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SIGNALNI I CITOKINSKI UTICAJ NA RAZVOJ MASNOG TKIVA, NASTANAK GOJAZNOSTI I DIJABETESA

Sažetak: Proces potrošnje energije je trajan, a obnavljanje energije putem unosa hrane samo povremeno. Stoga je tokom evolucije bilo neophodno oformiti sistem koji bi stvarao rezerve energije i čuvao ih za period između obroka – masno tkivo. Na razvoj masnog tkiva mogu uticati brojni signalni i hormonalni faktori, opredeljujući ukupnu količinu i distribuciju masnog tkiva na supkutano i visceralno. Pored nesporne uloge u energetskoj homeostazi, masno tkivo je i važan endokrini i parakrini organ koji oslobađa mnoge citokine i hormone, te presudno utiče na celinu metaboličkih i imunoloških procesa u organizmu. Tako primarno visceralno masno tkivo sintetiše značajne količine adipocitokina: leptina, adiponektina, tumor necrosis factor-ά, interleukina-6 i brojnih drugih. Masno tkivo tako u stvari može biti krucijalni alarmni sistem koji pobuđuje urođeni imunitet i akutnu fazu zapaljenja. Hronična inflamacija je najvažnija karakteristika metaboličkog sindroma, a inflamatorni signali uglavnom potiču iz visceralnog masnog tkiva. Stoga se eksces masnog tkiva lako može povezati sa nastankom brojnih metaboličkih poremećaja i razvojem dijabetesa, kako tipa 2, tako i tipa 1.

Ključne reči: razvoj masnog tkiva, visceralno masno tkivo, adipocitokini, metabolički sindrom, hibridni dijabetes

BELO MASNO TKIVO

Belo masno tkivo je glavni energetski organ u periodu nakon odojačkog, pa sve do pozne starosti (1). Raspoređeno je po celom telu kao visceralno (VMT) i supkutano (SMT). Visceralno („abdominalno“ ili „centralno“) lokalizovano je oko unutrašnjih

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organa, u mezenterijumu i omentumu i čini oko 20% ukupnog masnog tkiva. Supkutano tkivo čini oko 80% i distribuirano je dominantno u dve glavne regije, abdominalnoj i gluteofemoralnoj (2). Između ova dva tkiva postoje značajne razlike u histološkoj strukturi, u endokrinom profilu adipocita, lipolitičkoj aktivnosti, te reaktivnosti na insulin i druge hormone. SMT predstavlja fiziološko mesto za deponovanje masti u ekscesu, gde se višak glicerola i slobodnih masnih kiselina lako deponuje u obliku triglicerida u dosta inertne adipocite. VMT predstavlja metabolički jako aktivan deo masnog tkiva, čiji je višak presudan u konstituisanju brojnih metaboličkih abnormalnosti, uključujući dijabetes. Dve su osnovne vrste adipocita u masnom tkivu: mali – novosintetisani i oni veliki, maturisani. Kompozicija masnog tkiva zavisiće od balansa *de novo* sinteze malih adipocita i apoptoze onih velikih. Mali adipociti sekretuju insulin-senzitivišuće molekule kakav je adiponektin, mnogo su osetljiviji na insulin i imaju veliku sposobnost preuzimanja slobodnih masnih kiselina i triglicerida, čime se prevenira njihovo deponovanje u neadipocitnim tkivima (3). Veliki adipociti su disfunkcionalni, rezistentni na antilipolitičko dejstvo insulina i aktivni u lučenju brojnih adipocitokina koji uslovljavaju različite patološke poremećaje, pre svih metabolički sindrom. Rezistentnost na insulin velikih adipocita sprečava deponovanje triglicerida i favorizuje lipidnu oksidaciju, sa stvaranjem veoma toksičnih produkata kakvi su lipidni peroksidi.

Za razliku od SMT, koje je uglavnom građeno od malih adipocita, VMT sadrži dominantno velike adipocite, ali i makrofage i druge inflamatorne ćelije. Visceralno tkivo je i mnogo bolje inervisano i vaskularizovano. Za razliku od SMT koje sekretuje u sistemsku cirkulaciju VMT se zbog svoje anatomske pozicije drenira direktno u portnu cirkulaciju. Ovo omogućava direktan uticaj oslobođenih slobodnih masnih kiselina (SMK) i adipocitokina na jetru, pa upravo tako interleukina-6 (IL-6) iskazuje stimulativan uticaj na stvaranje C-reaktivnog proteina (CRP) u jetri (4).

Najveće rezerve energije u organizmu deponovane su u obliku triglicerida upravo u masnom tkivu. Pod dejstvom lipoproteinske lipaze (LPL) u endotelu kapilara trigliceridi iz hilomikrona i lipoproteina vrlo niske gustine (VLDL) se hidrolizuju na glicerol i SMK koje se zatim deponuju u masnim ćelijama, gde će se ponovo resterifikovati u trigliceride. Kada postoji potreba za energijom, deponovani trigliceridi iz adipocita se pod dejstvom hormone-senzitivne lipaze (HSL) razgrađuju ponovo do glicerola i SMK. Aktivacija HSL je posledica aktivacije AMP-aktiviranih protein kinaza (AMPK). AMPK aktiviraju kateholamini, dok glikokortikoidi, tiroidni hormoni i hormon rasta imaju permisivnu ulogu. Suprotno, AMPK inhibira insulin, a posredno i IGF-1, adenozin i prostaglandini. Povećano oslobađanje SMK u cirkulaciju posledično je praćeno kako povećanjem produkcije šećera i sinteze triglicerida u jetri, tako i sniženjem klirensa insulina, što favorizuje nastanak insulinske rezistencije.

