

**SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)**

**NOVEL METABOLIC EFFECTS OF GLYCOGEN  
PHOSPHORYLASE INHIBITORS**

Lilla Nikoletta Nagy



UNIVERSITY OF DEBRECEN  
DOCTORAL SCHOOL OF MOLECULAR MEDICINE

DEBRECEN, 2017

**SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PhD)**

**NOVEL METABOLIC EFFECTS OF GLYCOGEN  
PHOSPHORYLASE INHIBITORS**

Lilla Nikoletta Nagy

Supervisor: Dr. Péter Bay



UNIVERSITY OF DEBRECEN  
DOCTORAL SCHOOL OF MOLECULAR MEDICINE

DEBRECEN, 2017

# **NOVEL METABOLIC EFFECTS OF GLYCOGEN PHOSPHORYLASE INHIBITORS**

By Lilla Nikoletta Nagy, Molecular biology MSc degree

Supervisor: Péter Bay, PhD, DSc

Doctoral School of Molecular Medicine, University of Debrecen

Head of the **Examination Committee:** László Csernoch, PhD, DSc

Members of the Examination Committee: Szabolcs Sipeki, MD, PhD

András Mádi, PhD

The Examination takes place at Library of Department of Physiology, Faculty of Medicine, University of Debrecen, September 26, 2017 at 11:00 am.

Head of the **Defense Committee:** László Csernoch, PhD, DSc

Reviewers: András Balla, PhD

Péter Fülöp, MD, PhD

Members of the Defense Committee: Szabolcs Sipeki, MD, PhD

András Mádi, PhD

The PhD Defense takes place at the Lecture Hall of Bldg. A, Department of Internal Medicine, Faculty of Medicine, University of Debrecen, September 26, 2017 at 1:00 pm.

## **THEORITICAL BACKGROUND**

### **Glycogen metabolism**

Glycogen is a highly branched polymer of D-glucose that has a dominant role in glucose storage. When glucose is plentiful in the cell, it is stored as glycogen through a process called glycogenesis, which is catalyzed by glycogen synthase (GYS) enzyme in collaboration with the branching enzyme. When cells need glucose, glycogen can be converted back to glucose in the counter-process termed glycogenolysis that is catalyzed by two enzymes, glycogen phosphorylase (GP) and the debranching enzyme. GP occurs in three isoforms with different physiological roles encoded by separate genes. The muscle isozyme (PYGM) supports muscle contraction, the liver isotype (PYGL) plays a central role in regulation of blood glucose homeostasis, and finally the brain isoform (PYGB) expressed in adult brain and embryonic tissues, which is responsible for glucose supply in case of anoxia or hypoglycemia. Nevertheless, all GP isoforms catalyze the same reaction where GP hydrolyses monosaccharide units from the non-reducing ends of glycogen, which is the rate limiting step of glycogenolysis. Each sugar unit is released as G1P that can readily enter into catabolic pathways or can be converted to G6P providing substrate for ATP producing processes such as glycolysis. However, in the liver, glucose 6-phosphatase can remove the phosphate group of G6P in order to enable the transport of glucose through cell membrane into the bloodstream to support other tissues. This process is called hepatic glucose production (HGP).

### **Glycogen phosphorylase enzyme and its inhibition**

The dimeric GP enzyme can be phosphorylated by phosphorylase kinase (PhK) that makes the switch between two interconvertible forms of GP: a phosphorylated, normally active GP<sub>a</sub> and a dephosphorylated, normally inactive GP<sub>b</sub>. Conversely, protein phosphatase 1 (PP1) inactivates GP by dephosphorylation bringing about the inactive GP<sub>b</sub> form. GP enzyme bears seven binding sites: catalytic site, inhibitor site, allosteric site, glycogen storage site, newly discovered allosteric or indole binding site, benzimidazole site and finally, quercetin binding site. Each binding site offers potential target for allosteric GP modulation by pharmacological agents except for the glycogen storage site. High energy substrates, such as ATP, glucose and G6P are considered the physiological inhibitors of GP. GP inhibitors mimicking the action of glucose and G6P or bind to other sites acting synergistically with glucose are expected to inhibit glycogenolysis and stimulate

glycogenesis, the oppositely regulated process. Not surprisingly, there is a great deal of glucose analogue GP inhibitors. Glucose analogue inhibitors primarily bind to the catalytic site thus blocking access of the substrate to the catalytic site, however, these molecules were reported to bind to the allosteric site of GP, too. N-acetyl- $\beta$ -D-glucopyranosylamine was among the first efficient glucose analogue inhibitors. The glycogen phosphorylase inhibitors KB228, BEVA33, TH, NV50 and NV76 are glucose analogue compounds synthesized by the laboratory of László Somsák at Department of Organic Chemistry, University of Debrecen. CP-316819 has been reported to bind the novel allosteric site by enhancing the dephosphorylation of GP, converting it to the inactive form. CP-316819 belonging to the indole-2-carboxamide series of GP inhibitors and completed phase I in clinical trials confirming the safety of these drugs. Therefore, it was selected as a reference GP inhibitor in our studies.

GP, catalyzing glycogen breakdown, has a major contribution to HGP, therefore GP became a validated target to modulate glucose levels in type 2 diabetes mellitus and pharmacological GP inhibitors (GPis) are considered as potential antidiabetic agents.

### **The opposite regulation of glycogen phosphorylase and glycogen synthase**

GP and GYS, the two rate-limiting enzymes of glycogen metabolism are reciprocally regulated in response to changing physiological conditions by hormones and metabolic signals mainly through posttranslational modifications like phosphorylation and dephosphorylation, but also by action of allosteric effectors. These regulatory interactions ensure that carbohydrate metabolism responds appropriately to glucose overload and glucose deprivation, in other words the synthesis and degradation of glycogen does not occur simultaneously. GYS is also regulated either by allosteric regulators or by phosphorylation. GYS phosphorylation in response to hormonal signal is implemented on nine regulatory serine residues by several kinases, resulting in progressive inactivation of GYS, thereby obstructing glycogenesis under catabolic state. As for the reverse pathway, the direct dephosphorylation and subsequent activation of GYS is attributed to the serine-threonine phosphatase protein phosphatase 1 (PP1). The reciprocal control between GP and GYS is mainly accomplished by PP1 which activates GYS and simultaneously inactivates GP contributing to stimulation of glycogen deposition.

## **Glycosomes**

A population of proteins involved in glycogen metabolism associate with glycogen particles in order to maintain their structure and regulate their size, number and cellular location. These macromolecular complexes, also called glycosomes, as morphologically distinct cellular organelles enable the efficient regulation of glycogen metabolism. Furthermore, microscopic studies gave evidence for association of glycosomes with different cellular structures, such as the endoplasmic reticulum and mitochondria, or especially in muscle other spherical structures, including SR,  $\beta$ -actin,  $\alpha$ -actinin, and smooth muscle tropomyosin. Consequently, glycogen particles as sub-cellular compartments are capable of responding to metabolic and synthetic requirements of the cell in a spatially and temporally regulated way.

