Učinak intenzivne inzulinske terapije na koncentraciju dušikova oksida i aktivnost adenozin-deaminaze kod sekundarnog neuspjeha liječenja sulfonilurejom

Effect of intensive insulin therapy on systemic nitric oxide levels and adenosine deaminase activity in secondary sulfonylurea failure

Ayşe Binnur Erbağcı[1], Rukiye Deveci[1], Ersin Akarsu[2], Özlem Tiryaki[2], Ş. Nur Aksoy[1]

[1]Odjel za biokemiju i kliničku biokemiju, Medicinski fakultet, Sveučilište u Gaziantepu, Gaziantep, Turska

[1]Department of Biochemistry and Clinical Biochemistry, School of Medicine, University of Gaziantep, Gaziantep, Turkey

[2]Interna medicina, Medicinski fakultet, Sveučilište u Gaziantepu, Gaziantep, Turska

[2]Internal Medicine, School of Medicine, University of Gaziantep, Gaziantep, Turkey

Sažetak

Uvod: Vjerojatno je da upala, oksidativni stres i apoptoza imaju ulogu u sekundarnom neuspjehu liječenja sulfonilurejom, koji se povezuje s propadanjem beta-stanica i njihovom smanjenom funkcijom. Dušikov oksid (NO) stimulira otpuštanje inzulina, ima proupalne, apoptotičke učinke te djeluje kao slobodni radikal. Adenozin-deaminaza (ADA) regulira koncentraciju adenozina, koja utječe na otpuštanje inzulina i glukagona te na periferni metabolizam glukoze. Cilj ovoga istraživanja bio je ispitati aktivnost ADA i koncentraciju NO kao potencijalne posrednike intenzivne inzulinske terapije kod bolesnika sa sekundarnim neuspjehom liječenja sulfonilurejom.

Materijali i metode: U istraživanje su bila uključena 24 bolesnika sa šećernom bolešću tipa 2 i sekundarnim neuspjehom liječenja sulfonilurejom. Koncentracije NO u serumu i aktivnosti ADA određene su u uzorcima krvi uzetim prije liječenja, nakon trodnevne inzulinske infuzije i nakon šestomjesečne višestruke supkutane primjene inzulina. Aktivnost ADA određena je metodom prema Giustiu. Koncentracija NO izmjerena je kolometrijskom metodom prema Griessu.

Rezultati: Kod sekundarnog neuspjeha liječenja sulfonilurejom, bazalna je aktivnost ADA (17,0 [14,6-21,7] U/L) bila statistički značajno niža nego aktivnost ADA izmjerena trećeg dana (20,5 [16,2-23,4] U/L; P = 0,018) i u šestom mjesecu (21,2 [16,6-22,5] U/L; P = 0,010). Između vrijednosti NO određenih na početku (18,8 [11,6-28,4] μmol/L), trećeg dana (17,8 [9,7-33,6] μmol/L; P = 0,966) i u šestom mjesecu (21,7 [16,0-33,9] μmol/L; P = 0,230) nije bilo statistički značajne razlike.

Zaključak: Prema našem je istraživanju aktivnost ADA bila povećana u objema fazama, ranoj i kasnoj fazi intenzivne inzulinske terapije kod sekundarnog neuspjeha liječenja sulfonilurejom. O ulozi NO kod pogoršanja funkcije beta-stanica nismo puno saznali na razini perferne krvi.

Ključne riječi: adenozin-deaminaza; intenzivna inzulinska terapija; dušikov oksid; sekundarni neuspjeh liječenja sulfonilurejom

Abstract

Background: Inflammation, oxidative stress and apoptosis are suggested to take part in secondary sulfonylurea failure associated with β cell destruction and impaired β cell function. Nitric oxide (NO) stimulates insulin release, has proinflammatory, apoptotic and free radical effects. Adenosine deaminase (ADA) exerts control on adenosine levels, which affects insulin and glucagon release and peripheral glucose metabolism. In this study, we aimed to investigate ADA activity and NO levels as potential mediators of intensive insulin treatment in patients with secondary sulfonylurea failure.

Materials and methods: Twenty-four patients with type 2 diabetes mellitus and secondary sulfonylurea failure were enrolled in the study. Serum NO levels and ADA activity were determined in blood samples obtained prior to treatment, after three-day insulin infusion and after six-month multiple subcutaneous insulin administration. ADA activities were estimated by the method of Giusti. Nitric oxide levels were measured with colorimetric assay by the method of Griess reaction.

Results: In secondary sulfonylurea failure, baseline ADA activity (17.0 [14.6-21.7] U/L) was significantly lower than ADA activity measured on day 3 (20.5 [16.2-23.4] U/L; P = 0.018) and at 6 months (21.2 [16.6-22.5] U/L; P = 0.010). There was no statistically significant difference between NO levels determined before (18.8 [11.6-28.4] μmol/L), on day 3 (17.8 [9.7-33.6] μmol/L) and at 6 months (21.7 [16.0-33.9] μmol/L; P = 0.966 and P = 0.230, respectively).

Conclusions: According to our study, ADA activity is increased both in the early and late periods of intensive insulin therapy in secondary sulfonylurea failure. The role of NO in amelioration of β cell function is not supported at the level of peripheral blood.

Key words: adenosine deaminase; intensive insulin therapy; nitric oxide; secondary sulfonylurea failure

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Uvod

Sulfonilureja stimulira lučenje inzulina u beta-stanicama gušterače te je u pravilu lijek prvog izbora kod šećerne bolesti tipa 2. Međutim, nakon dugotrajnog liječenja sulfonilurejom neki bolesnici ne odgovaraju na liječenje, što je povezano s ponovnim povećanjem glukoze u krvi, tj. sekundarnim neuspjehom liječenja sulfonilurejom (1). Progresivno smanjenje količine beta-stanica, smanjenje sekretornih rezerva beta-stanica, inzulinska rezistencija i disfunkcija pretvorbe proinzulina su stanja povezana sa patogenezom sekundarnog neuspjeha liječenja sulfonilureje (1,2). Glavni čimbenici progresivnog gubitka funkcije i količine beta-stanica su glukotoksičnost, lipotoksičnost, proupalni citokini, reaktivni kisikovi spojevi i ubrzana apoptoza (3,4). Čini se da su poremećena funkcija beta-stanica i količina beta-stanica reverzibilni, naročito u ranijim fazama bolesti. Kratkotrajna intenzivna inzulinska terapija ubraja se među intervencije za očuvanje ili „pomlađivanje" beta-stanica i poboljšanje osjetljivosti na inzulin (2).

Dušikov oksid (NO) je glasnik koji slobodno difundira kroz membrane te je uključen u mnoga fiziološka i patološka stanja. Interakcija NO sa superoksidom koja rezultira stvaranjem peroksinitrita ($ONOO^-$) i inhibicija citokrom *c* oksidaze koja uzrokuje smanjenje potencijala membrane mitohondrija i oslobađanja citokroma *c* iz mitohondrija, smatraju se mehanizmima kojima NO izaziva apoptozu (5-7). Spominje se i utjecaj NO na lučenje inzulina ili na inzulinsku rezistenciju (8-12).

