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ISHEMIJSKI MOŽDANI UDAR U PACIJENATA SA TIPOM 2 DIJABETESA: POVEZANOST SNIŽENE INSULINSKE SENZITIVNOSTI I POREMEĆAJA FIBRINOLIZE

Sažetak: Uloga i značaj insulinske senzitivnosti (IS), kao i povezanost IS sa poremećajem fibrinolize za ispoljavanje ishemijskog moždanog udara (IMU) još uvek nije razjašnjen. Cilj istraživanja je bio da se analiziraju nivoi IS i insulinemije, kao i njihova povezanost sa nivoom plazminogen aktivator inhibitora 1 (PAI-1) u 34 pacijenta sa tipom 2 dijabetesa (T2D) i IMU (grupa A), 30 pacijenata sa T2D bez IMU (grupa B), 33 pacijenta sa IMU bez T2D (grupa C) i 33 zdrave kontrole (grupa D). Postojanje IMU potvrđeno je na osnovu kliničkog i nalaza neurovizuelizacionih procedura. IS je određivana metodom minimalnog modela (Si indeks). Nivo insulina je određen metodom radioimunoeseja, a nivo PAI-1 određivan je hromogenim plazminogen/plazmin supstrat esejom. Rezultati su pokazali da su nivoi Si bili značajno niži u grupi A u poređenju sa B (1.17+/-0.66 i 2.79+/-0.62 min^{-1}/mU/lx10^4; p<0.001), kao i u grupi C u odnosu na D (3.25+/-0.84 i 6.03+/-1.69 min^{-1}/mU/lx10^4; p<0.001). Grupa A je imala značajno više nivoe insulinemije u poređenju sa grupom B (19.46+/-4.11 i 14.79+/-1.75 mU/l; p<0.001), kao i grupa C u odnosu na D (15.16+/-2.23 i 7.54+/-2.03 mU/l; p <0.001). Nivo PAI-1 je bio značajno viši u grupi A u odnosu na B (7.78+/-1.05 i 4.56+/-0.71 mU/l; p<0.001) i u grupi C u odnosu na D (4.65+/-0.69 i 3.48+/-1.29 mU/l; p<0.001).

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Pokazana je korelacija Si sa nivoom PAI-1, u ispitanika sa i bez T2D. Rezultati ukazuju da snižena insulinska senzitivnost, udružena sa kompenzatornom hiperinsulinemijom, može ostvarivati svoj aterogeni uticaj na ispoljavanje IMU i preko sniženja fibrinolize.

Ključne reči: ishemijski moždani udar, tip 2 dijabetesa, insulinska senzitivnost, plazminogen aktivator inhibitora -1 (PAI-1)

Abstract: The role of insulin sensitivity (IS), as well as the association of IS with fibrinolysis impairment, in the occurrence of ischemic stroke, has not been clarified. The study was aimed to analyze IS, plasma insulin (PI) and plasminogen activator inhibitor (PAI)-1 levels in 34 type 2 diabetics (T2D) with ischemic stroke (group A), 30 T2D without ischemic stroke (group B), 33 nondiabetics with ischemic stroke (group C) and 33 healthy controls (group D). Ischemic stroke was confirmed by clinical and neuroimaging criteria. IS levels were determined by the minimal model analysis (Si index). Plasma insulin levels were measured by radioimmunoassay and PAI-1 activity was performed by the plasminogen chromogenic plasmin substrate assay. We found that Si levels were significantly lower in group A vs. B (1.17+/-0.66 vs. 2.79+/-0.62 $min^{-1}/mU/Lx10^4$; $p<0.001$) and in C vs. D (3.25+/-0.84 vs. 6.03+/-1.69 $min^{-1}/mU/Lx10^4$; $p<0.001$), while PI levels were higher in group A vs. B (19.46+/-4.11 vs. 14.79+/-1.75 mU/L; $p<0.001$) and in C vs. D (15.16+/-2.23 vs. 7.54+/-2.03 mU/L; $p <0.001$). Also, PAI-1 activity was significantly higher in group A vs. B (7.78+/-1.05 i 4.56+/-0.71 mU/L; $p<0.001$) and in C vs D (4.65+/-0.69 i 3.48+/-1.29 mU/L; $p<0.001$). Moreover, Si levels correlated with PAI-1, both in T2D and nondiabetics. Our results indicate that appearance of ischemic stroke was associated with decreased insulin sensitivity, together with compensatory hyperinsulinemia, both in T2D and nondiabetics. Our results imply that impaired insulin sensitivity exerts its atherogenic influence, at least in part, by decreased fibrinolysis.

Key words: ischemic stroke, type 2 diabetes, insulin sensitivity, plasminogen activator inhibitor -1 (PAI-1)

Uvod

Snižena insulinska senzitivnost (IS) je značajan činilac u razvoju ateroskleroze (1), i predstavlja poznati faktor rizika za ispoljavanje ishemijskog moždanog udara (IMU) (2,3). Iako mehanizmi putem kojih snižena IS utiče na razvoj vaskularnih događaja nisu u potpunosti rasvetljeni, smatra se da uključuju hiperglikemiju (4), dislipidemiju, hipertenziju, hipofibrinolizu i endotelijalnu disfunkciju (5). Istovremeno, snižena IS, odnosno insulinska rezistencija, predstavlja važan patofiziološki mehanizam u nastanku tipa 2 dijabetesa (T2D) (6).

Prethodna istraživanja su ukazala na postojanje direktne povezanosti između različitih podtipova IMU i insulinske rezistencije, određivane različitim metaboličkim testovima, kako u obolelih od T2D, tako i u osoba bez dijabetesa (2,3). Istovremeno, pokazano je da je insulinska rezistencija bila značajno viša u nedijabetičara sa razvijenom i ekstrakranijalnom i intrakranijalnom aterosklerozom u odnosu na ispitanike koji su imali samo intra-, odnosno samo ekstrakranijalnu aterosklerozu (7).

Takođe, hiperinsulinemija, koja se može koristiti kao parametar insulinske rezistencije u nedijabetičara, a često je, mada ne i obavezno, prisutna kao kompenzatorni odgovor na insulinsku rezistenciju u bolesnika sa T2D (8), prepoznata je kao nezavisan faktor rizika za ispoljavanje IMU (9,10).