## **Hormonal regulation of glycogen metabolism**

Dynamic changes of glycogen stores are regulated by several hormones. Insulin promotes glycogenesis and the counteracting hormones (glucagon, adrenaline, cortisol, and growth hormones) have glycogenolytic effects. Insulin mediates its biological effects via activation of insulin receptor (IR), which phosphorylates several downstream substrates and docking proteins, among them the phosphatidylinositol-3 kinase (PI3K) and protein kinase B (Akt) pathway. PI3K mediates the localization of Akt and (3-phosphoinositide dependent protein kinase-1) PDK1 to the cell membrane. PDK1 activates Akt on threonine-308 residue by phosphorylation. Other key residue in activation of Akt is serine-473, which is considered as an mTORC2-specific phosphorylation site. Activation of mammalian target of rapamycin complex 2 (mTORC2) induces nutrient storage (glycogen and fatty acid synthesis) and glycolysis through its downstream targets, among them Akt. Complete Akt activation requires phosphorylation of both Thr-308 and Ser-473, allowing AKT to be released into the cytosol mediating many effects of insulin, including glucose uptake and glucose excursion as well as glycogenesis.

## **Liver and muscle glycogen metabolism**

Glucose can be stored as glycogen in almost all cells, but in mammals the two major glycogen reserves are skeletal muscle and liver. Since liver and skeletal muscle have different roles in sustaining bodily functions, there are several differences in the character of their glycogen metabolism, as well. Liver glycogen provides energy for liver cells, but its primary role is supplying the other tissues with glucose. This function is permitted by large amount of G6Pase which allows

the release of free glucose from hepatocytes. Muscle lacks G6Pase, therefore, glucose is forced to remain inside the cells. Consequently, muscle glycogen is not generally available to other tissues, but dedicated as a local energy supply instead. However, muscle glycogen through glycolysis can be broken down to lactate and its transport to the liver contribute to HGP through gluconeogenesis (Cori cycle).

### **Physiology of pancreatic $\beta$ cell and the PDX1 transcription factor**

Pancreatic  $\beta$ -cells are specifically evolved to synthesize, store and secrete large amounts of insulin in order to respond to the nutritional status of the body. Pancreatic and duodenal homeobox factor-1 (PDX1) plays a crucial role in the early stage of pancreatic development,  $\beta$  cell differentiation and maintenance of mature  $\beta$  cell function by regulation of several  $\beta$  cell-related genes. In mature  $\beta$ -cells, PDX1 translocating to the nucleus (e.g. stimulated by Akt) transactivates genes involved in glucose sensing and metabolism, insulin and insulin secretion. On the course of  $\beta$  cell maturation several genes belonging to metabolically unfavorable pathways for insulin secretion (e.g. lactate production or pentose-phosphate pathway) are repressed early in life. This creates a specific gene expression pattern in  $\beta$  cell that. There is a tight coupling between glycolysis and mitochondrial metabolism essential for glucose sensing. Following glycolysis the TCA cycle induces the electron transport chain resulting in an increasing in  $O_2$  consumption, and ATP generation. In fact, mitochondria translate increased glucose levels into ATP signals. Intracellular ATP binding to ATP-sensitive  $K^+$  channels ( $K_{ATP}$ ) closes the channel and consequently depolarizes the plasma membrane which triggers the opening of voltage-gated  $Ca^{2+}$  channels.  $Ca^{2+}$  influx increases cytosolic free  $Ca^{2+}$  triggering exocytosis of insulin secretory granules. Insulin exerts its effect on insulin sensitive tissues through binding to its receptors. Considering that the insulin receptor (IR) is expressed in pancreatic  $\beta$ -cells as well, presumably insulin has an autocrine role for insulin signaling in  $\beta$  cell function and regulation

### **Type 2 diabetes mellitus and its metabolic consequences**

Diabetes mellitus is the most common endocrine and metabolic disorder with an escalating prevalence. Type 2 diabetes mellitus (**T2DM**) is responsible for 90–95% of diabetes cases worldwide. This multifactorial disorder results from an interaction between genetic and environmental conditions (sedentary lifestyle and obesity) and characterized by a peripheral insulin resistance, hyperglycaemia and  $\beta$  cell dysfunction. Insulin resistance is a complicated condition in

the insulin-sensitive metabolic tissues: skeletal muscle, liver and white adipose tissue. In insulin resistant state, adipocytes are blocked from glucose uptake and increased fatty acids released. Chronic elevation in the plasma FFA is involved in the etiology of obesity-associated insulin resistance. Mitochondrial dysfunction is associated with insulin resistance in liver and skeletal muscle, which can be attributable to FFA and glucose overload. Uncoupling protein 2 (**UCP2**) is a mitochondrial intermembrane ion transporter protein that induces proton leak uncoupling respiration from ATP production. UCP2 senses and negatively regulates superoxide production – derived from excess glucose influx or enhanced hepatic fatty acid accumulation and catabolism – exerting its cytoprotective effect. In line with these, increased UCP2 expression in white adipose tissue and skeletal muscle is associated with a reduced risk of developing obesity. Insulin deficiency or insulin resistance coexists with increased secretion of glucagon, even when blood glucose concentration is high because tissues do not take up glucose. Despite that insulin resistance is well established early in the emergence of T2DM, glucose tolerance remains normal because of a compensatory increase in insulin secretion of  $\beta$  cells. The process of  $\beta$  cell compensation is a combination of  $\beta$  cell mass expansion and an increase of acute glucose-stimulated insulin secretion. Moreover, the process is associated with an improved capacity of the secretory machinery to support increased insulin production. In contrast to these, chronic  $\beta$  cell exposure to high glucose and FFA levels can inhibit insulin secretion, impair  $\beta$  cell function and finally induce  $\beta$  cell apoptosis. The progressive  $\beta$  cell failure ultimately determines the rate of T2DM.

## AIM OF THE STUDY

GP inhibition has been shown to be a potential target for influencing blood glucose levels in T2DM. We set out to investigate the metabolic effects of GP inhibition in different models. In our experiments, we used an apparently potent glucose analog GP inhibitor, namely KB228 compound. On one hand, we utilized normal and high-fat diet-fed murine models in order to obtain evidences for KB228 applicability and efficiency. On the other hand, we used hepatic and muscle myoblast cells, as models of investigated metabolic organs (liver and skeletal muscle). To imitate the normal and diabetes-induced hyperglycemic conditions, experiments were implemented under normoglycaemic (5.5 mM) and hyperglycemic (25 mM) conditions.

### 1. Our aims were the following:

- to set out to find an appropriate dose and administration of KB228 for *in vivo* studies,
- to characterize the impact of KB228 on *in vivo* glucose metabolism including glucose tolerance, excursion and consumption,
- to explore the effects of KB228 on glucose metabolism in HepG2 cells,
- gene expression profiling of HepG2 cells to evaluate the effects of KB228 on key marker genes of major metabolic pathways,
- to investigate the activity of protein kinases involved in energy homeostasis.

Furthermore, taken in consideration that the pancreatic  $\beta$  cells are in the center of the diabetes research,  $\beta$  cells became our next model to investigate the effects of GP inhibition in details. MIN6 insulinoma cell, as a well-established model for  $\beta$  cells, was in the focus of the next chapter of our study. KB228 was further investigated in *in vivo* experiments and three structurally different GPis (KB228, BEVA335 and CP-3016819) were tested in *in vitro* experiments.

