

## TOPIC 06 – Diabetes, dyslipidemia, metabolism

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0094

### Chronic CT-1 treatment impairs stimulation of glucose transport in cardiomyocytes

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**Aims:** Cardiotrophin-1 (CT-1), a member of Interleukine-6 (IL-6) family, causes insulin resistance in adipocytes. However its effect on glucose metabolism in the heart remains unknown. Thus we proposed to test whether CT-1 induces insulin resistance in cultured cardiomyocytes.

**Methods:** Rat cardiomyocytes were cultured in presence or absence of 1nM CT-1. Thereafter glucose transport was measured in response to insulin or Oligomycin, a mitochondrial ATP synthase inhibitor, used as a surrogate of metabolic stress. Intracellular signaling triggered by glucose transport stimuli was analyzed by Western-Blot.

**Results:** Cardiomyocytes stimulated with increasing dose of insulin exhibited a dose response of glucose transport. However, when treated with CT-1 the response of relative glucose transport was markedly, indicating reduction in insulin responsiveness. CT-1 also reduced oligomycin-stimulated glucose transport. By Western-Blot we found lower phosphorylation of the signaling intermediates IR, Akt, and AS160 in CT-1 treated cardiomyocytes.

**Conclusion:** Chronic exposure of cardiomyocytes to CT-1 leads to a loss in the flexibility of glucose metabolism, by mechanisms that may involve a role for SOCS3 and STAT5, known insulin resistance inducers.

0378

### AMPK exerts an insulin-sensitizing effect on cardiac glucose uptake by multiple molecular mechanisms

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Insulin-resistant cardiomyocytes are characterized by a decreased ability of insulin to stimulate Akt phosphorylation and glucose uptake. We have previously shown that the activation of AMPK by 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. Indeed, this pathway is known to reduce insulin signaling *via* a negative feedback loop involving serine phosphorylation of IRS-1. As AMPK inhibits p70S6K, it is commonly accepted that AMPK increased insulin-sensitivity by inhibiting this p70S6K-mediated negative feedback loop. To confirm this hypothesis, we tested if rapamycin, a specific inhibitor of the mTOR/p70S6K pathway, was able to mimic AMPK activators. Similarly to phenformin, rapamycin increased the insulin-dependent phosphorylation of Akt and its substrate AS160, involved in the regulation of glucose uptake. Despite the ability of rapamycin to induce this Akt/AS160 over-phosphorylation, rapamycin was not able to restore the insulin-dependent stimulation of glucose uptake like phenformin did. In view of this result showing that the inhibition of the mTOR/p70S6K pathway had no effect on insulin-stimulated glucose uptake, we used leucine known to activate the mTOR/p70S6K pathway. The leucine-dependent p70S6K over-phosphorylation induced the stimulation of the negative feedback loop and the inhibition of both PKB and AS160 phosphorylation but did not change the insulin-stimulated glucose uptake. In conclusion, we showed a discrepancy between the regulation of the PKB/AS160 pathway and of glucose uptake in cardiomyocytes. Moreover, the inhibition of the p70S6K/IRS-1-mediated negative feedback loop is not sufficient to

explain the insulin-sensitizing effect of AMPK on glucose uptake demonstrating that AMPK exerts its insulin-sensitizing effect by multiple molecular mechanisms.

0376

### Angiotensin type II receptor (AT2R) increases reactive oxygen species levels and COX-2 derived vasoconstrictor prostanoids in diabetic mice resistance arteries

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**Background and objectives:** The renin-angiotensin system (RAS) is a key regulator of cardiovascular physiopathology. The effects of angiotensin II are mainly mediated by two receptors: Angiotensin type 1 (AT1R) and type 2 (AT2R) receptors. AT1R is well known for its hypertensive and trophic effects leading to most cardiovascular diseases. AT2R is described as opposing the effects of AT1R, with vasodilatory and antitrophic properties. AT2R opposes angiotensin II-dependent hypertension in female rats, not in males. We have also previously shown (Hypertension. 2010;55:339-44) that AT2R stimulation in mesenteric arteries from Zucker diabetic fatty (ZDF) rats increases reactive oxygen species (ROS) level and activates a COX-2-dependent production of thromboxane A2. Thus we aimed to determine the role of AT2R in endothelium-mediated dilation in a mouse model of diabetes using both male and female animals.

**Methods:** Male and female wild-type (WT) and AT2R KO mice were injected with Streptozotocin (150mg/kg) in order to induce diabetes. After 45 days, mice were sacrificed and mesenteric resistance arteries mounted on a wire myograph.

