Hormone resistances in type 2 diabetes

Ph.D. thesis summary

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1. Introduction

Around the world, there were approximately 537 million adults (20-79) living with diabetes in 2021, which is 1 out of 10 people [1].

Type 2 diabetes' pathogenesis is complex [2,3], but one of its main characteristics is peripheral chronic insulin resistance and the reduced uptake of glucose by adipose tissues [4]. The cause of insulin resistance is oxidative stress due to systemic subclinical inflammation and hormonal interactions, that by nicotinamide nucleotide (NAD(P)H) oxidase enzyme activation can cause oxidative stress [5].

Phenylalanine (Phe) is an essential amino acid, that is vulnerable to the non-enzymatic oxidative processes. In an enzymatic reaction, it is used for the production of para-tyrosine. Hydroxyl free radicals are able to convert Phe to meta-, orto- and para-tyrosine. In previous studies, our group has shown that m- and o-tyrosines can be the markers of hydroxyl free radicals in type 2 diabetes [6].

Patients' serum and urinary m-Tyr and o-Tyr levels are significantly elevated in diseases associated with hydroxyl free radicals. In patients with chronic renal disease, we can detect a decrease in the level of p-Tyr, while o-Tyr excretion is elevated [7].

Significantly lower p-Tyr level in dialyzed patients was found compared to the control group, but the o-Tyr level and o-Tyr/p-Tyr ratio were higher in dialyzed patients. Elevation of the ratio of o-Tyr/p-Tyr could be responsible for decreased ESA responsiveness in dialyzed patients [8].

Urinary excretion of o-Tyr can be decreased by the administration of resveratrol in patients with type 2 diabetes, and Akt phosphorylation and insulin senisitivy increases as shown by HOMA-IR [9].

In our study, we investigated the incorporation of Phe derivatives into proteins and their effect on glucose uptake and insulin signaling, namely the changes in the phosphorylation of insulin receptor substrate-1 (IRS-1) and Akt.

We know that other hormone resistances can also occur in insulin-resistant patients, such as leptin, erythropoietin, acetylcholine, triiodothyronine, and glucagon-like peptide-1 resistances [10]. It is also accepted, that the key reason behind insulin resistance is defective signaling. Therefore, we can assume that the same postreceptoral defect can occur in other hormones working with a similar pathway.

Metformin is used for the treatment of insulin resistance in prediabetes and type 2 diabetes, it lowers the plasma glucose and triglyceride levels, and leads to a decrease in glucotoxicity and lipotoxicity. Rapid lowering of glucotoxicity also affects the cell metabolism. This process is supposed to be the background of a 'breakthrough phenomenon', which is well described in connection with insulin resistance [11].

We assumed, that if metformin is able to sensitize patients to insulin, it can also work in the case of thyroid hormones. Our goal was to investigate the effect of metformin on the T3 resistance in euthyroid, metformin-naïve, newly diagnosed type 2 diabetic patients, by measuring insulin resistance, thyroid hormones, glucose-, and lipid metabolism, TSH, blood pressure, and heart rate.

2. Aims

1. In our first study our goal was to investigate the effect of metformin in euthyroid, metformin-naïve, newly diagnosed type 2 diabetic patients

2. In our second study we wanted to examine the role of o-Tyr and m-Tyr in insulin resistance

3. Methods

3./1./1. *Clinical protocol*

In our first study, 21 patients between 40 és 75 years of age were included, who were euthyroid and drug naïve, with newly diagnosed type 2 diabetes. Previously initiated metformin therapy or other antidiabetic therapy and known thyroid disease, or treatment with LT4 were the exclusion criteria.

After being informed about the study protocol, a signed informed consent was provided by the patients.