### 2. Our goals were the following:

- to assess glycogen metabolism and the localization of glycogen particles in MIN6 cells,
- to explore the glycogen-associated proteins by *in silico* screening,
- To evaluate the impact of GP inhibition on proliferation and on classical insulin secretion pathways in MIN6 cells and in mice.

## MATERIALS AND METHODS

### Materials

Unless otherwise stated, all chemicals were from Sigma-Aldrich (St. Louis, MO, USA), including CP-316819 GP inhibitor and wortmannin (WM) PI3K inhibitor.

Glucose analogue GP inhibitors – KB228, KB228, BEVA33, TH, NV50 and NV76 – were synthesized by the Laboratory of László Somsák, at Department of Organic Chemistry.

### Animal studies

C57/Bl6J male mice *ad libitum* access to water and food, and were kept on chow (10 kcal% of fat) (SAFE, Augy, France) or on high fat diet (HFD, hypercaloric diet, 60% fat content, Research Diets, Inc., New Brunswick, NJ, USA). Mice were administrated with vehicle (physiological saline + 1% DMSO) or with 90 mg/kg bodyweight of KB228 as a single intraperitoneal (i.p.) bolus once a week for 5 constitutive weeks. All measurements took place 2 hours after injection and tissues (liver, gastrocnemius muscle and pancreas) were collected and processed as specified. Mice were randomly incorporated into the vehicle or KB228 group.

### Intraperitoneal glucose tolerance test (ipGTT)

Animals were fasted for 8 hours prior to the experiment. Blood glucose levels were determined using a handheld Accu-Chek (Roche, CA, USA) device. Each animal received an intraperitoneal glucose (20% m/m) injection (10  $\mu$ L/body weight g). Subsequently, blood-glucose levels were monitored at regular intervals (time points).

### Indirect calorimetry

Indirect calorimetry experiments were performed in a CLAMS system (Columbus Instruments, Columbus, OH, USA). Mice were habituated to the new environment of the cages for 24 hours. Then at 8 a.m. mice received a bolus i.p. injection of KB228 (90 mg/kg) or vehicle then were returned to the measurement cages and for the following six hours oxygen consumption and carbon dioxide release was recorded.

### **Glucose uptake experiment**

In glucose uptake experiments mice were injected under isoflurane anesthesia with 120  $\mu\text{Ci/kg}$   $^{14}\text{C}$ -2-deoxyglucose and 20 U/kg insulin through the jugular vein. Blood glucose levels were monitored at the time of the suture, 15 and 30 minutes post intervention. The mice were sacrificed by cervical dislocation 30 minutes post intervention and the (liver, gastrocnemius muscle, white adipose tissue, pancreas and heart) were removed, weighed then lysed in 0.5 mL 1 M NaOH at 70 °C for 60 mins. Lysates were mixed with Aqualight HIBEX (PerkinElmer Life Sciences, The Netherlands) scintillation liquid and were measured in a Wallac scintillation counter (PerkinElmer, Waltham, MA, USA).

### **Histochemical assessment of glycogen content in pancreas**

Glycogen content of pancreatic tissue was determined by Periodic Acid Schiff (PAS) staining. Microscopic images were acquired with a Zeiss Axioscope 20 microscope using Plan NEOFLUOR 40x objective at 25°C. Images were processed using Leica Application Suite V4.8 software. Glycogen content was determined by measuring the intensity with Image J software, where more intensive PAS staining correlates with higher glycogen content.

### **Insulin immunohistochemistry and islet size determination**

To detect insulin, 5  $\mu\text{m}$  paraffin sections were incubated in guinea pig polyclonal insulin antibody (DAKO, Glostrup, Denmark; 1:100) overnight at 4 °C in 1% BSA diluted in PBS. Slides were stained with Envision one-step polymer HRP (BioGenex Fremont CA, USA) (90 minutes). Color reaction was developed for 2 minutes using nickel 3,3'-diaminobenzidine tetrachloride (Ni-DAB) as a substrate for antibody-coupled HRP. After rinsing sections in 50 mM Tris-HCl (pH 7.4), the color was enhanced by incubating the sections for 2 minutes in 2% cobalt chloride (in 50 mM Tris-HCl, pH 7.2). Sections were counterstained with 1% methyl green solution. A negative immunohistochemical control was included in each run. Microscopic images were acquired with a Zeiss Axioscope 20 microscope using Plan NEOFLUOR 40x objective at 25°C. Images were processed using Leica Application Suite V4.8 software. Islet size was determined in  $\mu\text{m}^2$  by using Image J software.

## **Cell culture**

HepG2 human hepatocarcinoma cell line and C2C12 mouse myoblast cell line cultured in DMEM, 10% fetal calf serum, 1% L-glutamine, 1% penicillin-streptomycin. Normoglycaemic condition was provided by using 5.5 mM glucose-containing medium, while for hyperglycemic conditions 25 mM glucose during the treatments. Cells were treated with 3  $\mu$ M KB228. MIN6 mouse insulinoma cell line was cultured in DMEM, 15% fetal calf serum, 1% L-glutamine, 1% penicillin-streptomycin, 50  $\mu$ M 2-mercapto-ethanol and 25 mM glucose. MIN6 cellular treatments and measurements took place in DMEM containing 5.5 mM glucose. The measurements took place 1 and 2 days after the addition of GP as indicated. Wortmannin was used in the last 1 hour of the GPi treatment. Control group was treated with 1% DMSO-containing medium. MIN6 cells were treated with 3  $\mu$ M KB228, or 1.5  $\mu$ M BEVA335, or 0.5  $\mu$ M CP-316819. Wortmannin treatment took place in the last hours of GPi treatments.

## **Biochemical glycogen determination**

KOH-ethanol glycogen extraction method was used for extraction of the glycogen. Phenol-sulfuric acid assay was applied for determination of glycogen content. The absorbance was measured at 450 nm. Readings were normalized to protein content or wet weight of the liver piece.

## **Total RNA isolation, reverse transcription and RT-qPCR**

Total RNA was prepared using TRIzol (Invitrogen, Carlsbad, CA, USA) reagent following the manufacturer's instructions. 2  $\mu$ g of RNA was used for reverse transcription (RT) (High Capacity cDNA Reverse Transcription Kit, Applied Biosystems, CA, USA). Reverse transcription was carried out in a PCR machine (Applied Biosystems Veriti 96-Well Thermal Cycler). 10x diluted cDNA samples were used for real-time quantitative PCR (qPCR) reactions which were performed using qPCR Supermix (qPCRBIO SyGreen Mix, Nucleotest Bio Kft., Hungary) with the primers and carried out in the Light-Cycler 480 II instrument (Roche Applied Science, Penzberg, Germany). Expression was normalized to the geometric mean of three control genes ( $\beta$ -actin, cyclophilin, 36B4 or/and GAPDH).

### **Protein extraction and Western blotting**

HepG2 cells and murine liver homogenates were lysed in RIPA lysis buffer. MIN6 cells were lysed in homogenizing lysis buffer. Supernatant contained the cytoplasmic fraction and the pellet contained the nuclear fraction. Pellet was further extracted using buffer “A” followed by buffer “B”. Proteins were separated by SDS-PAGE and transferred onto nitrocellulose membranes. Blots were probed with antibodies then signals were developed using SuperSignal™ WestPico Enhanced Chemiluminescent (ECL) Substrate (Pierce Biotechnology Inc., Rockford IL) and captured by a Fluorchem FC2 gel documentation system (Alpha Innotech, San Leandro, CA, USA). Densitometry was performed using the Image J software.

