetic and parasympathetic modulation, and the high frequency (HF) band (0.15-0.40 Hz) is related to parasympathetic effects. The ratio of LF to HF power is often used as a metric of sympathetic-parasympathetic balance. Low frequency and high frequency components of frequency domain parameters were also measured and LF/HF ratio was determined manually.

**Statistical Methods**

Descriptive statistics were given as mean±SD (Standard Deviation). Paired and unpaired Student's t tests were used for normally distributed data and Spearman analysis was used to establish correlations using the statistical package SPSS-10.0 for Windows. P value less than 0.05 was regarded as significant.

**Results**

Gender of the patients was 16 male and 13 female and their mean age was 8.08±3.8 (1-15). Twenty-seven had an acyanotic heart disease (15 ventricular septal defects, 12 atrial septal defects), 2 of them had a cyanotic heart disease (Tetrology of Fallot). None of the patients required any medications in the postoperative follow-up. Baseline HRV recordings were noted for seven days prior to the elective surgery. There was no operative or postoperative mortality. Sinusal tachycardia was encountered in two patients because of hypovolemia in the first hours of postoperative period and turned to normal with fluid and blood replacement. Low dose inotropic (dopamine 3 mcg/kg/min) support was required in 7 patients at the first two hours. Other operative data and postoperative data of all patients were shown in Table 1.

There was any significant difference between the heart rates of preoperative, postoperative first and third month (104±17, 106±20 and 103±20 respectively, p>0.05). Postoperative HRV recordings were obtained in one and three month period after surgery (Table 2). For all patients, SDNN (p=0.048) and HRV triangular index (p=0.017) were significantly lower in the postoperative first month than preoperatively (Figure 1). The magnitude of decrease was greater for HF than for LF power, resulting in a significantly (p=0.01) increase in LF/HF ratio (Figure 2). Mean RR values of third month were found significantly increased in order to preoperative and first month values (Table 2). There were no significant preoperative differences in other time or frequency domain measures of HRV between the preoperative recordings and postoperative for the first month.

SDNN (p=0.05) and LF (p=0.05) found significantly low when compared between the postoperative first and third month (Figure 3), although

<table>
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<th>Table 1. Operative and postoperative patient data</th>
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<td>Patient data (n=29)</td>
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<td>Cardiopulmonary bypass time (min)</td>
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<td>Cross clamp time (min)</td>
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<tr>
<td>Duration of ventilation (hour)</td>
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<tr>
<td>Number of patients with inotropic support</td>
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<td>Postoperative bleeding (ml)</td>
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<tr>
<td>Mean±SD</td>
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HRV IN CONGENITAL HEART DISEASE

Figure 1. Comparison of SDNN and HRV between preoperative and postoperative first month.

Figure 2. Comparison of LF, HF and LF/HF between preoperative and postoperative first month.

Table 2. Time related changes in HRV

<table>
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<tr>
<th></th>
<th>Preoperative</th>
<th>1 month</th>
<th>3 month</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
</tr>
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<tr>
<td>Mean RR</td>
<td>593±16</td>
<td>598±32</td>
<td>685±32</td>
<td>0.1</td>
<td>0.002</td>
<td>0.007</td>
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<tr>
<td>SDNN (ms)</td>
<td>24,91±2.4</td>
<td>24,33±5.7</td>
<td>32,8±7,4</td>
<td>0.048</td>
<td>0.04</td>
<td>0.05</td>
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<td>RMSSD (ms)</td>
<td>22,31±4.1</td>
<td>22,9±10</td>
<td>35,85±13,1</td>
<td>0.09</td>
<td>0.55</td>
<td>0.01</td>
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<tr>
<td>HRV triangular index</td>
<td>7.7±0.65</td>
<td>7.4±1.1</td>
<td>8.0±0.69</td>
<td>0.017</td>
<td>0.60</td>
<td>0.58</td>
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<tr>
<td>LF</td>
<td>303.6±44.1</td>
<td>244.4±37.3</td>
<td>186.79±22.1</td>
<td>0.64</td>
<td>0.69</td>
<td>0.05</td>
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<tr>
<td>HF</td>
<td>201.3±31.34</td>
<td>161.03±31.3</td>
<td>255.4±33.3</td>
<td>0.13</td>
<td>0.13</td>
<td>0.09</td>
</tr>
<tr>
<td>LF/HF</td>
<td>1.69±0.29</td>
<td>3.4±1.5</td>
<td>1.3±0.6</td>
<td>0.012</td>
<td>0.64</td>
<td>0.26</td>
</tr>
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</table>

Mean±SD
P1: P value of preoperative and first month comparison.
P2: P value of preoperative and third month comparison.
P3: P value of first and third month comparison.
operations. Despite its extensive use, CPB has been associated with various problems such as cardiac dysfunction, electrolyte disturbances, catecholamine stimulation, irritative scar and sutures in the myocardium, residual hemodynamic impairment, as well as pain and anxiety. These and other unknown factors may affect HRV.

There have been limited reports on HRV in pediatric patients with congenital heart disease before and after cardiopulmonary bypass. In the first study, Heragu and Scott found reduced HRV in acyanotic and cyanotic patients postoperatively. Similar to this study, recordings of patients operated for tetralogy of Fallot particularly with ventricular arrhythmia showed reduced HRV. Kaltman et al. evaluated only frequency domain HRV analysis in neonates with single ventricle and two ventricles physiology, despite early difference after cardiac surgery, HRV indices become indistinguishable between two groups by 3-6 months of age. In our study, reduced HRV was found in the first month according to preoperative measurements (Figure 1).

We know from the literature that measures of HRV are changing with the age and Heragu and Scott measured HRV in healthy controls and found that time domain measures of HRV increase with age throughout the pediatric age range, achieving adult values by adolescence and also found that the quotient of SDNN and mean RR intervals tended to remain stable across most of the pediatric age range. Their findings were similar to those of Massin and von Bernuth. In our study, spectral indices of HRV were measured in different congenital cardiac defects and compared with the same patient before and after cardiopulmonary bypass, therefore, we were not able to comment on the effect of age.

**Discussion**

CPB was introduced during the 1950's and has since then been used extensively in congenital cardiac operations. Despite its extensive use, CPB has been associated with various problems such as cardiac dysfunction, electrolyte disturbances, catecholamine stimulation, irritative scar and sutures in the myocardium, residual hemodynamic impairment, as well as pain and anxiety. These and other unknown factors may affect HRV.

There have been limited reports on HRV in pediatric patients with congenital heart disease before and after cardiopulmonary bypass. In the first study, Heragu and Scott found reduced HRV in acyanotic and cyanotic patients postoperatively. Similar to this study, recordings of patients operated for tetralogy of Fallot particularly with ventricular arrhythmia showed reduced HRV. Kaltman et al. evaluated only frequency domain HRV analysis in neonates with single ventricle and two ventricles physiology, despite early difference after cardiac surgery, HRV indices become indistinguishable between two groups by 3-6 months of age. In our study, reduced HRV was found in the first month according to preoperative measurements (Figure 1).

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**LF/HF ratio is an indirect indicator of sympathetic – parasympathetic balance.** Significant change in the LF/HF ratio of our patients were explained as an operative stress and sympathetic hyperactivity in the first month and turned to preoperative values in the third month measurement, so any significant change could not be found between preoperative and third month measurements.
Significant changes were found in parameters mean RR, SDNN between the first and third month. These results in the postoperative period suggest that further parasympathetic withdrawal is an important component of these changes. This could partly be the result of decreased sensitivity of the sinus node to autonomic nervous input immediately after surgery for congenital heart disease. Heragu and Scott found similar results in their study.4

Type of cardiac lesion, preoperative medications, type of surgical procedure, cardiopulmonary bypass time, cross clamp time, did not correlate with the HRV recordings because of our homogenous and small patient group but in the literature Gordon et al demonstrated in a heterogeneous population of congenital heart patients that decreased LF and decreased LF/HF ratio, in the postoperative time period, which was associated with cardiac arrest. These reduced spectral indices were postulated to represent a diminished capacity for cardiac autonomic control.11

Conclusion

Our findings showed that decreased HRV is a nonspecific marker of cardiovascular stress just after the cardiac operations, reflecting an alteration in autonomic nervous system input to the heart and turning to the normal in the third month.

References

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Faculty of Medicine  
The University of Hong Kong

The Thirteenth Annual Scientific Meeting

12 December 2009  
Hong Kong Convention and Exhibition Centre  
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SCIENTIFIC PROGRAMME

12 DECEMBER 2009 (SATURDAY)

07:30-08:30  Registration

08:30-10:00  Oral presentations for young investigator award  
Chairmen: Professor Stephen SM Chung, Dr Heather J Ballard

10:00-11:30  Coffee break, poster presentations for young investigator award

11:30-13:00  Morning session  
Chairmen: Professor Tak-Ming Wong, Professor Yu Huang

**IL1**  Endothelial dysfunction: the common consequence in diabetes and hypertension  
Professor Yu Huang, The Chinese University of Hong Kong, China

**IL2**  Vascular oxidative stress: the common link  
Professor Richard A Cohen, Boston University, USA

**IL3**  The TP-receptor: the common villain  
Professor Michel Félétou, Inst. Research Servier, France

13:00-14:00  Lunch

14:00-14:15  Opening ceremony  
Professor Paul M Vanhoutte

14:15-16:15  Afternoon session  
Chairmen: Professor Bernard MY Cheung, Dr Kathy LF Lee

**IL4**  The hypertension-diabetes continuum  
Professor Bernard MY Cheung, The University of Hong Kong, China

**IL5**  Importance of blood pressure lowering in type 2 diabetes  
Professor John Chalmers, University of Sydney, Australia

**IL6**  Glycemic interventions and vascular prognosis of people with diabetes  
Professor Michel Marre, University Paris VII, France

16:15-16:45  Coffee break, poster viewing and booth visit

16:45-17:45  Case-based interactive session  
Chairman: Professor Paul M Vanhoutte

17:45-18:00  Closing remark and young investigator award ceremony  
Professor Paul M Vanhoutte

18:00  Annual General Meeting
**ABSTRACTS**

Abstracts for Invited Lectures:

**IL1.**

**ENDOTHELIAL DYSFUNCTION: THE COMMON CONSEQUENCE IN DIABETES AND HYPERTENSION**

Y Huang  
Institute of Vascular Medicine & School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong

Endothelial dysfunction, caused by diminished impact of endothelium-derived relaxing factors and/or exaggerated influence of endothelium-derived contracting factors in the vascular wall, is the common consequence of diabetes and hypertension. The degree of endothelial dysfunction predicts future cardiovascular outcomes. We have examined the cellular mechanisms involved in endothelial dysfunction in diabetes and hypertension using *ex vivo* and *in vivo* models. We have identified an upregulation of renin-angiotensin system (RAS) components in both hypertension and diabetes. Our data show that the use of RAS blockers (including renin inhibitor, ARB and ACE inhibitors) effectively restores endothelial function in both animal models of diabetes and hypertension. Likewise, cyclooxygenase-2 (COX-2) is also implicated to be a critical player in endothelial dysfunction in both pathological conditions. Both RAS and COX mediate the detrimental effects of several risk factors on endothelium-dependent relaxations in mouse and rat arteries, including hyperglycemia, oxidative stress, inflammatory cytokines, angiotensin II, and advanced glycation end products. Recently, we have found that bone morphogenetic protein 4 (BMP4) is possibly one of the common upstream activators to induce endothelial dysfunction in both hypertension and diabetes. BMP4 activated NAD(P)H oxidases to induce oxidative stress and upregulated the expression and activity of COX-2 in endothelial cells, which could cause hypertension, impair endothelium-dependent relaxation, induce endothelium-dependent contraction, and cause endothelial cell apoptosis. Importantly, we found a crosstalk between the RAS and BMP4 in hypertension and diabetes. Better understanding of the cellular and molecular mechanisms of endothelial dysfunction shall help to identify more effective therapies to improve cardiovascular outcomes in patients with hypertensive and diabetes.

