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Metabolism

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Research advances in metabolism 2015

1. Introduction

In this era of numerous publications in the field of Metabolic Medicine, we have carefully selected and present here the most interesting and important original research studies published in *Metabolism* during 2015. They cover the whole spectrum of research -from cell and animal to human studies- and reflect the most current advances. These papers also provide a good base for new ideas, as they prompt the readers not only to expand their clinical research knowledge but also to hopefully incentivize them to design future research projects.

2. Experimental Studies

2.1. *Novel Therapeutic Agent with Multi-Targeted Anti-Diabetic Potential*

Lei et al [1] examined in mice the therapeutic effect of a novel agent, named SHP289-03, with dual activating action on both peroxisome proliferator activated receptor-γ (PPARγ) and glucokinase (GK), an important enzyme for glycolysis. The investigators assessed the glucose metabolism on hepatic cell lines from normal mice and they showed that SHP289-03 increased the glucose uptake significantly. Furthermore, they used pancreatic cells form mice without diabetes, both normal and insulinoma-derived, and showed that SHP289-03 stimulated the insulin secretion by increasing intracellularly the concentrations of ATP and calcium. Mice with experimentally induced type 2 diabetes were finally included in the study to assess various aspects of the new agent pharmacodynamics. It was found that SHP289-03 improved insulin sensitivity and subsequently reduced glucose levels with parallel reduction of lipid levels. The gene for GK was shown to be upregulated, while an increase in beta cell mass was observed too. The simultaneous multiple effects of this novel agent on insulin sensitivity, insulin secretion and lipid metabolism, without observation of serious side effects like hypoglycemia or weight gain provide hope for a new generation of anti-diabetic agents. Further experimental and possibly human studies will elucidate if such a drug will enrich in the future the therapeutic armamentarium for type 2 diabetes.

2.2. *Vildagliptin Reduces Apoptosis and Endoplasmatic Reticulum Stress in Mice*

DPP-4 (dipeptidyl peptidase-4) inhibitors are broadly used in the treatment of patients with type 2 diabetes, as they inhibit and delay the catabolism of the endogenous incretins. It has been also suggested that these agents may have beneficial actions on beta cells, reducing their apoptosis. Wu et al [2] sought to investigate if such a DPP-4 inhibitor, vildagliptin, has any effect on the endoplasmatic reticulum stress which is thought to precede the apoptosis of beta cells. Diabetic mice were randomized to receive orally vildagliptin or placebo for 6 weeks. After six weeks of treatment, the diabetic mice from vildagliptin group presented higher levels of plasma GLP-1 (22.63 ± 1.19 vs 11.69 ± 0.44, $p < 0.001$), while apoptosis of beta pancreatic cells, evaluated both with the TUNEL technique

Abbreviations: CRP, C-reactive protein; HDL, high density lipoprotein; LDL, low density lipoprotein; PPARγ, peroxisome proliferator activated receptor-γ; DPP-4, dipeptidyl peptidase-4; GLP-1, glucagon like peptide-1; TRIB3, tribbles homolog 3; ATF-4, activating transcription factor 4; SIRT1, sirtuin-1; LKB-1, liver kinase B1; CREB, cAMP response element binding protein; CK, creatine kinase; UP1, Uncoupling Protein 1; DHEA, Dehydroepiandrosterone; AMPK, AMP-activated protein kinase; PKC-δ, Protein Kinase C–δ; TNF, tumor necrosis factor; NAFLD, non-alcoholic fatty liver disease; DGAT2, diacylglycerol acyltransferase 2; CPT1, carnitine palmitoyltransferase 1; PBMCs, peripheral blood mononuclear cells; OGTT, oral glucose tolerance test; MetS, metabolic syndrome; MRI, magnetic resonance imaging; PCOS, polycystic ovary syndrome; PWS, Prader-Willi syndrome; PCI, percutaneous coronary intervention; VAT, visceral adipose tissue; BMI, body mass index; AUC-ROC, area under the curve–receiver operating characteristic; ATP III, Adult Treatment Panel III; PPL, postprandial lipemia; GWAS, Genome Wide Association Study; ET-1, endothelin-1; BAT, Brown adipose tissue; FFDG, fluoro-deoxy-D-glucose; HR, hazard ratio; CI, confidence interval; HOMA-IR, homeostatic model assessment-insulin resistance; eGFR, estimated glomerular filtration rate; COX-IV, cytochrome C oxidase-subunit 4; STAT-3, signal transducer and activator of transcription 3; LPS, lipopolysaccharides.

(0.37 ± 0.03 vs. 0.55 ± 0.03, $p < 0.01$) and the caspase 3 activity (1.48 ± 0.11 vs 2.67 ± 0.13, $p < 0.01$), was significantly reduced. These differences were accompanied by down-regulation of various genes associated with endoplasmatic reticulum stress, which was assessed by quantitative RT-PCR or immunohistochemistry. Specifically, tribbles homolog 3 (TRIB3), activating transcription factor 4 (ATF-4) and C/EBP homologous protein (CHOP) genes were significantly less activated in diabetic mice treated with vildagliptin (in all comparisons $p < 0.001$). These findings confirm the beneficial effects of DPP-4 inhibitors on beta-cell survival, highlighting a possible mechanism which involves the endoplasmatic reticulum.