### **Determination of oxygen consumption and extracellular acidification rate**

Oxygen consumption rate (OCR, reflecting mitochondrial oxidation) of HepG2 cells were measured using an XF96 oximeter (Agilent Technologies, Santa Carla, California, USA USA). After recording the baseline OCR, cells received a single bolus dose of 3  $\mu$ M KB228. Then, OCR was recorded every hour to follow the effects of GPis. Final reading took place at 8 hours post-treatment. Data were normalized to protein content.

OCR and changes in pH, termed extracellular acidification rate (ECAR, reflecting glycolysis) of MIN6 cells were assessed by using the mentioned XF96 oximeter. After recording the baseline, OCR and ECAR were recorded every 3 minutes up to 60 minutes to follow the effects of GPis. Antimycin (10  $\mu$ M) was used for distinguishing the mitochondrial from non-mitochondrial oxygen consumption.

### **Confocal microscopy**

MIN6 cells were grown on glass coverslips for one day then starved in glucose-free overnight. Next morning cells were charged with 500  $\mu$ M fluorescent D-glucose analogue [(2-NBDG), Cayman Chemicals Company, Ann Arbor, Michigan, USA] in uptake buffer pH 7.4 for 3 hours then incubated in 5.5 mM glucose-containing DMEM for one more day. Cells were fixed, blocked then incubated with primary monoclonal rabbit antibody (Insulin receptor beta (IR $\beta$ , Cell signaling, 1:50) in moist chamber overnight at 4°C. The secondary antibody (Alexa Fluor® 647 donkey anti-rabbit IgG (H+L), Life Technologies, 1:1000), diluted in blocking solution, was added for 1 hour at room temperature. Cell nuclei were stained with DAPI special formation (NucBlue® Fixed cell

Stain ReadyProbes™ reagent, Life Technologies, Carlsbad, CA, USA). Confocal images were acquired with a Leica TCS SP8 confocal microscope using HC PL APO CS2 63x/1.40 OIL immersion objective on a DMI6000 CS microscope at 25°C. Images were processed using LAS X 2.0.1.14392 software. Nonspecific binding of secondary antibodies were checked in control experiments.

### **Electron microscopy**

Pellets of cells were processed for electron microscopic investigation according to the ferrocyanide-reduced osmium method. Sections were investigated with a JEOL 1010 transmission electron microscope and photographed at a magnification of 6000-10000x with an Olympus Veleta CCD camera. Digitalized images were processed with Adobe Photoshop CS5 software. Morphometric assessment was done using the Image J software. To control the specificity of the staining, a group of cells underwent the same EM staining protocol without the addition of potassium ferrocyanide to osmium tetroxide.

### **Measurement of changes in intracellular Ca<sup>2+</sup> concentrations**

MIN6 cells on glass coverslips (25 mm diameter and 1 mm thickness; Thermo Fisher (Thermo Fisher Scientific Gerhard Menzel B.V. & Co. KG, Braunschweig, Germany) were charged with 5 μM of Fura-2AM fluorescent Ca<sup>2+</sup> indicator dye (Molecular Probes) for 90-120 minutes. Cells were illuminated alternatively by 340 and 380 nm light (excitation) and pictures were recorded at a wavelength above 510 nm (emission). After reaching a stable baseline (min. 60 seconds) cells were induced with 20 mM glucose.

### **Insulin release in MIN6 cells**

Insulin production of MIN6 cells was measured using a Mouse Insulin ELISA Kit (Mercodia, Winston Salem, N.C., USA) following the manufacturer's instructions. Insulin secretion was normalized for protein content.

### **Glucose-induced insulin release in MIN6 cells**

Cells were washed twice with glucose-free KRBH buffer, then preincubated for 1 h in 5% CO<sub>2</sub> at 37°C in KRBH (1 mM glucose). The pre-incubation medium was removed, and cells were washed, then incubated for 1 h in 20 mM glucose-containing KRBH in the absence (control, CTL) or presence of inhibitors (KB228, BEVA335, CP-316819 and/or Wortmannin). At the same time, a set of control cells were incubated for 1 h in 1 mM glucose-containing KRBH for determination of insulin baseline. Insulin concentration in diluted samples was determined using a Mouse Insulin ELISA Kit (Merckodia, Winston Salem, N.C., USA). Insulin secretion was normalized to protein content.

### **Total insulin protein in MIN6 cells**

Cells were extracted by using acid-ethanol extraction method, then measured using Mouse Insulin ELISA Kit (Merckodia, Winston Salem, N.C., USA) following the manufacturer's instructions. Insulin content was normalized for protein content where ng insulin / mg protein values were transformed to fold change compared to the controls.

### **Transfection and Luciferase assay**

MIN6 cells were transfected with 5 µg of corresponding PDX1 promoter construct (-6500STF-1luc plasmid a generous gift from Marc Montminy (Salk Institute, La Jolla, CA, USA)) and 1 µg of β-galactosidase expression plasmid in Pei (Polyethylenimine) transfection reagent. 48-hour post transfection cells were washed and collected into Eppendorf tubes by scraping in Luciferase lysis buffer. Samples were disrupted, then lysates were divided into two parts for determination of luciferase activity (Victor Luminescence Reader, PerkinElmer, Waltham, MA, USA) and β-galactosidase activity (absorbance at 405 nm). Luciferase activity was expressed as luciferase activity/β-galactosidase activity transformed to fold change respect of the control.

### **In silico screening for glycogen-binding proteins**

The database containing a list of proteins with potential association with glycogen was assembled from multiple queries using the UniProt database (<http://www.uniprot.org>). The resulting list was manually revised and redundancies in the list were removed manually.

### **Statistical analysis**

We determined the statistical significance between two groups regarding control/vehicle versus KB228 by using Student's *t*-test (unpaired, two-tailed) unless otherwise stated. Statistical significance of 3-5 group-designed measurements was determined using one-way ANOVA with Tukey HSD. Error bars represent  $\pm$  standard error of the mean (SEM) unless otherwise stated.

\*, \*\* and \*\*\* indicate statistically significant difference between vehicle and treated groups at  $p < 0.05$ ,  $p < 0.01$  or  $p < 0.001$ , respectively.

## RESULTS

### ***Characterization of the in vivo applicability of GP inhibitor KB228***

We wanted to set up an appropriate dose and administration of KB228 for *in vivo* studies. KB228 was administered to C57/Bl6 mice as a single intraperitoneal (i.p.) injection in 90 mg/kg dose (lower doses were ineffective). KB228 treatment reduced blood glucose levels 30 minutes post treatment and the effect remained for 6 hours after KB228 administration that coincided with an increment in hepatic glycogen content. KB228 treatment in high-fat diet (HFD)-fed mice increased hepatic glycogen content, as well. These data validated the applicability of KB228 under normoglycaemic and hyperglycaemic conditions in mice.