**IL2.**

**VASCULAR OXIDATIVE STRESS: THE COMMON LINK**

RA Cohen, XY Tong  
Vascular Biology Unit, Whitaker Cardiovascular Institute, Boston University School of Medicine, USA

Vascular disease in hypertension and diabetes is associated with systemic vascular inflammation and increased adhesion of leukocytes to activated vascular cells. Excess levels of oxidants are implicated both as a cause of damage to vascular constituents, but also as important pathogenic contributors to changes in cardiovascular cell structure and function. The excess oxidants arise from several sources including NADPH oxidase, xanthine oxidase, and mitochondria. Superoxide anion and hydrogen peroxide are produced by these systems in both leukocytes and vascular cells, and hypochlorous acid, another strong oxidant, is produced by leukocyte myeloperoxidase. Nitric oxide is also produced in excess by the inducible isoform of nitric oxide synthase in inflamed blood vessels. Peroxynitrite, formed by the rapid reaction of superoxide and nitric oxide is another potent endogenous oxidant in hypertensive and diabetic human and animal blood vessels. These oxidants cause damage, altering protein function, introducing DNA mutations, and increasing lipid peroxidation. Unlike that to DNA and lipids, the damage to proteins is selective, affecting specific oxidant-sensitive cysteine, tyrosine, methionine, and tryptophan amino acid residues. Altered structure and function of some proteins results, and if the oxidation is irreversible, then protein degradation is required for restoration of function. With some important vascular proteins, for example endothelial nitric oxide synthase, prostacyclin synthase, and superoxide dismutase, oxidation of a single susceptible amino acid inactivates the enzyme. The beneficial effects of antioxidants, at least in animal models of hypertension and diabetes, can in part be ascribed to protection of the function of these and other proteins. The pathogenic importance of oxidation of specific cysteine residues is reflected by the fact that in some proteins, such as the sarcoplasmatic reticulum calcium ATPase, expression of mutant proteins lacking the reactive cysteine thiol, recapitulates a disease phenotype, such as accelerated smooth muscle or impaired endothelial cell migration. Thus, many of the shared functional abnormalities of hypertensive and diabetic blood vessels may be caused by oxidants. Although studies using antioxidants have failed in patients, the successful treatment of vascular disease with HMG CoA reductase inhibitors, PPAR agonists, thromboxane A<sub>2</sub> antagonists, thrombin, antagonists, and polyphenols may depend upon their ability to decrease production of damaging oxidants.
Thromboxane $A_2$ is the preferential physiological ligand of the TP receptor but other prostaglandins as well as some prostanoids, which do not necessarily derived from cyclooxygenases (COX) such as isoprostanes, are also able to activate this receptor. The stimulation of TP receptors elicits diverse physiological/pathophysiological actions, including platelet aggregation and smooth muscle contraction. Furthermore, the activation of endothelial TP receptors promotes the expression of adhesion molecules and favours adhesion and infiltration of monocytes/macrophages. In various cardiovascular diseases, the endothelial dysfunction is due to the release of endothelium-derived contracting factors (EDCF) that counteract the vasodilatator effect of NO. Endothelium-dependent contractions involve the activation of COX, the production of reactive oxygen species along with that of EDCF, which diffuse toward the vascular smooth muscle cells and activate the TP receptors. Antagonists of the TP receptor curtail the endothelial dysfunction in diseases such as hypertension and diabetes, are potent antithrombotic agents and prevent vascular inflammation. Therefore, TP receptor antagonists, because of this triple activity, may demonstrate a unique potential for the treatment of cardiovascular disorders.

Hypertension and diabetes are both common chronic conditions that affect a major proportion of the general population. They tend to occur in the same individual, suggesting common predisposing factors, which can be genetic or environmental. While the genes causing hypertension or diabetes await elucidation, the environmental causes of these diseases are well known. Obesity and physical activity are the two leading factors that predispose to both diseases. Individuals with abdominal obesity are likely to develop lipid abnormalities and elevation of blood pressure and glucose. In time, hypertension and diabetes ensue. Because of the shared aetiology, there is substantial overlap between hypertension and diabetes. In the Hong Kong Cardiovascular Risk Factor Prevalence Study, 40% of the subjects in the community had either raised blood pressure or raised blood glucose. Only 42% of people with diabetes had normal blood pressure and only 52% of people with hypertension had normal glucose tolerance. The presence of hypertension or diabetes should alert the clinician to the possibility of the other condition. Obesity, lipid abnormalities, raised blood pressure and glucose are all components of the metabolic syndrome. The syndrome therefore implies a pathological process, which is potentially reversible in the early stages. Previous efforts targeting smoking, hypertension and hypercholesterolaemia have started to bear fruit. However, obesity is on the increase in developed and developing countries. It is now time to focus on obesity and the metabolic syndrome, which require more a public health than a pharmacological approach.
OC1.

NOX4 IS A NOVEL SOURCE OF INTRACELLULAR ROS REQUIRED FOR OXIDIZED LDL-INDUCED MACROPHAGE DEATH

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An elevated plasma level of oxidized low density lipoproteins (OxLDL) is a biomarker for cardiovascular diseases including atherosclerosis. OxLDL promotes macrophage death, a hallmark of atherosclerotic lesions. In human monocyte-derived macrophages (HMDM), OxLDL increases intracellular ROS formation, which is absolutely required for OxLDL cytotoxicity (Asmis et al, Circ. Res. 2003; Wang et al., FRBM, 2006). However, the source of these ROS was not known. We now identified a new member of the Nox family, Nox4, in HMDM, and hypothesized that Nox4 mediates OxLDL-induced ROS formation and macrophage death. We found that in HMDM OxLDL up-regulated Nox4 mRNA expression but not Nox1, 2, 3 or 5 mRNA. OxLDL concomitantly up-regulated Nox4 and p22phox protein levels. Confocal microscopy studies showed that Nox4 appears to co-localize with p22phox. Co-immunoprecipitation confirmed the association between p22phox and Nox4, suggesting that an active Nox4/p22phox complex is present in HMDM. Inhibition of MEK but not p38-MAPK or JNK prevented the up-regulation of Nox4 induced by OxLDL. Inhibition of MEK also prevented the OxLDL-induced increase in ROS formation and protected HMDM from OxLDL-mediated cell death, suggesting that Nox4 mediates both ROS formation and cell death induced by OxLDL. In contrast, inhibitors of p38-MAPK or JNK did not block OxLDL-induced ROS formation, and showed no protection against OxLDL. To confirm a mechanistic link between OxLDL-induced Nox4/p22phox induction, ROS production and macrophage death, we used gene silencing and over-expression approaches. Adenovirus-delivered siRNA directed against Nox4 suppressed OxLDL-induced ROS formation and macrophage death while ectopic Nox4 expression enhanced ROS formation and accelerated macrophage death induced by OxLDL. In summary, we demonstrate that the Nox4/p22phox complex is induced in HMDM in response to OxLDL stimulation via the MEK/ERK pathway. In conclusion, Nox4 mediates OxLDL-induced ROS formation and macrophage death, implicating monocytic Nox4 in the development and progression of atherosclerotic lesions.

OC2.

MATRIX METALLOPROTEINASE-9 (MMP-9) DELETION SLOWS CARDIAC AGING

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Cardiac aging is associated with decreased function of the left ventricle (LV) and higher prevalence of cardiovascular disease, but the mechanisms of cardiac aging are not fully understood. We have shown that matrix metalloproteinase-9 (MMP-9) regulates cardiac remodeling after myocardial infarction (MI), and LV MMP-9 levels increase from middle-aged to old mice. Accordingly, we tested the hypothesis that the age-related increase in MMP-9 regulates cardiac aging. We compared young (4-8 month old) and old (18-23 month old) wild type (WT) and MMP-9 null mice. For both WT and MMP-9 null mice, no increases in blood pressure were observed with age. By Doppler echocardiography, old WT mice showed lower early diastolic filling to atrial filling (E/A) ratios compared with young WT mice, indicating diastolic dysfunction with age. In contrast, old MMP-9 null mice showed similar E/A ratios as young WT and young null mice, suggesting that MMP-9 deletion preserves diastolic function with age. In addition, picrosirius red (PSR) staining showed increased perivascular collagen in WT LV with age, while old MMP-9 null mice appeared to have less perivascular collagen deposition. Together, these data suggest that blocking MMP-9 function may slow cardiac aging in mice.
ABSTRACTS

Abstracts for Oral Communications:

OC3.

TOLL-LIKE RECEPTOR 4 DEFICIENCY ATTENUATES INSULIN RESISTANCE AND ENDOTHELIAL DYSFUNCTION ASSOCIATED WITH OBESITY AND DIABETES IN MICE

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Background: Aging and obesity are major risk factors for endothelial dysfunction and metabolic syndrome. Endothelial dysfunction is characterized by an impaired release of endothelium-derived relaxing (EDRF) and hyperpolarizing (EDHF) factors and enhanced production of contracting factors (EDCF). The Toll-like receptor 4 (TLR4) is a major target for lipopolysaccharide and saturated fatty acids, both of which are potent inducers of inflammation and insulin resistance. The present study was designed to evaluate the role of TLR4 in modulating metabolism and endothelial function in mice with loss-of-function mutation of TLR4.

Methods: TLR4−/− (C3H/HeJ) and wild type (C3H/HeOuJ) mice were subjected to standard or high fat diet. A type-2 diabetes animal model, double knockout in leptin receptor (Lepr) and TLR4 (DKO), was obtained by crossing Lepr−/− and TLR4−/− mice. Glucose and insulin tolerance tests (GTT and ITT) were carried out. Systolic blood pressure was measured by tail-cuff method. The animals were sacrificed at the age of 12 weeks. Rings of aorta, carotid artery and mesenteric artery (with or without endothelium) were suspended in a wire-myograph for measuring changes in isometric tension. Endothelial function was assessed by recording the responses to endothelium-dependent vasodilator and vasoconstrictor agonists.

Results: TLR4−/− mice under high fat diet feeding showed a better insulin sensitivity and lower blood pressure than wild type mice. DKO mice also demonstrated a significantly lower fasting blood glucose, serum cholesterol, lower blood pressure and better insulin sensitivity than Lepr−/− control mice. Acetylcholine-induced EDCF-mediated responses in carotid arteries were enhanced by aging, high fat diet, and genetic obesity, but were significantly attenuated by TLR4 deficiency. The contractions were inhibited by indomethacin, SC560, and S18886, but not NS398, suggesting the involvement of COX-1. Apocynin, MnTMPyP, catalase but not deferoxamine also inhibited the contractions, suggesting that reactive oxygen species may play a role. Acetylcholine-evoked hyperpolarizations were estimated in mesenteric arteries as endothelium-dependent relaxations blocked by Tram-34 and UCL-1684. The acetylcholine-induced EDHF responses were potentiated in TLR4−/− mice fed with control and high fat diet. The acetylcholine and sodium nitroprusside-induced relaxations in the aorta were not different in wild type and TLR4−/− mice.

Conclusion: Toll-like receptor 4 deficiency can prevent aging and obesity-induced insulin resistance and endothelial dysfunction possibly by decreasing oxidative stress.

OC4.

ENHANCEMENT OF ENDOTHELIAL NITRIC OXIDE SYNTHASE PREVENTS ENDOTHELIAL DYSFUNCTION INDUCED BY ASYMMETRICAL DIMETHYLARGININE

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Background & Objectives: Asymmetrical dimethylarginine (ADMA) is an endogenous L-arginine analogue that may competitively inhibit nitric oxide synthase (NOS). A growing body of evidence suggests the association between an elevated plasma level of ADMA and endothelial dysfunction. ADMA is now recognized as a risk factor for several cardiovascular diseases, such as hypertension and coronary artery disease. In the present study, we investigated the efficacy and mechanisms of AVE3085, a newly developed transcription enhancer of endothelial NOS (eNOS), with regard to its protection against ADMA-induced coronary endothelial dysfunction.