Adenozin-deaminaza (ADA; EC 3.5.4.4) katalizira nereverzibilnu hidrolitičku deaminaciju adenozina i deoksiadenozina kako bi izlučili inozin, odnosno deoksiinozin, kao dio puta recikliranja purinskih baza. Doprinosi regulaciji unutarstaničnih i izvanstaničnih koncentracija adenozina i deoksiadenozina zajedno s 5' nukleotidazom i adenozin-kinazom (13). Iako je glavna funkcija ADA razvoj imunog sustava kod ljudi, čini se da je također povezana s diferencijacijom epitelnih stanica i monocita te s upalom (14). Sve je očitije da je adenozin pleotropna molekula uključena u upalni sustav, apoptozu, rast stanica i lučenje inzulina (14-16).

Cilj ovoga istraživanja bio je ispitati aktivnost ADA i koncentraciju NO kao potencijalnih posrednika intenzivnog inzulinskog liječenja kod bolesnika sa sekundarnim neuspjehom liječenja sulfonilurejom.

Materijali i metode

Ispitanici

Istraživanje je provedeno 2004. godine na Medicinskom fakultetu Sveučilišta Gaziantep, na Zavodu za biokemiju i kliničku biokemiju te na Klinici za unutarnje bolesti. Svi su ispitanici dali obaviješteni pristanak prema Helsinškoj

Introduction

Sulfonylurea stimulates insulin secretion by pancreatic β-cells and is generally used as a first-line treatment for type 2 diabetes. However, after long-term sulfonylurea therapy, some patients do not respond to treatment anymore, which is associated with an increase in blood glucose again, known as secondary sulfonylurea failure (1). Progressive decrease in β-cell mass, deterioration in β-cell secretory reserve, insulin resistance and dysfunction of the proinsulin conversion machinery have been suggested in the pathogenesis of secondary sulfonylurea failure (1,2). The major factors for progressive loss of β-cell function and mass are glucotoxicity, lipotoxicity, proinflammatory cytokines, reactive oxygen species, and accelerated apoptosis (3,4). Impaired β-cell function and possibly β-cell mass appear to be reversible, particularly at early stages of the disease. Short-term intensive insulin therapy is among the interventions to preserve or "rejuvenate" β-cells, and improve insulin sensitivity (2).

Nitric oxide (NO) is a diffusible messenger that has been implicated in numerous physiological and pathological conditions. The interaction of NO with superoxide resulting in the formation of peroxynitrite ($ONOO^-$) and NO inhibition of cytochrome *c* oxidase causing a decrease in mitochondrial membrane potential and cytochrome *c* release from mitochondria are proposed mechanisms by which NO induces apoptosis (5-7). An effect of NO on either insulin secretion or resistance is also suggested (8-12).

Adenosine deaminase (ADA; EC 3.5.4.4) catalyzes irreversible hydrolytic deamination of adenosine and deoxyadenosine to yield inosine to deoxyinosine, respectively, as part of purine salvage pathway. It contributes to the regulation of intracellular and extracellular concentrations of adenosine and deoxyadenosine, along with 5' nucleotidase and adenosine kinase (13). Although the main function of ADA is the development of the immune systems in humans, it also seems to be associated with the differentiation of epithelial cells and monocytes, and inflammation (14). It is increasingly apparent that adenosine is a pleotropic molecule implicated in inflammatory system, apoptosis, cell growth and insulin secretion (14-16).

In this study, we aimed to investigate ADA activity and NO levels as potential mediators of intensive insulin treatment in patients with secondary sulfonylurea failure.

Materials and methods

Subjects

The study was conducted at Gaziantep University, Faculty of Medicine, Department of Internal Medicine, and Department of Biochemistry and Clinical Biochemistry in 2004. Informed consent was obtained from all sub-

deklaraciji revidiranoj 1996. U istraživanje je uključeno 48 uzastopnih ambulantnih bolesnika koji su 2004. s uputnicom došli na Kliniku za endokrinologiju i metabolizam s dijagnozom šećerne bolesti tipa 2 (prema rezultatima glukoze natašte i oralnog testa opterećenja glukozom koje preporuča Američka udruga za šećernu bolest) te sekundarnim neuspjehom liječenja sulfonilurejom. Sekundarni neuspjeh u liječenju sulfonilurejom definira se kao nedovoljna kontrola glikemije ($HbA_{1c} \geq 7\%$) unatoč primanju maksimalne doze sulfonilureje (240 mg/dan gliklazida ili 6 mg/dan glimepirida) u trajanju od najmanje 4 tjedna, kod bolesnika koji su prethodno postigli zadovoljavajuću kontrolu glikemije sulfonilurejom u minimalnom trajanju od 6 mjeseci. Kriteriji za isključenje iz istraživanja bili su inzulinska terapija, neredovito uzimanje oralnih antidijabetika, uzimanje dijabetogenih lijekova poput glukokortikoida, diuretika i beta-blokatora, stanja povezana s medicinskim stresom, uzimanjem alkohola, trudnoća ili dojenje, primarni neuspjeh liječenja sulfonilurejom te neka dodatna sistemska bolest, npr. zatajenje bubrega, endokrini poremećaji. Bolesnici su praćeni 24 tjedna i 24 bolesnika su isključena iz istraživanja prema navedenim kriterijima. Istraživanje je tako provedeno na 17 žena i 7 muškaraca sa sekundarnim neuspjehom liječenja sulfonilurejom (min-max: 42-73; medijan: 56; interkvartilni raspon: 53-64 godina). Raspon trajanja šećerne bolesti kod ispitanika bio je 4-32 godine. Početno se koncentracija glukoze u plazmi kontrolirala tri dana inzulinskom infuzijom od 8,88 mmol/L. Nastavilo se višestrukim injekcijama inzulina kroz 6 mjeseci; bolesnici su dobivali redovite supkutane inzulinske injekcije tri puta na dan i jednu supkutanu injekciju NPH (engl. *Neutral Protamine Hagedorn*) - srednjedugodjelujućeg inzulina prije spavanja. Tijekom istraživanja bolesnici su nastavili sa svojim režimom prehrane i programom vježbanja. Praćenje ispitanika provodilo se ambulantnim kontrolnim pregledima dva puta u prvom mjesecu i jednom u slijedećih pet mjeseci.

Inzulinska rezistencija izračunala se pomoću procjene modela homeostaze (engl. *homeostasis model assessment,* HOMA-IR), koji su prvotno opisali Mathew i sur. (17). HOMA-IR se izračunala formulom:

$$\text{HOMA-IR} = \text{glukoza natašte (mmol/L)} \times \text{inzulin natašte (}\mu\text{mol/mL)}/22{,}5.$$

Proteinurija je definirana kao koncentracija ukupnih proteina ≥ 0,15 g u uzorku 24-satne mokraće.