### ***Effect of KB228 on energy balance***

Given the long lasting effect of KB228 we performed indirect calorimetry experiments on C57/Bl6J mice that were on chow diet, or on HFD. KB228 treatment enhanced oxygen consumption in chow-fed mice suggesting increased oxidative metabolism that was correlated with higher RQ in chow-fed mice indicative of higher glucose oxidation rates. In line with these data KB228 treated mice displayed better glucose tolerance in ipGTT assays. Glucose uptake assays suggested that the main organ responsible for glucose excursion is the liver. KB228 treatment in HFD-fed animals had similar effects than in chow fed animals in terms of oxygen consumption, RQ and glucose tolerance. However, the improvement of their metabolic properties was less pronounced (e.g. there was only a slight increase in RQ on HFD instead of the significant enhancement on chow, or the weaker improvement of ipGTT) that was in line with the lower potency of KB228 in hyperglycaemic mice.

### ***KB228 treatment induces UCP2 expression***

*In vivo* data suggested metabolic rearrangements in the hepatocytes. Therefore, we performed a set of experiments on HepG2 cells under normoglycaemic (5.5 mM glucose) and hyperglycaemic conditions (25 mM glucose). The expression of GP isoforms were unaltered and glycogen build-up was induced upon KB228 treatment in both under conditions suggesting effective GP inhibition in this cellular model. The administration of KB228 to HepG2 cells enhanced mitochondrial oxidation under normoglycaemia. Treatments under hyperglycaemia exerted negligible effects on mitochondrial oxidation, similarly to the *in vivo* experiments.

We have observed that the mRNA and protein expression of uncoupling protein2 (UCP2) was induced upon KB228 treatment in a time-dependent manner in HepG2 cells. Induction of UCP2 were not as pronounced in hyperglycemic conditions as in normoglycaemia. We investigated whether the same molecular events take place similarly *in vivo*. Hepatic UCP2 expression was induced by KB228 in chow and high fat-fed mice both on the mRNA and protein level. We assessed the expression of UCP2 in skeletal muscle samples. Although we did not detect changes in UCP2 expression in chow-fed mice, we have observed 2-fold induction of UCP2 mRNA levels in HFD-fed mice. Interestingly, in C2C12 myoblasts a much larger 6-fold enhancement of UCP2 expression was observed. We tested other potent GPis (TH,  $K_i=5.1 \mu\text{M}$ , NV50,  $K_i=3 \mu\text{M}$  and NV76,  $K_i=0.47 \mu\text{M}$ ) on HepG2 cells cultured under normoglycaemic conditions. These GPis efficiently inhibited GP as demonstrated by increases in cellular glycogen content. Furthermore, the treatment of cells led to a ~2 fold induction of UCP2 expression similarly to KB228. These data demonstrate that the induction of UCP2 is not limited to KB228 only but can be elicited by other GP inhibitors, too. Furthermore, potency of the drugs to induce UCP2 expression correlated with their respective  $K_i$ 's.

#### ***KB228 treatment also induces mTORC2 activity***

To assess further metabolic rearrangements triggered by KB228, we examined the activity of certain protein kinases involved in energy homeostasis, such as mTORC2. Akt2 phosphorylation on serine-473 was enhanced upon KB228 treatment in chow-fed and HFD-fed mice despite lower Akt2 protein content, highlighting marked activation of mTORC2

#### ***The presence of glycogen in pancreatic $\beta$ cells***

To get insight into the relevance of glycogen metabolism in  $\beta$  cells we investigated murine pancreas and MIN6 insulinoma cells. To confirm the existence of active glycogen metabolism and to assess the presence of glycogen granules in MIN6 cells we used confocal microscopy. Living MIN6 cells were charged with fluorescent labelled derivative of D-glucose (2NBDG) in order to incorporate it into glycogen. 2NBDG fluorescence appeared in MIN6 cells and displayed a punctate pattern in various sizes in the cytosol MIN6 cell were treated with structurally unrelated GP inhibitors, such as glucose-based inhibitors (KB228, BEVA335) and a heterocyclic derivative (CP-316819). We assessed total glycogen content using biochemical methods. All GPis enhanced cellular glycogen content, supporting an active glycogen metabolism in MIN6 cells. We investigated the effects of

the KB228 administration in mice kept either on chow diet or HFD. PAS staining showed elevated glycogen content in Langerhans-islets upon KB228 treatment under both feeding regimens. As a next step, we evaluated glycogen content in MIN6 cells by image analysis of electron microscopic sections. This way we were able to quantify glycogen content in cells and to approximate the size of glycogen particles. We have observed increase in cellular glycogen content upon GP inhibition. In a similar fashion, the circumference of glycogen particles also increased in the presence of KB228, BEVA335 and CP-316819. Taken together, the application of GPis increases cellular glycogen content and the circumference of the glycogen particles.

### ***Assessment of the role of glycogen in MIN6 cells***

To explain these data, we hypothesized that increases in glycogen content and size of glycogen granules may provide additional surface area for binding and interaction with proteins involved in the regulation of  $\beta$  cell function. We performed an *in silico* screen and identified more than 100 potential glycogen bound proteins. Importantly, the list was dominated by members of the IR signaling pathway, mTOR and Akt were found among the bound proteins. The overrepresentation of the IR signaling pathway among the glycogen-bound proteins prompted us to assess the expression and localization of IR in MIN6 cells. We tested the localization of IR $\beta$  in untreated, intact MIN6 cells by immunofluorescence microscopy that displayed a punctate pattern, similarly to other reports of non-stimulated MIN6 cells. We also aimed to test whether the *in silico*-detected interaction between the glycogen particles and the IR exists in cells. We found that the  $\beta$  subunit of the IR (IR $\beta$ ) showed a colocalization with glycogen particles at the cell membrane

### ***Glycogen phosphorylase inhibitors induce insulin receptor signaling***

| In GPi-treated MIN6 cells, we have observed elevated phosphorylation of IR $\beta$  on tyrosine-1345 as compared to control cells. Downstream effectors of IR $\beta$ , such as mTORC2 and mTORC1, were also activated regarding to phosphorylation of Akt on serine-473 and of p70S6K protein on threonine-389. Furthermore, we investigated the expression of PDX1, a downstream effector of IR/PI3K/Akt pathway and the master regulator of pancreatic  $\beta$  cell. We observed enhanced *Pdx1* promoter activity and mRNA expression upon GPi treatment that subsequently led to a large increase in PDX1 protein content both in the cytoplasmic and nuclear fraction. Observing induced growth and survival signaling, we investigated the MIN6 cell proliferation and size of Langerhans

islets in mice. Although the GPi treatment had no effect on MIN6 cell proliferation, KB228 treatment resulted in a significant increase in the size of the Langerhans islets in mice kept on HFD. The size of Langerhans islet also tended to grow during KB228 treatment in chow-fed mice, however, the difference did not reach the levels of significance. The next focus of our investigations was insulin production and secretion. Insulin expression and protein levels were enhanced upon GPi treatment. Non-glucose-stimulated insulin release also showed a tendency towards increase as well as glucose-induced insulin release in GPi-treated groups of MIN6 cells. To verify the involvement of insulin receptor signaling in GP inhibitor-evoked biochemical changes wortmannin (WM, a specific inhibitor of PI3K) was used. Treatment of MIN6 cells with applying WM abolished the GPi-induced increases in mRNA and protein levels of PDX1 and insulin, as well as insulin secretion.