Istovremeno, sa insulinskom rezistencijom su povezani i drugi relevantni aterogeni faktori kao što su hiperkoagulabilnost i hipofibrinoliza (11). Tako je pokazana pozitivna korelacija između snižene IS i poremećaja fibrinolize u koronarnoj bolesti, u pacijenata sa ili bez T2D (12). Međutim, povezanost IS sa nivoom plazminogen aktivator inhibitora 1 (PAI-1) u IMU još uvek nije razjašnjena.

Uzimajući u obzir sve navedeno, ispitivali smo povezanost insulinske senzitivnosti i poremećaja fibrinolize kao potencijalnog mehanizma koji leži u osnovi ispoljavanja IMU u obolelih od T2D, kao i u nedijabetičara.

Cilj

Ispitati povezanost insulinske senzitivnosti i nivoa fibrinolize u ispoljavanju IMU u obolelih od T2D.

Metode

Ispitanici

U istraživanje je bilo uključeno 64 obolela od T2D koji su bili podeljeni u dve grupe, T2D sa (N=34) i bez IMU (N=30), 33 nedijabetičara sa IMU i 33 zdrava ispitanika.

Dijagnoza T2D je utvrđena prema kriterijumima Svetske zdravstvene organizacije (13).

Dijagnoza IMU je utvrđena od strane neurologa, na osnovu kliničkog i nalaza vizuelizacionih metoda, kompjuterizovane tomografije endokranijuma i magnetne rezonance, sprovedenih tokom dva uzastopna pregleda, u toku prvih sedam dana od nastanka IMU u Institutu za neurologiju Kliničkog centra Srbije (14).

U istraživanje su uključeni oboleli sa IMU kod kojih nisu registrovani znaci kardioembolijskog moždanog udara, kao ni koronarne bolesti, utvrđene na osnovu

podataka o prethodnom infarktu miokarda sa potvrđenim porastom kardijalnih enzima ili na osnovu rezultata koronarne angiografije. U studiju su bili uključeni oboleli od T2D na terapiji peroralnim antihiperglikemijskim agensima, dok su bili isključeni pacijenti na insulinskoj terapiji, kao i oni sa drugim endokrinološkim, infektivnim ili malignim oboljenjima. Svi ispitanici, sa ili bez IMU, pokazivali su sličan nivo fizičke aktivnosti. Metabolička evaluacija je sprovedena na Institutu za endokrinologiju, dijabetes i bolesti metabolizma Kliničkog centra Srbije, nakon što su ispitanici u potpunosti obavešteni i dali informisani pristanak za učešće u studiji.

Plan testiranja ispitanika

U toku jednodnevnog ispitivanja, sproveden je fizikalni pregled, metaboličko testiranje i uzeti su podaci koji su se odnosili na prethodna oboljenja, aktuelnu medikaciju i navike. U svakog ispitanika obavljeno je merenje telesne težine (TT) i telesne visine (TV) i na osnovu toga izračunat je indeks telesne mase (ITM) prema sledećoj formuli:

$$\text{ITM (kg/m}^2\text{)} = \text{TT (kg)} / \text{TV (m)}^2$$

Dijagnoza hipertenzije utvrđena je na osnovu kriterijuma Svetske zdravstvene organizacije (sistolni/dijastolni krvni pritisak ≥140/≥90 mm Hg) ili na osnovu podataka o uzimanju antihipertenzivne terapije) (15).

Metaboličko ispitivanje

Metaboličko ispitivanje je sprovedeno nakon najmanje 6 meseci od pojave IMU i to nakon 12h gladovanja. Insulinska senzitivnost evaluirana je testom intravenske glukozne tolerancije sa učestalim uzimanjem uzoraka (frequently sampled intravenous glucose tolerance – FSIGT) i kompjuterskom obradom dobijenih rezultata korišćenjem metode minimalnog modela radi određivanja parametra, odnosno indeksa insulinske senzitivnosti (Si) (16). U toku ovog oblika FSIGT testa, uzorci za određivanje nivoa glukoze i insulina u plazmi uzimani su neposredno pre i u 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 20, 23, 24, 25, 27, 30, 40, 50, 60, 70, 80, 90, 100, 120, 160 i 180 minutu posle stimulacije sa 0.3g/kgTT iv. glukoze. U cilju dobijanja odgovarajuće krive opadajućih vrednosti glikemije i insulinemije, koja omogućava adekvatnu obradu podataka kompjuterskim modelom, primenjena je kontinuirana iv. infuzija insulina kroz poseban venski pristup, brzinom od 4mU/kg/min, u trajanju od 5 minuta, između 20. i 25. minuta testa.

Obrada vrednosti glikemije i insulinemije obavljena je korišćenjem kompjuterskog programa minimalnog modela (MINMOD), dobijenog ljubaznošću dr Ričarda Bergmana iz Univerziteta Južne Kalifornije u Los Anđelesu.

Laboratorijske analize

Vrednost nivoa glikemije u serumu određena je metodom korišćenja enzima glikozo-oksidaze (pribor Beckman), dok je vrednost nivoa insulinemije u serumu određena metodom radioimunoeseja (pribor INEP – Zemun). Vrednost nivoa aktivnosti PAI-1 u plazmi određena je metodom hromogenog plazminogen/plazmin supstrat eseja (pribor Boehringer).

Statističke analize

Podaci su prikazani kao aritmetička sredina ± standardna greška (SE). Za kontinuirane varijable u svakoj grupi ispitanika primenjena je analiza varijanse (ANOVA) sa post hok Bonferoni (Bonferonni) testom, dok je za diskontinuirane varijable korišćen χ^2 test. Statistička značajnost razlike definisana je na nivou $p<0.05$. Statističke analize izvršene su pomoću SPSS softvera za personalne računare.

Rezultati

Kliničke karakteristike ispitanika

Kliničke karakteristike ispitanika prikazane su u tabeli 1. Starosna dob, trajanje dijabetesa i period od ispoljavanja IMU nisu se značajno razlikovali između grupa. Svi ispitanici su bili umereno gojazni (ITM: A: 27.56±3.11 vs B: 27.62±3.70 vs C: 26.21±4.15 vs D: 26.34±2.36 kg/m^2, p=NS), a u obolelih od T2D registrovana je zadovoljavajuća glikoregulacija pre metaboličkog testiranja. Pacijenti sa IMU, kako oni sa T2D tako i nedijabetičari, imali su višu prevalencu hipertenzije u poređenju sa zdravim ispitanicima, uz usaglašene vrednosti sistolnog i dijastolnog krvnog pritiska.