Metode

Venska krv uzorkovana je standardno u prijepodnevnim satima između 9.30 i 11.00 nakon 12-satnog gladovanja. Uzorci seruma su odmah odvojeni nakon desetominutnog centrifugiranja na 4 °C, 2000 g i pohranjeni na -20 °C

jects according to the Helsinki Declaration as revised in 1996. Forty eight consecutive outpatients referred to Endocrinology and Metabolism Clinic in 2004 with a diagnosis of type 2 diabetes mellitus (according to fasting plasma glucose or oral glucose tolerance test as recommended by the American Diabetes Association) and secondary sulfonylurea failure were enrolled in the study. Secondary sulfonylurea failure is defined as insufficient glycemic control (HbA1c ≥ 7%) despite receiving maximal dosage of sulfonylureas (240 mg/day gliclazide or 6 mg/day glimepiride) for at least four weeks in patients that have previously achieved sufficient glycemic control with sulfonylureas for a minimum of six months. Exclusion criteria were insulin therapy, irregular use of oral antidiabetics, diabetogenic medications like glucocorticoids, diuretics and β-blockers, conditions associated with medical stress, alcohol intake, pregnancy or lactation, primary sulfonylurea failure, and an additional systemic illness, e.g. renal failure, endocrine disorders, etc. Patients were followed for 24 weeks and 24 were excluded according to the criteria mentioned above. Seventeen female and seven male secondary sulfonylurea failure patients (min-max: 42-73, median: 56, interquartile range: 53-64 years) were recruited. In the study group, diabetes duration ranged from 4 to 32 years. Initially, plasma glucose level was regulated below 8.88 mmol/L by insulin infusion for three days, thereafter maintained with multiple subcutaneous insulin injections for six months: subcutaneous regular insulin injection three times a day plus subcutaneous NPH insulin injection at bed time. During the study period, patients continued their previous diet and exercise programs. Follow-up was performed by outpatient control visits twice in the first month and once in the next five months.

Insulin resistance was assessed using the homeostasis model assessment (HOMA-IR) originally described by Mathew *et al*. (17). HOMA-IR was calculated using the following formula:

$$\text{HOMA-IR} = \text{fasting glucose (mmol/L)} \times \text{fasting insulin (}\mu\text{mol/mL)}/22.5.$$

Proteinuria was defined as total protein ≥ 0.15g/24-h urine.

Methods

Blood samples were collected using standard venipuncture technique between 9.30 to 11.00 a.m. after 12-h fast. Serum samples were separated immediately after centrifugation at 4 °C, 2000 g for 10 min and stored at -20 °C until analysis, which was performed in the same run to avoid inter-run analytical variation.

do analize, što je načinjeno u jednoj seriji, kako bi se izbjegla analitička varijacija unutar serije.
Ukupna aktivnost ADA u serumu određena je na 37 °C prema metodi koju su opisali Giusti i Galanti, temeljenoj na reakciji po Bertholetu, odnosno nastanku obojenih kompleksa indofenola iz amonijaka oslobođenog iz adenozina, te je kvantificirana spektrofotometrijski (13). Optička je gustoća izmjerena spektrofotometrijski na 625 nm u reakcijskoj smjesi (konačan volumen 1 mL) koja je sadržavala 12 mM adenozin hemisulfata, 50 mM fosfatnog pufera (pH 6,5) i 0,05 mL seruma. Jedna jedinica ADA definirana je kao količina enzima potrebna za otpuštanje 1 µmol/min amonijaka iz adenozina u standardnim uvjetima testa. Preciznost testa za određivanje ADA unutar serije te iz dana u dan određena je iz *poola* seruma na 20 ponavljanja u jednoj seriji i 10 različitih serija, uz CV za preciznost unutar serije 2,53% i za preciznost iz dana u dan 3,37%.
Koncentracija dušikova oksida izmjerena je kolometrijskom metodom prema Griessu, a koncentracije NO_2^- i NO_3^- izmjerene su u serumu prema uputama proizvođača (pribor za kolometrijski test Nitrate/Nitrite, Cayman Chemical Co, Ann Arbor, MI, SAD). Proteini u mokraći su analizirani pomoću turbidimetrijske metode na analizatoru Roche/Hitachi modular P analyzer prema uputama proizvođača (U/CSF protein, Roche Diagnostics, Mannheim, Njemačka).

Statistička analiza

Podaci su izraženi kao medijan; interkvartilni raspon i srednja vrijednost ± standardna devijacija (SD). Normalnost raspodjele ispitana je Kolmogorov-Smirnovljevim testom. Usporedbe uzastopnih mjerenja za neparametrijske podatake (NO, ADA, inzulin natašte, HOMA-IR, C-peptid, HbA_{1c}, ukupni kolesterol, LDL kolesterol, trigliceridi) ispitane su Friedmanovim testom, a *post hoc* testiranje načinjeno je Wilcoxonovim testom sume rangova. Usporedbe uzastopnih mjerenja parametrijskih podataka (glukoza natašte) napravljene su jednosmjernom analizom varijance (engl. *one-way ANOVA*), a *post hoc* testiranje je načinjeno Tukeyevim *post hoc* testom. Dvostrana vrijednost $P < 0,05$ smatrala se statistički značajnom. Analize i ilustracije napravljene su statističkim programom SPSS 9.0 (SPSS Inc., Chicago, IL, SAD).

Rezultati

Osnovne značajke ispitanika, tj. dob, spol, indeks tjelesne mase (engl. *body mass index*, BMI), srednja vrijednost trajanja šećerne bolesti, krvni tlak, prisutnost mikroproteinurije, sedimentacija eritrocita, ukupan broj leukocita i limfocita prikazane su u tablici 1.
Metaboličke promjene povezane s kontrolom šećerne bolesti sažete su u tablici 2. Vrijednosti glukoze natašte

Serum total ADA activity was determined at 37 °C according to the method of Giusti and Galanti based on the Bertholet reaction, i.e. the formation of colored indophenol complexes from ammonia liberated from adenosine, quantified spectrophotometrically (13). Optical density was measured spectrophotometrically at 625 nm in an assay mixture (final volume, 1 mL) containing 12 mM adenosine hemisulfate, 50 mM phosphate buffer (pH 6.5), and 0.05 mL of serum. One unit of ADA is defined as the amount of enzyme required to release 1 µmol/min of ammonia from adenosine at standard assay conditions. Intra-assay and inter-assay precision of the ADA assay was determined from serum pool on 20 replicates in a single run and in 10 different runs yielding CVs of 2.53% and 3.37%, respectively.
Nitric oxide levels were measured with colorimetric assay by the method of Griess reaction as the levels of NO_2^- and NO_3^- in serum according to the manufacturer's instructions (Nitrate/Nitrite colorimetric assay kit, Cayman Chemical Co, Ann Arbor, MI, USA). Urine protein was analyzed with turbidimetric method on a Roche/Hitachi modular P analyzer according to the manufacturer's instructions (U/CSF Protein, Roche Diagnostics, Mannheim, Germany).