Methods: Porcine coronary small arteries (diameter 600 to 800 μm) were studied in a myograph for bradykinin (-10−6 to -6.5 Log M)-induced, endothelium-dependent relaxation as well as endothelium-independent relaxation to sodium nitroprusside (-11−4.5 Log M). Western blot experiments were performed to determine the protein expression of eNOS and the phosphorylation of eNOS at serine 1177 (p-eNOSSer1177) whereas increased the expressions of p-eNOSThr495 and nitrotyrosine (p<0.05). The decreased expression of eNOS and eNOS phosphorylation at serine 1177 was reversed by AVE3085. The increased expression of p-eNOSThr495 and nitrotyrosine was however, lowered by AVE3085 treatment (p<0.05).

Conclusions: AVE3085 protects against ADMA-induced endothelial dysfunction in coronary arteries. Enhancement of eNOS expression and activation as well as reduction of oxidative stress may account for the protective effect of AVE3085.

Acknowledgments: This study was supported by Hong Kong RGC grant (CUHK4651/07M) and CUHK direct grants 2041388 & 2041384.
OC5.

POLYOL PATHWAY CONTRIBUTES TO THE ACUTE HYPERGLYCEMIA-INDUCED CONTRACTILE DYSFUNCTION IN PERFUSED HEART FROM RAT

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The polyol pathway, consisting of aldose reductase (AR) and sorbitol dehydrogenase (SDH), is an alternate metabolic route that converts excess glucose to fructose. It has been implicated in the development of various diabetic complications including nephropathy, retinopathy and cardiovascular disease. Previously, inhibition of AR was found to protect diabetic mice hearts from contractile dysfunction, suggesting that the polyol pathway contributes to hyperglycemia-induced contractile dysfunction. In this report we show that this glucose metabolic shunt also contributes to acute hyperglycemia-induced cardiac contractile dysfunction. Rat hearts were isolated and retrogradely perfused with either Krebs' buffer containing 10 µM AR inhibitor, Fidarestat, or 1 µM SDH inhibitor, CP-170,711, and subjected to acute hyperglycemia by perfusing with high glucose medium (33.3 mM) for 2 hours. The polyol pathway activity was measured by high performance liquid chromatography. Changes in the oxidative stress were determined by biochemical assays. We find that acute hyperglycemia-induced contractile dysfunction of the isolated perfused hearts is improved by pharmacological inhibition of the polyol pathway. The acute hyperglycemia-induced contractile dysfunction is most likely contributed by the changes in the activities of sacro/endoplasmic reticulum Ca2+-ATPase (SERCA) and sodium calcium exchanger (NCX), two key players in Ca2+ regulation. Under acute hyperglycemia, SERCA is inactivated by the tyrosine nitration, which is contributed by high level of peroxynitrite. However, the activity of NCX is significantly increased, and the activation is probably contributed by reactive oxygen species. All these abnormalities were significantly attenuated by treatment with ARI or SDI. Thus, during acute hyperglycemia, polyol pathway-induced depletion of glutathione and increased level of superoxide probably inactivate SERCA, and stimulate NCX, leading to the abnormalities in contractile function.

OC6.

PROTECTIVE EFFECTS OF GINSENOSIDES AGAINST ENDOTHELIAL DYSFUNCTION IN TYPE 2 DIABETIC MICE

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Diabetes is associated with endothelial dysfunction which contributes to the increased cardiovascular risks. The present study investigated whether or not ginseng extracts (PPD- or PPT-type ginsenosides) could restore endothelial function in the lepr−/− db/db mouse and the possible underlying cellular mechanisms. Db/db mice of 12-week-old were treated with PPD- or PPT-type ginsenosides (20 mg/kg/day) and vehicle for 14 days. Oral glucose tolerance test was performed after chronic administration of ginsenosides. Vascular function was assessed in aortas and femoral arteries. The expression of total and phosphorylation form of AMP-activated protein kinases (AMPK) and endothelial nitric oxide synthase (eNOS) were detected by Western blotting method. The present results show that PPD-type but not PPT-type ginsenosides improved oral glucose tolerance in db/db mice. Endothelium-dependent relaxations induced by acetylcholine were markedly impaired in diabetic mice and rescued by chronic treatment with PPD- or PPT-type ginsenosides. Acute inhibition of AMPK by compound C prevented the improvement of acetylcholine-induced relaxations in aortas and femoral arteries of PPD-treated but not PPT-treated db/db mice. Western blot analysis revealed that PPD increased the phosphorylation of AMPK and eNOS in db/db mouse aortas without affecting the total amount of AMPK or eNOS. PPT did not change the protein expression of AMPK phosphorylation. The present findings demonstrate that consumption of PPD- and PPT-type ginsenosides reverses endothelial dysfunction in diabetic mice. More importantly, PPD-type ginsenosides increase nitric oxide bioavailability through the increased phosphorylation of AMPK. These vasoprotective effects may account for a significant reduction of cardiovascular events in type 2 diabetic patients who receive ginsenoside therapy. (Supported by GRF/465308 and HKBU1/06C).
ABSTRACTS

Abstracts for Oral Communications:

OC7.
BINDING OF GENISTEIN WITH MEMBRANE ESTROGEN RECEPTOR AND THE POTENTIATING EFFECT OF GENISTEIN IN RAPID, NON-GENOMIC VASCULAR ACTION

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Background: Genistein is a phytoestrogen which enhances endothelial functions in a receptor-mediated manner. The present study was designed to characterize the mechanism involved in the rapid vascular actions of genistein and to determine whether genistein share the same receptor with estrogen in its non-genomic action.

Methods: Using tissue bath studies, isometric tension was measured in aortic rings isolated from 32-week-old male spontaneously hypertensive rats. The nuclear and membranous isoforms of estrogen receptor (ER)-α, ER-α66 and ER-α46, were cloned and expressed using a cell-free expression system. Binding study was performed subsequently.

Results: Genistein acutely potentiated acetylcholine-induced relaxation. This effect was insensitive to the transcription and translation inhibitors, actinomycin D and cycloheximide, respectively. The potentiation of acetylcholine and A23187-induced relaxation by genistein was inhibited by NF023 and GP antagonist-2A, the selective G i and Gq α-subunit antagonists, respectively, but not by NF449, a selective Gi α-subunit antagonist. ER-α66 and ER-α46 were successfully cloned and expressed in vitro, with molecular sizes confirmed by Western blotting. 17β-estradiol bound to the ER-α66 and ER-α46 with similar affinity and genistein competed with 17β-estradiol for binding to both receptors.

Conclusion: The tissue bath studies demonstrate that rapid potentiating effect of genistein in acetylcholine-induced relaxation is non-genomic and G protein-coupled. In addition, our data also suggests that genistein may bind to nuclear and membranous estrogen receptors. Further studies are required to reveal whether the non-genomic vascular effect of genistein is mediated through the membranous estrogen receptors.

OC8.
CHRONIC TREATMENT OF VITAMIN D DERIVATIVES REDUCE ENDOTHELIUM-DEPENDENT CONTRACTIONS IN THE AORTA OF THE SPONTANEOUSLY HYPERTENSIVE RAT

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The available evidences suggest that vitamin D has cardiovascular effects besides regulating calcium homeostasis. Previous studies demonstrated that 1,25-dihydroxyvitamin D3, the major metabolite of vitamin D, acutely reduce endothelium-dependent contractions induced by acetylcholine. To examine the chronic effect of 1,25-dihydroxyvitamin D3, rats were treated with the vitamin D derivative for 6 weeks. The serum 1,25-dihydroxyvitamin D3 level was significantly higher than the control while the mean arterial blood pressure was significantly lower. Aortic rings with or without endothelium were used for organ bath experiments. The release of prostacyclin and thromboxane A2, after acetylcholine or A23187 stimulation were measured. The cytosolic-free calcium concentration was measured by confocal microscopy with the fluorescent dyes Fluo-4. Real time PCR was used to compare the mRNA level of COX-1, prostacyclin synthase, thromboxane synthase and eNOS between the control and treated groups. Both acetylcholine- and A23187-induced endothelium-dependent contractions were reduced significantly in the treated group. The acetylcholine-induced release of prostacyclin and the A23187-induced thromboxane A2 was reduced in the treated group. There was no significant difference in cytosolic free calcium concentration caused by acetylcholine or A23187 between control and treated groups. COX-1 mRNA level was significantly inhibited in the treated SHR. These results demonstrate that chronic treatment of 1,25-dihydroxyvitamin D3 modulates vascular tone by inhibiting the expression level of COX-1 mRNA which is a completely different mechanism as in the acute treatment. This chronic effect to EDCF may account for one of the factors that reducing the mean arterial blood pressure in the SHR rats.
Abstracts for Posters:

**P1.**

**LUTEOLIN REDUCES CARDIAC DYSFUNCTIONS AND MITOCHONDRIAL OXIDATIVE STRESS IN STREPTOZOTOCIN-INDUCED DIABETIC RATS**

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**Aim:** To investigate the effects of luteolin on cardiac functions and mitochondrial oxidative stress in streptozotocin (STZ)-induced diabetic rats.  

**Methods:** Male Sprague-Dawley rats were randomly divided into a normal control group, a luteolin control group, a diabetic group, and diabetic groups orally administered with a low dose (10 mg/kg/d) or a high dose of luteolin (100 mg/kg/d) for eight weeks. The body weight, blood glucose, cardiac functions, left ventricular weight, myocardial collagen, and reactive oxygen species (ROS) levels were assayed. The cardiac mitochondrial ROS level, superoxide dismutase (SOD) activity and the mitochondrial swelling were measured.  

**Results:** Treatment with luteolin had no effect on the blood glucose but reduced the losing of body weight in diabetic rats. High dose of luteolin markedly reduced the ratio of ventricular weight and body weight, increased the left ventricular develop pressure, and decreased the left ventricular end diastolic pressure in diabetic rats. The myocardial levels of ROS and collagen, the cardiac mitochondrial ROS level, and the mitochondrial swelling in diabetic rats were all markedly reduced by high dose of luteolin. Furthermore, high dose of luteolin significantly increased the mitochondrial SOD activity in diabetic rat hearts.  

**Conclusion:** Treatment with luteolin for 8 weeks markedly improves the cardiac function, which may be related to reducing mitochondrial oxidative stress and mitochondrial swelling, in diabetic rats.  

(This work was supported by grants from the National Natural Science Foundation of Zhejiang Province (Y206179))

**P2.**

**MATERNALLY INHERITED HYPERTENSION IS ASSOCIATED WITH THE MITOCHONDRIAL tRNA\textsubscript{Ile} A4295G MUTATION IN A CHINESE FAMILY**

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**Objective:** Mutations in mitochondrial DNA have been associated with cardiovascular disease. We report here the clinical, genetic, and molecular characterization of one three-generation Han Chinese family with maternally transmitted hypertension.  

**Methods:** Members of this Chinese family underwent a physical examination, laboratory assessment of cardiovascular disease risk factors, and routine electrocardiography. Genomic DNA was isolated from whole blood and the entire mitochondrial gene was amplified by PCR. PCR fragments were purified and subsequently analyzed by direct sequencing analysis.  

**Results:** Sequence analysis of the complete mitochondrial DNA in this pedigree revealed the presence of the known hypertension-associated tRNA\textsubscript{Ile} A4295G mutation and 33 other variants, belonging to the Asian haplogroup D4j. The A4295G mutation, which is extraordinarily conserved from bacteria to human mitochondria, is located at immediately 30 end to the anticodon, corresponding to conventional position 37 of tRNA\textsubscript{Ile}. The occurrence of the A4295G mutation in several genetically unrelated pedigrees affected by cardiovascular disease but the absence of 242 Chinese controls strongly indicates that this mutation is involved in the pathogenesis of cardiovascular disease. Of other variants, the tRNA\textsubscript{Glu} A14693G and ND1 G11696A mutations were implicated to be associated with other mitochondrial disorders. The A14693G mutation, which is a highly conserved nucleoside at the TwC-loop of tRNA\textsubscript{Glu}, has been implicated to be important for tRNA structure and function. Furthermore, the ND4 G11696A mutation was associated with Leber's hereditary optic neuropathy.  