RAZVOJ MASNOG TKIVA

Masno tkivo čine tri grupe ćelija: stem ćelije, preadipociti i zreli unilokularni adipociti sa deponovanim trigliceridima. Preadipociti se morfološki i biohemijski gotovo ne razlikuju od fibroblasta, ali se njihova diferencijacija u pravcu adipocita odvija pod određenim fiziološkim uticajima celog života. Broj preadipocita može biti neograničen i zavisi od broja i aktivnosti stem ćelija, iz kojih mogu nastati i osteoblasti, hondroblasti i mioblasti. U kome će pravcu poći diferencijacija stem ćelija – prema adipocitnim ili neadipocitnim linijama, zavisiće od čitavog niza hormonskih, citokinskih i signalnih uticaja.

Adipociti se normalno razvijaju iz prekurzornih mezenhimalnih ćelija pod uticajem različitih hormonalnih i signalnih mehanizama (5). Kakva će biti diferencijacija mezenhimnih stem ćelija zavisiće od mnogih hormona: tako će testosteron inhibirati razvoj masnog tkiva i favorizovati nastanak mišićnog. Diferencijacija stem ćelija zavisiće i od različitih signalnih, transformacionih faktora kakvi su *Notch1c* (pomeraju diferencijaciju osteoblasta ka adipocitima) ili *RhoGTPasa* (potenciraju diferencijaciju prema miogenezi).

U nastanku masnog tkiva važnu ulogu ima i angiogeneza – adipogeneza i neovaskularizacija suštinski su povezani procesi. Bez angiogeneze nema diferencijacije preadipocita. Stoga preadipociti snažno sekretuju VEGF (vaskularni endotelijalni faktor rasta) te TGF-β (transformišući faktor rasta beta) i direktno stimulišu angiogenezu. Ova činjenica objašnjava vezu između obeziteta i kancera: s obzirom na to da je i rast tumora duboko zavisan od angiogeneze, moguće je da gojaznost prekomernom sintezomVEGF i TGF-β utiče na tumorsko nastajanje i napredovanje.

Jednom nastali preadipociti prolaze kroz faze proliferacije i diferencijacije.

Stimulacija mirujućih preadipocita odvija se pod uticajem insulina, glikokortikoida i faktora koji uvećavaju nivo cAMP, a koji tako postaju snažni adipogeni stimulusi.

Nakon klonalne ekspanzije preadipocita, njihova terminalna diferencijacija je pod uticajem nuklearnog receptora koji funkcioniše kao transformacioni faktor i označava se kao PPARγ (receptor aktivacije proliferacije peroksizoma gama). Na aktivnost PPARγ utiču prirodni (SMK) i sintetski ligandi (tiazolidindioni) uslovljavajući njegovu aktivaciju i omogućavajući terminalnu diferencijaciju adipocita. Faktor diferencijacije mogu biti i glikokortikoidni receptori.

Neporeciva fenotipska sličnost Kušingovog sindroma i pacijenata sa prostom centralnom gojaznošću objašnjava suštinsku povezanost glikokortikoidnog ekscesa i nastanka obeziteta. Glikokortikoidni receptori imaju centralnu ulogu u klonalnoj ekspanziji i terminalnoj diferencijaciji adipocita, ali i na distribuciju masnog tkiva i njegovu metaboličku aktivnost. Najveći broj glukokortikoidnih receptora otkriva se u VMT (6). Presudan uticaj na aktivnost glikokortikoida u masnom tkivu ima enzim 11β-hidroksi steroid dehidrogenaza (11β-HSD) koji reguliše prevođenje aktivne forme

glikortikoida (kortizol) u neaktivnu (kortizon) i obrnuto (7). Dok je tip 2 ovog enzima predominantno eksprimiran u bubrezima, gde ima ulogu u homeostazi natrijuma i regulaciji krvnog pritiska, tip 1 je aktivan u masnom tkivu i jetri. Mada je ovo „dvosmerni“ enzim (prevodi aktivnu u neaktivnu formu i obrnuto) u adipocitima i jetri dominantno aktivira kortizon do kortizola! Ovo ima značajne metaboličke konsekvence – u jetri stimuliše glikoneogenezu, a u adipocitima ekspanziju i diferencijaciju. Nije nelogično zaključiti da je centralna gojaznost moguća posledica pojačane supkutane ekspresije tipa 1 11β-HSD u adipocitima.

Prvi adipociti formiraju se oko 15. nedelje gestacije, a zatim se uvećava i njihov broj i njihova zapremina, dominantno pod uticajem fetalnog insulina i placentarnog laktogena (8). Kod donesenog novorođenčeta masno tkivo čini oko 12% ukupne telesne mase – razvijenost ovog tkiva više zavisi od trajanja trudnoće a manje od ishrane majke. Dalji porast masnog tkiva ne prati idealno telesni rast – dolazi do naglog uvećanja masnog tkiva sa vrhuncem u šestom mesecu života – tada masno tkivo čini čak 25% telesne mase. Nadalje postoji tendencija smanjivanja količine masnog tkiva sve do puberteta (9). Onda dolazi do drugog kruga rasta masnog tkiva – mnogo intenzivnije kod devojčica (zbog manjeg rasta mišićnog tkiva), nego kod dečaka. U kasnoj adolescenciji, masne rezerve čine 15–18% od mase muškaraca i 25–28% žena. U istom periodu ukupna težina tela raste samo 10–15%, što ukazuje da je uvećanje težine u pubertetu žena uglavnom posledica rasta masnog tkiva i smanjenja „mršave telesne mase” (“lean body mass” – anglosaksonski termin koji kalkuliše razliku ukupne mase tela i mase masnog tkiva).