Statistical analysis

Data are presented as median and interquartile range and mean ± SD. Kolmogorov-Smirnov test was applied to test normality of the distributions. Comparison of repeated measures for non-parametric data (NO, ADA, fasting insulin, HOMA-IR, C-peptide, HbA1c, total cholesterol, LDL-c, triglycerides) was performed with Friedman test and *post hoc* testing with Wilcoxon Signed Rank Test. Comparison of repeated measures for parametric data (FBG) was performed with one-way ANOVA and *post hoc* testing with Tukey's post hoc test. Two-tailed $P < 0.05$ was considered significant. Analyses and illustrations were performed with the SPSS 9.0 (SPSS Inc., Chicago, IL, USA) statistical software.

Results

Baseline characteristics of the study subjects, i.e. age, sex, body mass index (BMI), mean diabetes duration, blood pressure (BP), presence of microproteinuria, erythrocyte sedimentation rate (ESR), white blood cell (WBC) and lymphocyte count are presented in Table 1.
Metabolic changes about diabetes control are summarized in Table 2. Fasting blood glucose (FBG) gradually and significantly decreased during intensive insulin therapy. On day 3 and at 6 months, fasting insulin levels and HOMA-IR decreased as compared to baseline values. The decrease in C-peptide levels became obvious at 6 months. HbA_{1c} and exogenous insulin dose needed to achieve good control significantly decreased. There was no

Tablica 1. Osnovne značajke ispitanika (medijan; interkvartilni raspon)

Table 1. Baseline characteristics of study subjects (median; 25th-75th percentile)

	Secondary Sulfonylurea Failure N = 24
Age (years)	56 (53-64)
Gender	18F/6M
Mean Diabetes Duration (years)	9 (6-14)
BMI (kg/m2)	28.24 (26.10-31.04)
Diastolic BP (mmHg)	90 (70-90)
Systolic BP (mmHg)	150 (140-165)
Proteinuria positive cases	11, 46%
WBC ($\times 10^9$ /L)	9.120 (6.135-10.570)
Lymphocyte ($\times 10^9$ /L)	2.220 (1.335-3.140)
ESR mm/hour	8 (3-37)
Data are presented as median and interquartile range.	

Tablica 2. Metabolički parametri kontrole šećerne bolesti

Table 2. Metabolic parameters of diabetes control

	Basal (N = 24)	3rd day (N = 24)	6th month (N = 24)	P (total group)	P (basal vs. 3rd d)	P (basal vs. 6th m)	P (3rd d vs. 6th m)
FBG (mmol/L)	17.9±3.5 17.3 (15.2-20.4)	6.7±1.0 6.9 (5.9-7.6)	9.8±1.3 9.7 (9.0-10.3)	< 0.001	< 0.001	< 0.001	< 0.001
Fasting insulin (μU/mL)	21.8±13.8 19.0 (11.0-29.2)	15.9±9.2 13.1 (10.3-21.9)	12.7±8.7 10.9 (6.4-17.4)	0.009	0.010	0.011	0.331
HOMA-IR	17.5±12.2 14.3 (9.4-21.9)	4.8±3.1 4.3 (2.7-6.7)	5.4±3.7 2.8 (5.3-7.6)	< 0.001	< 0.001	< 0.001	0.391
C-peptide (nmol/L)	1.06±0.52 0.96 (0.67-1.44)	0.88±0.42 0.83 (0.44-1.13)	0.72±0.28 0.63 (0.53-1.01)	0.005	0.128	0.005	0.117
Exogenous Insulin (U)	101.7±24.5 100 (80-120)	/	75.8±24.3 72 (64-96)	/	/	0.001	/
HbA_{1c} (%)	11.1±1.9 10.8 (9.6-12.9)	/	7.8 ±1.1 7.6 (7.0-8.8)	/	/	< 0.001	/
Total Cholesterol (mmol/L)	4.85±0.87 4.69 (4.38-5.50	/	4.83±0.90 4.70 (4.34-5.17)	/	/	0.920	/
LDL-c (mmol/L)	3.30±0.67 3.12 (2.78-4.01)	/	3.30±0.65 3.21 (2.91-3.71)	/	/	0.988	/
Triglycerides (mmol/L)	1.98±1.29 1.75 (1.14-2.03)	/	1.82±0.69 1.88 (1.21-2.12)	/	/	0.072	/
Data are presented as median and interquartile range or as mean ± SD.							

su se postupno i statistički značajno snizile tijekom intenzivne inzulinske terapije. Trećeg dana i u šestom mjesecu snizile su se vrijednosti inzulina natašte i HOMA-IR u usporedbi s početnim vrijednostima. Sniženje vrijednosti C-peptida postalo je očito na kontroli u šestom mjesecu.

statistically significant difference in total cholesterol and LDL-c. The decrease in plasma triglycerides did not reach statistical significance.

In secondary sulfonylurea failure, baseline ADA activity (17.0 [14.6-21.7] U/L) was significantly lower than ADA ac-

HbA1c i doza egzogeno davanog inzulina potrebnog za postizanje dobre kontrole značajno su se snizili. Nije zabilježena statistički značajna razlika za ukupni kolesterol i LDL kolesterol. Sniženje triglicerida u plazmi nije doseglo statistički značajnu razinu.

Kod sekundarnog neuspjeha liječenja sulfonilurejom bazalna je aktivnost ADA (medijan; interkvartilni raspon, 17,0 [14,6-21,7] U/L) bila statistički značajno niža, nego aktivnosti ADA izmjerene trećega dana (20,5 [16,2-23,4] U/L; P = 0,018) i nakon šest mjeseci (21,2 [16,6-22,5] U/L; P = 0,010) (tablica 3., slike 1. i 2).

Između koncentracija NO određenih na početku istraživanja (medijan; interkvartilni raspon, 18,8 [11,6-28,4] μmol/L), trećega dana (17,8 [9,7-33,6] μmol/L; P = 0,966) i u tivity measured on day 3 (20.5 [16.2-23.4] U/L; P = 0.018) and at 6 months (21.2 [16.6-22.5] U/L; P = 0.010) (Table 3, Figures 1 and 2).

There was no statistically significant difference between NO levels determined before (18.8; [11.6-28.4] μmol/L), on day 3 (17.8 [9.7-33.6] μmol/L; P = 0.966) and at 6 months of intensive insulin therapy (21.7 [16.0-33.9] μmol/L; P = 0.230) (Table 3, Figures 3 and 4).