**Conclusion:** The combination of the A4295G mutation in the tRNA\textsubscript{Ile} gene with the ND4 G11696A mutation and tRNA\textsubscript{Glu} A14693G mutation may contribute to the high penetrance of hypertension in this Chinese family.
ICSM, THE THIRTEENTH ANNUAL SCIENTIFIC MEETING

ABSTRACTS

Abstracts for Posters:

P3.

VOLTAGE-DEPENDENT ANION CHANNEL (VDAC) IS INVOLVED IN APOPTOSIS OF CELL LINES CARRYING THE MITOCHONDRIAL DNA A4263G MUTATION

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The mitochondrial voltage-dependent anion channel (VDAC) is increasingly implicated in the control of apoptosis. We have studied the effects on mitochondrial DNA (mtDNA) A4263G tRNAIle  mutation on VDAC expression, localization, and apoptosis. Lymphoblastoid cell lines were derived from 3 symptomatic and 1 asymptomatic members of a family with hypertension associated with the A4263G tRNAIle mutation as well as from control subjects. Mitochondrial potential (∆Ψm) and apoptosis were measured by flow cytometry; co-localization of VDAC and Bax was evaluated by confocal microscopy. Expression of VDAC and Bax in mtDNA A4263G cell lines was found to be increased compared to controls, while expression of the small conductance calcium-dependant potassium channel (sKCa) was unchanged. Confocal imaging revealed co-localization of VDAC/Bax on the outer mitochondrial membrane of A4263G cell lines but not from controls. Flow cytometry indicated that the mitochondrial potential was decreased by 32% in A4263G cells versus controls while rates of apoptosis were increased (P<0.05). The difference was attenuated by Cyclosporin A (CsA, 2 μM), a blocker of VDAC. We conclude that increased expression of mitochondrial VDAC and subconscious co-localization of VDAC/Bax increases mitochondrial permeability and apoptosis in cell lines carrying the mtDNA tRNAIle A4263G mutation.

P4.

THE ROLE OF PPAR-GAMMA IN TELMISARTAN-INDUCED INHIBITION OF CONTRACTIONS IN MOUSE MESENTERIC RESISTANCE ARTERIES

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Upon the activation of renin-angiotensin system (RAS), angiotensin II stimulates the angiotensin II type 1 (AT1) receptor through triggering NAD (P)H oxidase to produce superoxide anions, also referred to as reactive oxygen species (ROS) in vascular cells. The elevated level of ROS dramatically reduces the bioavailability of nitric oxide (NO), leading to endothelial dysfunction. Recently, telmisartan has been suggested to possess a partial peroxisome proliferator-activated receptor-γ (PPAR-γ) activity in addition to being a general angiotensin II receptor blocker (ARB). Since the studies of the role of PPAR-γ in telmisartan have been vastly lacking in blood vessels, the present study is to examine the hypothesis that telmisartan-induced inhibition of agonist-elicited contractions in mouse mesenteric resistance arteries is due to an increase in basal NO level which is mediated via a PPAR-γ-dependent mechanism. The arteries taken from C57 mouse were suspended in myograph for isometric tension measurement. Incubation of telmisartan (0.1-10 μmol/L) for 24 hours reduces 9,11-dideoxy-11α,9α-epoxymethano-prostaglandin F2α (U46619)-elicited contractions in a concentration-dependent manner. Co-treatment of the rings with 300 nmol/L GW9662 (a PPAR-γ antagonist) antagonized the effect of 10 μmol/L telmisartan. The inhibitory effect of telmisartan (10 μmol/L) on contractions was prevented by actinomycin D (10 μmol/L). Both a NO synthase inhibitor, Nω-nitro-L-arginine methyl ester, and a guanylyl cyclase inhibitor 1H[1,2,4] oxadizolo-[4,3-a]quinoxalin-1-one, abolished the telmisartan (10 μmol/L)-induced inhibition of U46619-elicited contractions. The present results suggest that additional endothelial NO production is probably the mechanism that accounts for the telmisartan-induced inhibition of vasoconstriction and the NO production is likely to be PPAR-γ-dependent. (Supported by GRF grants and CUHK LKS Institute of Health Sciences)
P5.

**DPP4 INHIBITOR SITAGLIPTIN PROTECTS AGAINST ENDOTHELIAL DYSFUNCTION IN SPONTANEOUSLY HYPERTENSIVE RATS**

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Sitagliptin, a highly selective DPP-4 inhibitor, acts by inhibiting the inactivation and degradation of glucagon like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), is an effective anti-diabetic drug for the treatment of type 2 diabetes. Little is known about the beneficial effects of sitagliptin against vascular dysfunction associated with hypertension. The present study aimed to investigate whether sitagliptin can protect endothelial function in spontaneously hypertensive rat (SHR). Changes in vascular tone were studied in myograph and the protein expression was detected by Western blotting. The level of nitric oxide was determined by confocal microscopy using DAF-FM fluorescent dye in primary culture of SHR aortic endothelial cells. 12-hr treatment with 10 µM sitagliptin or 10 nM GLP-1 agonist exendin-4 both augmented the acetylcholine-induced endothelium-dependent relaxations in SHR renal arteries and these effects were abrogated by GLP-1 receptor antagonist exendin 9-39. Three-week treatment with sitagliptin led to a significant improvement in endothelium-dependent relaxations in SHR renal arteries. The protective effect of sitagliptin was abolished by exendin 9-39, while exendin 9-39 had no effect on acetylcholine-induced endothelium-dependent relaxations in SHR and WKY renal arteries. Sitagliptin treatment also increased phosphorylation of eNOS at Ser1177 in cultured endothelial cells. Moreover, sitagliptin and exendin-4 enhanced nitric oxide production in primary culture of SHR aortic endothelial cells, which was blocked by exendin 9-39. In conclusion, the novel results suggest that sitagliptin improves endothelial function in SHR by increasing NO bioavailability, which provides functional implications of the GLP-1 signaling pathway in the cardiovascular system. (Supported by GRF and CUHK LKS Institute of Health Sciences)

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P6.

**PHARMACOLOGICAL INHIBITION OF ADIPOCYTE-FATTY ACID BINDING PROTEIN (A-FABP) IMPROVES ENDOTHELIAL FUNCTION IN MALE APOLIPOPROTEIN E-KNOCKOUT MICE**

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Adipocyte-fatty acid binding protein (A-FABP) modulates inflammatory responses in macrophages and may play a role in formation of foam cells and atherosclerotic plaques. A-FABP is markedly upregulated in regenerated porcine coronary arterial endothelial cells. The project were designed to investigate the presence (or not) of A-FABP as well as endothelial function at early stages of atherosclerosis in the aorta of 8, 12 and 18 weeks old male C57 apolipoprotein E-knockout (ApoE−/−) mice. The effect was determined by treatment with a selective A-FABP inhibitor, BMS 309403, in 12-weeks old ApoE−/− mice. A-FABP was detected by immunofluorescent staining in the endothelium of the aorta at 12, but not 8 weeks. In myograph experiments, the endothelium-dependent relaxations to acetylcholine and UK14304 (a selective α1-adrenoceptor agonist) were reduced significantly in the ApoE−/− mice at 8 and 12 weeks on, respectively, compared to those obtained in wild type mice. Relaxations to the calcium ionophore A23187 were diminished significantly only from 18 weeks. Treatment with the A-FABP inhibitor significantly improved the relaxation to acetylcholine and UK14304, but not that to A23187 without affecting the plasma lipid profile. In conclusion, A-FABP was detected in male atherosclerotic-prone ApoE−/− mice since the age of 12 weeks. Endothelial dysfunction was observed as early as at 8 weeks of age and deteriorated until 18 weeks, as judged from the reduced relaxations to acetylcholine, UK14304 and A23187. Endothelial dysfunction can be alleviated by treatment with an A-FABP inhibitor, suggesting that A-FABP may be a novel target for the treatment of endothelial dysfunction.
ABSTRACTS

Abstracts for Posters:

P7.
INVOLVEMENT OF CFTR AND KATP CHANNEL IN ACIDOSIS-INDUCED ATP RELEASE FROM L6 CELLS
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Introduction: In our earlier study in dog skeletal muscle, muscle contractions brought about an increase in interstitial adenine nucleotides and a decrease in muscle pH. Depression of the pH of rat soleus or EDL muscle with lactic acid infusion also stimulated ATP release, which could be inhibited by CFTRinh172, an inhibitor of CFTR, or glibenclamide, an inhibitor of both KATP channels and CFTR.

Objectives: In this study, we used RNA interference to selectively silence expression of the channel proteins, in order to confirm whether both CFTR and KATP channels are involved in pH-depression-induced ATP increase.

Methods: Specific siRNAs for CFTR and the KATP channel were transfected into L6 cells, followed by lactic acid treatment for 3 hours. As a control, other L6 cells were transfected with the siRNA for MAPK, a protein unrelated to ATP release. The protein expression of MAPK, CFTR and the KATP channel was determined by Western Blotting, and the effects of pH depression on the accumulation of ATP and adenosine in the culture medium were determined using HPLC.

Results: The protein expression of MAPK, CFTR and KATP channel was decreased significantly after siRNA knock down. Lactic acid treatment significantly increased the accumulation of ATP and adenosine in the medium surrounding the L6 cells in non-transfected cells. Silencing of MAPK did not affect the lactic-acid-induced ATP release, but silencing of either CFTR or the KATP channel abolished the lactic-acid-induced increases in extracellular ATP and adenosine.

Conclusions: Lactic-acid-induced pH depression stimulated ATP release from L6 cells, which may involve both CFTR and the KATP channel.

P8.
Puerarin Protects Against High Glucose-Induced Apoptosis by Inhibiting Calpain Activation in HUVECs
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Objectives: The aim of this study is to investigate whether puerarin could protect against high glucose-induced apoptosis by suppressing calpain activation in human umbilical vein endothelial cells (HUVECs).

Methods: HUVECs were exposed to normal glucose (5.5 mmol/L) or high glucose (33 mmol/L) for 48 h. Then cell apoptosis and caspase-3 activity were determined. The expression of heme oxygenase-1 (HO-1) mRNA was evaluated by RT-PCR analysis. The activation of calpain and HO activity were also detected.

Results: Compared with the normal glucose group, exposure of HUVECs with high glucose for 48 h resulted in the significant increases in calpain and caspase-3 activity, and apoptosis, which were prevented by co-incubation with puerarin (10⁻⁶, 10⁻⁵, or 10⁻⁴ mol/L) in a concentration-dependent manner. HO-1 mRNA expression and HO activity were decreased in HUVECs treated with high glucose for 48 h. Compared with high glucose group, co-incubation HUVECs with puerarin and high glucose induced the increases in HO-1 mRNA expression and HO activity. HO-1 inhibitor protoporphyrin IX zinc (II) abolished the inhibitive effect of puerarin on high glucose-induced calpain and caspase-3 activation, and apoptosis.

Conclusion: The data show that puerarin protects against high glucose-induced endothelial cells apoptosis by a mechanism involving upregulation of HO-1 expression and inhibition of calpain activity.
P9.
HIGH CONCENTRATIONS OF EPIGALLOCOATECHIN GALLATE INDUCE CONTRACTIONS OF THE RAT AORTA DUE TO PRODUCTION OF REACTIVE OXYGEN SPECIES, ACTIVATION OF CYCLOOXYGENASE, PRODUCTION OF PROSTANOIDS AND STIMULATION OF TP-RECEPTORS
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Objective: Although regular consumption of green tea is believed to be beneficial for the cardiovascular system, a previous study revealed that high concentrations of the green tea catechin epigallocatechin gallate (EGCG) causes contractions of the aorta of spontaneously hypertensive rats (SHR). The present studies were aimed to investigate the mechanisms underlying these EGCG-induced contractions.