### ***KB228 and CP-316819 improves $\beta$ cell function***

We next turned to investigate the effects of GP inhibition on the “classical” pathway of insulin release. Our first observation was that BEVA335 did not influence most features of the “classical” insulin secretion pathway, therefore, we omitted it from further investigations and used only KB228 and CP-316819. Interestingly, the effects of KB228 and CP-316819 on the classical pathway lasted longer (48 hours) than their effects on IR signaling (all experiments described above with MIN6 cells were performed after 24 hours of treatment). KB228 and CP-316819 GP inhibitors enhanced glycolysis and mitochondrial oxidation in  $\beta$ -cells. In pancreatic  $\beta$ -cells increased ATP levels lead to depolarization by closing the ATP-sensitive potassium channels which triggers opening of voltage-gated calcium channels. Consequently, we have observed enhanced glucose-induced calcium influx upon treatment suggesting MIN6 cell depolarization. In pancreatic  $\beta$ -cells  $\text{Ca}^{2+}$ -influx leads to the fusion of insulin-rich granules with the cell membrane leading to insulin secretion. GPi treatment enhanced insulin secretion. Therefore, we investigated the glucose-stimulated insulin secretion in MIN6 cells and we observed that GPi treatment enhanced insulin secretion. We were curious whether the administration of KB228 had effect on insulin secretion in mice. We have observed that KB228 had a tendency to improve glucose-induced insulin release mainly in HFD-mice

## DISCUSSION

We characterized the metabolic effects of potent glycogen phosphorylase inhibitors. In our first study we investigated KB228. Similar to previously described observations with other GPis, KB228 increased oxygen consumption, reduced serum glucose levels and enhanced hepatic glycogen content. However, the potency of KB228 was reduced under hyperglycemic conditions. Our assumption is that KB228, which has a glucose moiety, competes with glucose for binding to the catalytic center of GP enzyme, hence high glucose levels may reduce KB228's affinity to GP. Changes in the uptake of radioactive 2-deoxyglucose clearly pointed out the principle role of the liver in glucose clearance upon KB228 treatment. Nevertheless, we provided evidence for the involvement of skeletal muscle in glucose clearance that was highlighted by induction of UCP2 in murine gastrocnemius muscle and in C2C12 cells.

UCP2 mRNA is present in many tissues and its regulation is implemented by at both transcriptional and translational levels. UCP2 has debated evolutionary role and biological function, although the short half-life of this protein suggests its suitability for regulating rapid biologic responses. Increased UCP2 expression has shown to be an adaptive response to increased fatty acid  $\beta$ -oxidation and ROS production that occurs during obesity. Furthermore, evidences also suggest the beneficial role of UCP2, such as promoting mitochondrial respiration, reducing ROS production and fatty acid accumulation that might contribute to its anti-steatotic and anti-inflammatory effects. In our view, the UCP2 induction might help to understand the metabolic rearrangements generated by KB228-induced GP inhibition in mice. Under hyperglycemic conditions, excess glucose and fatty acid influx contributes to production of mitochondrial free radicals by obstructing the mitochondrial electron transport chain (ETC). UCP2 is able to mediate proton leaks across the mitochondrial inner membrane in mammalian cells and uncouples fuel oxidation from ATP synthesis. Therefore, UCP2 keep the membrane potential sufficiently low via abolishing high proton gradients built across the inner membrane to minimize superoxide production. Mitochondrial dysfunction - including reduction of fatty acid oxidation, lower expression of genes involved in oxidative activity, as well as decrease or blockade of ETC function - has been described in the development of metabolic syndrome, such as T2DM. It is presumable that the upregulated UCP2 by KB228 treatment may release the blockade of ETC by channeling excess proton load to the matrix thereby protecting cells from oxidative stress. Hence, GP inhibition

not only enhanced glucose uptake and mitochondrial oxidation but it contributed to the elimination of oxidative damage through UCP2 induction.

Several GPis were designed and tested for the treatment of T2DM. Former studies demonstrated that pharmacological GP inhibition improves hyperglycemia in animal models of diabetes, although long term application was reported to have adverse effects characterized by hepatic lipid and glycogen accumulation symptoms, characteristic of glycogen storage diseases. The induction of mitochondrial oxidation or UCP2 activation may be beneficial over these adverse effects that could be exploited to counteract hepatic glycogen and lipid accumulation upon prolonged GP inhibition. We uncovered another molecular event induced by KB228, the induction of mTORC2 activity. mTOR signaling is associated with numerous metabolic disorders such as obesity, diabetes, and cancer. mTORC2 functions as a key activator of protein kinases, including PKB (Akt), that act downstream of insulin and growth factor signaling. Studies with liver-specific *ricor* knockout (LiRiKO) mice demonstrated the functional importance of hepatic mTORC2 in the regulation of glucose and lipid homeostasis via insulin-induced Akt signaling. These liver-specific defects consequently induced systemic hyperglycemia and hyperinsulinemia, featuring glucose intolerance and insulin resistance. Other studies revealed that chronic administration of the mTOR inhibitor rapamycin dramatically worsened the metabolic syndrome in T2DM. Long-term application of rapamycin substantially impaired glucose tolerance and insulin action by disrupting not only mTORC1 but mTORC2 as well, which in turn obstructed insulin-mediated suppression of hepatic gluconeogenesis.

How KB228 treatment leads to mTORC2 activation in liver is yet unknown, but it could be explained by alterations in insulin signaling. Alternatively, glucose influx could simply rearrange mTOR signaling, whereby cells sense glucose plenitude and turn towards storage. The activation of mTORC2, or more importantly, of mTORC1 induction by GP inhibition was observed in pancreatic  $\beta$  cell model, MIN6 cell. KB228, BEVA335 and CP-316819 treatment induced the insulin receptor signaling, which was shown in mTORC2, Akt and mTORC1 activation. KB228 and BEVA335 proved to be more effective in this regard, however, in almost all respects, the effects of the reference inhibitor CP-316819 were far behind the KB228 or even BEVA335. Several studies confirmed the concept that mTORC1 is critical for  $\beta$  cell expansion, cell growth, proliferation and regeneration, as well as for  $\beta$  cell adaptation to hyperglycemia and insulin resistance. The mTORC2 pathway could be linked indirectly to  $\beta$  cell proliferation and insulin

secretion through Akt activation. In line with that, GP inhibition induced Akt and PDX1, which are known to be survival and growth signals. Enhanced growth and survival signaling may explain the increase in size of Langerhans islets in mice upon KB228 treatment.

The major goal in diabetes research is to find ways to improve the regeneration and function of insulin secreting pancreatic  $\beta$  cells to prevent and/or delay diabetes. We suggest that not only the liver and skeletal muscle are targeted by GP inhibition, but pancreatic  $\beta$  cells, too. Our data point out the beneficial effects of mild increases in the glycogen content of  $\beta$  cells and demonstrate that GP inhibitors have positive impact on  $\beta$  cell function. The pro-proliferative effects of GPis, however, raise safety concerns. Although confirmatory studies do not exist, there are studies that assessed the effects of GPis on cancer cell proliferation. Two studies showed that GPis are anti-proliferative in pancreatic adenocarcinoma cells and other tumor cells as well, suggesting that GPis may have a cytostatic effect on cancer cells. Our results demonstrate that, although KB228 increased to a small extent but significantly the size of the islets of Langerhans in mice, we were unable to detect even a little change in the proliferation of MIN6 insulinoma cells under KB228, BEVA335 and CP-316819 treatment for several days (1-14 days). In our investigations, beyond the proliferative effects, insulin transcription and translation were induced by GP inhibition in  $\beta$  cells, which was further evidenced by elevated non-stimulated insulin secretion. Here, I have to note that the induction of PDX1 and mTORC1 greatly contribute to these processes.