Blood was taken from the patients before starting metformin treatment and after 4 weeks of treatment. During the 4 weeks, the metformin was up titrated (for the first 2 weeks of 500 mg, and up to 4 weeks of 1000 mg). We measured and calculated the following parameters: TSH, T3, T4, T3/T4 ratio, thyrotroph thyroid hormone sensitivity index (TTSI = 100 × TSH (mU/L) × fT4 (pmol/L)/assay-specific upper limit of the reference interval for fT4 concentration (pmol/L)), thyroid feedback quantile-based index (TFQI = population empirical cumulative distribution function to hormone concentration (cdf) fT4 − (1−cdf TSH)), fructosamine, triglyceride, HOMA-IR (= fasting plasma glucose (mmol/L) × fasting plasma insulin (pmol/L)/22.5), plasma glucose, and plasma insulin.

TTSI: We can get a more realistic picture of the state of the thyroid gland function by measuring the effect of the hormones (thyroid gland hormone sensitivity), instead of monitoring the hormone levels in the serum. The upper-mentioned index shows the level of thyroxine resistance. It is elevated when the thyroid hormone resistance is the result of THRB gene mutation [12].

TFQI: a new marker of resistance to thyroid hormone index to quantify deviations from the median pituitary response (inhibition) to thyroid hormones [13]. There are recent studies that reported an association between TFQI and metabolic diseases, and cardiovascular disease risk and found that increased TFQI was related to the increased risk for high blood pressure metabolic syndrome criterion [14].

We chose to measure fructosamine level instead of HbA_{1C} because of the 4-week duration of our study, as HbA_{1C} shows the average glucose levels of the last 3 months, while fructosamine reflects recent changes (2-3 weeks) in blood glucose levels. Serum samples were analyzed by routine laboratory tests using fully automated instrumentation (Roche) in our University's accredited centralized laboratory (PTE, Department of Laboratory Medicine).

Blood pressure and heart rate were measured using an ambulatory blood pressure monitor (ABPM04, ABPM Art Ltd.). For the statistical analysis, we used the following blood pressure (BP) and heart rate parameters: mean systolic BP, mean diastolic BP, mean arterial pressure, and mean pulse pressure, as well as mean, maximum, minimum, and standard deviation of the heart rate.

3./1./2. In vitro study

To investigate the possible mechanism behind T3 resistance, we performed an *in vitro* study. We used a human embryonal kidney (HEK293) cell line. Cells were grown on Dulbecco's modified Eagle's medium (DMEM) containing 5 mM glucose, supplied with 10% fetal bovine serum (FBS), 100 U/ml penicillin, 0,1 mg/ml streptomycin and 2 µg/ml Fluconazole. Cells were maintained in a humidified incubator at 37°C and 5% CO_2. Later, this medium was changed to media containing 5 or 25 mM glucose and the cells were incubated under these circumstances for 5 days. Cells were serum deprived for one night, then

cells in 5 and 25 mM glucose were treated with T3 (0, 0.1, 0.3, 1 nM), and another group of cells in 25 mM glucose with T3 (0, 0.1, 0.3, 1 nM) and metformin (10 mM) for 20 min. The treatment was followed by a washing step (twice with 4 ºC PBS), then the cells were mechanically scraped off in the presence of radio-immunoprecipitation assay (RIPA) lysis buffer and were frozen at −80°C.

We performed Western blot analysis using p-Akt and total Akt staining. Protein content was measured with the Bradford method, and bovine serum albumin (BSA) was used as standard. Samples were solubilized in a buffer of 100 mmol/L Tris–HCl (pH 6.8), 4.0% sodium dodecyl sulfate (SDS), 20% glycerol, 200 mmol/L DTT, and 0.2% bromophenol blue. Samples (80–120 μg protein) were electrophoretically resolved on polyacrylamide gels (10%) and transferred in a buffer (pH 8.3) containing 38 mmol/L glycine, 48 mmol/L Tris-base, and 20% methanol to PVDF membranes for 90 min at 250 mA. Membranes were washed in 0,1%, TBST blocked in 5% BSA, then incubated overnight in anti-phospho-(Ser473)-Akt antibody diluted in TBST containing 5% BSA, then after washing for 1 hour in peroxidase-conjugated IgG secondary antibody diluted in TBS-T with 5% BSA. To reprobe Western blots with alternative primary antibodies for total PKB/Akt, membranes were stripped as follows: membranes were washed in stripping buffer, containing 1.5% glycine, 0.1% SDS, 1% Tween-20 at pH 2.2, for 2 × 10 min, then washed in PBS 0.1% for 2 × 5 min, then washed in TBS-T 0.1% for 2 × 5 min. Membranes were incubated for 1 hour in the total Akt-containing solution, then after washing steps for another 1 hour in peroxidase-conjugated IgG secondary antibody. We used enhanced chemiluminescence for to visualize the bands. We used Scion Image for Windows Software to analyze the specific bands.