U prvom ciklusu (šesti mesec života) uvećanja masnog tkiva posledica je uglavnom povećanje zapremine adipocita – *hipertrofije*. „Gojaznost” u ovom periodu gotovo da je fiziološka (čak 60% sve dece) i priprema odojče za rizik nedovoljne ili neadekvatne ishrane nakon prekida dojenja. Prosečne dimenzije adipocita dece na kraju prve godine života ne razlikuju se bitnije od veličine adipocita negojazne odrasle osobe – veličina adipocita, dakle, ostaje nepromenjena tokom detinjstva. Rast masnog tkiva u pubertetu odlikuje pre svega povećanje broja adipocita – *hiperplazije*. Dakle, gojaznost u detinjstvu inicijalno je posledica uvećanja volumena adipocita (“hipertrofična gojaznost”), a onda kada adipociti postignu kritični volumen (maksimalni kapacitet deponovanja) nastupa i njihovo umnožavanje – „hiperplastična gojaznost” (10).

ENDOKRINA AKTIVNOST MASNOG TKIVA

Masno tkivo nije samo prosti organ za skladištenje masti – ono je ustvari aktivan endokrini organ i deo urođenog imunog sistema koji utiče na mnoge fiziološke i patološke mehanizme, kakvi su homeostaza glukoze, inflamacija, angiogeneza, ćelijska proliferacija i diferencijacija (11). Ovu endokrinu ulogu ostvaruju pre svega sami adipociti ali i aktivirani makrofagi infiltrovani u masno tkivo – značajan izvor

proinflamatornih citokina. Adipociti eksprimiraju niske nivoe monocitno hemotaktičkog proteina 1 (MCP-1). Sa nastankom gojaznosti i nagomilavanjem MCP-1 dolazi i do privlačenja i nagomilavanja makrofaga koji najčešće okružuju mrtve adipocite u obliku krune ("crown-like structure"). Dok je na filogenetskom početku prisustvo makrofaga u masnom tkivu bilo izraz potrebe odbrane od infekcije ili povrede, danas makrofagi ovde imaju ulogu da uklanjaju nekrotične adipocite (12). Ako je adipocitna nekroza inicijalni događaj u infiltraciji makrofaga, onda je hipoksija verovatni vodeći uzrok nekroze adipocita. Sa gojaznošću i progresivnim uvećanjem adipocita njihovo snabdevanje krvlju postaje redukovano, razvija se hipoksija i nekroza, dolazi do infiltracije makrofaga i pokretanja inflamacije.

Uvećanje adipocita i akumulacija triglicerida može biti benigni fenomen jer će prevenirati nagomilavanje masti u jetri, mišićima i drugim ektopičnim tkivima. Inflamacija će se pojaviti kada je ekspanzija adipocita limitirana zbog poremećenog razvoja masnog tkiva (poremećaj PPAR-γ), ili zbog poremećaja u sintezi masti, te će se slobodne masne kiseline nagomilavati u jetri i mišićima što će biti praćeno insulinskom rezistencijom. Agonisti PPAR-γ (tiazolidindioni) unapređuju adipogenezu, povećavaju produkciju adiponektina i imaju jasan antinflamatorni efekt na rezidentne makrofage masnog tkiva, redukujući tako insulinsku rezistenciju.

Tumor necrosis factor-α

Principalni citokin masnog tkiva je tumor necrosis faktor-α (TNF-α). Mada TNF-α sekretuju uglavnom makrofagi infiltrovani unutar masnog tkiva, određena količina mRNA za ovaj citokin detektuje se i u adipocitima – utoliko više ukoliko je gojaznost izraženija (11). Cirkulišuće koncentracije TNF-α rastu sa gojaznošću i korelišu sa stepenom insulinske rezistencije.

TNF-α primarno obara aktivnost insulinskih receptora: aktiviranjem svojih TNF-R2 receptora redukuje se fosforilizacija tirozina i indukuje insulinska rezistencija. Stimulacijom TNF-R1 receptora preko nuklearnog faktora kapa B (NF-kB) inhibira se aktivnost PPAR-γ, što ne samo da zaustavlja diferencijaciju preadipocita u adipocite, već i uslovljava reverziju adipocita u manje diferentovane forme. Što je adipocit veći produkuje više TNF-α, a što uzvratno limitira veličinu adipocita i indukuje apoptozu. Na ovaj način – indukujući apoptozu preadipocita, blokirajući njihovu diferencijaciju i podstičući dediferencijaciju zrelih adipocita TNF-α u stvari omogućava deponovanje masti u neadipoznim tkivima, sa forsiranjem insulinske rezistencije i konstituisanjem metaboličkog sindroma.

Nadalje, TNF-α indukuje lipolizu i povećava cirkulišuće nivoe SMK čime podstiče hepatičnu lipogenezu i dalje pogoršava insulinsku rezistenciju.

I na kraju – konačno konstituisanje izražene insulinske rezistencije u gojaznosti pod povećanim koncentracijama TNF-α posledica je konstituisane inflamacije zbog redukcije stvaranja antiinflamatornog adiponektina i pojačane aktivacije NF-kB.