GPi-induced biochemical modifications involve several downstream targets of insulin signaling and within that, the elements of IR-PI3K cascade in MIN6 cells. GP inhibition mildly induced IR activity, furthermore, we have evidenced the involvement of PI3K by using its specific inhibitor, WM. Inhibition of PI3K blunted GPi-induced PDX1 and insulin expression, as well as non-stimulated insulin secretion.

Another hallmark of GP inhibition was the increased glucose-induced insulin secretion. Here, we showed that KB228 and CP-316819 induced glycolysis, mitochondrial oxidation and glucose-induced calcium transients. During the study CP-316819 induced significant changes only in case of glycolysis and mitochondrial activity in MIN6 cells after 1-day treatment. But the expected consequences, including enhanced  $\text{Ca}^{2+}$ -influx and glucose-induced insulin release, were absent. Surprisingly, BEVA335 did not influence most features of the “classical” insulin secretion pathway that has resulted in its exclusion from these experiments with MIN6 cells. Glucose-induced insulin release was significantly enhanced *in vitro*, while the *in vivo* experiments did not

produce the expected results, however, tendency is promising for the future. Presumably, further adjustment of KB228 formulation and dosing are indispensable in the future. We cannot explain how GPis induce glucose-induced insulin release in MIN6 cells, although in current study and the results of others reported the induction of mitochondrial oxidation by GP inhibition. Along these lines, it would be an easy explanation that IR signaling can enhance mitochondrial oxidation and consequently glucose-induced insulin release. However, the activation of the insulin secretion cascade lasted longer (up to 2 days of treatment) than the induction of IR signaling (up to 1 day of treatment), suggesting the involvement of other, yet unknown, pathway(s). Furthermore, PI3K inhibition only modestly reduced GPi-mediated increases in glucose-induced insulin release, suggesting again additional mechanism(s).

Several studies emphasize the physiological role of glycogen in pancreatic  $\beta$  cells, although there are many contradictions. Earlier studies suggest that glycogen breakdown provides glucose for glycolysis and mitochondrial oxidation in  $\beta$  cells under starvation and thereby facilitates insulin release. However, chronic exposure of  $\beta$  cells to high concentrations of glucose, as in diabetes, provokes accumulation of glycogen in islet cells by inhibiting glycogenolysis, which did not appear to adversely affect the utilization of glucose resulting in a paradoxical decrease in glycolytic rate. Therefore, under hyperglycemia, glycogen accumulation in  $\beta$  cells may lead to glucotoxicity. However, latest research provided that glycogen accumulation alone is not sufficient to trigger dysfunction, apoptosis and progression to diabetes in  $\beta$  cells, and glycogen metabolism is not required for the maintenance of  $\beta$  cell function. GPi-induced increases in glycogen is likely lower in our study than in others that may obstruct different pathways. Similar differences in glycogen accumulation may account for the lack of glucotoxicity in our models.

In summary, our data highlight that structurally different GPis exert more effects than a simple inhibition of catalytic activity. These data raise the possibility of repurposing GP inhibitors from the original indication as agents to inhibit hepatic glucose output in T2DM to a new indication that is the improvement of  $\beta$  cell function in the context T2DM. However, GPi clinical studies were halted after phase II, though the reasons were not communicated. Our experiments suggest that infrequent or low dose-administration of the GPis can target metabolic regulatory pathways without promoting the typical adverse effects of GP inhibition, such as hypoglycemia or glycogen storage disorder.

## SUMMARY

Glycogen phosphorylase, engaged in the regulation of glycogen metabolism, has a major role in hepatic glucose production. Therefore, glycogen phosphorylase has become a validated target to modulate glucose levels in type 2 diabetes mellitus and pharmacological glycogen phosphorylase inhibitors are considered as potential antidiabetic agents.

KB228, a glucose analogue glycogen phosphorylase inhibitor, was tested *in vitro* and *in vivo* under normal and hyperglycemic conditions on two major glycogen stores, liver and skeletal muscle. KB228 enhanced hepatic glycogen content *in vivo* that coincided with significantly increased glucose excursion to the liver. KB228 affected the energy balance as well by enhancing oxygen consumption and improving glucose tolerance in mice. However, changes were smaller in high-fat diet-fed mice as compared to the chow-fed animals, probably because high glucose levels, which may reduce the potency of KB228. *In vitro* studies on HepG2 hepatocytes presented KB228-induced mitochondrial activity, which was paralleled by an increase in UCP2 mRNA and protein levels. The unexpected induction of UCP2 was detected in murine liver and skeletal muscle, as well as C2C12 cells, suggesting the involvement of skeletal muscle in increased glucose oxidation upon KB228 treatment. To further assess metabolic rearrangements triggered by KB228 we investigated certain signaling pathways involved in insulin action and energy homeostasis. We detected induced mTORC2 activity, which modulating hepatic glucose production and glycolysis, as well as inducing glycogen storage and glucose import, may improve diabetes.

We also tested whether glycogen phosphorylase inhibition affects  $\beta$  cells *in vivo* and *in vitro* using KB228 and another glucose-based glycogen phosphorylase inhibitor BEVA335, and the structurally different CP-316819. Increased glycogen content led to elevated surface of glycogen particles. Glycogen phosphorylase inhibition induced IR/PI3K/Akt signaling cascade. We also showed that IR $\beta$  co-localized with glycogen particles, and components of IR signaling were identified as glycogen-bound proteins. Glycogen phosphorylase inhibition also induced glucose-stimulated insulin secretion marked by enhanced glycolysis, mitochondrial oxidation, and calcium signaling. Finally, glycogen phosphorylase inhibitor treatment increased the size of the islets of Langerhans.

Taken together these data, we conclude that glycogen phosphorylase might be involved in a complex metabolic regulatory network in the liver, the skeletal muscle and even in the  $\beta$  cells that could be exploited in the management of diabetes.

## **CONCLUSIONS**

- GP inhibition induced mitochondrial oxidation and improved glucose tolerance in mice.
- In model of pancreatic  $\beta$  cells the pharmacological inhibition of GP and the consequent increase in cellular glycogen content was associated with induction of the insulin signaling pathway and glucose-induced insulin release.
- GP might be involved in a complex metabolic regulatory network in liver, muscle and even  $\beta$ -cells.

## **KEYWORDS**

glycogen, glycogen phosphorylase, glycogen phosphorylase inhibitors, type 2 diabetes mellitus, mitochondria, UCP2, AKT2, mTORC2, pancreatic  $\beta$ -cell, PDX1, insulin, insulin receptor, insulin receptor signaling.