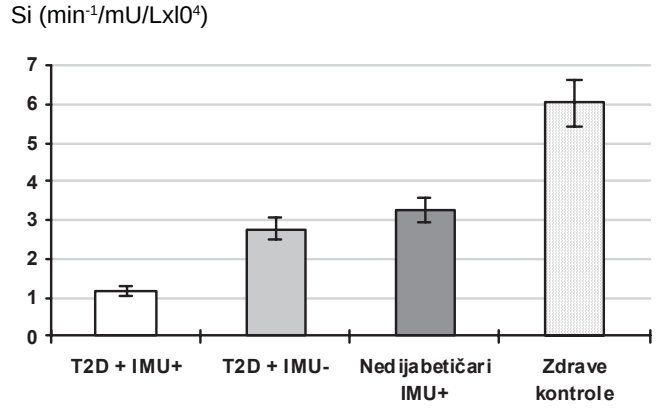
Svi oboleli od T2D su bili na monoterapiji preparatom metformina ili na kombinovanoj terapiji, metformin i sulfonilureja, bolesnici sa hipertenzijom su dobijali antihipertenzivnu terapiju (inhibitore angiotenzin-konvertujućeg enzima, blokatore kalcijumskih kanala, ili njihovu kombinaciju), bolesnici sa IMU uzimali su antiagregacionu terapiju.

Tabela 1. Kliničke karakteristike pacijenata sa tipom 2 dijabetesa (T2D) i nedijabetičara sa ili bez ishemijskog moždanog udara (IMU). Podaci su n, aritmetičke sredine ± SE. *p<0.001 A, B, C u poređenju sa zdravim kontrolama

	T2D $^{+}$ IMU^{+} A	T2D^{+} IMU^{-} B	Nedijabetičari IMU^{+} C	Zdrave kontrole D
n (M/Ž)	34 (16/18)	30 (15/15)	33 (16/17)	33 (15/18)
Starost (godine)	57.01 ± 2.20	58.10 ± 2.57	57.63 ± 2.79	57.87 ± 2.63
Trajanje dijabetesa (godine)	4.82 ± 1.78	5.84 ± 2.4	-	-
Vreme proteklo od nastanka IMU (godine)	1.14 ± 0.39	-	1.01 ± 0. 21	-
HbA1c (%)	7.35 ± 0.31*	7.23 ± 0.24*	5.67 ± 0.48	4.9 ± 0.4
Hipertenzija	22 (64.7%)	19 (63.3%)	19 (57.6%)	5 (15.2%)*
Sistolni krvni pritisak (mm Hg)	152.4 ± 4.2	154.1 ± 4.4	151.1 ± 2.9	135 ± 3.0*
Dijastolni krvni pritisak (mm Hg)	90.4 ± 5.7	92.9 ± 4.9	88.6 ±.3.1	80 ± 1.2*
Pušenje	13 (38.2%)	11 (36.7%)	12 (36.4%)	10 (30.3%)
Metformin/Metformin+sulf.	6 / 28	8 / 22	-	-
Antiagregacioni agensi	34 (100%)	30 (100%)	33 (100%)	0 (0%)
Hipolipemici	11 (33%)	5 (16.6%)	4 (12.1%)	0 (0%)

Insulinska senzitivnost

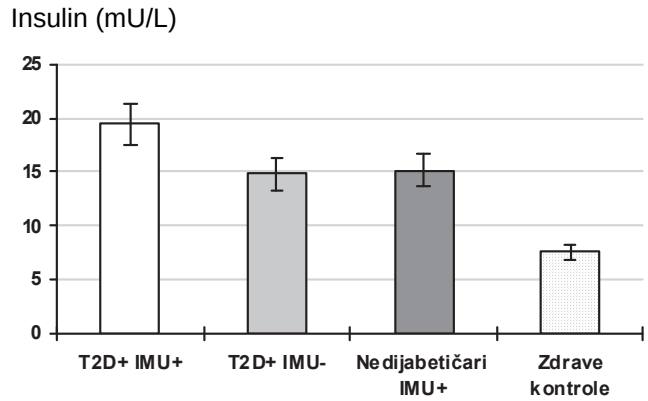
Rezultati našeg istraživanja su pokazali da su nivoi Si bili značajno niži u obolelih od T2D i IMU, u poređenju sa obolelima od T2D bez IMU (1.17+/-0.66 i 2.79+/-0.62 $min^{-1}/mU/lx10^{4}$; p<0.001). Takođe, ispitanici bez T2D a sa IMU su imali značajno niže nivoe Si u odnosu na kontrolnu grupu (3.25+/-0.84 i 6.03+/-1.69 $min^{-1}/mU/lx10^{4}$; p<0.001) (grafikon 1). Najniži nivo insulinske senzitivnosti registrovan je u obolelih od T2D i IMU.



Grafikon 1. Vrednosti su aritmetička sredina ± SE. Stubići pokazuju vrednosti Si određene metodom minimalnog modela.

Nivoi insulinemije

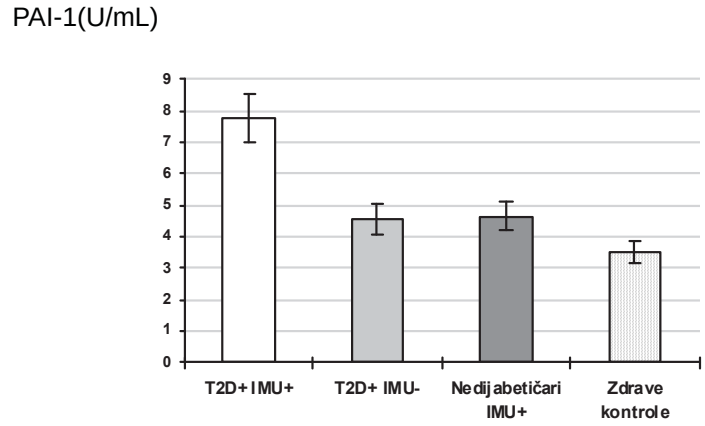
Nivoi insulinemje su bili značajno viši u grupi obolelih od T2D i IMU, u odnosu na obolele od T2D bez IMU (19.46+/-4.11 i 14.79+/-1.75 mU/l; p<0.001), kao i u nedijabetičara sa IMU u poređenju sa zdravim ispitanicima (15.16+/-2.23 i 7.54+/-2.03 mU/l; p<0.001). Takođe, oboleli od T2D, sa ili bez IMU, imali su značajno više vrednosti insulinemija u odnosu na kontrolnu grupu (p<0.001), dok su među ispitanicima sa IMU značajno viši nivoi insulinemije registrovani kod obolelih od T2D, u odnosu na nedijabetičare (p<0.001). Sa druge strane, nije bilo značajne razlike u nivoima insulinemije u grupi obolelih od T2D bez IMU u poređenju sa bolesnicima sa IMU, ali bez dijabetesa (grafikon 2).



