**Topic 5 – Diabetes, lipids, metabolism – A**

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0341

AMPK exerts an insulin-sensitizing effect on cardiac glucose uptake by multiple molecular mechanisms including cytoskeleton reorganization

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**Background:** Insulin-resistant cardiomyocytes are characterized by a decreased ability of insulin to stimulate glucose uptake. We have previously shown that the activation of AMPK by metformin or phenformin restores insulin-sensitivity in insulin-resistant cardiomyocytes. The aim of our present work is to understand by which molecular mechanisms AMPK exerts its insulin sensitizing effect. In this study we focused on the mTOR/p70S6K pathway and on cytoskeleton reorganization. mTOR/p70S6K, which is known to be inhibited by AMPK, is able to reduce insulin signaling via a negative feedback loop involving serine phosphorylation of IRS-1. On the other hand, cytoskeleton reorganization, which is a known target of AMPK, is responsible for the translocation of the glucose transporter GLUT4 to the plasma membrane.

**Methods:** Adult rat cardiomyocytes were primary cultured and treated with different agents including insulin, AMPK activator (phenformin), mTOR inhibitor rapamycin and/or actin cytoskeleton inhibitor latrunculin B. Glucose uptake was assessed by deitritiation of 2-3H-glucose.

**Results:** First, we tested if rapamycin was able to mimic AMPK activators. Similarly to phenformin, rapamycin increased the insulin-dependent phosphorylation of Akt involved in the regulation of glucose uptake. Despite the ability of rapamycin to induce this Akt over-phosphorylation, rapamycin was not able to restore the insulin-dependent stimulation of glucose uptake like phenformin did. On the other hand, latrunculin B abolished the insulin-sensitizing action of phenformin on glucose uptake, in insulin-sensitive as well as in insulin-resistant cells.

**Conclusions:** actin cytoskeleton reorganization but not mTOR/p70S6K, is involved in the insulin-sensitizing effect of AMPK on cardiac glucose uptake. The role played by Small G proteins, known to be involved in the regulation of actin cytoskeleton is under investigation.

0366

A role for focal adhesion kinase in the stimulation of glucose transport in cardiomyocytes

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**Background:** Stimulation of glucose transport in response to insulin or metabolic stress is an important determinant of cardiomyocytes function and survival, particularly during ischemia-reperfusion episodes. Stimulation of glucose transport is markedly impaired in cardiomyocytes exposed to free fatty acids (FA), despite relative preservation of insulin- or metabolic stress signaling.

**Aim:** To determine whether Focal Adhesion Kinase (FAK) activity is required for stimulation of glucose transport in cardiomyocytes, and whether FAK downregulation participates in FA-induced impairment of glucose transport stimulation.

0048

Deranged myofilament O’GlcNacylation and function in myocardium of obese patients

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The obesity-related cardiomyopathy is a poorly explained disease. There is no data available on myofilaments sensitivity to calcium and post-transla- 
tional modification or isoform shifts of sarcomeric proteins that could be 
involved in the contractile impairment. We conducted a study on obese 
and non obese human atrial trabeculae of the right atrium collected in the surgery 
room during cardiopulmonary bypass. We studied the contractile force of the 
trabeculae, the sensitivity of the myofilaments to Ca2+. Western blots were 
performed in order to explore the post-translational modification of sarco-
meric proteins including phosphorylation and O’GlcNacylation. Finally we explored the acute ex vivo effect of the modulation of O’GlcNac on the myofilament sensitivity to calcium. There was a significant contractile dysfunction in obese subjects compared to normal subjects, in both the force (p=0.01) and myofilaments sensitivity to calcium concerning the Fmax (p=0.03). No change in the expression of genes encoding sarcomeric proteins or enzymes involved in post-translational modification; nor phosphorylation modification of sarcomeric proteins like CML2 or cTnI in obese patients was observed. Conversely, we showed in obese patients a decreasing O’GlcNacylation of proteins of 25 kDa (p=0.007) and 130 kDa (p=0.03) which include the sarcomeric proteins MLC2 and Troponin I. Finally, we pointed out that alteration of the O’GlcNac level by Azaserin decreased the cardiac myofilaments sensitivity to calcium pCa50 (p=0.05). This study highlights before clinical and echocardiographic onset, an association between impaired contractile function, an altered sensitivity to calcium, and a decreased O’GlcNacylation of possible sarcomeric proteins in humans.