Methods & Results: Isometric tension was measured in isolated aortic rings from 36-week-old male SHR. From $10^{-6}$ to $10^{-4}$ M EGCG induced concentration-dependent contractions in preparations both with and without endothelium, which were potentiated by L-NAME (inhibitor of endothelial NO synthase) and abolished by indomethacin (inhibitor of cyclooxygenases) or the thromboxane-prostanoid (TP) receptors antagonist S18886. The extracellular antioxidants SOD and catalase and the intracellular antioxidants apocynin, DETCA and deferoxamine, but not tiron partly reduced the response, while the combined treatment with all intracellular antioxidants abolished the contractions. The release of prostanoids end-products [including prostaglandin F$_{20}$, prostaglandin F$_{30}$ and thromboxane B$_{2}$] was significantly increased by EGCG as measured by enzyme immunoassay kits.

Conclusion: These results demonstrate that hKv4.3 channel is regulated by both EGFR kinase and Src-family kinases. EGFR and Src-family kinases favor tyrosine phosphorylation of the channel, and therefore may affect the cardiac electrophysiology.

P10.
PROTEIN TYROSINE KINASES REGULATE HUMAN CARDIAC Kv4.3 CHANNEL
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Background. The transient outward K⁺ current I$_{to}$ (encoded by Kv4.3) plays an important role in the phase 1 rapid repolarization of cardiac action potentials in the heart. Modulation of I$_{to}$ by intracellular signal transduction is not understood. The present study was designed to determine whether hKv4.3 channel ($\alpha$-subunit of human cardiac I$_{to}$) is regulated by protein tyrosine kinases (PTKs) in HEK 293 cells stably expressing human Kv4.3 gene using a whole-cell patch clamp technique.

Results. It was found that human cardiac Kv4.3 current amplitude was remarkably inhibited by the broad-spectrum PTK inhibitor genistein (10 μM), and the inhibition was partially antagonized by the protein tyrosine phosphoatases (PTPs) inhibitor orthovanadate (1 mM). It is interesting that the selective EGFR (epidermal growth factor receptor) kinase inhibitor AG556 (10 μM) reversibly reduced Kv4.3 current, and the inhibitory effect was almost fully countered by orthovanadate. In addition, the Src-family kinase inhibitor PP2 (10 μM) also decreased hKv4.3 current and the effect was partially antagonized by orthovaanadate. Immunoprecipitation and Western blot analysis revealed that tyrosine phosphorylation level of hKv4.3 channel was reduced by genistein, AG556 or PP2. Their reduction of hKv4.3 channel phosphorylation level was reversed by orthovanadate.

Conclusion: These results demonstrate that hKv4.3 channel is regulated by both EGFR kinase and Src-family kinases. EGFR and Src-family kinases favor tyrosine phosphorylation of the channel, and therefore may affect the cardiac electrophysiology.
ABSTRACTS

Abstracts for Posters:

P11.
LARGE-CONDUCTANCE Ca$^{2+}$-ACTIVATED POTASSIUM AND ETHER-$\alpha$-GO-GO POTASSIUM CHANNELS REGULATE PROLIFERATION OF HUMAN MESENCHYMAL STEM CELLS

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Background: Bone marrow-derived mesenchymal stem cells (MSCs) are a promising cell source for regenerative medicine; however, cellular physiology is not fully understood in human MSCs. The present study was to explore the potential role of the dominant functional ion channels, large-conductance Ca$^{2+}$-activated potassium (BKCa) channel, ether-$\alpha$-go-go potassium (hEAG1) channel, and sodium channel, in regulating proliferation of human MSCs using whole-cell patch clamp and cell proliferation assay approaches.

Results: We found that the BKCa channel blocker paxilline (1 µM) almost fully inhibited BKCa current (from 6.76±0.99 pA/pF of control, to 0.02±0.09 pA/pF at +100 mV, n=5, P<0.05) in human MSCs. The hEAG1 channel blocker astemizole (0.5 µM) significantly reduced hEAG1 current from 4.28±1.86 pA/pF to 1.40±1.13 pA/pF at +50 mV, n=6, P<0.05). The MTT experiment showed that paxilline at 0.3, 1.0, and 3.0 µM reduced cell proliferation to 97.2, 84.4, and 48.7% of control, respectively, and astemizole (from 4.28±1.86 to 1.40±1.13 pA/pF at +50 mV, n=6, P<0.05). The MTX experiment showed that paxilline at 0.3, 1.0, and 3.0 µM reduced cell proliferation to 97.2, 84.4, and 48.7% of control, respectively, and astemizole (from 4.28±1.86 to 1.40±1.13 pA/pF at +50 mV, n=6, P<0.05). The MTT experiment showed that paxilline at 0.3, 1.0, and 3.0 µM reduced cell proliferation to 97.2, 84.4, and 48.7% of control, respectively, and astemizole (from 4.28±1.86 to 1.40±1.13 pA/pF at +50 mV, n=6, P<0.05). The MTT experiment showed that paxilline at 0.3, 1.0, and 3.0 µM reduced cell proliferation to 97.2, 84.4, and 48.7% of control, respectively, and astemizole (from 4.28±1.86 to 1.40±1.13 pA/pF at +50 mV, n=6, P<0.05). The MTT experiment showed that paxilline at 0.3, 1.0, and 3.0 µM reduced cell proliferation to 97.2, 84.4, and 48.7% of control, respectively, and astemizole (from 4.28±1.86 to 1.40±1.13 pA/pF at +50 mV, n=6, P<0.05). The MTT experiment showed that paxilline at 0.3, 1.0, and 3.0 µM reduced cell proliferation to 97.2, 84.4, and 48.7% of control, respectively, and astemizole (from 4.28±1.86 to 1.40±1.13 pA/pF at +50 mV, n=6, P<0.05). The MTT experiment showed that paxilline at 0.3, 1.0, and 3.0 µM reduced cell proliferation to 97.2, 84.4, and 48.7% of control, respectively, and astemizole (from 4.28±1.86 to 1.40±1.13 pA/pF at +50 mV, n=6, P<0.05). The MTT experiment showed that paxilline at 0.3, 1.0, and 3.0 µM reduced cell proliferation to 97.2, 84.4, and 48.7% of control, respectively, and astemizole (from 4.28±1.86 to 1.40±1.13 pA/pF at +50 mV, n=6, P<0.05). The MTT experiment showed that paxilline at 0.3, 1.0, and 3.0 µM reduced cell proliferation to 97.2, 84.4, and 48.7% of control, respectively, and astemizole (from 4.28±1.86 to 1.40±1.13 pA/pF at +50 mV, n=6, P<0.05). The MTT experiment showed that paxilline at 0.3, 1.0, and 3.0 µM reduced cell proliferation to 97.2, 84.4, and 48.7% of control, respectively, and astemizole (from 4.28±1.86 to 1.40±1.13 pA/pF at +50 mV, n=6, P<0.05). The MTT experiment showed that paxilline at 0.3, 1.0, and 3.0 µM reduced cell proliferation to 97.2, 84.4, and 48.7% of control, respectively, and astemizole (from 4.28±1.86 to 1.40±1.13 pA/pF at +50 mV, n=6, P<0.05).

Conclusion: Our results demonstrate that BKCa and hEAG1 channels, but not sodium channel, participate in the regulation of cell proliferation by promoting G0/G1 into cell cycling progression.

P12.
AMELIORATION OF HYPERGLYCEMIA-INDUCED MITOCHONDRIAL ROS GENERATION OF SINGLE PANCREATIC ISLET $\beta$-CELLS OF OBESE/DIABETIC MICE BY CHRONIC N-ACETYL-L-CYSTEINE

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Background: Mitochondria are the principal source of reactive oxygen species (ROS) in pancreatic islets $\beta$-cells and impairment of mitochondrial functions is intrinsically related with diabetes mellitus. Hyperglycemia-induced ROS production by mitochondria is an important aspect in $\beta$-cell glucose toxicity. However, most previous studies were performed in either normal islets/single $\beta$-cells or insulinoma cells which were bathed in high glucose medium which could not mimic the pathophysiological conditions. Objectives: To compare and measure hyperglycemia-induced mitochondria ROS generation of primary pancreatic islet $\beta$-cells of obese/diabetic (+/db/+db) and lean/control (+/db/+m) mice, and the effects (acute and chronic) of N-acetyl-L-cysteine (NAC) on ROS generation.

Methods: Collagenase-dissociated single pancreatic islet $\beta$-cells of C57BL/6J obese/diabetic (+/db/+db) mice which exhibit phenotypes of the human T2DM were harvested, and the effects (acute, 10 min; chronic, 24 h) of NAC (20 mM) on high glucose-induced mitochondrial ROS generation were evaluated. Mitochondrial ROS levels were estimated by MitoTracker Red (reduced form) (a selective fluorescence probe for mitochondrial ROS measurement) using confocal microscope.

Results: A trend of, but a non-significant, higher resting/basal ROS level was observed in single pancreatic $\beta$-cells of +/db/+db mice compared to +/db/+m mice. High glucose (15 mM) application gradually caused an increase in ROS levels in single pancreatic $\beta$-cells of +/db/+db mice whereas no apparent change was observed in +/db/+m mice. Chronic (24 h), but not acute (10 min), treatments with NAC (20 mM) ameliorated high-glucose induced ROS generation in single pancreatic $\beta$-cells of +/db/+db mice.

Conclusions: Hyperglycemia elicited mitochondrial ROS generation only in single pancreatic $\beta$-cells of +/db/+db mice. Chronic NAC (a well known anti-oxidant) pre-treatment eradicated high glucose-induced ROS generation. Current study is underway to elucidate the underlying mechanism(s) involved in the differential effects of high glucose on ROS generation as well as the identification of the particular mitochondrial ROS generating system.

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P13.
APIGENIN AMELIORATES VASORELAXATION IN DIABETIC RATS INDUCED BY STREPTOZOTOCIN
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Objective: To explore the effect of apigenin on endothelium-dependent vasorelaxation in isolated rat aortic rings from streptozotocin (STZ)-induced diabetic rats.

Methods: Diabetes was induced in male Sprague-Dawley rats by STZ treatment (60 mg/kg i.p.) and all rats were randomly divided into a normal control group, an apigenin control group, a diabetic group, and diabetic groups orally administered with a low dose (10 mg/kg/d), a medium dose (50 mg/kg/d) or a high dose (100 mg/kg/d) of apigenin for eight weeks. Then the thoracic aorta was rapidly dissected out and the acetylcholine (ACh)-induced endothelium-dependent vasorelaxation and phorbol 12-myristate 13-acetate (PMA)-induced constriction was measured on the organ bath system. The levels of reactive oxygen species (ROS) and the activity of nitric oxide synthase (NOS) were measured in aortas.

Results: The blood glucose was elevated compared to citrate treated control rats (30.2±4.4 mM vs. 4.9±0.9 mM, P<0.01) and there was an increased aortic generation of ROS (191.5±21.1% of control, P<0.01) and a decreased aortic constitutive NOS activity (4.0±0.5 U/mg protein in control group vs. 0.7±0.2 U/mg protein in diabetic group, P<0.01) in diabetic rats after eight weeks of STZ treatment. Acetylcholine (ACh)-induced relaxation was impaired (E_max: 84.3±3.6% in control group vs. 46.4±6.0% in diabetic group, P<0.01) whereas PMA (1 µM)-induced constriction was increased (E_max: 105.9±9.2% of KCl in control group vs. 129.3±12.2% of KCl in diabetic group, P<0.01) in aortic rings. Treatment with apigenin dose-dependently enhanced vasorelaxation to ACh (P<0.01), markedly decreased aortic ROS production (P<0.01) and increased NOS activity (P<0.01) in diabetic rats. The increase of constriction to PMA was also markedly inhibited by apigenin in diabetic rats (P<0.05).

Conclusion: The results indicate that apigenin dose-dependently reverses the decrease of endothelium-dependent vasorelaxation in diabetic rat aortic rings, which may be mediated by reducing PKC activity and ROS production induced by diabetes and maintaining the activity of constitutive NOS.