5./1./3. Statistical analysis

We used the Statistical Package for the Social Sciences (SPSS) software, version 24.0 (IBM Corporation) for the statistical analysis. The Kolmogorov–Smirnov test was used to test normality, in cases of normal distribution mean** ± SD was given, and a paired sample *t*-test was applied, while in the case of non-normal distribution, the Mann–Whitney *U*-test was used. Values of $P < 0.05$ were considered as significant.

5./2./1. Podocyte cell culture

We cultured immortalized podocyte cell culture (Moin Saleem, Bristol University, UK) on RPMI1640, supplemented the medium with 10% FBS, insulin-transferrin-selenium, 100 U/ml penicillin, 0,1 mg/ml streptomycin and 112.5 nM p-, m- or o-Tyr (same, as the p-Tyr content of the medium). Cells were grown at 33 ºC and 5% CO_2. When they reached 40-60% confluency, they were transferred to 37 ºC to differentiate. After the thermoswitching, cells were kept on RPMI1640 medium containing 2% FBS, antibiotics, and different tyrosines. Cells were incubated in a serum-deprived medium overnight, before every treatment.

5./2./2. HPLC analysis

Methods were based on earlier publications with minor modifications [7].

To measure the total protein-bound cellular tyrosine concentration, cells were lysed in 200 µl distilled water, then they were kept at -70 ºC. After thawing, next step was centrifugation for 10 minutes at 4000 rpm. 200 µL supernatant was mixed with 200 µL 60% trichloroacetic, then the samples were incubated on ice for 30 min to precipitate proteins. They were centrifuged for another 10 minutes at 4000 rpm, then the sediment was resuspended in 1% trichloroacetic acid and 4 µL of 400 mmol/L desferrioxamine. Then, 40 µL of 500 mmol/L

butylated hydroxytoluene was added to the samples to avoid possible free radical formation during hydrolysis. A total of 200 µL of 6 N hydrochloric acid was added in order to hydrolyze the proteins at 120 °C overnight. The hydrolysate was then filtered through a 0.2 µm filter and 20 µL of the filtrate was injected onto the HPLC column of a Shimadzu Class LC-10 ADVP HPLC system using a Rheodyne manual injector.

To measure the p-, o- és m-Tyr content of the samples their autofluorescence was measured, derivatization or staining was not needed. Samples were run on a Shimadzu Class 10 HPLC system equipped with an RF-10 AXL fluorescent detector. The mobile phase consisted of 1% sodium acetate and 1% acetic acid dissolved in water. The separation took place on a LiChroCHART 250-4 column in an isocratic run. To assess p-, o- and m-Tyr, the following wavelengths were used: 275 nm for excitation and 305 nm for emission, while Phe was detected at 258 nm excitation and 288 nm emission wavelengths. Determination of the area under the curve (AUC) plus external standard calibration was used to calculate the exact concentrations of the amino acids.