**Results:** Acetylcholine induced a concentration-dependent relaxation. Diabetes induced a reduction in relaxation from 80±5% to 71±9% in WT female but not significantly in WT male. Blockade of AT2R (PD123319), ROS reduction (tempol + catalase) or COX2 inhibition (CAY10404) improved significantly relaxation in diabetic male and female mice arteries. In mesenteric arteries from diabetic AT2R KO mice, acetylcholine-mediated relaxation was equivalent to that in non-diabetic mice in both male and female animals. Phenylephrine-mediated contraction and endothelium-independent relaxation to sodium nitroprusside were not affected.

**Conclusions and perspectives:** AT2R opposes endothelium-dependent relaxation in a mouse model of type 1 diabetes, through the activation of ROS and COX2-derived prostanoid(s) without major difference between male and female mice.

0176

### Gender differences in type 2 diabetes: experimental study in the type 2 diabetic Goto-Kakizaki (GK) rat heart

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**Background:** Aging and diabetes in women increase their susceptibility to myocardial ischemic injury but the molecular mechanisms involved are not well understood. Consequently, we have investigated the effect of gender on cardiac function, energy metabolism, endothelial function and NO pathway in the aging type 2 diabetic Goto-Kakizaki (GK) rat heart.

**Materials and Methods:** Age-matched (8 months) Control Wistar (male n=11, female n=9) and GK (male n=11, female n=12) isolated rat hearts were perfused during 28min with a physiological Krebs-Henseleit buffer containing 0.4mM palmitate, 3% albumin, 11mM glucose, 3U/L insulin, 0.8mM lactate and 0.2mM pyruvate before freeze-clamping for biochemical assays. High energy phosphate compounds and intracellular pH (pHi) were followed using 31P magnetic resonance spectroscopy with simultaneous measurement

of contractile function. NO pathway was studied by total nitrate concentration (NOx) as well as total level and phosphorylation of eNOS and Akt. In parallel, endothelium-dependent and independent vasodilatations were measured in other hearts (male Control n=8, male GK n=8, female Control n=10, female GK n=11), using 5-hydroxytryptamine and papaverine to assess endothelial and smooth muscle functions. Results: Phosphocreatine, ATP, PME and Pi were not significantly different in Control and diabetic groups. Total pool of creatine (creatine and phosphocreatine) and total adenine nucleotides content were similar in all groups. Myocardial function was significantly impaired in male and female diabetic *versus* Control groups ( $p < 0.05$ ). Endothelium-dependent and independent vasodilatations were not different in male Control and GK rat hearts. By contrast, endothelium-dependent and independent vasodilatations were significantly impaired in female GK compared with male GK ( $p < 0.05$ ) and female Control ( $p < 0.05$ ) rat hearts. **Conclusion:** We reported here an impaired endothelial and smooth muscle functions but normal energy metabolism in the female GK rat hearts. These results could be related to higher risk of cardiovascular disorders in type 2 diabetic female.

### 0307

#### Aldosterone protects the insulin-activated AKT pathway in heart of diabetic type 2 mice

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**Introduction:** The pathogenesis of diabetic cardiomyopathy is not fully elucidated. Numerous factors may contribute to the development of heart failure in a diabetic context, including the scarcity of microvasculature and neuro-humoral dysregulation, particularly the renin-angiotensin-aldosterone-system. We have previously shown that a modest increase of intracardiac aldosterone prevents the development of cardiomyopathy in mice with type 1 diabetes, possibly through a prevention of cardiac capillary dropout (Messaoudi et al, FASEB J 2009). This study aimed to determine the pathophysiological effects and to study transduction pathways of insulin in the case of a cardiac hyperaldosteronism with or without type 2 diabetes (T2D).

**Methods:** 3 week-old mice overexpressing aldosterone synthase (AS), n=9, and their wild-type (WT) n=5 were fed a high fat, high sucrose diet (HFHSD) or a standard diet *ad libitum*. After 4 months of diet, glucose and insulin tolerance tests were performed. Mice were sacrificed and tissue samples were used for RT-PCR. In addition, to analyze the signaling pathways dependent of the insulin receptor and insulin growth factor receptor, some of these mice (n=5) received a dose of insulin (1UI/kg bw) 30 minutes before the sacrifice.

**Results:** After 4 months of HFHSD, both WT-D and AS-D mice had hyperglycemia (+55%, 56%,  $P < 0.05$  vs WT and AS, respectively) and body overweight (+20%,  $P < 0.001$ ; +30%,  $P < 0.05$  vs WT and AS, respectively). Both WT and AS displayed glucose intolerance and insulin resistance. Surprisingly, VEGFa and insulin receptor substrate 1 (IRS1) mRNAs were upregulated in AS-D mice (+44%,  $P < 0.05$  vs WT-D; +20%  $P < 0.05$  vs AS). Besides, NOS3 (Nitric Oxide Synthase 3) and IRS2 were increased in both diabetic groups. In basal conditions, as shown by the decreased p-AKT/AKT ratio on Western blot the AKT kinase activity was decreased in hearts of WT-D mice only. Interestingly, acute stimulation of AKT by insulin revealed an increase of the p-AKT/AKT ratio in AS-D only.