Acknowledgement: This work was funded by the grant from Zhejiang Provincial Natural Science Foundation of China (Y206179).

P14.
CAROTID PLAQUES AND LEFT VENTRICULAR HYPERTRROPHY IN OLDER PATIENTS WITH HYPERTENSION AND DIABETES: ASSOCIATION WITH BLOOD PRESSURE AND GLYCEMIC CONTROL STATUS
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Background and Objective: Hypertension and type 2 diabetes mellitus, which are frequently co-exist, are leading risk factors of coronary heart disease (CHD) and stroke. Both carotid artery plaques and left ventricular (LV) hypertrophy are well-established predictors for the occurrence of cardiovascular events. Our study aimed to investigate the relationship between prevalence of carotid plaque, LV mass and the control status of blood pressure and glycemia in older patients with hypertension and type 2 diabetes.

Methods: From March 2009 to August 2009, a total of 10,468 people aged 60 years or over participated in “Health Promotion Sojourn for Retired Cadres”, a program sponsored by the China National Committee on Aging. Before their traveling, all participants undertook a comprehensive health examination which, in addition to a range of procedures commonly covered by a typical annual check-up in China (measurement of blood pressure, height and weight, fasting lipids and glucose, complete blood cell count, urinalysis, kidney, liver, and thyroid function testing, chest X-ray, electrocardiography), also included ultrasonography of the carotid artery and echocardiograph examination. For subjects with a history of diabetes, HbA1c was also measured. Data of 678 participants (512 males and 156 females, mean age 68.3 years) with self-reported hypertension and diabetes but without history of CHD or stroke were analyzed. A carotid plaque was defined as a localized protrusion of the internal part of the vessel wall into the lumen, and LV mass was determined according to the formula introduced by Devereux et al: 0.80x[septal thickness + LV internal diameter + posterior wall thickness]² - (LV internal diameter)² + 0.6 g. Blood pressure control status was categorized into 4 groups: ideal: ≤120 mmHg, adequate: 121-140 mmHg, inadequate: 141-160 mmHg, and poor: ≥161 mmHg; according to patient's systolic blood pressure; and glycemic control status was categorized into 3 groups: ideal: <6.5%, adequate: 6.5%-7.5%, and poor: >7.5%, according to the HbA1c level. Differences between groups were tested with ANCOVA. The independent relationship between plaque and blood pressure, HbA1c level and other risk factors was tested by logistic regression analysis. Multiple linear regression analysis was performed to evaluate the impact of risk factors on LV mass.

Results: Among 678 participants, 392 (57.8%) had carotid plaques. Prevalence of carotid plaque increased with poorer hypertension control (23.3% in the ideal group to 72.8% in the poor group, P=0.003) but not glycemic control (P=0.14). Logistic regression showed that systolic blood pressure but not HbA1c level was significantly associated with carotid plaque after adjustments for age, sex, body mass index, duration of smoke, total serum cholesterol and HDL cholesterol. The mean LV mass was 176 ± 44 g. Multiple linear regression analysis showed systolic blood pressure as the only variable significantly associated with LV mass.

Conclusion: In older patients with hypertension and diabetes, blood pressure control rather than glycemic control, is associated with carotid artery plaques and LV hypertrophy.
P15.
OXIDATIVE STRESS AND LOCAL INFLAMMATION IN RAT ADRENAL MEDULLA IN CHRONIC HYPOXIA
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**Background:** Chronic hypoxia (CH) leads to cardiopulmonary changes in subjects sojourning at high altitude, and pathophysiological changes in patients with chronic obstructive pulmonary disease. Sympathetic activation of the adrenal medulla plays an important role in the cardiovascular response to hypoxia. Oxidative stress triggered by a variety of stimuli including hypoxia can mediate cellular damages with increased productions of reactive oxygen species and free radicals in local tissues.

**Hypothesis:** oxidative stress induced by CH, leads to local inflammation and cellular injury in the rat adrenal medulla.

**Methods:** Normoxic (N) and CH rats were exposed to air and 10% O₂ for 7 days, respectively. The adrenal medulla was harvested for the measurement of markers for oxidative stress, malondialdehyde (MDA) and nitrotyrosine (NTR), and for the histological analysis of macrophages infiltration and TUNEL staining for apoptosis. Also, the expressions of NADPH oxidase subunits p22(phox) and NOX-4 were examined by RT-PCR.

**Results:** The MDA level was significantly increased in the CH group, when compared with the Nx control. Image analysis also showed significantly more % adrenal medulla area with positive immunostaining of NTR than that of the Nx group. In addition, macrophage marker ED1-immunoreactivity was remarkable in the CH group, suggesting a local inflammation. Also, there was an increase in apoptotic cells in the adrenal medulla of CH rats. Moreover, the mRNA expression of p22(phox) was increased in the CH group, suggesting an involvement of NADPH oxidase in the oxidative stress.

**Summary:** Results support our hypothesis that CH-induced oxidative stress is involved in the local inflammation and apoptosis in the adrenal medulla. The role of the NADPH oxidase in the CH-induced oxidative stress awaits further investigation.

P16.
CHRONIC INTERMITTENT HYPOXIA INDUCES OXIDATIVE STRESS AND DECREASES NO PRODUCTION IN THE CAROTID ARTERY OF RATS
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Obstructive sleep apnea (OSA) syndrome is a risk factor of hypertension and stroke. Chronic intermittent hypoxia (CIH) leads to oxidative stress and tissue injury. We examined the hypothesis that CIH-induced oxidative stress plays a pathophysiological role in the endothelial dysfunction in rat carotid artery. Adult Sprague-Dawley rats were exposed to IH treatment mimicking a severe OSA condition for 14 days. The carotid arteries were harvested for the malondialdehyde assay, PCR and Western-blotting analysis, and the measurement of nitric oxide (NO) with electrochemistry. Levels of malondialdehyde were significantly elevated in the hypoxic group when compared to the normoxic control. Also, the mRNA expressions of NADPH oxidase (gp91(phox), p22(phox)) were markedly increased in the hypoxic group, indicating an involvement in the CIH-induced oxidative stress. In addition, the protein level of phosphorylated eNOS (ser1177) and the NO levels were notably lowered in the hypoxic group. These results suggest that oxidative stress induced by the CIH treatment deteriorates the endothelial function of the carotid artery with decreased NO bioavailability. These data may be clinically relevant to the increased risk for cerebrovascular disease in OSA patients.
ABSTRACTS

Abstracts for Posters:

**P17. RESPONSE OF TRPC3 CHANNELS TO ACUTE HYPOXIA**

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**Background & Objectives:** Transient receptor potential channels (TRPs) have been recognized as novel players in the regulation of intracellular Ca^{2+} concentration ([Ca^{2+}]_{i}) that is essential to cell function. The TRPC3 channel is an important family member of TRPs and sensitivity of these channels to reactive oxygen species has been demonstrated in recent studies. However, little has been known regarding the effect of ischemia/hypoxia on these channels. Therefore, in this study, we investigated the response of the TRPC3 channel to acute hypoxia.

**Methods:** Human embryonic kidney cells (HEK293 cells) were transiently overexpressed with TRPC3 gene and exposed to either normoxia or acute hypoxia (10 min, PO_{2}<10 mmHg). Patch-clamp study of ionic currents was performed in whole-cell configuration. Protein expression was determined by western blot.

**Results:** Application of 1-oleyl-2acetyl-sn-glycerol (OAG, 100 µM), the membrane permeable DAG analogue, evoked significant cation current in TRPC3-overexpressing HEK293 cells (3.1±0.4 vs. 1.9±0.3 pA/pF, p<0.01) but not in wild-type cells (2.0±0.4 vs. 1.9±0.4, p>0.05). Acute exposure to hypoxia for 10 min enhanced the increase of current induced by OAG (5.7±0.9 vs. 3.0±0.4 pA/pF in normoxia cells, p<0.05). OAG failed to induce current change with the presence of specific anti-TRPC3 antibody in both normoxia (1.9±0.2 vs. 2.1±0.1 pA/pF, p>0.05) or hypoxia-exposed cells (2.0±0.4 vs. 2.4±0.4 pA/pF, p>0.05). Protein expression of TRPC3 was not altered by acute hypoxia.

**Conclusions:** Acute hypoxia enhances the electrophysiological activity of the TRPC3 channel. This may be a mechanism involved in [Ca^{2+}]_{i} dysregulation under hypoxic / ischemic states.

**Acknowledgments:** This study was supported by Hong Kong RGC grant (CUHK4651/07M) and CUHK direct grants 2041388 & 2041384.

**P18. NITRIC OXIDE DOES NOT AFFECT THE RELEASE OF ENDOTHELIUM-DERIVED CONTRACTING FACTOR PGF2α IN THE HAMSTER AORTA**

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We have recently observed endothelium-dependent contractions in the young hamster aorta treated with N^ω-nitro-L-arginine methyl ester (L-NAME, 100 µM), an inhibitor of nitric oxide (NO) production and identified a likely endothelium-derived contracting factor (EDCF), prostaglandin F_2α (PGF_2α). However, it remains unsolved whether the masking effect of endothelium-derived NO on endothelium-dependent contractions is due to its inhibition to the EDCF release which is mediated by COX-2 or NO plays a dominant role countering the EDCF-mediated contractions of vascular smooth muscle cells. The present study investigates the interaction between NO and EDCF. Aortic rings from young golden hamsters (aged ~3 months) were suspended between two stainless steel wires in myograph for the measurement of isometric tension. The release of PGF_2α was measured by enzyme immunoassay. Endothelium-dependent contractions were elicited by acetylcholine (ACh) only in the presence of L-NAME. Incubation of 1H-[1,2,4]oxadiazolo[4,3-g]quinioxalin-1-one (ODQ, 3 µM) also unmasked ACh-induced contractions. Sodium nitroprusside (SNP, 1 µM) inhibited endothelium-dependent contractions which were unmasked by L-NAME but not by ODQ. Co-incubation of L-arginine (1 mM) with L-NAME abolished ACh-induced endothelium-dependent contractions. PGF_2α elicited greater contractions in hamster aortas without endothelium or with endothelium treated with L-NAME. SNP attenuated the PGF_2α-induced contractions. ACh-stimulated release of PGF_2α was similar regardless of the presence of L-NAME and SNP. Our results indicate that the presence of NO dose not affect the PGF_2α release, but inhibits ACh- or exogenous PGF_2α-induced contractions, it is thus likely that NO exerts a downstream effect on vascular smooth muscle cells to inhibit endothelium-dependent contractions, instead of a direct inhibition on the COX-2 activity and subsequent PGF_2α production. (Supported by GRF and CUHK LKS Institute of Health Sciences)
P19.

**PPARδ ACTIVATION PROTECTS ENDOTHELIAL FUNCTION IN DIABETES**

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Recent evidence highlights the therapeutic potential of peroxisome proliferator-activated receptor-δ (PPARδ) agonists to increase insulin sensitivity in diabetes. The implication of PPARδ activation in the regulation of cardiovascular function is unclear. The present study investigates whether PPARδ activation can improve endothelial function under hyperglycemic conditions and in type 2 diabetes. Vascular reactivity of mouse aortas was studied in myograph. Protein expressions were detected by Western blotting. Reactive oxygen species (ROS) production was measured by dihydroethidium fluorescence, and nitric oxide (NO) production was quantified by DAF-FM dye using confocal microscopy. GW0742 and GW501516 (1 μM, PPARδ agonists) improved endothelium-dependent relaxations, inhibited endothelium-dependent contraction induced by acetylcholine, and suppressed the cyclooxygenase-2 (COX-2) up-regulation in the aortas of diabetic db/db mouse. PPARδ agonists prevented hyperglycemia-induced impairment of endothelium-dependent relaxation in aortas of wild type mice, but not in PPARδ−/− mice. PPARδ agonists reduced the ROS production induced by hyperglycemia and in db/db mouse aortas.