Grafikon 2. Vrednosti su aritmetička sredina ± SE. Stubići pokazuju vrednosti bazne insulinemije.

Fibrinoliza

Nivoi PAI-1 su bili značajno viši u obolelih od T2D i IMU u poređenju sa obolelim od T2D bez IMU (7.78+/-1.05 i 4.56+/-0.71 mU/l; p<0.001), kao i u nedijabetičara sa IMU u odnosu na kontrolnu grupu (4.65+/-0.69 i 3.48+/-1.29 mU/l; p<0.001) (grafikon 3). Sa druge strane, u grupi ispitanika bez IMU, nije bilo značajne razlike u nivoima PAI-1 između pacijenata sa i bez T2D.



Grafikon 3. Vrednosti su aritmetička sredina ± SE. Stubići pokazuju vrednosti nivoa PAI-1.

Korelacija

Rezultati naše studije su pokazali korelaciju između nivoa Si i PAI-1 kako u obolelih od T2D (r=-0.690, p<0.0001), tako i u nedijabetičara (r=-0.437, p<0.001) (tabela 2).

T2D	*r*	*p*	*Nedijabetičari*	*r*	*p*
PAI-1			*PAI-1*		
Si	-0.690	0.0001	Si	-0.437	0.001

Tabela 2. Korelacija između Si i nivoa PAI-1 u pacijenata sa T2D i nedijabetičara

Diskusija

Naši rezultati su pokazali postojanje snižene insulinske senzitivnosti u obolelih sa IMU, kako u T2D tako i u nedijabetičara, ukazujući na insulinsku rezistenciju kao važnu determinantu u razvoju IMU. Ova studija je takođe pružila dokaz da je najniži nivo insulinske senzitivnosti prisutan u obolelih od T2D sa IMU. Istovremeno, naj-

više vrednosti bazne insulinemije registrovane su u obolelih od T2D i IMU, što bi se moglo objasniti da su ispitanici obuhvaćeni ovom studijom imali uglavnom očuvan kapacitet insulinske sekrecije u vreme metaboličkog testiranja, pa je hiperinsulinemija odražavala sniženu insulinsku senzitivnost. Kompenzatorna hiperinsulinemija, udružena sa sniženom insulinskom senzitivnošću, registrovana je i u grupi ispitanika bez T2D sa IMU, naglašavajući ulogu i značaj insulinske rezistencije u patogenezi IMU, što je i u saglasnosti sa rezultatima Shinozaki i sar. (17).

U našem radu koristili smo metodu minimalnog modela (intravenski test tolerancije glukoze sa učestalim uzimanjem uzoraka), s obzirom na to da je prethodno pokazana izuzetno dobra korelacija ovog testa i testa euglikemijskim klampom (18), koji se smatra „zlatnim standardom" za određivanje nivoa insulinske senzitivnosti, ali je značajno zahtevniji za izvođenje (19).

Ranije studije su sugerisale povezanost insulinske rezistencije i različitih podtipova IMU u obolelih od T2D, ali uz primenu drugačijih metaboličkih testova (kratak test tolerancije insulina, homeostazni model, određivanje insulina imunoreaktivnom metodom u 2h OGTT) u odnosu na metodu minimalnog modela primenjenu u našoj studiji (2). Takođe, rezultati novijih istraživanja su potvrdili postojanje insulinske rezistencije, u bolesnika sa IMU, ali bez prethodno dokumentovanog poremećaja tolerancije glukoze i to u akutnoj fazi IMU (3).

U cilju smanjenja poznatog štetnog efekta „glukozne toksičnosti" na insulinsku senzitivnost (20), u grupi obolelih od T2D, i sa i bez IMU, uključili smo ispitanike sa sličnom dužinom trajanja dijabetesa i zadovoljavajućom metaboličkom kontrolom pre evaluacije nivoa insulinske senzitivnosti. Takođe, s obzirom na to da je poznato da starosna dob snažno i nezavisno korelira sa pojavom IMU, u istraživanje smo uključili ispitanike mlađe od 65 godina. Merenje insulinske senzitivnosti sprovedeno je najmanje 6 meseci nakon ispoljavanja IMU, omogućavajući ostvarivanje maksimalnog oporavka, kao i sličnog nivoa fizičke aktivnosti među ispitanicima.

Rezultati Studije rizika ateroskleroze u zajednicama (Atherosclerosis Risk in Communities (ARIC) Study) ukazali su na pozitivnu korelaciju između relativnog rizika za IMU i povišenih nivoa bazalne insulinemije u nedijabetičara, podržavajući pretpostavku o ulozi insulinske rezistencije u nastanku IMU (10), što je u saglasnosti i sa podacima dobijenim iz starije populacije ispitanika u Finskoj kohortnoj studiji koja je obuhvatila kako obolele od T2D, tako i ispitanike bez dijabetesa (21).

Sa druge strane, veliki broj dokaza ukazuje da PAI-1 ima važnu ulogu u razvoju makrovaskularnih komplikacija u T2D (22). Međutim, mehanizmi koji se nalaze u osnovi ateroskleroze, naročito u smislu povezanosti snižene IS i poremećaja fibrinolize u razvoju IMU, nisu u potpunosti razjašnjeni. Uzimajući u obzir da se oštećena fibrinoliza smatra mogućim faktorom koji povezuje sniženu IS i hiperinsulinemiju sa razvojem ateroskleroze, ispitivali smo povezanost IS i PAI-1, kao važnog činioca u patogenezi ateroskleroze (23).

U našoj studiji je pokazano da su oboleli od T2D sa IMU imali više nivoe PAI-1, dok oni sa T2D ali bez IMU nisu imali različite vrednosti PAI-1, u odnosu na nedijabetičare sa IMU. S obzirom na nalaze studija u kojima su izneti drugačiji rezultati i koji su ukazivali da su oboleli sa T2D bez IMU imali više nivoe PAI-1 u poređenju sa nedijabetičarima (24), naši rezultati su pokazali da sa pojavom IMU u T2D ne dolazi do daljeg oštećenja fibrinolize. Pokazano je da u određenim etničkim grupama u prvih rođaka bolesnika sa IMU postoji povišen nivo IS, insulinemije i PAI-1, ukazujući na mogućnost da oštećena fibrinoliza prethodi IMU (25).