5./2./3. Western blot analysis

Cells were treated with 400 nM insulin, then washed in cold saline twice, and scraped off mechanically in lysis buffer (1 mol/l tris base, 1,15% Triton-X, 0,2 mol/l EGTA, 0,5 mol/l EDTA, 5 mg/l phenylmethylsulfonyl fluoride (PMSF), 0,1 mol/l dithiothreitol (DTT), 0,1 mol/l Na_3VO_4, 5mg/ml aprotinin, 5mg/ml leupeptin and phosphatase inhibitor cocktail 1 and 2). The lysates were vortexed and centrifuged for 10 min at 13,000 rpm, 4 °C. Their protein content was determined by the Lowry method using bovine serum albumin as a standard. Samples were solubilized in a loading buffer containing 100 mmol/L Tris-HCl (pH 6.8), 4.0% sodium dodecyl sulfate (SDS), 20% glycerol, 200 mmol/L DTT, 0.2% bromophenol blue, then the solution containing 80-120 µg protein was loaded to 7.5% polyacrylamide gels and were electrophoretically resolved and transferred to PVDF membranes. To ensure the transfer

was successful, membranes were stained with Ponceau dye. Non-specific binding sites were blocked in 5% BSA in TBS-T solution at room temperature for one hour.

Membranes were incubated in the primary antibody anti-phospho-(Tyr612)-IRS-1 in a final dilution of 1:2000 overnight at 4 ºC. The next day the membranes were washed three times in TBS-T, then they were incubated with HRP-conjugated anti-rabbit IgG secondary antibody diluted in the blocking solution (1:4000) for one hour at room temperature and membranes were washed again three times for 5 min in TBS-T. To re-probe Western blots with alternative primary antibodies, first, we added a stripping step as follows: membranes were washed in 0.1% TBS-T for 10 min, then merged in stripping buffer containing 0.1% SDS, 1.5% glycine, and 1% Tween-20 at pH 2.2, twice for 10 min, then they were washed in PBS twice for 5 min, and in TBS-T 0.1% twice for 5 min. The membranes were blocked in 5% BSA in TBS-T solution at room temperature for one hour and then incubated with the total IRS-1antibody in a final dilution of 1:1000 for 1 hour at room temperature. The membranes were washed three times in TBS-T for 5 minutes, then incubated with HRP-conjugated secondary antibody diluted in the blocking solution (1:2000) for one hour at room temperature. Membranes were washed in TBS-T (three times for 5 minutes). Afterward, they were incubated in enhanced chemiluminescence HRP substrate. Scion Image for Windows software was used to analyze the specific bands. Protein signals were corrected for total IRS-1 protein levels and adjusted to controls.

5***./2./4. Immunofluorescence***

Podocyte cells were cultured in a 6-well plate on glass coverslips previously washed with alcohol and dried under UV light. The cells were grown at 33 °C, 5% CO_2 on RPMI1640 medium supplemented with 10% FBS until they reached 60% confluency, then they were transferred to 37 °C, 5% CO_2 to allow the cells to differentiate and the medium was switched to RPMI1640 medium containing 2% FBS and 112.05 nM of the different tyrosine isoforms.

Before being incubated with 400 nmol/l insulin for 10 min, cells were serum-deprived overnight. Afterward, the medium was removed and the coverslips were washed twice with PBS. They were fixed at room temperature in 2% paraformaldehyde and 4% sucrose for 8 min, then permeabilized using 0.3% Triton X-100 in 1xPBS for 20 min and blocked in 2.5% BSA for 45 min [15].

The cells were incubated in humid conditions for 60 minutes with the following primary antibodies: mouse anti-WT1 antibody (H-1) (1:100), chicken anti-vimentin antibody (1:100), rabbit anti-glucose transporter GLUT4 antibody (1:100), mouse Insulin Receptor Substrate-1 antibody (1:100) and rabbit anti-phospho-Insulin Receptor Substrate-1 (pTyr612) antibody (1:10), all diluted in 1xPBS. After the incubation the coverslips were washed in PBS three times for 5 min in PBS and incubated in the fluorophore-conjugated secondary antibodies anti-goat, anti-chicken, secondary antibody Alexa fluor 647, anti-rabbit Alexa fluor 350, and anti-mouse Alexa Fluor 488 (1:10) for 60 min. Then after another washing steps (3 times in PBS) the samples were mounted in Vectashield. Nikon Eclipse Ti2 microscope was used for taking the microscopic images.