Discussion

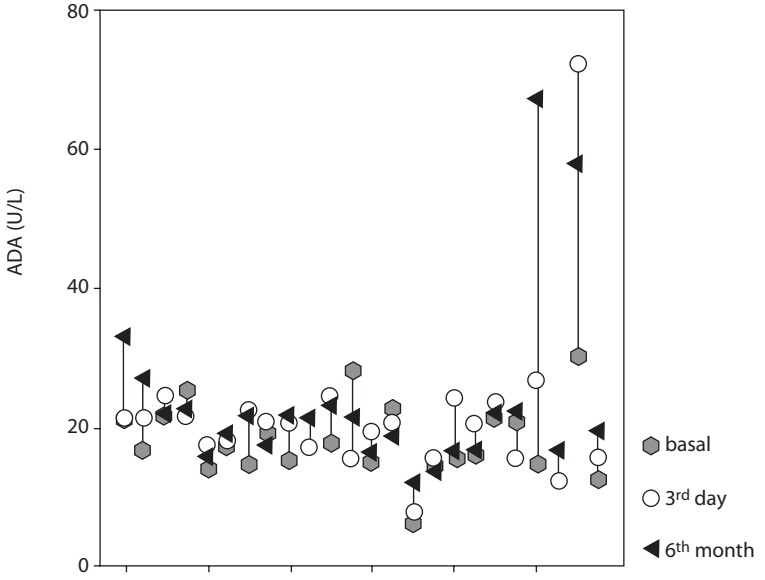
Despite great progress in the treatment of diabetes, late diabetic complications still remain the principal cause of morbidity and mortality in patients with diabetes melli-

Tablica 3. Aktivnost adenozin deaminaze (ADA) i koncentracija dušikova oksida

Table 3. Adenosine deaminase (ADA) activity and nitric oxide (NO) level

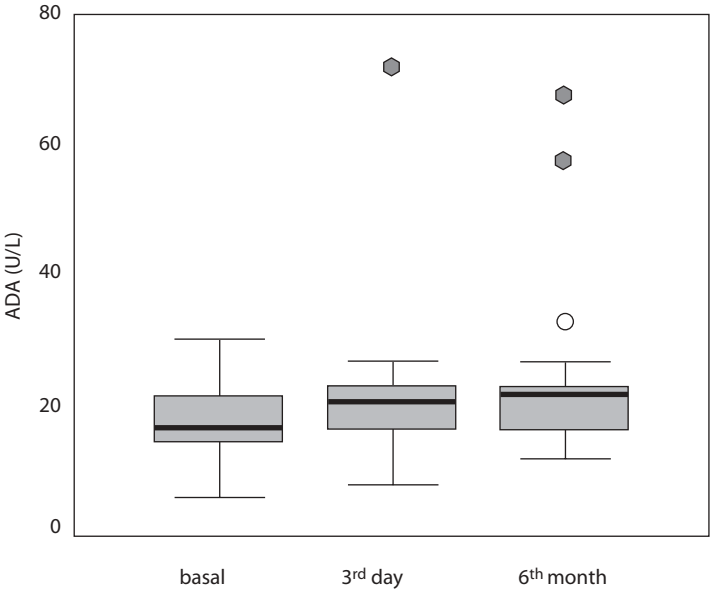
	Basal (N = 24)	3rd day (N = 24)	6th month (N = 24)	P (total group)	P (basal vs. 3rd d)	P (basal vs. 6th m)	P (3rd d vs. 6th m)
NO (μmol/L)	22.5±13.9 18.8 (11.6-28.4)	22.7±15.6 17.8 (9.7-33.6)	24.1±9.2 21.7 (16.0-33.9)	0.311	0.966	0.230	0.637
ADA (U/L)	18.0±5.4 17.0 (14.6-21.7)	21.6±11.6 20.5 (16.2-23.4)	23.5±12.9 21.2 (16.6-22.5)	0.011	0.018	0.010	0.558

Data are presented as median and interquartile range or as mean ± SD.



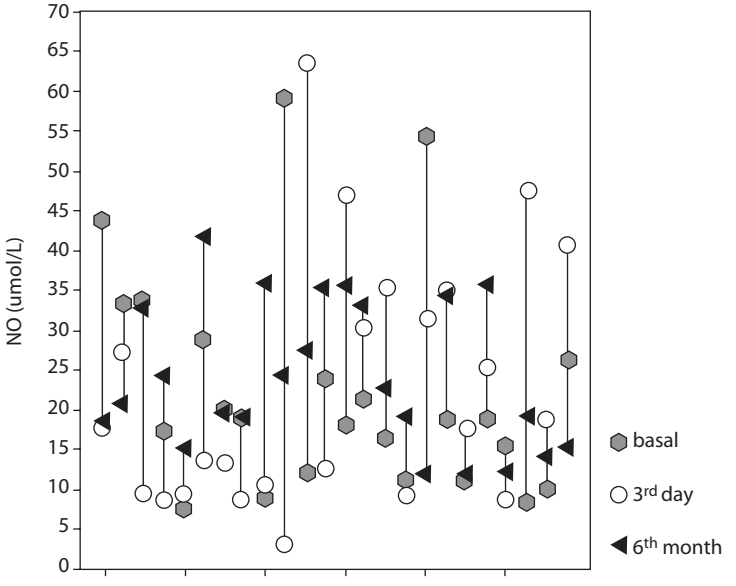
Slika 1. Aktivnost adenozin deaminaze (ADA) u tri različita vremenska razdoblja.

Figure 1. Adenosine deaminase (ADA) activity in individual cases at three different time points.



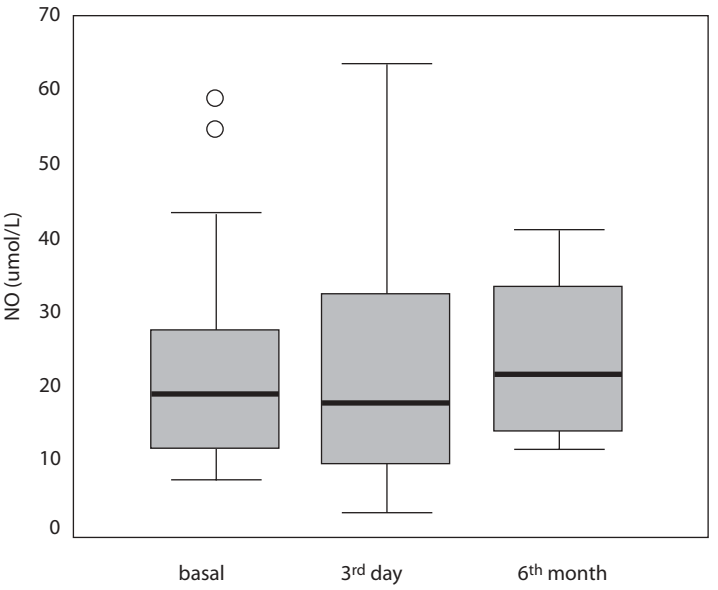
Slika 2. Aktivnost adenozin deaminaze (ADA) u serumu bolesnika u tri različita vremenska razdoblja. Središnji pravokutnik predstavlja vrijednosti od niže do više kvartile (25. i 75. percentila). Središnja linija predstavlja medijan. Linija se širi od najniže do najviše vrijednosti isključujući ekstremne i atipične vrijednosti koje su označene odvojenim točkama. Atipična vrijednost (engl. *outlier*) definirana je kao vrijednost udaljena između 1,5 i 3 duljine pravokutnika, a ekstremna vrijednost kao vrijednost udaljena više od 3 duljine pravokutnika od gornje ili donje granice pravokutnika. Duljina pravokutnika je interkvartilni raspon.

Figure 2. Serum adenosine deaminase (ADA) activity in study patients at three different time points. Central box represents the values from the lower to the upper quartile (25th to 75th percentile). Middle line represents the median. The line extends from the minimum to the maximum value excluding extreme cases and outliers that are displayed as separate points. An outlier is defined as a value between 1.5 and 3 box lengths and an extreme value is defined as more than 3 box lengths from the upper or lower edge of the box. The box length is the interquartile range.