S obzirom na registrovanu povećanu aktivnost PAI-1 u obolelih od T2D bez IMU, koji se, sa druge strane, nisu značajno razlikovali u poređenju sa vrednostima nedijabetičara sa IMU, moguće je da abnormalnosti sistema fibrinolize prethode ispoljavanju IMU (12). U tom smislu, novija istraživanja ukazuju na postojanje izvesne nasledne predispozicije za poremećaj fibrinolitičke aktivnosti (26). U našoj studiji ispitivali smo poremećaj aktivnosti PAI-1 tokom prve godine nakon IMU, odnosno posle akutne faze. Dobijeni rezultati pokazuju da povišeni nivoi PAI-1 u pacijenata sa IMU, sa ili bez T2D, predstavljaju pojačanu inhibiciju fibrinolize, što je prethodno i pokazano u nedijabetičara u periodu od čak dve do četiri godine nakon nastanka IMU (27).

Smatra se da je dopunski faktor, koji značajno doprinosi porastu aktivnosti PAI-1, gojaznost, s obzirom na povećanu ekspresiju PAI-1 u masnom tkivu (28). Naši rezultati su pokazali da su nivoi Si korelirali sa PAI-1 u umereno gojaznih ispitanika, sa ili bez T2D. Rezultati naše studije su u saglasnosti sa prethodnim u smislu da je insulinska senzitivnost bila nezavisno povezana sa nivoima PAI-1 u pacijenata sa T2D, kao i u gojaznih ispitanika, sa i bez dijabetesa (29). Takođe, poznato je da su nivoi PAI-1 povišeni već u ranim fazama poremećaja tolerancije glukoze, implicirajući povezanost porasta aktivnosti PAI-1 i rizika za ispoljavanje T2D, nezavisno od drugih faktora rizika, kao što su gojaznost, insulinska rezistencija, endotelna disfunkcija i inflamacija (30).

U tom smislu, insulinska rezistencija bi mogla imati značajnu ulogu u patogenezi IMU, kako u T2D, tako i u nedijabetičara.

U celini, naši rezultati ukazuju da bi snižena insulinska senzitivnost, udružena sa kompenzatornom hiperinsulinemijom, mogla, preko poremećaja fibrinolize, ostvarivati aterogeni uticaj na ispoljavanje IMU.

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ISCHEMIC STROKE IN PATIENTS WITH TYPE 2 DIABETES: RELATIONSHIP BETWEEN DECREASED INSULIN SENSITIVITY AND FIBRINOLYSIS IMPAIRMENT

Abstract: The role of insulin sensitivity (IS), as well as the association of IS with fibrinolysis impairment, in the occurrence of ischemic stroke, has not been clarified. The study was aimed to analyze IS, plasma insulin (PI) and plasminogen activator inhibitor (PAI)-1 levels in 34 type 2 diabetics (T2D) with ischemic stroke (group A), 30 T2D without ischemic stroke (group B), 33 nondiabetics with ischemic stroke (group C) and 33 healthy controls (group D). Ischemic stroke was confirmed by clinical and neuroimaging criteria. IS levels were determined by the minimal model analysis (Si index). Plasma insulin levels were measured by radioimmunoassay and PAI-1 activity was performed by the plasminogen chromogenic plasmin substrate assay. We found that Si levels were significantly lower in group A vs. B (1.17+/-0.66 vs. 2.79+/-0.62 $min^{-1}/mU/Lx10^4$; $p<0.001$) and in C vs. D (3.25+/-0.84 vs. 6.03+/-1.69 $min^{-1}/mU/Lx10^4$; $p<0.001$), while PI levels were higher in group A vs. B (19.46+/-4.11 vs. 14.79+/-1.75 mU/L; $p<0.001$) and in C vs. D (15.16+/-2.23 vs. 7.54+/-2.03 mU/L; $p <0.001$). Also, PAI-1 activity was significantly higher in group A vs. B (7.78+/-1.05 i 4.56+/-0.71 mU/L; $p<0.001$) and in C vs D (4.65+/-0.69 i 3.48+/-1.29 mU/L; $p<0.001$). Moreover, Si levels correlated with PAI-1, both in T2D and nondiabetics. Our results indicate that appearance of ischemic stroke was associated with decreased insulin sensitivity, together with compensatory

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hyperinsulinemia, both in T2D and nondiabetics. Our results imply that impaired insulin sensitivity exerts its atherogenic influence, at least in part, by decreased fibrinolysis.

Keywords: ischemic stroke, type 2 diabetes, insulin sensitivity, plasminogen activator inhibitor -1 (PAI-1)

Sažetak: Uloga i značaj insulinske senzitivnosti (IS), kao i povezanost IS sa poremećajem fibrinolize za ispoljavanje ishemijskog moždanog udara (IMU) u potpunosti, još uvek nije razjašnjen. Cilj istraživanja je bio da se analiziraju nivoi IS i insulinemije, kao i njihova povezanost sa nivoom plazminogen aktivator inhibitora 1 (PAI-1) u 34 pacijenta sa tipom 2 dijabetesa (T2D) i IMU (grupa A), 30 pacijenata sa T2D bez IMU (grupa B), 33 pacijenta sa IMU bez T2D (grupa C) i 33 zdrave kontrole (grupa D). Postojanje IMU potvrđeno je na osnovu kliničkog i nalaza neurovizuelizacionih procedura. IS je određivana metodom minimalnog modela (Si indeks). Nivo insulina je određen metodom radioimunoeseja, a nivo PAI-1 određivan je hromogenim plazminogen/plazmin substrat esejom. Rezultati su pokazali da su nivoi Si bili značajno niži u grupi A u poređenju sa B (1.17+/-0.66 i 2.79+/-0.62 min^{-1}/mU/Lx10^4; p<0.001), kao i u grupi C u odnosu na D (3.25+/-0.84 i 6.03+/-1.69 min^{-1}/mU/Lx10^4; p<0.001). Grupa A je imala značajno više nivoe insulinemije u poređenju sa grupom B (19.46+/-4.11 i 14.79+/-1.75 mU/L; p<0.001), kao i grupa C u odnosu na D (15.16+/-2.23 i 7.54+/-2.03 mU/L; p <0.001). Nivo PAI-1 je bio značajno viši u grupi A u odnosu na B (7.78+/-1.05 i 4.56+/-0.71 mU/L; p<0.001) i u grupi C u odnosu na D (4.65+/-0.69 i 3.48+/-1.29 mU/L; p<0.001). Pokazana je korelacija Si sa nivoom PAI-1, u ispitanika sa i bez T2D. Rezultati ukazuju da snižena insulinska senzitivnost, udružena sa kompenzatornom hiperinsulinemijom, može ostvarivati svoj aterogeni uticaj na ispoljavanje IMU i preko sniženja fibrinolize.