## THE THESIS IS BASED ON THE FOLLOWING PUBLICATIONS



UNIVERSITY OF DEBRECEN  
UNIVERSITY AND NATIONAL LIBRARY



Registry number: DEENK/115/2017.PL  
Subject: PhD Publikációs Lista

Candidate: Lilla Nagy  
Neptun ID: JLM2H9  
Doctoral School: Doctoral School of Molecular Medicine  
MTMT ID: 10037938

### List of publications related to the dissertation

1. **Nagy, L.**, Márton, J., Vida, A., Kis, G., Bokor, É., Kun, S., Gónczi, M., Docsa, T., Tóth, A., Antal, M., Gergely, P., Csóka, B., Pacher, P., Somsák, L., Bai, P.: Glycogen phosphorylase inhibition improves [béta]-cell function.  
*Br. J. Pharmacol. [Epub ahead of print], 2017.*  
IF: 5.259 (2015)
2. **Nagy, L.**, Docsa, T., Szántó, M., Brunyánszki, A., Hegedűs, C., Márton, J., Kónya, B., Virág, L., Somsák, L., Gergely, P., Bai, P.: Glycogen Phosphorylase Inhibitor N-(3,5-Dimethyl-Benzoyl)-N'-(β-D-Glucopyranosyl)Urea Improves Glucose Tolerance under Normoglycemic and Diabetic Conditions and Rearranges Hepatic Metabolism.  
*PLoS One. 8 (7), e69420-, 2013.*  
DOI: <http://dx.doi.org/10.1371/journal.pone.0069420>  
IF: 3.534

### List of other publications

3. Abdul-Rahman, O., Kristóf, E., Doan-Xuan, Q. M., Vida, A., **Nagy, L.**, Horváth, A., Simon, J., Maros, T. M., Szentkirályi, I., Palotás, L., Debreceni, T., Csizmadia, P., Szerafin, T., Fodor, T., Szántó, M., Tóth, A., Kiss, B. K., Bacsó, Z., Bai, P.: AMP-Activated Kinase (AMPK) Activation by AICAR in Human White Adipocytes Derived from Pericardial White Adipose Tissue Stem Cells Induces a Partial Beige-Like Phenotype.  
*PLoS One. 11 (6), e0157644, 2016.*  
DOI: <http://dx.doi.org/10.1371/journal.pone.0157644>  
IF: 3.057 (2015)

Address: 1 Egyetem tér, Debrecen 4032, Hungary Postal address: Pf. 39. Debrecen 4010, Hungary  
Tel.: +36 52 410 443 Fax: +36 52 512 900/63847 E-mail: [publikaciok@lib.unideb.hu](mailto:publikaciok@lib.unideb.hu) Web: [www.lib.unideb.hu](http://www.lib.unideb.hu)

## ORAL AND POSTER PRESENTATIONS

## Presentations

Mikó E, Vida A, Kovács T, Újlaki Gy, Kovács P, Lovas B, Sári Zs, Jankó L, Márton J, **Nagy L**, Bai P (2016) *Diverse ideas around the mitochondria*. Biotechnology days at the University of Debrecen

Marton J, **Nagy L**, Fodor T, Bhattoa Pal H, Bai P (2016) *PARP2 interferes with skeletal muscle cholesterol homeostasis and steroid metabolism*. PARP Family and Friends, Cold Spring Harbor, NY, USA

Fodor T, Mikó E, Vida A, Kovács T, Abdul-Rahman O, **Nagy L**, Trencsényi Gy, Bai P (2015) *New ways to revert Warburg-type metabolism*. FEBS3+ meeting, Portoroz, Slovenia

**Nagy L**, Docsa T, Tóth A, Somsák L, Fodor T, Gergely P, Bai P (2014) *Glycogen phosphorylase inhibitors enhance pancreatic  $\beta$  –cell function and hepatic mitochondrial metabolism*. Annual Meeting of the Hungarian Biochemical Society, Debrecen, Hungary.

Fodor T, Szántó M, Márton J, **Nagy L**, Bai P (2014) *The analysis of AMPK activation in MCF7 cell model*. Seahorse training, Cambridge, UK

Szántó M, Fodor T, **Nagy L**, Márton J, Csomita M, Kiss B, Gergely P, Virág L, Bai P (2013) *Poly(ADP-ribose) polymerase enzymes in metabolic regulation*. Conference of the Hungarian Society for Physiology, Budapest, Hungary

## Poster presentations

**Nagy L**, Márton J, Vida A, Kis G, Bokor É, Kun S, Gönczi M, Docsa T, Tóth A, Antal M, Gergely P, Csóka B, Pacher P, Somsák L, Bai P (2017) *The role of glycogen phosphorylase in  $\beta$ -cells*. HunLifeSci 2017 Eger 2017

**Nagy L**, Docsa T, Tóth A, Somsák L, Fodor T, Gönczi M, Gergely P, Bai P (2015) *Glycogen phosphorylase inhibition has positive effect on metabolism*. FEBS3+ Portoroz, Szlovénia

Szántó M, Brunyánszki A, Márton J, **Nagy L**, Fodor T, Kiss B, Bai P (2014) *The role of poly(ADP-ribose) polymerase enzymes in the regulation of cholesterol homeostasis* Frontiers in Metabolism: From Molecular Physiology to Systems Medicine EMBL Heidelberg 2014

Márton J, Szántó M, **Nagy L**, Fodor T, Kiss B, Bai P (2014) *The role of poly(ADP-ribose) polymerase-2 in the cholesterol homeostasis*. MBKE Debrecen 2014

Fodor T, Szántó M, Márton J, **Nagy L**, Bai P (2014) *Study of anti-Warburg effect of AMPK activators and antimetabolite drugs on MCF7 cell model*. MBKE Debrecen 2014

**Nagy et al.**, (2014) *Beneficial metabolic changes induced by glycogen phosphorylase inhibitor treatment*. The FEBS-EMBO 2014 Conference, Paris, France.

Docsa T, **Nagy L**, Szántó M, Hegedűs Cs, Márton J, Kónya B, Virág L, Somsák L, Gergely P, Bai P (2013) *Glycogen phosphorylase inhibitor N-(3,5-dimethyl-benzoyl)-N'-(β-D-glucopyranosyl)urea improves glucose tolerance under normoglycemic and diabetic conditions*. Europhosphatase 2013 Rehovot, Israel

T, Szántó M, **Nagy L**, Brunyánszki A, Kiss B, Gergely P, Virág L, Bai P (2013) *The role of PARP10 in metabolic regulation*. Conference of the Hungarian Society for Physiology, Budapest, Hungary.

Fodor T, Szántó M, **Nagy L**, Brunyánszki A, Gergely P, Virág L, Bai P (2013) *The role of PARP10 enzyme in mitochondrial metabolism*. Hungarian Molecular Life Sciences 2013, Siófok, Hungary

**Nagy et al.**, (2012) *Investigation of the effect of glycogen phosphorylase inhibitor N-(3,5-dimethyl-benzoyl)-N'-(β-D-glucopyranosyl)urea on energy homeostasis*. MBKE Conference on Signal Transduction, Esztergom, Hungary.

**Nagy L**, Docsa T, Szántó M, Brunyánszki A, Hegedűs Cs, Márton J, Kónya B, Virág L, Somsák L, Gergely P, Bai P (2012) *Glycogen phosphorylase inhibitor N-(3,5-dimethyl-benzoyl)-N'-(β-D-glucopyranosyl)urea induces energy expenditure*. EMBL Diabetes and Obesity Symposium, Heidelberg, Germany

The work/publication is supported by the GINOP- 2.3.2-15-2016-00006 project. The project is co-financed by the European Union and the European Regional Development Fund.