Slika 3. Koncentracija dušikova oksida (NO) u pojedinačnih ispitanika u tri različita vremenska razdoblja.

Figure 3. Nitric oxide (NO) level in individual cases at three different time points.



Slika 4. Koncentracija dušikova oksida (NO) u serumu bolesnika u tri različita vremenska razdoblja. Središnji pravokutnik predstavlja vrijednosti od niže do više kvartile (25. i 75. percentila). Središnja linija predstavlja medijan. Linija se širi od najniže do najviše vrijednosti isključujući ekstremne i atipične vrijednosti koje su označene odvojenim točkama. Atipična vrijednost (engl. *outlier*) definirana je kao vrijednost udaljena između 1,5 i 3 duljine pravokutnika, a ekstremna vrijednost kao vrijednost udaljena više od 3 duljine pravokutnika od gornje ili donje granice pravokutnika. Duljina pravokutnika je interkvartilni raspon.

Figure 4. Serum nitric oxide (NO) level in study patients at three different time points. Central box represents the values from the lower to the upper quartile (25th to 75th percentile). Middle line represents the median. The line extends from the minimum to the maximum value excluding extreme cases and outliers that are displayed as separate points. An outlier is defined as a value between 1.5 and 3 box lengths and an extreme value is defined as more than 3 box lengths from the upper or lower edge of the box. The box length is the interquartile range.

šestom mjesecu (21,7 [16,0-33,9] μmol/L; P = 0,230) intenzivne inzulinske terapije nije zabilježena statistički značajna razlika (tablica 3., slike 3. i 4.).

Rasprava

Usprkos velikom napretku u liječenju, kasne komplikacije šećerne bolesti još su uvijek glavni uzroci smrtnosti i pobola kod ovih bolesnika. Istraživanje kontrole i komplikacija šećerne bolesti (engl. *The Diabetes Control and Complications Trial*, DCCT) dokazalo je da se intenzivnom inzulinskom terapijom može postići stroga kontrola glikemije i rezultirajuće dugotrajne prednosti (18). Ova analiza potvrđuje prethodne podatke o poboljšanju kontrole glikemije intenzivnom inzulinskom terapijom kod sekundarnog neuspjeha liječenja sulfonilurejom, što podupiru i podaci o statistički značajnom sniženju koncentracije glukoze u krvi natašte, HbA1c, HOMA-IR, C-peptida te doze inzulina potrebne za održavanje dobre metaboličke kontrole. Stroga kontrola glukoze u krvi u istraživanju provedenom na kirurškom odjelu smanjila je pobol i smrtnost (19), dok je u drugom istraživanju na odjelu intenzivne njege, došlo do smanjenja pobola (20). Smanjile su se komplikacije kao što su teške upale i zatajenje organa. Nekoliko potencijalnih mehanizama moglo bi objasniti ove pozitivne učinke,

tus. The Diabetes Control and Complications Trial has demonstrated that tight glycemic control and the resulting long term benefits can be achieved by intensified insulin therapy (18). The present analysis confirmed previous data that intensive insulin therapy can improve glycemic control in secondary sulfonylurea failure as supported by the significant decrease in FBG, HbA1c, HOMA-IR, C-peptide levels and insulin doses required to achieve good metabolic control. In previous studies, one conducted in a surgical and the other in a medical intensive care unit, strict control of blood glucose levels with insulin reduced morbidity plus mortality and morbidity, respectively (19,20). Complications such as severe infections and organ failure were reduced. Several potential mechanisms may explain these benefits, i.e. reduction of systemic inflammation, prevention of immune dysfunction, and protection of the endothelium and mitochondrial ultrastructure (20-25). However, an epidemiological study suggested that the use of intensive therapy as compared with standard therapy increased mortality and did not significantly reduce major cardiovascular events (26).

ADA contributes to the regulation of intracellular and extracellular concentrations of adenosine and deoxyadenosine, along with 5' nucleotidase and adenosine kinase, and increased ADA activity is considered to be a sign of

kao što su smanjenje sistemske upale, prevencija disfunkcije imunosnog sustava, zaštita endotela i mitohondrijskih ultrastruktura (20-25). Međutim, jedno epidemiološko istraživanje ukazuje na to da je intenzivna terapija u usporedbi sa standardnom terapijom povećala smrtnost i nije značajno smanjila veće kardiovaskularne ispade (26).

ADA doprinosi regulaciji unutarstaničnih i izvanstaničnih koncentracija adenozina i deoksiadenozina zajedno s 5' nukleotidazom i adenozin-kinazom te se povećana aktivnost ADA smatra znakom snižene koncentracije adenozina (13,27). Koncentracija adenozina je povišena na ozlijeđenim mjestima i mjestima zahvaćenim upalom te ima središnju ulogu u regulaciji upalnih odgovora i u smanjenju oštećenja tkiva zahvaćenog upalom (14). Adenozin modulira proliferaciju, preživljavanje i apoptozu različitih tipova stanica, od epitelnih, endotelnih i glatkomišićnih stanica do stanica imunog i neuralnog podrijetla (15). Naknadno je potvrđeno zaštitno djelovanje izvanstaničnog adenozina u staničnim i organskim sustavima uključujući mozak, bubrege, skeletne mišiće i masno tkivo. Prema našem istraživanju, aktivnost ADA porasla je i u ranoj i u kasnoj fazi intenzivne inzulinske terapije kod sekundarnog neuspjeha liječenja sulfonilurejom. Prema prijašnjim rezultatima pretpostavljamo da se povećana aktivnost ADA u ovom istraživanju može pripisati izostanku inicirajućeg medicinskog stresa, tj. oksidacijskog stresa, lokalne upale ili stanične smrti. Pretpostavljamo da se adenozin otpušta kao odgovor na široku lepezu podražaja koji dovode do ozljeda i posreduje u autoregulatornom mehanizmu uloga kojega je zaštititi organe. Newby i sur. su smislili naziv osvetnički metabolit (engl. *retaliatory metabolite*) kako bi opisali zaštitnu funkciju adenozina (14,28).

Učinci purinergičnih agonista na lučenje inzulina opisani u literaturi su proturječni. U jednom eksperimentalnom istraživanju u kojem su se rabile stanice INS-1, ali i Langerhansovi otočići gušterače štakora, adenozin je spriječio oslobađanje inzulina ovisno o koncentraciji. Inhibicijski učinak visoke koncentracije ATP pripisuje se njegovim razgradnim proizvodima, odnosno adenozinu, jer je ADA (1 U/mL) prekinula inhibicijski učinak ukazujući pritom na utjecaj adenozina (27). Nadalje, pokazalo se da adenozin može djelovati na površinu alfa-stanica endokrinog dijela gušterače, kako bi se pojačalo lučenje glukagona (16). Pretpostavlja se da adenozin utječe i na metabolizam glukoze. Adenozin pojačava glikogenolizu i otpuštanje glukoze iz jetre (29-31). Stoga pretpostavljamo da bi povećana aktivnost ADA zabilježena u ovoj studiji te popratno sniženje koncentracije adenozina moglo doprinijeti korisnosti intenzivne inzulinske terapije oslobađanjem kapaciteta lučenja inzulina iz beta-stanica, poboljšanjem lučenja glukagona i utjecajem na periferni metabolizam glukoze.