Ključne reči: ishemijski moždani udar, tip 2 dijabetesa, insulinska senzitivnost, plazminogen aktivator inhibitora -1 (PAI-1)

Introduction

Decreased insulin sensitivity (IS) plays an important role in the development of atherosclerosis (1), and is recognized risk factor for ischemic stroke (2,3). The mechanisms underlying the association between diminished insulin sensitivity and vascular events are not completely revealed but may involve hyperglycemia (4), dislipidemia, hypertension, hypofibrinolysis and endothelial dysfunction (5). At the

same time, decreased IS, e.g. insulin resistance, is an important pathophysiological mechanism in type 2 diabetes (T2D) (6).

The previous studies suggested that insulin resistance, measured by different metabolic tests, was directly related to different subtypes of ischemic stroke in both T2D patients and nondiabetics (2, 3). Moreover, higher levels of insulin resistance were demonstrated in nondiabetics with both intra and extra cranial atherosclerosis compared to those who had either intra or extra cranial atherosclerosis alone (7).

Hyperinsulinemia, which may be used as a parameter of insulin resistance in nondiabetics, and which is often, although not obligatory, present in patients with T2D as a compensatory response to insulin resistance (8), was also recognized as an independent risk factor for ischemic stroke (9,10).

Also, other relevant atherogenic factors such as hypercoagulability and hypofibrinolysis were related to the insulin resistance (11), and positive relationship between decreased IS and fibrinolysis impairment was demonstrated in patients with T2D and nondiabetics with coronary artery disease (12). However, the association between changes in IS and plasminogen activator inhibitor (PAI)-1 levels, in respect of occurrence of ischemic stroke, has not been elucidated yet.

Therefore, we have explored the relationship between IS and fibrinolysis impairment as the potential mechanism underlying ischemic stroke in T2D patients, as well as in nondiabetics.

Aim

To analyse the association between insulin sensitivity level and fibrinolysis activity in the occurence of ischemic stroke in patients with T2D.

Methods

Patients

A sixty four patients with T2D were classified into two groups, T2D with (N=34) and without ischemic stroke (N=30), in comparison to nondiabetics with ischemic stroke (N=33) and healthy controls (N=33).

T2D was diagnosed according to the criteria of the World Health Organization (13).

Diagnosis of ischemic stroke was set by neurologist in Institute for Neurology, Clinical Center of Serbia, according to clinical features and brain imaging methods, cranial computerized scan and magnetic resonance imaging, in two consecutive examinations, during the first 7 days from the occurrence of ischemic stroke (14).

The patients with ischemic stroke were included in the study providing that did not show signs of cardioembolic cerebral infarction or coronary heart disease, based on a history of myocardial infarction confirmed either by elevation of serum cardiac enzymes or coronary angiography. T2D patients were treated with oral antidiabetic agents while patients treated with insulin therapy as well as patients who had other endocrine, infectious or malignant disease, were excluded.

All the patients, with or without ischemic stroke, showed the similar level of physical activity. The metabolic evaluation was performed in the Clinic for Endocrinology, Diabetes and Metabolic Diseases, after all the patients were fully informed and gave the inform consent to participate in the study.

Research design

The interview, containing the questions about medical conditions, current medication and habits, physical examination and metabolic test were conducted in all of the patients included in the study (all completed at the same day). Body weight (BW) and body height (BH) were measured in each subject and used to calculate the body mass index (BMI), according to following formula:

$$BMI\ (kg/m^2) = BM\ (kg) / BH^2\ (m^2)$$

Hypertension was diagnosed according to World Health Organization criteria (systolic/diastolic blood pressure $\geq$140/$\geq$90 mm Hg) or by the use of antihypertensive agents (15).

Metabolic investigation

The metabolic evaluation was conducted after at least 6 months from occurrence of ischemic stroke and after at least 12h of fasting. Insulin sensitivity was evaluated by frequently sampled intravenous glucose tolerance (FSIGT) test with computerized minimal model analysis to determine the insulin sensitivity index (Si) (16). During this form of FSIGT test, the blood samples for measurement of plasma glucose (PG) and plasma insulin (PI) levels were taken immediately before and 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 20, 23, 24, 25, 27, 30, 40, 50, 60, 70, 80, 90, 100, 120, 160 and 180 minutes after stimulation with 0.3g/kg body weight of intravenous glucose. The continous intravenous infusion of 4mU/kg/min of insulin was adminstered during 5 minutes, between 20. and 25. minute of test. Si was calculated from the results of PG and PI levels by computerized minimal model analysis, using the MINMOD program (kindly provided by Dr Richard Bergman from the University of Southern California (Los Angeles).

Laboratory analyses

PG was measured by glucose oxidase method using a Beckman Glucose Analyser, while PI was determined by radioimmunoassay (INEP-Zemun). Plasma PAI-1 activity was determined by plasminogen chromogenic plasmin substrate assay (Boehringer).

Statistical analyses

Data are presented as mean ± standard error (SE). The continuous variables within each group of patients were analyzed using analysis of variance (ANOVA) with a post hoc Bonferroni test, while the categorical variables were analyzed with χ^2 test. Differences were considered statistically significant at $p< 0.05$. Statistical analysis was performed with the SPSS statistical software package for personal computers.

Results

Clinical characteristics

The clinical characteristics of study subjects are shown in Table 1. The age, duration of diabetes, and time period from the onset of ischemic stroke were similar within the groups. All subjects were moderately obese (BMI: A:27.56±3.11 vs B: 27.62±3.70 vs C: 26.21±4.15 vs D: 26.34±2.36 kg/m^2, p=NS) and T2D patients showed similar HbA_1c levels, implying satisfying metabolic control before metabolic investigation. The prevalence of hypertension in patients with ischemic stroke, both diabetics and nondiabetics, was significantly higher comparing to healthy controls, and subjects were matched in respect to the level of systolic and diastolic blood pressure before the metabolic testing was performed.