5./2./5. Statistical analysis

SPSS Statistics 27 was used to carry out the statistical analysis. The data was checked for normality of distribution using the Kolmogorov–Smirnov test. Those with normal distribution were analyzed using parametric tests, while non-parametric tests were used for non-normally distributed data.

4. Results

4./1./1. Metformin reduces T3, T3/T4 ratio, fructosamine, and HOMA-IR

After 4 weeks of metformin therapy, the T3 level, T3/T4 ratio, fructosamine level, and HOMA-IR all changed.

We could detect a significant decrease in the level of T3 (before treatment: 5.24±0.7 pmol/l; after metformin treatment: 4.71±0.6 pmol/l; $p<0.001$).

The ratio of T3/T4 was also diminished (before treatment: 0.33±0.065; after metformin treatment: 0.3±0.065; p=0.038].

We measured fructosamine instead of HbA_{1c} because of the short duration of the study, its values were also lower after metformin therapy (before treatment: 300±74.7 mmol/l; after metformin treatment: 274.6±52.8; P=0.008)

HOMA-IR representing insulin resistance showed a decrease, as well (before treatment: 2.6±1.2; after metformin treatment: 2.05±1.0; p=0.022).

4./1./2. Unchanged parameters

There were parameters, that remained unchanged, the levels of TSH, T4, TTSI, TFQI triglyceride, plasma glucose, plasma insulin, and the values of blood pressure and heart rate.

4./1./3. Akt phosphorylation in HEK cells

Our *in vitro* experiment was conducted using the HEK293 cell line. T3 treatment (0.1, 0.3, and 1 nM)-induced Akt phosphorylation in cells grown on a medium containing 5 mM glucose (control: 100±18.12; 0.1 nM T3 treatment: 227.27±21.2 p=0.013; 0.3 nM T3

treatment: 158.74±25.97 p=0.045; 1 nM T3 treatment: 173.88±24.92 p=0.038). But triiodothyronine had no effect on p-Akt/Akt ratio in cells cultured in 25 mM glucose (control: 100±8.77; 0.1 nM T3 treatment: 114.97±17.84, p=0.421; 0.3 nM T3 treatment: 102.74±9.87, p=0.813; 1 nM T3 treatment:105.93±9.28, p=0.732) and 25 mM glucose with metformin (10 mM) (control: 100±3.67; 0.1 nM T3 treatment 106.83±2.92, p=0.763; 0.3 nM T3 treatment: 104.9±4.15, p=0.712; 1 nM T3 treatment: 101.16±2.43, p=0.911).

4./2./1. Both o- and m-Tyr can be taken up by podocyte cells and are incorporated into cellular proteins

Insulin signaling may be altered by the incorporation of abnormal amino acids into their proteins. We tested whether the abnormal amino acids could be taken up by the cells and if the cells could incorporate o- and m-Tyr into their proteins.

The ratio of p-Tyr/Phe remained unchanged or was lower, and the protein-bound o-Tyr/p-Tyr and m-Tyr/p-Tyr ratio were increased in the cells grown on o- or m-Tyr. These experiments suggested that abnormal amino acids can be incorporated into podocyte cell proteins.

4./2./2. Phosphorylation of IRS-1 and AKT in podocyte cells grown on o- or m-Tyr

Key steps of insulin signaling responsible for glucose uptake, the phosphorylation levels of the insulin-receptor substrate-1 (IRS-1) and Akt (protein kinase B) were studied, so we could detect the mechanism behind the inhibitory effect of o- and m-Tyr on insulin-induced glucose uptake. In p-Tyr containing medium, insulin treatment caused an approximately two-fold increase in the activating phosphorylation of IRS-1 [p-Tyr control: 100±26.3; insulin-treated: 192.6±44.83, p=0.048]. We couldn't verify this change in the phosphorylation when m- or o-Tyr was added to the media. [m-Tyr control: 194.8±71.7;

insulin-treated: 255.8±89.4, p=0.315; o-Tyr control: 251.5±118.9; insulin-treated: 256.2±141.2, p=0.808].