Abstract 0048 – Figure: Level of O’GlcNac of myofilaments proteins

0470
Role of Lipocalin 2 (LCN2) in cardiovascular remodeling induced by aldosterone


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Neutrophil Gelatinase Associated Lipocalin or Lipocalin 2 (LCN2) is a circulating protein, member of the lipocalin family, which binds MMP9 and modulates its stability and activity. We have recently shown that LCN2 could be a mediator of aldosterone/MR fibroproliferative and proinflammatory effects in the cardiovascular system. Wild type (WT) and LCN2 Knock Out (KO) mice were subjected to an un-in nephrectomy aldosterone salt challenge (NAS, 200μg/kg/day of ald, 1% NaCl in tap water) for 4 weeks. Blood pressure (SBP) was measured by tail cuff method. Cardiomyocyte fibrosis and inflammation were analyzed by RT-PCR, western blot, immunohistochemistry and ELISA. There was no difference in SBP between transgenic mice compared to WT mice in basal condition. With NAS challenge, SBP was increased only in WT mice in basal condition (SBP: CT 107±3, CT NAS 135±3, KO 109±3, KO NAS 115±3 mmHg). Quantification of pro collagen I N-terminal peptide (PINP) in plasma showed an increase of PINP due to NAS treatment in WT that was prevented by LCN2 inactivation (CT 83±15, CT NAS 129±10, KO 70±19, KO NAS 59±12 μg/l). In myocardium, NAS treatment increased collagen type I and perivascular fibrosis in WT whereas KO were resistant to fibrosis (Collagen Volume Fraction; CT 19±3, CT NAS 28±2, KO 20±3, KO NAS 20±3%). In aorta, collagen type I, vascular fibrosis and osteopontin were also increased by NAS in WT. These increases were prevented by LCN2 inactivation (CVP; CT 24±4, CT NAS 34±5, KO 21±2, KO NAS 28±2%). Our results show that LCN2 plays a key role in aldosterone/MR-mediated vascular fibrosis and inflammation, but not in cardiac interstitial fibrosis and vascular dysfunction. We are now analyzing 1) the specificity of ald/or MR versus other pro-fibrotic challenges (AngII, catecholamines) as well as 2) the role of inflammation in the effects mediated by Lcn2.

0253
Sympathetic overactivity: a very early manifestation of metabolic syndrome

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Sympathetic overactivity is a hallmark of metabolic syndrome (MS). Data showing sympathetic overactivity in patients with early cardiovascular and metabolic disorders, i.e., prodromal forms of MS, are lacking. Sympathetic activity was measured by microneurography (MSNA), heart rate variability (HRV), blood pressure variability (BPV) and plasma and urinary catecholamines. β1-adrenergic receptor (β1-AR) and the G-protein coupled receptor kinase 2 (GRK2) mRNA expression levels were tested as possible markers of sympathetic activity in blood mononuclear cells (PBMCs). 40 healthy volunteers and 16 patients with established (3 components of the MS) and 23 incomplete (2 components) MS were compared. MSNA was not only increased in patients with overt MS but also among patients with incomplete MS (P<0.001). In PBMCs of patients with incomplete MS, a significant 3.4 fold increase in the β1-AR over GRK2 mRNAs expression ratio was observed (P=0.001); this ratio correlated well with MSNA. (Funded by the Clinical Research program of the French Ministry of Health)

0426
Admission glycemia: the crystal ball to assess prognosis value after STEMI

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Introduction: High glycemia at admission in STEMI patients is common and associated with an increased risk of in-hospital and post-discharge death.

Aim: To evaluate the impact of admission glycemia in the short prognosis of diabetic and non-diabetic patients admitted for STEMI and to identify independent predictors of post-ACS mortality.

Population and methods: This study included 567 patients admitted to a single coronary care unit for STEMI, between January 2004 and June 2012. Our population was divided in three groups according to the tertiles of glycemia at admission (T1<7; T2=7-11 e T3>11 mmol/l). Rates of success after revascularisation, in-hospital mortality, and ventricular arrhythmias were collected

Results: Hyperglycemia at admission was associated to worse cardiovascular risk profile, more severe coronary disease (more 3 vessel disease), incomplete revascularization, higher creatinine levels and more life threatening ventricular arrhythmias (VT/VF). In the predefined tertiles, in-hospital mortality was 4%, 5.2% and 14% (p<0.001). Life threatening arrhythmias were respectively 2.4%; 4% and 9% (p<0.001). In the group of patients with glycemia >11 mmol/l (224 patients), outcomes were similar between the diabetic and non diabetic patients with death rates respectively 13.8% and 13.9% (p=0.988). In a multivariate analysis, predictor factors of in-hospital mor.