Interleukin-6

I interleukin-6 (IL-6), drugi najvažniji citokin masnog tkiva, prati ponašanje TNF-α i leptina, te obezitet uvećava i njegovu produkciju. Za razliku od TNF-α koga uglavnom sekretuju makrofagi, IL-6 dominantno sintetišu zreli adipociti. Oko 30% ukupnog serumskog IL-6 poreklom je iz masnog tkiva. Cirkulišući IL-6 je najvažniji faktor koji kontroliše sintezu reaktanata akutne faze. Pored sinteze CRP, IL-6 kontroliše i produkciju fibrinogena, uzrokuje porast broja i aktivnosti trombocita, kao i ekspresiju adhezionih molekula na endotelu, što ukupno povećava rizik ka stvaranju tromba. Pored insulinske rezistencije slično TNF-α, IL-6 indukuje i lipidne abnormalnosti – povećavajući aktivnost HSL mobiliše lipide iz adipocita i povećava koncentracije SMK, što zajedno sa povećanom koagulabilnošću krvi uslovljava pokretanje ateroskleroze. U CNS-u deluje potpuno identično leptinu vezujući se za iste anoreksigene neurone.

Insulin i glikokortikoidi, kao i sam TNF-α povećavaju stvaranje IL-6. Stoga će se u uslovima obeziteta i naglašene hiperinsulinemije povećavati cirkulišuće koncentracije ovih citokina. Konstituisanje inflamacije i insulinske rezistencije pomognuto je i inhibitornim dejstvom TNF-α na ekspresiju adiponektinskih gena (13).

Povećani trigliceridi i SMK moćni su induktori stvaranja TNF-α.

Leptin

Leptin (grč. „leptos", mršav) je peptid supkutanog masnog tkiva sa 167 aminokiselina kojeg kodiraju "OB" geni na dužem kraku sedmog hromozoma. Njegova sekrecija proporcionalna je veličini masnog tkiva. Osnovna uloga ovog lipostatskog hormona je da smanjuje apetit i povećava potrošnju energije, te da mehanizmom negativne povratne sprege „komunicira" sa višim nervnim centrima i održava masu nepromenjenom. Leptin, dakle, informiše hipotalamus o veličini energetskih depoa na periferiji vezujući se za svoje receptore, klasa 1 citokinskih receptora, na nivou hematoencefalne barijerije, a kasnije i u nucleus arcuatusu. OBR geni, koji kodiraju aktivnost leptinskog receptora, nalaze se na kratkom kraku prvog hromozoma. Način sekrecije leptina je pulsativan stimulišući anoreksigene a inhibirajući oreksigene neurone (14). Na periferiji leptin iskazuje snažan inhibitorni uticaj na oreksigeni molekul grelin – pad grelina ovde nije posledica direktnog dejstva leptina već predstavlja fiziološku adaptaciju na uslove pozitivnog energetskog balansa udruženog sa gojaznošću.

Količina leptina je strogo uslovljena količinom belog masnog tkiva i sadržajem triglicerida, ali i akutnim promenama u kalorijskom unosu (gladovanje ili prejedanje). U gladovanju se smanjuje „stepen adipoznosti" i koncentracija leptina u krvi, što podstiče apetit i smanjuje potrošnju energije čime se sprečava dalji gubitak mase – već par sati nakon početka gladovanja naglo pada koncentracija leptina u krvi. Obrnuto,

povećani unos hrane preko „povećanja adipoznosti" uvećava koncentracije leptina u krvi, što smanjuje apetit i uvećava potrošnju energije, prevenirajući time dalju gojaznost (negativni feedback). Što je period koji protiče od prethodnog obroka duži, to je brzina i intenzitet pada serumskih koncentracija leptina veći – ova redukcija u stvari igra ulogu tranzicionog signala iz stanja sitosti prema osećaju gladi (15). Drugim rečima, nizak leptin predstavlja signal za glad a visok leptin signal sitosti.

U fiziološkim koncentracijama leptin lako prolazi hematoencefalnu barijeru i vezuje se za anoreksigene receptore u hipotalamusu, a pre svih onih u proopiomelankortinskim i kokain-amfetamin neuronima dok inhibira centre koji podstiču apetit – one u neuropeptidu Y i Agouti povezanom peptidu. Leptin takođe povećava potrošnju energije na periferiji: preko aktivacije kortikotropnog rilizing hormona (CRH) i simpatikusom indukovane lipolize, ali i preko ushodne regulacije uncoupling proteina 3 (UCP3) zaduženog za regulisanje nivoa potrošnje energije u mitohondrijama perifernog tkiva. Leptin, dakle, efikasno redukuje i apetit i telesnu masu.

Međutim, u gojaznosti izostaju ovakvi efekti leptina i razvija se rezistencija na leptin. Ovakva rezistencija može biti posledica defekta receptora za leptin jednako kao i poremećenog transporta leptina kroz hematoencefalnu barijeru. Stalnim prehranjivanjem, zbog sve izraženijeg smanjenja senzitivnosti hipotalamičnih receptora sitosti (*"down" regulacija*) dolazi do stalnog i progredijentnog povećanja produkcije leptina, odnosno do razvoja leptinske rezistencije (16). Takođe, koncentracije leptina u cerebrospinalnom likvoru u odnosu na serumske mnogo su niže u gojaznih nego u mršavih. Ovo stoga što će u gojaznosti doći do hiperprodukcije CRP od strane adipocita, te će CRP vezujući leptin onemogućiti njegov transport kroz hematoencefalnu barijeru do centra sitosti. *De facto*, u gojaznosti bilo kog porekla instalirana leptinska rezistencija onemogućava stimulaciju proopiomelankortinskih i kokain-amfetaminskih neurona (time i sekreciju onih peptida koji smanjuju apetit kakvi su proopiomelanokortin, CRF i α-MSH) i dozvoljava dominaciju oreksigenih hipotalamičnih uticaja, primarno neuropeptida Y (17).