The same pattern could be seen in the case of Akt [p-Tyr control: 100±20.3; treated: 182±18.12; p=0.0065]. In cells grown on o- or m-Tyr, this significant increase in phosphorylation of Akt can not be detected [m-Tyr control: 155±21.97; insulin-treated: 202±24.78, p=0.442; o-Tyr control: 170.8±32.61; insulin-treated: 208.087±20.65, p=0.256]. It is interesting, that in cells grown on o- and m-Tyr containing medium, the basal levels of Akt phosphorylation were higher and could not be further raised by insulin treatment [p-Tyr control: 100±20.3; m-Tyr control:155±21.97; p=0.046; p-Tyr control: 100±20.3; o-Tyr control: 170.8±32.61; p=0.048].

4./2./3. Microscopical analysis

In p-Tyr containing medium phosphorylation of IRS-1 leads to its membrane translocation, but this change in localization can not be detected if the cells are cultured on m- and o-Ty containing medium. The intensity of p-IRS-1 is also lower in the cells, that incorporated m- and o-Tyr.

The same membrane translocation can be seen in the case of a GLUT4, which is responsible for insulin-dependent glucose transport, but only if the cells were grown on p-Tyr-containing medium. In cells grown on o-Tyr és m-Tyr containing medium, GLUT4 is localized in the perinuclear region. Vimentin has a role in the transport of GLUT4, we could detect changes in the vimentin filaments also. In cells grown on p-Tyr-containing medium the filaments were thin, they were slightly thicker when cells were grown with m-Tyr and even thicker when they were grown in the presence of o-Tyr.

5. Discussion

5./1. Metformin decreases T3 resistance in euthyroid, type 2 diabetic patients

In euthyroid, newly diagnosed, type 2 diabetic, drug naïve patients metformin reduced both the level of T3 and the ratio of T3/T4 and even fructosamine and HOMA-IR, while TSH, T4, blood pressure, and heart rate remained unchanged.

In the human cell line high glucose concentration decreased T3-induced Akt phosphorylation, which could not be reversed by metformin.

Previous studies have investigated the effect of metformin on the thyroid gland, but in those studies, they examined metformin's effect in either subclinical hypothyroid or treated overt hypothyroid patients.

5./2. Association between non-physiological tyrosine isoforms and insulin resistance

In our second study our goal was to investigate the effect of orto- and meta-tyrosine on the insulin response of a podocyte cell line.

We performed our study on a podocyte cell line, protocols were based on our group's previous research with the 3T3-L1 cell line. By using HPLC we observed that o- and m-Tyr could be taken up by the cells incorporating them into cellular proteins.

We report for the first time, that the abnormal amino acids, o- and m-Tyr inhibit insulin-induced glucose uptake of podocyte cells. Using Western blot we proved that insulin signaling is altered in cells grown on an o- or m-Tyr-containing medium.

Our microscopic investigation showed that adding different tyrosine isoforms to the cells leads to changes in the localization of IRS-1 and pIRS-1 and their ratio. The localization of GLUT4 is also changed (which means the glucose uptake is altered) and the vimentin filaments morphology was also different.

Our data suggests that while in cells grown on p-Tyr containing culture medium, insulin is able to induce glucose uptake to approximately two-fold; in cells grown on medium containing glucose, either o- or m-Tyr, glucose uptake is reduced. This effect could be observed after one day, and it is visible for at least 12 days. These observations prompt for a direct role of o- and m-Tyr itself in the glucose uptake and not the role of hydroxyl free radicals, as in this setting o- and m-Tyr were applied in the absence of an obvious hydroxyl free radical.

Our present data suggest that similarly to a high glucose environment, supplementation of podocyte cells with o- or m-Tyr leads to insulin resistance in the cells. In cells grown with o- or m-Tyr, insulin failed to increase IRS-1 phosphorylation.