Moreover, PPARδ activation increases eNOS phosphorylation at Ser1177 and Akt phosphorylation at Ser473, without modulating the protein expression of total eNOS and Akt. Importantly, PPARδ activation enhanced the NO production in cultured endothelial cells. Taken together, the present study provides novel evidence for the endothelial protective effects of PPARδ activation in diabetes through triggering Akt/eNOS signaling cascade, reducing oxidative stress, and inhibiting COX-2 upregulation. The novel findings of the present investigation provide useful insights into new therapeutic strategies against the development of vascular dysfunction in diabetes.

P20.

**CYCLOOXYGENASE-DERIVED PROSTANOIDS MEDIATE ENDOTHELIAL DYSFUNCTION INDUCED BY ADVANCED GLYcation END PRODUCTS**

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Diabetes increases the formation of advanced glycation end products (AGEs) on the vascular wall and AGEs are closely linked to the development and progression of diabetic atherosclerosis. The present study tests the hypothesis that AGEs can have a direct effect on blood vessels to reduce endothelium-dependent relaxations through an increase in the production of cyclooxygenase (COX)-derived prostanooids. Aortas from both non-diabetic db/m+ and diabetic db/db mice were studied. Mouse aortas were incubated for 24 hours with AGEs and their impact on vascular reactivity was assessed in myograph. AGEs impaired acetylcholine-induced endothelium-dependent relaxations of non-diabetic mouse aortas without affecting sodium nitroprusside-induced relaxations. Treatment with aminoguanidine (100 μM, AGE inhibitors) (1) prevented AGE-induced endothelial dysfunction in non-diabetic mouse aortas; and (2) improved endothelium-dependent relaxations in db/db mouse aortas. AGE-induced effects were abolished by indomethacin and COX-2 inhibitor (NS398, 3 μM) but not COX-1 inhibitor (sc-560, 0.3 μM), suggesting an involvement of COX-derived prostanooids in mediating AGEs-induced damaging effects. S18886 (100 μM, thromboxane-prostanoid receptor antagonist) also abrogated the AGE-induced attenuation in endothelium-dependent relaxations. EIA assay further revealed that AGEs increased PGF2α production in mouse aortas under the stimulation of acetylcholine. Importantly, AL8810 (1 μM, FP receptor antagonist) could prevent AGE-induced effects. Taken together, the present results demonstrate that AGEs impair endothelial function possibly through an increased production of COX-2 derived PGF2α. The novel findings of the present investigation may provide useful insights into new therapeutic strategies against diabetic atherosclerosis by targeting COX-2-derived PGF2α and FP receptor. (Supported by GRF and CUHK LKS Institute of Health Sciences)
P21. DIFFERENTIAL EFFECTS OF DPP4 INHIBITOR SITAGLIPTIN IN MODULATING VASCULAR TONE

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Sitagliptin, a newly developed anti-diabetic drug, inhibits the activity of dipeptidyl peptidase-4 (DPP-4) which improves glucose homeostasis in diabetes. Little is known about the effects of sitagliptin in the modulation of vascular function. The present study examined its impact on the vascular tone in several types of blood vessels isolated from male Sprague-Dawley rats. The artery was suspended in organ bath or in myograph for measurement of isometric force. Sitagliptin causes concentration-dependent relaxations in phenylephrine-contracted aortas, carotid, femoral, and renal arteries and the presence of endothelium plays a role in aortas and femoral arteries but not in other arteries. In contrast, sitagliptin produces a contractile effect in coronary arteries, which can be reversed by nifedipine (voltage-sensitive calcium channel blocker) and pinacidil (potassium channel activator). Sitagliptin-induced contraction in coronary artery is dependent on extracellular calcium ions, but is independent of the presence of endothelium. The results suggest that sitagliptin may exert a direct effect on arteries, probably by elevating intracellular free calcium concentration in rat coronary artery smooth muscle while producing the opposite effects in smooth muscle cells of other systemic arteries. The detailed mechanisms are being investigated. The novel results obtained from the present study may help to define the safety profile of sitagliptin in combating against vascular complications in diabetes. (Supported by GRF and CUHK LKS Institute of Health Sciences)

P22. RELATIONSHIP OF GENETIC VARIANTS IN GENE ENCODING ADRENOMEDULLIN WITH HYPERTENSION AND DYSGLYCAEMIA IN HONG KONG CHINESE

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Introduction: Adrenomedullin (AM) is a vasodilatory peptide that acts directly via cAMP and indirectly via endothelial nitric oxide. It also facilitates the differentiation of pre-adipocytes and affects lipolysis and glucose uptake. Therefore, we investigated the association of common genetic variants in the gene encoding adrenomedullin (ADM) with hypertension and dysglycaemia in the Hong Kong Chinese population.

Methods: We genotyped 4 SNPs of ADM, rs3814700, rs11042725, rs34354539 and rs4910118, in 1936 subjects from the Hong Kong Cardiovascular Risk Factor Prevalence Study-2 (CRISPS-2), which has a median follow-up time of 6.4 years. Dysglycaemia includes impaired fasting glucose (≥6.1 mmol/L), impaired glucose tolerance (2h glucose ≥7.8 mmol/L) and diabetes.

Results: The minor T allele of SNP rs4910118 was significantly associated with lower systolic blood pressure (β=-0.057, P=0.0079) and mean arterial pressure (β=-0.054, P=0.014) at baseline after adjusting for covariates, but not at follow-up. However, none of the SNPs was significantly associated with prevalent or incident hypertension. Although dysglycaemia was not significantly associated with any of the SNPs at baseline, the minor A allele of the SNP rs11042725 was significantly associated with the development of dysglycaemia during follow-up (OR=1.30, P=0.018) and dysglycaemia at follow-up (OR=1.24, P=0.0093), after adjusting for covariates.

Conclusion: Our study provides preliminary evidence for a role of the adrenomedullin gene in influencing blood pressure and the development of diabetes.
ABSTRACTS

Abstracts for Posters:

P23.

PHOSPHODIESTERASE INHIBITION AMELIORATES TP RECEPTOR-MEDIATED IMPAIRMENT OF VASORELAXATION INDUCED BY CYCLIC AMP-ELEVATING DILATORS

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Objects: To examine whether stimulation of TP receptors impairs endothelium-independent relaxations to cyclic AMP-elevating agents via increasing the activity of phosphodiesterases (PDE).

Methods: Rat carotid arteries without endothelium were isolated and suspended in myograph for the measurement of changes in isometric tension; the tissue content of cyclic AMP was assayed by enzyme immunoassay kit; and TP receptor was detected in vascular wall by immunohistochemistry.

Results: In phenylephrine-contracted rings, relaxations induced by isoprenaline (receptor-mediated) and forskolin (receptor-independent) were markedly reduced by the presence of U46619; the attenuated relaxations were prevented by acute treatment with S18886, the selective TP receptor antagonist but not by protein kinase C inhibitors. The reduced relaxations were partially restored by IBMX (non-selective PDE inhibitor), cilostazol (PDE3 inhibitor), rolipram (PDE4 inhibitor) or by Y27632 (RhoA/Rho kinase inhibitor), but not by T0156 (PDE5 inhibitor). U46619 diminished isoprenaline- or forskolin-stimulated rise in cyclic AMP and this effect was inhibited by cilostazol or rolipram.

Conclusions: The present results suggest that activation of TP receptors impairs cyclic AMP-dependent vasorelaxations partly via PDE- and RhoA/Rho kinase-dependent mechanisms.

P24.

RELATIONSHIP BETWEEN ABACAVIR AND RISK FACTORS OF CARDIOVASCULAR DISEASES

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The use of abacavir (a nucleoside reverse transcriptase inhibitor), in the treatment of HIV infection, has been shown to increase the risks of stroke and myocardial infarction.1 The underlying mechanism is hitherto unclear. It is known that thrombosis and endothelial dysfunction are closely related to the development of these cardiovascular disorders. Therefore, we hypothesized that administration of abacavir may result in the damage on endothelial functions or acceleration of thrombotic process.

Sprague-Dawley rats (330-350 g) were treated with abacavir (16 mg/kg/day) for 28 days by gavage. Isometric tensions of basilar artery and mesenteric artery were measured. Messenger RNA and proteins expressions of factors related to endothelial function and inflammation were measured by QPCR and Western blotting, respectively. In addition, the plasma level of CD40L, a platelet-derived factor which is commonly used as a marker of platelet activation, was measured by ELISA kit.

Our results showed that the maximum relaxations of both basilar artery and mesenteric artery by acetylcholine were not different between the control group and abacavir-treated group, though the value of IC50 was larger in the control. The data of QPCR and Western blotting showed that there were no significant change in the mRNA and protein levels of eNOS, COX-1, COX-2 and ICAM-1 in aorta after the treatment with abacavir. However, a higher plasma level of CD40L was detected in the abacavir-treated group.

The results of this study suggested that abacavir upregulates the platelet activity, which may increase the chance of thrombosis and result in a higher risk of cardiovascular events.
P25. MELAMINE AND ITS DERIVATIVE CYANURIC ACID IMPAIR RENOVASCULAR FUNCTION AND REDUCE RENAL BLOOD FLOW IN RATS

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The contamination of milk products with melamine in Mainland China caused a widespread public health concern. The present study aims to examine whether ingestion of melamine and cyanuric acid can impair renovascular function and reduce renal blood flow in rats. Melamine (60, 300 or 600 mg/kg/day) and cyanuric acid (150 mg/kg/day) were administered to 5-week-old rats daily. Vascular function of isolated intralobal renal arteries was assessed in myograph. Renal blood flow was examined by functional magnetic resonance imaging (fMRI). Chronic administration of melamine at 600 mg/kg/day to rats for 3 months significantly reduced acetylcholine-induced endothelium-dependent relaxations (EDR) in renal arteries without altering sodium nitroprusside-induced endothelium-independent relaxations. Chronic exposure to melamine also augmented the endothelium-dependent contractions (EDC) in renal arteries. Acute 30-min incubation of thromboxane-prostanoid (TP)-receptor antagonist (S18886, 100 nM) in melamine-treated arteries rescued the impaired EDR and abolished the augmented EDC. By contrast, S18886 did not affect EDR in vehicle-treated arteries. Combined treatment of melamine and cyanuric acid for 5 days led to a significant reduction of renal blood flow as detected by fMRI, while melamine or cyanuric acid alone had no effect. The results from the present study suggest that chronic exposure to high dose of melamine can damage renovascular function, probably through increases in cyclooxygenase-derived prostanoids which act on the TP-receptor to reduce the EDR and cause EDC in renal arteries. The detailed mechanisms underlying renovascular dysfunction caused by melamine and its derivative cyanuric acid are currently under investigation. (Supported by HKSAR Food and Health Bureau Grant)

P26. ROS DOES NOT CONTRIBUTE TO THE ACUTE DEVELOPMENT OF NITROGLYCERINE TOLERANCE IN RAT AORTAS

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The development of nitrate tolerance limits the clinical efficacy of nitric oxide (NO) donors. Several cellular mechanisms have been proposed to explain nitrate tolerance and the increased production of ROS is one of the possibilities. The present study examined if ROS participated in nitroglycerine tolerance which occurs acutely in aortas isolated from male Sprague-Dawley rats. The aortas were exposed to 30 µM nitroglycerine (with and without pre-incubation of ROS inhibitors) for 90 min and rinsed out four times before phenylephrine was added to cause a steady contraction. Subsequently, nitroglycerine was added cumulatively to the bathing solution to induce relaxations. Nitroglycerine-induced relaxations were severely reduced after 90 min-nitroglycerine exposure. Treatment with apocynin, tempol and tiron plus DETCA did not rescue the impaired relaxations. Angiotensin II, H2O2 and hypoxanthine plus xanthine oxidase (which can generate ROS as determined by electron paramagnetic resonance) did not impair nitroglycerine-induced relaxations. The studies on changes in the activity of NAD(P)H oxidase and ROS levels in the vascular wall are being carried out. The preliminary results indicate that ROS may not be involved in the acute occurrence of nitroglycerine tolerance. However, it is still unclear whether chronic treatment with antioxidants can delay the development of nitroglycerine tolerance (supported by CUHK LKS Institute of Health Sciences).
ABSTRACTS

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P27.
INVOLVEMENT OF PLASMA MEMBRANE MONOAMINE TRANSPORTER IN SEROTONIN UPTAKE IN VASCULAR SMOOTH MUSCLE CELLS
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Serotonin (5HT) is a potent vasoconstrictor. It has been reported that 5HT can be taken up by the rat aortas through the serotonin transporters (SERT). This 5HT uptake mechanism may play a crucial role in fine-tuning the availability of 5HT at its cognate receptors. However, many studies have demonstrated that a significant part of 5HT uptake in blood vessels is insensitive to the blockade by SERT inhibitor fluvoxamine, suggesting that other transport system(s) are also involved in the 5HT uptake in blood vessels.