Vrlo je specifičan odnos dva glavna „hormona gojaznosti" insulina i leptina i na periferiji, i na nivou CNS-a. Insulin stimuliše stvaranje leptina, najverovatnije preko svog trofičkog efekta na adipocite. U fiziološkom stanju leptin inhibira insulinsku sekreciju (i insulinom posredovano periferno preuzimanje glikoze, sintezu glikogena i lipogenezu), ali autonomno, *per se*, može delom povećavati preuzimanje glikoze od strane skeletnih mišića, smeđeg masnog tkiva, mozga i srca (18). Ovi efekti, logično, izostaju u slučajevima leptinske rezistencije – funkcionalno inaktivne hiperleptinemije kakva postoji u gojaznosti. Za razliku od insulina, leptin u skeletnoj muskulaturi povećava i stepen oksidacije SMK. Procenjuje se da ovde leptin može atenuirati 50% antioksidativnog i lipogenetičkog efekta insulina. Jednom rečju, insulin je anabolik i trudi se da mobilizuje energiju (glikozu, slobodne masne kiseline) u mišićima – leptin je katabolik, mobiliše trigliceride i oksidiše slobodne masne kiseline povećavajući potrošnju energije.

Dominantan uticaj leptina ostvaruje i na angiogenezu, proliferaciju i migraciju vaskularnih glatko-mišićnih ćelija, agregaciju trombocita i arterijalnu trombozu.

TNF-ά i IL-6 povećavaju ekspresiju leptinskih gena i nivoe cirkulišućeg leptina.

Adiponektin

Adiponektin je citokin kojeg takođe stvaraju adipociti po jedinstvenom regulacionom principu: cirkulišući nivoi adiponektina inverzno korelišu sa količinom masnog tkiva a proporcionalni su stepenu insulinske senzitivnosti. Suprotno od leptina, u gojaznosti pada produkcija adiponektina uslovljena redukcijom ekspresije adiponektinskih gena ali i hiperinsulinemijom, budući da insulin inhibira oslobađanje adiponektina.

Svoje receptore ima u jetri i mišićima. U jetri, potencirajući insulinsko kočenje glikoneogeneze, popravlja insulinsku senzitivnost, a time i periferno preuzimanje glikoze. U mišićima deluje lipolitički – posredstvom AMP-aktiviranih protein kinaza stimuliše β-oksidaciju masnih kiselina što redukuje količinu triglicerida pre svega u skeletnoj muskulaturi i time smanjuje insulinsku rezistenciju (19). Povećanjem ekspresije PPAR-γ adiponektin popravlja i insulinsku senzitivnost povećavajući potrošnju energije. Kao i agonisti PPAR-γ, adiponektin popravlja metabolički profil omogućavajući ekspanziju masnog tkiva i smanjujući time prepoznatljivu infiltraciju masnog tkiva makrofagima. Uticaj tiazolidindiona na insulinsku senzitivnost značajno je umanjena u odsustvu adiponektina, što sugeriše ključnu ulogu adiponektina u redukciji lipotoksičnosti i inflamaciji povezanoj sa obezitetom (20). Njegova smanjena aktivnost mogući je permisivni uzrok nastanka metaboličkog sindroma.

Ovaj važan hormon adipocita ima jasnu antiinflamatornu ulogu, budući da je moćni inhibitor stvaranja TNF-α a stimulator sinteze interleukina 10. Adiponektin takođe suprimira aktivnost NF-kB i „prirodnih ubica" (naturalnih kilera) stimulisanih preko IL-2. Stoga je logično da je nivo adiponektina nizak u dijabetesu i koronarnoj bolesti – dakle, u sindromima gde inflamacija može imati ulogu u patogenezi. Statistički dokazani vrh incidence dijabetesa u pubertetu može se objasniti činjenicom da sa seksualnom maturacijom pada produkcija adiponektina, a fakat da je to češće u dečaka zbog dominantnog inhibitornog dejstva testosterona (21). Niski nivoi adiponektina u predijabetesu imaju veliki prediktivni značaj, omogućavaju održavanje inflamacije „niskog stepena" i nagoveštavaju klinički manifestni početak bolesti. Antidijabetogeni uticaj adiponektina reprezentovan je njegovim uticajem na povećanje insulinske senzitivnosti, povećanje oksidacije SMK i smanjenje glikoneogeneze.

Osim antiinflamatorne, adiponektin ima i vazoprotektivni i antiaterogeni efekat jer inhibira ekspresiju adhezionih molekula, proliferaciju glatko mišićnih ćelija krvnih sudova i transformaciju makrofaga u penaste ćelije (22).

Povećanje aktivnosti transkripcionog faktora PPAR-γ povećava serumske nivoe adiponektina. Oksidativni stres, simpatikus, TNF-ά i IL-6 suprimiraju ekspresiju adiponektina.