While insulin was able to stimulate phosphorylation of Akt in p-Tyr grown cells in normal glucose media, in the presence of o- and m-Tyr, insulin didn't have any effect, similarly to the high glucose environment. Inhibition of Akt-phosphorylation in a high glucose environment was detected in other studies also, but there the 25mM glucose-containing medium was used as a control and 60mM glucose-containing medium as high glucose medium [16]. The effect of different tyrosine isoforms was not studied before.

Immunofluorescent staining showed that the incorporation of mTyr or o-Tyr altered the localization of IRS-1, pIRS-1, GLUT4, and vimentin. Total IRS-1 is located in the perinuclear region. pIRS-1 could be found near the membrane, but only in cells grown on p-

Tyr. In cells grown on m-Tyr or o-Tyr-containing medium, pIRS-1 was scattered in the cytoplasm. Our conclusion is, that adding m-Tyr or o-Tyr to the medium lowers insulin response due to the lower phosphorylation of IRS-1.

We also investigated the effect of non-physiological tyrosines on GLUT4 and vimentin localizations. When the cells were grown on p-Tyr containing medium, in the insulin-treated cells, the GLUT4 aggregates were localized near the membrane as described by others [17]. The transportation of GLUT4 to the membrane is not as prominent in cells grown on m-Tyr and is completely absent when the cells were grown on o-Tyr, where GLUT4 could be found in the perinuclear region. As we could expect, GLUT4 and vimentin showed colocalization [18].

An interesting observation was, that the morphology of the vimentin filament was different when the cells were grown on a non-physiological tyrosine-containing medium. In m- and o-Tyr-containing media, cells' vimentin filaments became thicker.

The changes in the morphology of vimentin are interesting when because there are studies that show the overexpression of vimentin in cancerous diseases, cataracts, Crohns disease, and rheumatoid arthritis. There is increased vimentin mRNA and protein in aging cells [19].

6. List of the Ph.D. theses

1. Our new concept is that in type 2 diabetic patients T3 resistance could be present

2. Supplementing podocyte cells with m- or o-Tyr leads to insulin resistance, just like high glucose does.

3. Localization of IRS-1 and pIRS-1, GLUT4, and vimentin is altered in cells when non-physiological tyrosines are incorporated into their proteins.

7. List of publications used for the theses

I. Kertész, Melinda ; Kun, Szilárd ; Sélley, Eszter ; Nagy, Zsuzsanna ; Kőszegi, Tamás ; Wittmann, István. A breakthrough-like effect of metformin reduces peripheral resistance to triiodothyronine in euthyroid, non-insulin-resistant, type 2 diabetic patients.

ENDOCRINE CONNECTIONS 10(7):782–788, 2021. **IF: 3,221**

II. Judit Mohás-Cseh, Gergő Attila Molnár, Marianna Pap, Boglárka Laczy, Tibor Vas, **Melinda Kertész**, Krisztina Németh, Csaba Hetényi, Orsolya Csikós, Gábor K. Tóth, Attila Reményi and István Wittmann. Incorporation of Oxidized Phenylalanine Derivatives into Insulin Signaling Relevant Proteins May Link Oxidative Stress to Signaling Conditions Underlying Chronic Insulin Resistance

BIOMEDICINES 10(5):975, 2022. **IF: 4.757**

Total IF: 7.978

List of publications not related to the thesis

Molnar, GA ; Kun, S ; Selley, E ; **Kertesz, M** ; Szelig, L ; Csontos, C ; Boddi, K ; Bogar, L ; Miseta, A ; Wittmann, I. Role of Tyrosine Isomers in Acute and Chronic Diseases Leading to Oxidative Stress - a review CURRENT MEDICINAL CHEMISTRY 23: 7 pp. 667-685. , 19 p. (2016) IF: 3.249

Sélley, E ; Kun, Sz ; Szijártó, IA ; **Kertész, M** ; Wittmann, I ; Molnár, GA. Vasodilator effect of glucagon. Receptorial crosstalk among glucagon, GLP-1 and receptor for glucagon and GLP-1. HORMONE AND METABOLIC RESEARCH 48: 7 pp. 476-483. , 8 p. (2016) IF: 2.268

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Total IF: 6.027

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