2.3. *Ezetimibe Directly Stimulates GLP-1 Secretion and Improves Glycemic Control*

Ezetimibe is used as a second line therapy for dyslipidemia, but very recent experimental data indicate that it may have a beneficial effect on glycemic control too, by increasing the levels of GLP-1. Chang et al [3] aimed to investigate if this increase is the result of direct stimulation of the secretion of GLP-1 or of inhibition of its endogenous catabolism by activation of the enzyme DPP-4. Mice with type 2 diabetes were divided into groups which received or not ezetimibe for 6 weeks. Treatment with ezetimibe resulted in reduction of glucose levels and insulin resistance, while concentrations of both serum and intestinal GLP-1 were increased too. In vivo and in vitro DPP-4 inhibition assays were also performed and did not reveal any inhibition of this enzyme. Then, the detailed molecular mechanisms were examined with the use of a human enteroendocrine cell line. It was shown that the increase in GLP-1 levels was accompanied by activation of the MEK/ERK (mitogen activated protein/extracellular signal-regulated kinase) signaling pathway. Furthermore, when this pathway was experimentally inhibited, the GLP-1 levels increase by ezetimibe was lost. These novel findings confirm the beneficial effect of ezetimibe in glycemic control and elucidate the mechanism of this action, suggesting a possible new promising role for ezetimibe in the prevention or treatment of type 2 diabetes.

2.4. *High Fat Diet Induces Cardiac Hypertrophy and Fibrosis in Mice*

Wang et al [4] set out to investigate the impact of high fat diet (HFD) on the cardiac muscle of mice. They randomly assigned mice to high fat diet, intermittent fasting and normal chow and collected the left ventricles of their hearts 11 months later. Tissue extracted from mice fed with HFD displayed increased cardiomyocyte cross-section area, widely distributed fibrosis and greater weight compared to control mice. In the molecular level, active caspase 3 and the ratio of microtubule-associated protein 1A/1B-light chain 3 (LC3 II/LC3I) were decreased, indicating attenuation of apoptosis and autophagy cellular pathways. On the other hand, phosphoglycogensynthase kinase-3β (GSK-3β) phosphorylated at Ser9 (inactivated form) was found to be increased as were two of its targets, nuclear GATA-binding protein 4 and YES-associated protein (YAP), which function as transcriptional factors inducing cardiac hypertrophy. Even though mice in the intermittent fasting group displayed increased active caspase 3 and decreased ratio of LC3II/LC3I, no other molecular phenomenon or indicator of hypertrophy was observed. These findings elucidate possible mechanisms underlying cardiac hypertrophy and outline the direct detrimental effects of high fat diet on the heart muscle.

2.5. *Treatment with Curcumin Enhances the Effect of Exercise on Mitochondrial Biogenesis*

Curcumin is a natural antioxidant with potential therapeutic uses. Hamidie et al [5] aspired to inquire into the combined effect of curcumin and endurance exercise (endurance training-eTR) on mitochondrial biogenesis in the skeletal muscle. They randomly assigned ten-week-old male Wistar rats to either the eTR or the non-eTR group and injected them with either low (50 mg kg^{-1} day^{-1}) or high (100 mg kg^{-1} day^{-1}) doses of curcumin intraperitoneally for 28 days. Subsequently Western blotting and immunoprecipitation were used to track selected molecular biomarkers. The expression of COX-IV (cytochrome C oxidase-subunit 4), OXPHOS (oxidative phosphorylation) subunits, mitochondrial DNA copy number and CS (citrate synthase) activity in the gastrocnemius and soleus muscles, AMPK (AMP activated protein kinase) phosphorylation, NAD + 7/NADH (nicotine amide adenine dinucleotide) ratio, SIRT1 (sirtuin-1) expression, and PGC-1α8 (peroxisome proliferator-activated receptor gamma coactivator-1alpha) deacetylation were found to be increased in the eTR group, indicating activation of mitochondrial biogenesis. Moreover, curcumin seemed to display a cumulative to the eTR effect on the aforementioned biomarkers proliferation. Curcumin increased the levels of cAMP and phosphorylation of CREB (cAMP response element binding protein) and LKB-1 (liver kinase B1), which are all involved in the regulation of cellular production of mitochondria. As curcumin seems to amplify the effect of exercise on the triggering of mitochondrial biogenesis, a potential future therapeutic role could be attributed to this new molecule.

2.6. *Increased Irisin Levels in Mice with Thyroid Dysfunction*

Irisin is a new myokine related to exercise and thought to participate in the regulation of body energy expenditure. As thyroid hormones play a critical role in thermogenesis and metabolic rate adjustments, Samy et al [6] investigated the possible changes in the levels of irisin in male rats with experimentally induced hyperthyroidism and hypothyroidism. They also sought such changes after acute or chronic swimming exercise. Sedentary rats both with hyperthyroidism and hypothyroidism presented higher levels of irisin compared to euthyroid ones (45% increase, $p < 0.001$ and 30% increase, $p < 0.001$, respectively). Acute forced swimming lead to increase of irisin levels ($p < 0.001$), while chronic swimming did not result in any changes. Irisin levels were positively correlated with CK (creatine kinase) a marker of muscle damage or myopathy, conditions observed after exercise or in patients with thyroid disorders. Irisin was not found to be associated with any other metabolic parameters, such as glucose, insulin resistance or lipids. Further studies are needed to confirm the association between thyroid dysfunction and irisin levels, as