Plazminogen aktivator inhibitor-1

Globalna fibrinolitička aktivnost u gojaznosti je često smanjena. Aktivator inhibitora plazminogena 1 (PAI-1) je povećan što redukuje prevođenje plazminogena u plazmin. Mada povećana sinteza PAI-1 u jetri može biti i posledica permanentne hiperinsulinemije, najveće količine PAI-1 sekretuju sami adipociti pod stimulacijom TNF-α. Dodatno, postoji i disfunkcija endotela – karakteristična je povećana aktivnost endotelina-1, markera endotelne disfunkcije i ozlede. Budući da je endotelin-1 moćan vazokonstriktorni peptid, njegovo povećanje jeste rani znak abnormalne vaskularne reaktivnosti, što sa kasnijom disfunkcijom i dislipidemijom čini osnovu pokretanja ateroskleroze. Dakle, u gojaznosti postoji povećana produkcija PAI-1 (23), a samo njegovo povećanje može biti dobar prediktor razvoja dijabetesa tipa 2.

Renin-angiotenzin sistem

Zreli adipociti eksprimiraju sve komponente renin-angiotenzin sistema, uključujući renin, angiotenzinogen, konvertujući enzim – čak i tip 1 angiotenzin receptora! Sa prevođenjem preadipocita u zrele adipocite raste ekspresija angiotenzinogena koji se može smatrati kasnim markerom adipocitne diferencijacije. Ekspresija gena za angiotenzinogen raste sa obezitetom što je važno u regulaciji krvnog protoka kroz masno tkivo, kao i pravilan rast i diferencijaciju adipocita (24).

UTICAJ GOJAZNOSTI NA KONSTITUISANJE DIJABETESA

Kada se adipociti prepune trigliceridima raste sekrecija leptina u pokušaju da se prevenira deponovanje lipida u za to neosposobljena neadipozna tkiva: skeletne mišiće, jetru, miokardijum ili beta-ćelije. U gojaznosti, kada postoji leptinska deficijencija ili rezistencija, funkcionalni nedostatak leptina dovešće do generalizovane steatoze, lipotoksičnosti i lipoapoptoze. Lipotoksičnost beta-ćelija, miokarda ili skeletne muskulature vode u dijabetes tipa 2, kardiomiopatiju i insulinsku rezistenciju, dakle u najveći globalni problem savremene civilizacije – metabolički sindrom (25)! Nakon preterane akumulacije masti u neadipoznim tkivima, SMK preko povećanog stvaranja azot-monoksida uzrokuju i apoptozu beta ili miokardnih ćelija – stoga važno mesto u prevenciji nastanka ovog sindroma pripada restriktivnoj dijeti i određenim medikamentima (tiazolidindioni).

Pod uticajem leptina dolazi i do smanjenja ekspresije $CD4^+CD25^+$ ćelija – takozvanih „T regulatornih ćelija“, koje nastaju pod uticajem IL-2 i transformacionog faktora rasta β (TGFβ), a koje jasno inhibiraju imuni odgovor (26). U gojaznosti usled hiperprodukcije leptina i pada aktivnosti T regulacionih ćelija dolazi do aktivacije citokinskog Th1 odgovora, sa nagomilavanjem makrofaga u masnom tkivu i povećanom produkcijom interferona-γ (INF-γ), IL-6 i TNF-α. Nagomilavanje ovih Th1 citokina preko mehanizma oksidativnog stresa ili apoptoze može direktno oštetiti beta-ćelija (TNF-ά) ili modulirati beta-ćelijsku proliferaciju (INF-γ). Dokazano je da se dijetom i gubitkom u telesnoj masi smanjuje nivo leptina što uslovljava i sniženje nivoa INF-γ. Obrnuto sa gojaznošću raste ne samo nivo INF-γ nego i titar za tip 1 dijabetesa specifičnih antitela na glutamin-acid dekarboksilazu (GAD).

Nema bilo kakve dileme da je uvećanje masnog tkiva praćeno mobilizacijom makrofaga i aktivacijom Th1-citokinskog puta i konstituisanjem inflamacije – inflamacija „niskog stepena“ zaštitni je znak preuhranjenosti i gojaznosti (27)! Vrlo je verovatno da oba osnovna tipa dijabetesa upravo nastaju po patogenetskom modelu inflamacije. U tipu 1 posredi je hronična inflamacija unutar pankreasnih ostrvaca sa detektibilnim autoimunim antitelima na periferiji, dok se u tipu 2 dijabetesa radi, *de facto*, o modelu prave sistemske inflamacije sa reaktantima akutne faze zapaljenja u cirkulaciji. Pouzdani markeri nastupajuće bolesti biće povećanje CRP, ali prediktivni značaj imaće i određivanje fibrinogena, plazminogena, PAI-1 i ceruloplazmina. Postoji stroga korelacija nivoa CRP i ITM – indeks telesne mase u stvari je najbolji prediktor nivoa CRP u dece. Činjenica da takođe postoji visoka korelativnost nivoa CRP i insulinemija na gladno može upućivati ne samo na prediktivnu, već i uzročnu povezanost CRP i insulinske rezistencije.

Prvi dokaz o tome da je sistemska inflamacija u osnovi dijabetesa star je više od 130 godina – profesor Ebstain je tada visokim dozama salicilata uspeo da redukuje glikozuriju u obolelih! Salicilati inhibiraju aktivnost molekula koji ima ključnu ulogu u regulaciji inflamacije, imunog odgovora i apoptoze – transkripcionog NF-κB. Ovaj se faktor aktivira pod uticajem različitih stimulusa – od “toll like“ receptora (važno u patogenezi dijabetesa tipa 1) do slobodnih radikala i citokina (važno u patogenezi i dijabetesa tipa 2 i tipa 1). Pripada grupi „brzodelujućih“ transkripcionih faktora koji za svoju akciju ne zahtevaju sintezu novih proteina već u pokušaju zaštite ćelije od štetnih stimulusa direktno može aktivirati različite gene – citokinske, hemokinske, apoptotične, adhezione... Aberantna ekspresija i aktivacija NF-κB može biti odgovorna za generaciju sistemske inflamacije i nastanak čitavog niza autoimunih bolesti – pa i dijabetesa tipa 1 (27). Takođe, povećavajući aktivnost serum-kinaze ovakva patološka ekspresija NF-κB dovodi i do povećane fosforilacije serina a smanjene fosforilacije tirozina što inhibira enzim tirozin-kinazu, čime se obara aktivnost insulinskih receptora i uslovljava insulinska ezistencija – što je osnov nastanka dijabetesa tipa 2.