All T2D patients were treated either with metformin monotherapy or combination of metformin and sulphonilurea agents, patients with hypertension were administered antihypertensive medication (angiotensin-converting enzyme inhibitors, calcium channel blockers, or their combination), while those with ischemic stroke were receiving antiplatelet agents.

Table 1. Clinical characteristics of type 2 diabetic (T2D) patients and nondiabetics with and without ischemic stroke Data are n, means ± SE. *p<0.001 A,B,C vs. healthy controls

	T2D + Ischemic stroke+ A	T2D+ Ischemic stroke- B	Nondiabetics Ischemic stroke+ C	Healthy Controls D
n (M/F)	34 (16/18)	30 (15/15)	33 (16/17)	33 (15/18)
Age (years)	57.01 ± 2.20	58.10 ± 2.57	57.63 ± 2.79	57.87 ± 2.63
Duration of diabetes (years)	4.82 ± 1.78	5.84 ± 2.4	-	-
Duration from onset of ischaemic stroke (years)	1.14 ± 0.39	-	1.01 ± 0. 21	-
HbA1c (%)	7.35 ± 0.31*	7.23 ± 0.24*	5.67 ± 0.48	4.9 ± 0.4
Hypertension	22 (64.7%)	19 (63.3%)	19 (57.6%)	5 (15.2%)*
Systolic blood pressure (mm Hg)	152.4 ± 4.2	154.1 ± 4.4	151.1 ± 2.9	135 ± 3.0*
Diastolic blood pressure (mm Hg)	90.4 ± 5.7	92.9 ± 4.9	88.6 ±.3.1	80 ± 1.2*
Smoking	13 (38.2%)	11 (36.7%)	12 (36.4%)	10 (30.3%)
Metformin/Metformin+sulph.	6 / 28	8 / 22	-	-
Antiplatelet agents	34 (100%)	30 (100%)	33 (100%)	0 (0%)
Lipid lowering agents	11 (33%)	5 (16.6%)	4 (12.1%)	0 (0%)

Insulin sensitivity

We found that Si levels were significantly lower in T2D patients with ischemic stroke compared to T2D patients without ischemic stroke (1.17+/-0.66 vs. 2.79+/-0.62 $min^{-1}/mU/Lx10^4$; p<0.001). Also, nondiabetics with ischemic stroke showed significantly lower Si levels compared to healthy controls (3.25+/-0.84 vs. 6.03+/-1.69 $min^{-1}/mU/Lx10^4$; p<0.001) (Figure 1). The lowest insulin sensitivity level was present in patients with T2D and ischemic stroke.

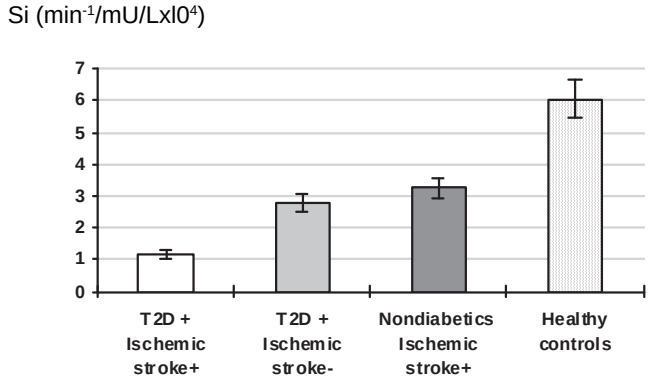

Figure 1. Values are mean ± SE. Bar graphs show the values of Si determined by minimal model analysis.

Insulin levels

PI levels were significantly higher in patients with T2D and ischemic stroke compared to T2D patients without ischemic stroke (19.46+/-4.11 vs. 14.79+/-1.75 mU/L; $p<0.001$) as well as in nondiabetics with ischemic stroke in comparison to healthy controls (15.16+/-2.23 vs. 7.54+/-2.03 mU/L; $p<0.001$). Also, T2D patients with and without ischemic stroke had higher PI levels than healthy controls ($p<0.001$), while in group with ischemic stroke T2D patients showed significantly higher PI levels compared to nondiabetics ($p<0.001$). On the other hand, T2D patients without ischemic stroke and nondiabetics with ischemic stroke did not significantly differ in respect to PI levels (Figure 2).

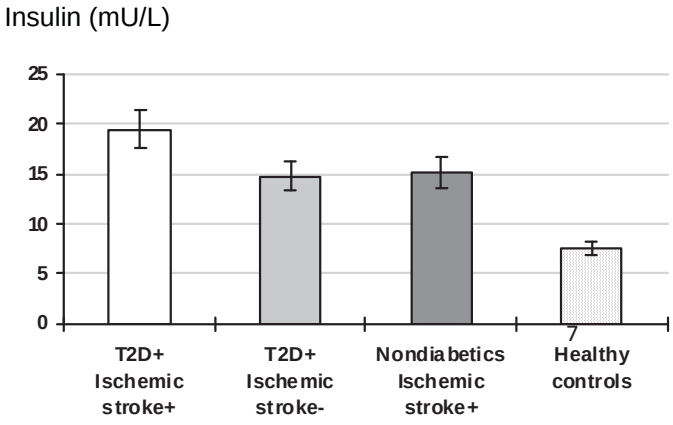

Figure 2. Values are mean ± SE. Bar graphs show the values of basal PI level.

Fibrinolysis

In the T2D group, PAI-1 levels were significantly higher in patients with ischemic stroke compared to those without ischemic stroke (7.78+/-1.05 vs. 4.56+/-0.71 mU/l; p<0.001), as well as in nondiabetics with ischemic stroke compared to healthy controls (4.65+/-0.69 vs. 3.48+/-1.29 mU/l; p<0.001) (Figure 3). Nevertheless, in the group without ischemic stroke, there was no statistically significant difference in PAI-1 levels between T2D patients and nondiabetics.

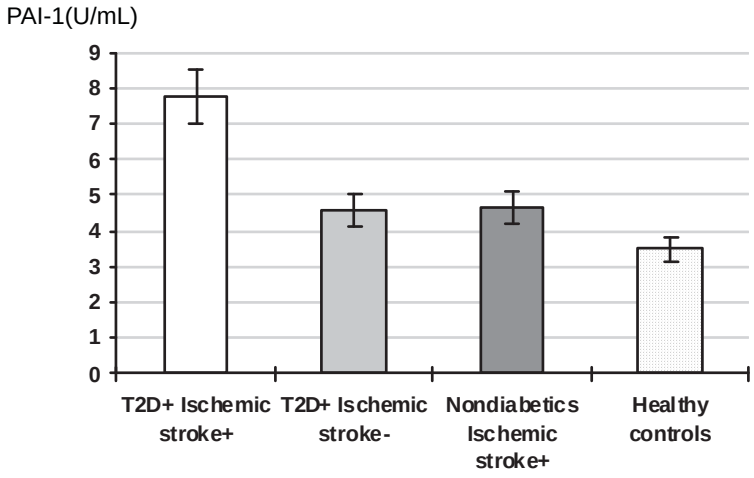

Figure 3. Values are mean ± SE. Bar graphs show the values of PAI-1 levels.