NO utječe na oslobađanje inzulina i inzulinsku rezistenciju, ima proupalno i apoptotičko djelovanje te djeluje kao slobodni radikal. Mehanizam djelovanja uključuje pro-

decreased adenosine levels (13,27). Adenosine is elaborated at injured and inflamed sites, and has a central role in the regulation of inflammatory responses and in limiting inflammatory tissue destruction (14). Adenosine modulates the proliferation, survival and apoptosis of many different cell types, ranging from epithelial, endothelial and smooth muscle cells, to the cells of the immune and neural lineages (15). Subsequently, evidence was obtained for extracellular adenosine protective actions in cellular and organ systems, including the brain, kidney, skeletal muscle and adipose tissue. According to our study, ADA activity is increased both in the early and late periods of intensive insulin therapy in secondary sulfonylurea failure. Based on the previous evidence, we suggest that increased ADA activity in this study may have represented disappearance of the initiating medical stress, i.e. oxidative stress, local inflammation, or cell death. It is hypothesized that adenosine is released in response to a wide range of injurious stimuli and mediates an autoregulatory loop, the function of which is to protect organs (14). The term 'retaliatory metabolite' was coined by Newby to describe the protective function of adenosine (28).

Literature data on the effects of purinergic agonists on insulin release are controversial. In an experimental study mainly using INS-1 cells, but also using rat pancreatic islets, adenosine inhibited insulin release in a concentration-dependent manner. The inhibitory effect of high ATP concentrations was attributed to its degradation product, namely adenosine because ADA (1 U/mL) abolished the inhibitory effect, indicating involvement of adenosine (27). Furthermore, it was shown that adenosine could act on the endocrine pancreas alpha cell surface to increase glucagon secretion (16). An effect of adenosine on peripheral glucose metabolism has also been suggested. Adenosine increases glycogenolysis and glucose release from the liver (29-31). Therefore, we suggest that increased ADA activity recorded in this study and the accompanying decrease in adenosine concentration may contribute to the benefits of intensive insulin therapy by relieving beta cells' insulin secretion capacity, amelioration of glucagon secretion and affecting peripheral glucose metabolism.

Nitric oxide affects insulin release, insulin resistance, has proinflammatory, apoptotic and free radical effects through signal transduction and direct posttranslational modifications of proteins, reacting with reactive oxygen species, and interacting with proteins that contain a heme moiety (5,32). Nakada *et al*. have provided evidence for a concentration-dependent dual effect of NO, i.e. a stimulatory effect at low concentrations and an inhibitory one at high concentrations, on insulin secretion (8). It has been proposed that NO mediates exercise-stimulated glucose transport in skeletal muscle (9,10). Exogenously administered NO, which is generated from the NO donor,

vođenje signala i direktne posttranslacijske modifikacije proteina preko reakcije sa slobodnim kisikovim radikalima i proteinima koji sadrže hem (5,32). Nakada i sur. su opisali dokaz dualnog učinka NO ovisnog o koncentraciji, tj. o stimulacijskom učinku kod niskih koncentracija i o inhibicijskom kod visokih koncentracija na lučenje inzulina (8). Pretpostavlja se da NO posreduje kod transporta glukoze stimuliranog vježbanjem u skeletne mišiće (9,10). Vanjskim unosom dušikovog oksida putem NO donatora (npr. natrijev nitroprusid), NO potiče prijenos glukoze u izoliranim skeletnim mišićima povećavajući koncentraciju GLUT4 na površini stanice (9-11). Dio mehanizma kojim inzulin povećava transport glukoze *in vivo* uključuje pojačani protok krvi i opskrbu mišića glukozom, proces kojim posreduje otpuštanje NO iz endotela (9,12). U ovom smo ispitivanju istražili sistemske koncentracije NO zbog njegove potencijalne uloge kod sekundarnog neuspjeha liječenja sulfonilurejom. Međutim, naše je istraživanje pokazalo da dobra kontrola glikemije postignuta intenzivnom inzulinskom terapijom kod sekundarnog neuspjeha liječenja sulfonilurejom nije imala utjecaja na sistemske koncentracije NO. Stoga se može zaključiti da podaci dobiveni našim istraživanjem ne podupiru ulogu NO u pogoršanju funkcije beta-stanica na razini sistemskih koncentracija NO.

Ovo je istraživanje imalo metodoloških ograničenja koja su mogla pridonijeti činjenici da nismo utvrdili ima li intenzivna inzulinska terapija utjecaja na koncentraciju NO. Izmjerili smo koncentracije NO u uzorcima plazme; daljnja istraživanja koja će obuhvaćati mehanizme na razini tkiva mogla bi pružiti uvid u točnu ulogu NO kod pojave sekundarnog neuspjeha liječenja sulfonilurejom. Drugo moguće ograničenje bila je mala skupina ispitanika. Potrebna je velika populacija ispitanika i sveobuhvatnija klinička procjena kako bi se razjasnilo jesu li ti rezultati dosljedni i klinički značajni. Mjerenje koncentracije adenozina moglo biti također pružiti vrijedne podatke.

Da zaključimo, ovo istraživanje pokazuje da je aktivnost ADA porasla u objema fazama primanja intenzivne inzulinske terapije kod sekundarnog neuspjeha u liječenju sulfonilurejom, u ranoj i u kasnijoj fazi. Utjecaj NO na intenzivnu inzulinsku terapiju kod sekundarnog neuspjeha u liječenju sulfonilurejom nije potvrđen ovim istraživanjem.

sodium nitroprusside, stimulates glucose transport in isolated skeletal muscles by increasing GLUT4 levels at the cell surface (9-11). Part of the mechanism by which insulin increases glucose transport *in vivo* involves enhanced blood flow and glucose delivery to the muscle, a process mediated by the release of NO from the endothelium (9,12). In this study, systemic NO levels were investigated for its potential role in secondary sulfonylurea failure. However, our study showed that good glycemic control achieved by intensive insulin therapy in secondary sulfonylurea failure had no effect on systemic NO levels. Therefore, the role of NO in amelioration of β-cell function is not supported by the data on systemic NO levels.

The present study had some methodological limitations that may have contributed to the fact that we did not find an impact of intensive insulin therapy on NO levels. We measured NO levels in plasma samples; further studies comprising the mechanisms at the tissue level may provide an insight into the exact role of NO in the setting of secondary sulfonylurea failure. Another potential limitation was the small number of the study subjects. A larger study population and clinical assessment are needed to elucidate whether these results are consistent and have clinical relevance. Also, measurement of adenosine levels may be informative.

In summary, the present study demonstrated that ADA activity is increased both in the early and late periods of intensive insulin therapy in patients with secondary sulfonylurea failure. The implication of NO on intensive insulin therapy in secondary sulfonylurea failure was not verified in this study.