Drugim rečima, gojaznost preko patološke aktivacije NF-κB može biti odgovorna za nastanak i beta ćelijske destrukcije i insulinske rezistencije, čime se potvrđuje teza

o povezanosti epidemije gojaznosti u mladih sa nastankom novog tipa dijabetesa: i 1 i 2 – „duplog“ ili „hibridnog“ (28).

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INFLUENCE OF SIGNALING FACTORS AND CYTOKINES ON THE DEVELOPMENT OF ADIPOSE TISSUE, OBESITY AND DIABETES

Abstract: The process of energy consumption is ongoing, while energy recovery through food intake occurs only occasionally. Therefore, during evolution, it was necessary to form a system that would create energy reserves, and preserve them for periods between food intake–adipose tissue. The development of adipose tissue is affected by numerous signaling and hormonal factors, which in turn determine the distribution of adipose tissue into subcutaneous and visceral fat. Besides its indisputable role in energy homeostasis, adipose tissue is an important endocrine and paracrine organ that releases many hormones and cytokines, and crucially affectsall metabolic and immunological processes in the body. As such, primarily visceral adipose tissue synthesizes significant amounts of adipocytokines: leptin, adiponectin, tumor necrosis factor-α, interleukin-6 and many others. Fat can actually be a crucial alarm system that triggers innate immunity and acute phase inflammation. Chronic inflammation is the hallmark of the metabolic syndrome, and inflammatory signals originate mainly from visceral adipose tissue. Therefore, excess adipose tissue can easily be linked to the emergence of numerous metabolic disorders and the development of diabetes, type 2 as well as type 1.

Key words: adipose tissue development, visceral adipose tissue, adipocytokines, metabolic syndrome, hybrid diabetes

WHITE ADIPOSE TISSUE

White adipose tissue serves as the main source of energyfollowing theperiod of infancy, and into lateryears (1). It is distributed throughout the body as visceral (VAT)

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and subcutaneous (SAT). Visceral ("abdominal" or "central") adipose tissue is localized around internal organs, the mesenterium and omentum, and accounts for about 20% of total body fat. Subcutaneous adipose tissue accounts for about 80% and is predominantly distributed in two main regions, the abdominal and gluteofemoral (2). Between these two forms of adipose tissue exist significant differences in histological structure,adipocyte endocrine profile, lipolytic activity, as well as responsiveness to insulin and other hormones. SAT is the physiological sitefor deposition of excess fat, where an excess of glycerol and free fatty acids are easily deposited in the form of triglycerides in otherwise inert adipocytes. VAT is the metabolically very active part of the adipose tissue, which in surplus is crucial in establishing a number of metabolic disorders, including diabetes. There are two basic types of adipocytes in the adipose tissue: small - newly synthesized,and large- mature cells. The composition of adipose tissue depends on the balance between de novo synthesis of small adipocytes and apoptosis of large cells. Small adipocytes secrete insulin sensitizing moleculessuch as adiponectin, they are much more sensitive to insulin and have a greaert ability to take on free fatty acids and triglycerides, thus preventing their deposition in nonadipocyte tissues(3). Large adipocytes are dysfunctional, resistant to the antilipolytic effects of insulin and actively secrete numerous adipocytokines that cause a variety of pathological conditions, notably the metabolic syndrome. Insulin resistance of large adipocytes prevents deposition of triglycerides while favouring lipid oxidation, thereby creating highly toxic products such as lipid peroxides.

Unlike SAT, which is mostly composed of small adipocytes, VAT contains predominantly large adipocytes, macrophages and other inflammatory cells. Visceral adipose tissue is much better innervated and vascularized. Unlike SAT, which secretes into the systemic circulation, VMT due to its anatomical position drains directly into the portal circulation. This allows direct impact of the released free fatty acids (FFA) and adipocytokines on the liver, this being the way interleukin-6 (IL-6) plays a stimulating role in the production of C-reactive protein (CRP) in the liver (4).

The largest energy reserves in the body are in the form of triglycerides deposited in adipose tissue. Under the action of lipoprotein lipase (LPL) in the endothelium of capillaries triglyceridesfrom chylomicrons and very low density lipoproteins (VLDL) are hydrolyzed to FFA and glycerol, which are then deposited in fatty cells, where they will be reesterified into triglycerides. When there is a need for energy, the triglycerides deposited in adipocytes, under the influence of hormone sensitive lipase (HSL),are broken down again to glycerol and FFA. HSL is activated by means of AMP-activated protein kinases (AMPK). AMPK are activated by catecholamines, while glucocorticoids, thyroid hormones and growth hormone have an inhibitory role. Conversely, AMPK inhibit insulin and, indirectly, IGF-1, adenosine and prostaglandins. The increased release of FFA into the circulation is consequently followed by an increase insugar and triglyceride synthesis in the liver, as well as a decrease in insulin clearance, which favors the development of insulin resistance.