**Conclusion:** The results indicate that in mice developing T2D the AKT signaling pathway in heart could be stimulated by insulin only when cardiac hyperaldosteronism was present. This aldosterone-dependent activation of the AKT pathway might play a role in the induction of VEGFa in heart.

### 0057

#### Effects of a multidisciplinary rehabilitation program in diabetics patients with peripheral arterial disease

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**Aim:** Peripheral arterial disease (PAD) is a peripheral arterial complication resulting in a decrease in the arterial calibre. Despite the importance of its prevalence and morbid-mortality, PAD remains too often underestimated and is insufficiently managed. Rehabilitation is recommended as a reference treatment during PAD. This study examines the impact of a multidisciplinary rehabilitation program on some vascular and hemodynamic parameters, on glycemic control and on levels of anxiety and depression in PAD patients.

**Methods:** 58 patients with diabetic and peripheral arterial disease (PAD) were enrolled in a 6 week tailored multidisciplinary cardiac rehabilitation program. This program includes medical supervision, educational sessions (7 hours per week) and adapted physical activity (13 hours per week).

Glycated haemoglobin, fasting blood glucose, small artery elasticity index, ankle brachial index, distance performed during a 6min walk test (6MWT), performance during progressive exercise on treadmill and the level of anxiety and depression were measured before and at the end of their cardiac rehabilitation.

**Results:** The cardiac rehabilitation program increased significantly distance performed at the 6MWT ( $p = 0.038$ ), performance during the progressive exercise ( $p = 0.005$ ), the ankle brachial index right ( $p = 0.003$ ) and left ( $p = 0.001$ ), small artery elasticity index ( $p < 0.001$ ), and decreased significantly glycated haemoglobin ( $p = 0.008$ ), fasting blood glucose ( $p = 0.003$ ), level of anxiety ( $p < 0.001$ ) and the level of depression ( $p = 0.002$ ).

**Conclusion:** The cardiac rehabilitation program had a significant positive effect on some vascular and hemodynamic parameters, the control of glycaemia and the levels of anxiety and depression of PAD patients. These parameters are considered prognosis. In view of our results and recent data from the literature, we can think that exercise in rehabilitation could be involved in development of collateral circulation.

### 0303

#### The efficacy of treatment with alpha-lipoic acid in the evolution of metabolic syndrome in rats

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**Objectives:** The metabolic syndrome (MS) is frequently met nowadays, being characterized by a perturbation of glucides' and lipids' metabolism in association with free radicals' synthesis and an oxidative stress installation. Alpha-lipoic acid (AL) is an endogenous antioxidant which is able to scavenge free radicals, chelate transition metals (iron and copper) or regenerate the reduced forms of the same antioxidants (vitamin E, vitamin C and glutathione). The purpose of this study was to monitor the benefits provided by an antioxidant therapy with alpha-lipoic acid in rats which present symptoms of MS.

**Methods:** 48 male Sprague-Dawley rats were randomized into two series: rats fed for 3 months with standard chow (Control) or with standard chow supplemented with fructose (60%). In each series, a group of rats was treated intraperitoneally during 14 days/month with NaCl 0.9% and another group with 50mg/kg/day AL. Throughout the treatment the body weight, glycaemia and systolic blood pressure were being monitored. At the end of the 3 months, we assessed: 1) plasmatic lipid profile, 2) products of lipid peroxidation: malondialdehyde (MDA) and 3) the endogenous antioxidant status: total glutathione.

**Results:** The glycaemia and the systolic blood pressure had significantly ( $p < 0.05$ ) increased in the fructose-fed rats since the 2 weeks of diet. After 3 months of fructose-enriched diet, the level of triglycerides (x5.35 times) and LDL-Cholesterol (x7.82 times) was significantly ( $p < 0.001$ ) higher as compared to the Control group, without influencing the rats' body weight. Also, the level of MDA and oxidized glutathione (GSSG) had significantly ( $p < 0.05$ ) increased. At the same time, in fructose-fed rats treated with AL, the MS characteristic parameters were reduced: glycaemia ( $119.92 \pm 2.43$  vs  $126.33 \pm 1.91$  mg/dl,  $p < 0.05$ ), systolic blood pressure ( $123.95 \pm 0.95$  vs  $132.98 \pm 1.15$  mmHg,  $p < 0.01$ ), triglycerides ( $151.73 \pm 8.32$  vs  $331.20 \pm 23.15$  mg/dl,  $p < 0.001$ ) and LDL-Cholesterol ( $35.08 \pm 4.70$  vs  $81.00 \pm 18.33$  mg/dl,  $p < 0.01$ ). A decrease in lipid and glutathione oxidation was also noted.