Plasma membrane monoamine transporter (PMAT) is a novel polyspecific organic cation transporter that can transport organic cations such as 5HT. PMAT is strongly expressed in kidney and brain. However, it is hitherto unclear whether PMAT is present in blood vessels.

The aim of this work was to study the role of PMAT in 5HT uptake in vascular cells. Results of RT-PCR demonstrated the presence of mRNA of PMAT in human brain microvascular smooth muscle cells (HBMSMCs) but not in human brain microvascular endothelial cells (HBMECs). The [3H]5HT uptake in HBVSMCs was increased with time and was saturable with a Michaelis-menten constant of 50.36±10.2 mM. This low affinity of 5HT transport was consistent to the characteristics of PMAT. Moreover, 30% of the [3H]5HT uptake in HBMSMCs was inhibited after the PMAT expression is silenced by siRNA. Interestingly, the result of semi-quantitative RT-PCR showed that mRNA expression of PMAT in basilar arteries of spontaneous hypertensive rats is higher than that of normal Wistar Kyoto rats. Taken together, our study suggests that PMAT is present and is involved in the 5HT uptake in the vascular smooth muscle cells. Upregulation of PMAT may be associated with hypertension and it warrants further investigation.

P28.
ACUTE VASCULAR EFFECT OF ALDOSTERONE ON RESISTANCE ARTERIES FROM NORMAL AND HEART FAILURE RATS
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Heart failure is a complex disease which involves numerous genetic, neuroendocrine and environmental factors. Some evidences have emerged in resent years to support the direct role of aldosterone in heart failure, independent of its regulation on blood volume and fluid, electrolyte metabolism. This study was designed to investigate the effect of aldosterone on resistance arteries from normal and heart failure rats. Acute heart failure of rat was induced by coronary artery ligation. Five weeks after the surgery, echocardiography showed the cardiac dysfunction. The hearts were then excised and Masson trichrome staining of cross sections revealed a visible myocardial infarction and fibrosis. Segments of third-order branches of the mesenteric arteries were isolated for isometric tension recording. We found that in normal rats, aldosterone (10^{-9} - 10^{-7} M) caused further contraction in mesenteric arteries precontracted by phenylephrine (PE, 10^{-6} mol/L), but aldosterone did not cause vasoconstriction in the arteries from heart failure rats. In the mesenteric arteries from normal rats, pre-incubation of aldosterone (3×10^{-8} M) for 10 min decreased the contractile response to low concentration of PE (1×10^{-7} - 1×10^{-5} M), but enhanced the contractile response to high concentration of PE (3×10^{-5} - 3×10^{-4} M). This effect was abolished by eplerenone (2×10^{-4} M), an inhibitor of aldosterone receptor. However, in the arteries from heart failure rats, aldosterone (3×10^{-8} M) decreased the contraction induced by PE (1×10^{-7} - 3×10^{-5} M), which was partly blocked by eplerenone (2×10^{-8} M). These results indicate that aldosterone has biphasic effect on contractile response to PE of normal rat arteries, mediated by aldosterone receptor. The effect of aldosterone on heart failure rat arteries is monophasic, reducing the sensitivity to PE, which is partly mediated by aldosterone receptor.
ABSTRACTS

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P29.
ION CHANNELS AND THEIR ROLE IN CELL PROLIFERATION OF 3T3-L1 PREADIPOCYTES

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Background: Mouse 3T3-L1 peradipocytes are widely used for metabolic study; however, cellular physiology (e.g. functional ion channel expression) is not fully understood. The present study was to investigate ion channel expression and functional role of them in regulating cell proliferation using whole cell patch voltage clamp technique, RT-PCR, Western blot, and cell proliferation assay in undifferentiated 3T3-L1 preadipocytes.

Results: We found that three types of ionic currents were present in 3T3-L1 preadipocytes, including a Ca2+-activated K+ current (IKCa) in 39% cells, an inwardly-rectifying K+ current (IKir) in 15% cells, and a chloride current (ICl) only in 8% cells under isotonic conditions. Interestingly, IKir was observed in all cells with hyptonotic (0.8T) insulin, suggesting that it is a volume-sensitive IClvol (IKClvol). IKir was inhibited by Ba2+, and IKCa was inhibited by the intermediate conductance IKCa channel blocker clotrimazole. ICl was reduced by the chloride channel blockers DIDS. RT-PCR revealed significant expression of mRNAs: KCa3.1 for IKCa, Kir2.1 for IKir, and Clcn3 for IClvol. Proteins of these channels were detected using Western blot analysis. Proliferation assay demonstrated that blockade of IKCa with clotrimazole or IClvol with DIDS inhibited cell proliferation in a concentration-dependent manner. Flowcytometry analysis showed that clotrimazole (3 µM) and DIDS (200 µM) accumulated the cells at G0/G1 phase (from control 49.91±2.8% to 57.05±3.6% for clotrimazole, P<0.05; to 61.08±4.3% for DIDS, P<0.05).

Conclusions: These results demonstrate the first information that three types of functional ion channel currents, including intermediate-conductance IKCa, IClvol, and IKir, are heterogeneously present in 3T3-L1 preadipocytes. IKCa and IClvol participate in the regulation of cell proliferation.

P30.
DISTINCT EFFECTS OF SIMVASTATIN ON CYTOSOLIC Ca2+ CHANGES AND Ca2+-SENSING RECEPTOR expression OF ISOLATED PANCREATIC ISLETS ß CELLS OF OBESE/DIABETIC MICE

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Background: Diabetics often have hyperlipidemia as a co-morbidity. In previous clinical and animal studies, statins (HMG CoA reductase inhibitors) provided cholesterol-independent beneficial effects in diabetic patients/animal models. Activation of Ca2+-sensing receptor (CaR) resulted in insulin release in human isolated pancreatic islets.

Objectives: To compare and measure the effects of simvastatin (SIM, a HMG CoA reductase inhibitor) (10 nM, 24 h incubation) on protein expression of CaR and ionomycin- and caffeine-elicted [Ca2+]-changes of single pancreatic islet ß-cells of obese/diabetic (+db/+db) and lean/control (+db/+m) mice.

Methods: Collagenase-dissociated single pancreatic islet ß-cells of C57BL/KsJ obese/diabetic (+db/+db) mice (which exhibit phenotypes of human T2DM) were harvested. The protein expression of CaR was evaluated using Western Blot. Ionomycin- and caffeine-induced [Ca2+]i changes were measured using Fluo-4 fluorescent imaging techniques. Glucose (5 and 15 mM)-induced insulin release was measured (by ELISA).

Results: Protein expression of CaR, but not HMG CoA reductase, was lowered in pancreatic islets of +db/+db mice (~60% of +db/+m mice) compared to +db/+m mice, and it was partially restored by SIM. A relatively small ionomycin (1 µM)- and caffeine (5 µM)-induced [Ca2+]-change was observed in single pancreatic β cells of +db/+db mice compared to +db/+m mice, and it was restored after SIM treatment. An attenuated glucose (5 and 15 mM)-induced insulin release was consistently observed in the pancreatic islets of +db/+db mice and the suppressed glucose (15 mM)-induced insulin release in pancreatic islets of +db/+db mice was partially restored by SIM.

Conclusions: The biochemical existence of HMG CoA reductase and CaR in pancreatic islets of +db/+db and +db/+m mice was confirmed. The suppressed ionomycin-induced [Ca2+]-i and glucose-mediated insulin release of pancreatic islets ß-cells +db/+db mice could be restored by SIM suggesting that SIM could be used in treating T2DM.

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P31.
MODULATORY EFFECTS OF SIMVASTATIN ON INSULIN RELEASE OF PIG PANCREATIC ISLETS OF LANGERHANS
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Background: Type 2 diabetes mellitus (T2DM) is a metabolic disease and many T2DM patients have hyperglycemia as a result of deficiencies in insulin secretion (β-cells dysfunction). A locus on chromosome 9 is linked to cholesterol levels and DM, and diabetic patients with dyslipidemia are now receiving HMG CoA reductase inhibitors (statins which are mainly for lowering blood cholesterol). However, there is no consensus on whether statins consumption can modulate insulin release in patients and animals with DM.

Objectives: To evaluate the effects of simvastatin (SIM, a HMG CoA reductase inhibitor) on insulin release of pig isolated pancreatic islets bathed in glucose medium (5.6 and 25 mM), and the underlying cellular mechanisms involved.

Methods: Fresh pig pancreases were collected from a local slaughterhouse. Pancreas was finely cut into small pieces and isolated pancreatic islets were handpicked under the dissecting microscope. The collected pancreatic islets were cultured in RPMI solution supplemented with glucose (5.6 and 25 mM) with and without SIM (24 h incubation) before they were subjected to different assays (Western blot, and insulin release using ELISA).

Results: The biochemical existence of HMG CoA reductase was confirmed, and only the protein expression of p-HMG CoA reductase was elevated in high glucose medium. Changing glucose from 5.6 to 25 mM resulted in ~5-fold increase in insulin release. Under normal glucose (5.6 mM) condition, SIM (10 µM) treatment (24 h) markedly enhanced (~9-fold) glucose-induced insulin release. In contrast, SIM pre-treatment did not modify insulin release from pancreatic islets bathed in high glucose (25 mM) medium.

Conclusions: The biochemical existence of HMG CoA reductase in pig pancreatic islets was confirmed. The expression of the inactivated form of HMG CoA reductase (p-HMG CoA reductase) was elevated under hyperglycemic conditions. SIM pre-treatment only enhanced insulin release from pancreatic islets bathed in normal but not high glucose medium suggesting that SIM may not provide beneficial effects in patients with T2DM.

Acknowledgements: This project was financially supported by GRF Grant (to YWK) (Reference number: 2410565).

P32.
THE FLAVONOID KAEMPFEROL ENHANCES SODIUM NITROPRUSSIDE-INDUCED RELAXATION IN PORCINE CORONARY ARTERIES VIA ACTIVATION OF POTASSIUM CHANNELS
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Kaempferol is a major flavonoid component of the Chinese medicine, Carthamus tinctorius, which has been used to treat cardiovascular diseases. Previous studies demonstrated that kaempferol potentiated sodium nitroprusside (SNP)-induced relaxation at concentrations that did not directly relax porcine coronary arteries. This study aimed to investigate the mechanisms of this vascular action of kaempferol using organ bath technique. In the presence of indomethacin, SNP-induced relaxation was significantly enhanced by kaempferol in porcine coronary arteries with and without endothelium. These potentiations were partially inhibited by iberiotoxin, a big conductance calcium-activated potassium channel (BKCa) blocker, but was not affected by TRAM-34 or UCL-1684, selective inhibitors of intermediate and small conductance calcium-activated potassium channels (IKca and SKca), respectively. Carbenoxolone, a gap junction inhibitor, and KT5720, a protein kinase A inhibitor, also did not affect the potentiation by kaempferol in arteries with or without endothelium. These findings suggest that kaempferol activates BKCa in vascular smooth muscle to enhance SNP-induced relaxation in porcine coronary arteries, while IKca, SKca, protein kinase A and gap junctional proteins do not play a role.
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