Adresa za dopisivanje:

Ayşe Binnur Erbağcı
Gaziantep Üniversitesi
Tıp Fakültesi, Biyokimya AD
TR-27310, Gaziantep
Türkiye
e-pošta: aerbagci@gantep.edu.tr

Corresponding author:

Ayşe Binnur Erbağcı
Gaziantep Üniversitesi
Tıp Fakültesi, Biyokimya AD
TR-27310, Gaziantep
Türkiye
e-mail: aerbagci@gantep.edu.tr

Literatura/References

1. Chen YN, Chen SY, Zeng LJ, Ran JM, Xie B, Wu MY, Wu YZ. Secondary sulphonylurea failure: what pathogenesis is responsible? Br J Biomed Sci 2003;60:9-13.
2. Rattarasarn C, Thamprasit A, Leelawattana R, Soonthornpun S, Setasuban W. The role of diminished beta cell reserve and insulin resistance in secondary sulfonylurea failure of type 2 diabetes mellitus. J Med Assoc Thai 2001;84:1754-62.
3. Wajchenberg BL. β-Cell failure in diabetes and preservation by clinical treatment. Endocr Rev 2007;28:187-218.
4. Fridlyand LE, Philipson LH. Does the glucose-dependent insulin secretion mechanism itself cause oxidative stress in pancreatic beta-cells? Diabetes 2004;53:1942-8.
5. Lee VY, McClintock DS, Santore MT, Budinger GR, Chandel NS. Hypoxia sensitizes cells to nitric oxide-induced apoptosis. J Biol Chem 2002;277:16067-74.
6. Borutaite V, Morkuniene R, Brown GC. Nitric oxide donors, nitrosothiols and mitochondrial respiration inhibitors induce caspase activation by different mechanisms. FEBS Lett 2000;467:155-9.
7. Shen YH, Wang XL, Wilcken DE. Nitric oxide induces and inhibits apoptosis through different pathways. FEBS Lett 1998;433:125-31.
8. Nakada S, Ishikawa T, Yamamoto Y, Kaneko Y, Nakayama K. Constitutive nitric oxide synthases in rat pancreatic islets: direct imaging of glucose-induced nitric oxide production in beta-cells. Pflugers Arch 2003;447:305-11.
9. Higaki Y, Hirshman MF, Fujii N, Goodyear LJ. Nitric oxide increases glucose uptake through a mechanism that is distinct from the insulin and contraction pathways in rat skeletal muscle. Diabetes 2001;50:241-7.
10. Balon TW, Nadler JL. Evidence that nitric oxide increases glucose transport in skeletal muscle. J Appl Physiol 1997;82:359-63.
11. Etgen GJ Jr, Fryburg DA, Gibbs EM. Nitric oxide stimulates skeletal muscle glucose transport through a calcium/contraction- and phosphatidylinositol-3-kinase-independent pathway. Diabetes 1997;46:1915-9.
12. Baron AD, Steinberg HO, Chaker H, Leaming R, Johnson A, Brechtel G. Insulin-mediated skeletal muscle vasodilation contributes to both insulin sensitivity and responsiveness in lean humans. J Clin Invest 1995;96:786-92.
13. Giusti G, Galanti B. Colorimetric method. In: Bergmeyer HU, editor. Methods of enzymatic analysis. Weinheim: Verlag Chemie, 1984. pp. 315-23.
14. Haskó G, Cronstein BN. Adenosine: an endogenous regulator of innate immunity. Trends Immunol 2004;25:33-9.
15. Jacobson KA, Hoffmann C, Cattabeni F, Abbracchio MP. Adenosine-induced cell death: evidence for receptor-mediated signalling. Apoptosis 1999;4:197-211.
16. Loubatieres-Mariani MM, Chapal J. Purinergic receptors involved in the stimulation of insulin and glucagon secretion. Diabet Metab 1988;14:119-26.
17. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412-9.
18. DCCT Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med 1993;329:977-86.
19. Van den Berghe G, Wouters PJ, Bouillon R, Weekers F, Verwaest C, Schetz M, et al. Outcome benefit of intensive insulin therapy in the critically ill: insulin dose versus glycemic control. Crit Care Med 2003;31:359-66.
20. Van den Berghe G, Wilmer A, Hermans G, Meersseman W, Wouters PJ, Milants I, et al. Intensive insulin therapy in the medical ICU. N Engl J Med 2006;354:449-61.
21. Weekers F, Giulietti AP, Michalaki M, Coopmans W, Van Herck E, Mathieu C, Van den Berghe G. Metabolic, endocrine, and immune effects of stress hyperglycemia in a rabbit model of prolonged critical illness. Endocrinology 2003;144:5329-38.
22. Hansen TK, Thiel S, Wouters PJ, Christiansen JS, Van den Berghe G. Intensive insulin therapy exerts anti-inflammatory effects in critically ill patients and counteracts the adverse effect of low mannose-binding lectin levels. J Clin Endocrinol Metab 2003;88:1082-8.
23. Van den Berghe G. How does blood glucose control with insulin save lives in intensive care? J Clin Invest 2004;114:1187-95.
24. Langouche L, Vanhorebeek I, Vlasselaers D, Vander Perre S, Wouters PJ, Skogstrand K, et al. Intensive insulin therapy protects the endothelium of critically ill patients. J Clin Invest 2005;115:2277-86.
25. Vanhorebeek I, De Vos R, Mesotten M, Wouters PJ, De Wolf-Peeters C, Van den Berghe G. Protection of hepatocyte mitochondrial ultrastructure and function by strict blood glucose control with insulin in critically ill patients. Lancet 2005;365:53-9.
26. Gerstein HC, Miller ME, Byington RP, Goff DC Jr, Bigger JT, Buse JB, et al. Effects of intensive glucose lowering in type 2 diabetes. Action to Control Cardiovascular Risk in Diabetes Study Group. N Engl J Med 2008;358:2545-59.
27. Verspohl EJ, Johannwille B, Waheed A, Neye H. Effect of purinergic agonists and antagonists on insulin secretion from INS-1 cells (insulinoma cell line) and rat pancreatic islets. Can J Physiol Pharmacol 2002;80:562-8.
28. Newby AC. Adenosine and the concept of retaliatory metabolites. Trends Biochem Sci 1984;9:42-4.
29. Töpfer M, Burbiel CE, Müller CE, Knittel J, Verspohl EJ. Modulation of insulin release by adenosine A(1) receptor agonists and antagonists in INS-1 cells: the possible contribution of (86)Rb(+) efflux and (45) Ca(2+) uptake. Cell Biochem Funct 2008;26:833-43.
30. Buxton DB, Fisher RA, Robertson SM, Olson M. Stimulation of glycogenolysis and vasoconstriction by adenosine analogues in the perfused rat liver. Biochem J 1987;248:35-41.
31. Mc Lane M, Black PR, Law WR, Raymond RM. Adenosine reversal of in vivo hepatic responsiveness to insulin. Diabetes 1990;39:62-9.
32. Stamler JS. Redox signaling: nitrosylation and related target interactions of nitric oxide. Cell 1994;l 78:931-6.