**Conclusions:** In our experimental conditions, the MS disturbances and the oxidative stress associated were significantly ameliorated by an antioxidant therapy with alpha-lipoic acid.

## 0372

### Physiological concentration of glutamine increases exogenous fatty acid utilization in the ex vivo working heart: Role of the hexosamine biosynthetic pathway

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Glutamine (GLN) is the most abundant amino acid in the plasma, and has been shown to exert cardiac effects but the underlying mechanisms remained unclear. We assessed the metabolic effects of a physiological concentration of GLN and tested the involvement of the following mechanism: i) anaplerosis (ANA) via conversion to citric acid cycle (CAC) intermediates or ii) activation of the hexosamine biosynthetic pathway (HBP). Working rat hearts were perfused with physiological concentrations of <sup>13</sup>C-labeled glucose and a long chain fatty acid (LCFA; oleate) with or without 0.5mM GLN under various conditions. We measured include functional and metabolic flux as well as tissue levels of CAC intermediates. GLN had little impact in working hearts, but reversed the decreased cardiac output (65%) induced by restricted CHO supply (without pyruvate and insulin to enhance demand on GLN ANA). At the metabolic level, our data do not support a role of GLN as ANA substrate: i) measured tissue levels of CAC intermediates, which were decreased (control) or unaffected (low CHO) by GLN addition, or ii) the absence of <sup>13</sup>C-labeling of these CAC intermediates when hearts were perfused with [U-<sup>13</sup>C]GLN. GLN addition increased the contribution of oleate β-oxidation to acetyl-CoA (+51%) and to triglyceride formation (+180%) albeit only under the control condition. This effect cannot be explained by mechanisms regulating β-oxidation, namely level of malonyl-CoA, acetyl-CoA carboxylase or AMP kinase (by Western). The effect on oleate oxidation was reversed by the addition of 20 μM azaserine an HBP inhibitor. In hearts from spontaneously hypertensive rats, (with a defective CD36 - a LCFA transporter (restricted exogenous LCFA utilization), GLN addition did not increase exogenous oleate utilization. These results demonstrate that a physiological concentration of glutamine can modulate energy substrate selection in the heart. The metabolic effect appears to involve the HBP and CD36 rather than ANA.

## 0067

### LDL-cholesterol lowering effect of a dietary supplement with plant extracts on moderate hypercholesterolemia subjects: a randomized controlled study

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**Purpose:** Red Yeast Rice (RYR), Sugar Cane-derived Policosanols (SCdP) and Artichoke Leaf Extracts (ALEs) are currently incorporated alone or in combination into dietary supplements for their potential Low-density-Lipoprotein-Cholesterol (LDL-C) lowering effects. To date, there is no information supporting the efficacy of this association on the reduction of LDL-C. The main objective of this study was to investigate the effects of a new dietary supplement with RYR, SCdP and ALEs on LDL-C on moderate untreated hypercholesterolemic subjects. The specificity of this supplement is the use of low doses of monacolins and ALEs to limit adverse effects.

**Methods:** It is a mono-centric, double-blind, placebo-controlled, parallel group study. Thirty-nine hypercholesterolemic subjects were randomly assigned to 2 groups, one consuming a Dietary Supplement (DS) containing RYR, SCdP and ALEs and the other consuming a placebo, during 16 weeks. Plasma levels of lipids (LDL-C, Total Cholesterol (TC), high-density-lipoprotein cholesterol, triacylglycerols (TG)), vitamins C and E, total polyphenols and malondialdehyde were determined at baseline and after 4, 8, 12 and 16 weeks of supplementation.

**Results:** LDL-C and TC were significantly reduced by respectively 21.44±9.75% and 14.05±8.12% at week 16 compared to baseline in DS group. Similar results were obtained already at weeks 4, 8 and 12. TG were decreased significantly at week 16 (-12.16±25.37%) in DS group. For the vitamin E/TC ratio, a meaningful difference was observed between groups at week 16. Other parameters were not modified substantially. Compliance was high (>91%) and products were well tolerated.

**Conclusions:** Daily consumption of a dietary supplement with a combination of RYR, SCdP and ALEs decreased significantly LDL-C and TC and is therefore an interesting and convenient aid in managing mild to moderate hypercholesterolemia.

**Trial registration:** ClinicalTrials.gov, www.clinicaltrials.gov, NCT01354327.