Correlations

Our results showed that Si levels correlated with PAI-1 levels both in T2D (r= -0.690, p<0.001) and nondiabetic subjects (r= -0.437, p<0.001) (Table 2).

T2D	*r*	*p*	*Nondiabetics*	*r*	*p*
PAI-1			*PAI-1*		
Si	-0.690	0.0001	Si	-0.437	0.001

Table 2. Correlation between Si and PAI-1 levels in patients with Type 2 diabetes and nondiabetics

Discussion

Our results showed decreased insulin sensitivity in both T2D patients and nondiabetics with ischemic stroke implying insulin resistance to be an important determinant in development of ischemic stroke. This study provided the evidence that the lowest level of insulin sensitivity is present in T2D patients with ischemic stroke. At the same time, we detected the highest level of fasting insulinemia in T2D patients with ishemic stroke, probably due to the fact that our patients had mostly preserved insulin secretion capacity at the time of metabolic investigation, so hyperinsulinemia reflected the decreased insulin sensitivity level. Compensatory hyperinsulinemia, together with decreased insulin sensitivity levels, was also found in nondiabetics with ishemic stroke, emphasising the role of insulin resistance in patogenesis of ischemic stroke, which is in agreement with previous study (17).

We decided to measure insulin sensitivity level by using FSIGT with minimal model analysis, due to previosly described excellent correlation between this test and hyperinsulinemic euglycemic clamp (18), which is concidered to be a "gold standard" for determining insulin sensitivity level, but is significantly more demanding and techically diffucult (19).

The association of insulin resistance and different subtypes of ischemic stroke in patients with T2D has been suggested in the previous studies, but by using other metabolic tests to assess insulin sensitivity (the short insulin tolerance test, homeostasis model, immunoreactive insulin after glucose loading in 2h OGTT) than the minimal model method we used (2). Also, the novel study confirmed the existence of insulin resistance in patients with ischemic stroke but without previously documented abnormal glucose tolerance, assessed in the acute phase of ischemic stroke (3).

In order to minimize the previously confirmed harmful effect of »glucose toxicity« to the insulin sensitivity level (20), we selected T2D patients with or without ischemic stroke matched in respect to duration of disease and showing satisfying metabolic control before the evaluation of insulin sensitivity level. Moreover, since age is known to be strongly and independently correlated with the occurrence of ischemic stroke, we selected patients less than 65 years old. Measurements of insulin sensitivity were made after at least 6 months after the stroke, providing sufficient time for investigated patients to approach maximum recovery, showing similar level of physical activity.

The results from Atherosclerosis Risk in Communities (ARIC) Study documented positive correlation beetwen relative risk for ischemic stroke and increased basal insulin levels among nondiabetics, supporting supposed role of insulin resistance in the ischemic stroke pathogenesis (10), which is in agreement with results from the elderly patient population of the Finnish cohort study that included both diabetic and nondiabetic patients (21).

On the other side, a growing body of evidence implicates the important role PAI-1 plays in the development of diabetic macrovascular complications (22). However, the underlying mechanisms in pathogenesis of atherosclerosis, especially in aspect of relationship between decreased insulin sensitivity and fibrinolysis impairment in the onset of ischemic stroke, have not been elucidated yet. Taking into concideration that impared fibrinolysis is concidered to be a possible link between decreased insulin sensitivity and hyperinsulinemia on the one side, and atherosclerosis on the other, we investigated the relationship of insulin sensitivity and PAI-1 levels as an important factor contributing to the pathogenesis of atherosclerosis (23).

Our study demonstrated that T2D patients with ischemic stroke had higher levels of PAI-1, while those with T2D without ischemic stroke did not have significantly different levels of PAI-1 compared to nondiabetics with ischemic stroke. According to some opposite findings that patients with T2D without ischemic stroke had higher PAI-1 levels than nondiabetics (24), our results suggested that further impairment of fibrinolysis does not occur with ischemic stroke in T2D. There were observations that in some ethnic group first relatives of patients with ischemic stroke exhibited increased insulin resistance, hyperinsulinemia and increased PAI-1, implying that impairment in fibrinolysis preceded cerebral infarction (25).

It is possible that abnormalities in the fibrinolytic system may occur even before the onset of ischemic stroke, regarding the increased level of PAI-1 detected in group of T2D diabetics without ischemic stroke, but not significantly different in comparison to nondiabetics with ischemic stroke (12). Novel data emphasized the inherited predisposition of certain genetic susceptibility related to impairments in fibrinolityc activity (26). In addition, we investigated the patients who were not in the acute phase of ischemic stroke in order to clarify the role of increased PAI-1 during the first year after the ischemic stroke. The results imply that the high PAI-1 level in patients with ischemic stroke, both T2D and nondiabetics, represent increased inhibition of fibrinolysis, which was previously reported in nondiabetics up to 2 to 4 years after stroke onset (27).

Obesity is also thought to contribute significantly to elevated plasma PAI-1 levels, taking into consideration the over expression of PAI-1 in adipose tissue (28). Our results demonstrated that Si levels correlated with PAI-1 levels in moderately obese, both T2D and nondiabetic subjects, which is consistent with previous findings that insulin sensitivity was independently related to PAI-1 levels in patients with T2D, as well as in obese subjects with or without T2D (29). Also, the elevated PAI-1 levels were demonstrated already in the early stage of impaired glucose tolerance, implying the relationship between increased PAI-1 activity and risk for T2D, independently of other risk factors, as obesity, insulin resistance, endothelial dysfunction and inflammation (30).

Therefore, insulin resistance might play an important role in the pathogenesis of ischemic stroke, both in T2D patients and nondiabetics.

In conclusion, our results imply that decreased insulin sensitivity, in association with compensatory hyperinsulinemia, might exert atherogenic influence on the occurrence of ischemic stroke, at least in part through impairment of fibrinolysis.

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