DEVELOPMENT OF ADIPOSE TISSUE

Adipose tissue is composed of three groups of cells: stem cells, preadipocytes, and mature unilocular adipocytes with deposited triglycerides. Preadipocytes are morphologically and biochemically almost indistinguishable from fibroblasts, but their differentiation toward adipocytes occurs under certain physiological conditions during life. Preadipocytes can be unlimited in number,however, this depends on the number and activity of stem cells, from which osteoblasts, hondroblastsand mioblasts can also develop. In which direction will differentiation of stem cells turn - towards adipocytes or nonadipocytic lines, will depend on a range of hormonal, cytokine and signal influences.

Adipocytes normally develop from precursor mesenchymal cells under the influence of various hormonal and signaling mechanisms (5). What path will differentiation of mesenchymal stem cells take will depend on many hormones: testosterone will inhibit the development of adipose tissue and favor the emergence of muscle tissue. Differentiation of stem cells will also depend on different signaling, transformational factors such as Notch1c (shifts osteoblast differentiation toward adipocytes) or RhoGTPase (potentiates differentiation towards miogenesis).

In the formation of adipose tissue, angiogenesis plays an important role - adipogenesis and neovascularization are intrinsically linked processes. Without angiogenesis, differentiation of preadipocytes will not take place. Therefore preadipocytes strongly secrete VEGF (vascular endothelial growth factor) and TGF-β (transforming growth factor beta) and directly stimulate angiogenesis. This fact explains the link between cancer and obesity: since tumor growth is deeply dependent on angiogenesis, it is possible that obesity through excessive synthesis of VEGF and TGF-β affects tumor formation and progression.

Once created, preadipocytes undergo proliferation and differentiation.

Stimulation of inert adipocytes takes place under the influence of insulin, glucocorticoids and factors that increase cAMP levels, thus becoming powerful adipogenic stimuli. After clonal expansion of preadipocytes, their terminal differentiation is influenced by a nuclear receptor which functions as a transformational factor and is referred to as PPARγ (peroxisome gamma proliferation activating receptor). The activity of PPARγ is infuenced by natural (SMK) and synthetic ligands (thiazolidinediones) causing its activation and allowing terminal differentiation of adipocytes. Theglucocorticoid receptors could also serve as a differentiating factor.

Undeniable phenotypic similarity of Cushing's syndrome and patients with simple central obesity explains the essential connection between an excess in glucocorticoids and the occurrence of obesity. Glucocorticoid receptors play a central role in the clonal expansion and terminal differentiation of adipocytes, but also in the distribution of adipose tissue and its metabolic activity. The largest number of glucocorticoid receptors is revealed in VAT (6). Decisive influence on the activity of glucocorticoids in adipose tissue belongs to the enzyme 11β-hydroxy steroid dehydrogenase (11β-HSD),

which regulates the translation of the active form of glucocorticoids (cortisol) into the inactive form (cortisone) and vice versa (7). While type 2 of this enzyme is predominantly expressed in the kidney where it has a role in the homeostasis of sodium and regulation of blood pressure, type 1 is active in adipose tissue and the liver. Although this is a "two-way" enzyme (transforms active into inactive forms and vice versa) in adipocytes and the liver it predominantly activates cortisone to cortisol! This has important metabolic consequences - stimulates gluconeogenesis in the liver, while promoting adipocyte expansion and differentiation. It seemsrational to conclude that central obesity is a possible consequence of increased subcutaneous expression of type 1 11β-HSD in adipocytes.

The first adipocytes are formed around the 15th week of gestation, followed by an increase in number and volume, predominantly under the influence of fetal insulin and placental lactogen (8). In the term infant,body fat makes up about 12% of total body weight –maturation of adipose tissue depends more onthe duration of pregnancy and much less on the mothers nutrition. Further increase in adipose tissue does not ideally follow body growth - there is a sudden increase in adipose tissue with a peak in the sixth month of life –at this point in time fat makes up 25% of body weight. Furthermore there is a tendency to reduce the amount of fatty tissue until puberty (9). Then there is a second round of fat tissue growth - much more intense in females (due to a lesser growth of muscle tissue) than in boys. In late adolescence, fat reserves make up 15-18% of the body mass of males and 25-28% of females. In the same period, total body weight increases only 10-15%, suggesting that the increase in weight during female puberty is a result of increase in quantity of adipose tissue and reduction in "lean body mass".

In the first cycle (sixth month of life) an increasein fat is mainly due to the increasing volume of adipocytes - *hypertrophy*. "Obesity" in this period is almost a physiological occurrence (as much as 60% of all children) and prepares the infant for the risk of insufficient or inadequate diet after stopping breastfeeding. Average size of adipocytesin children at the end of the first year of life does not differ significantly from the size of adipocytes in non-obese adults - adipocyte size, therefore, remains unchanged during childhood. The growth of adipose tissue in puberty is characterised by an increase in the number of adipocytes - *hyperplasia*. Thus, childhood obesity is initially the result of an increase in the volume of adipocytes ("hypertrophic obesity"), later, when adipocytes reach a critical volume (maximum capacity of deposit) adipocytes tend to multiply - "hyperplastic obesity'' (10).

ENDOCRINE ACTIVITY OF ADIPOSE TISSUE

Adipose tissue is not simply an organ meant for fat storage - it is actually an active endocrine organ and part of the innate immune system that affects many physiological and pathological mechanisms, such as glucose homeostasis, inflammation,

angiogenesis, cell proliferation and differentiation (11). This endocrine role primarily belongs to adipocytes, but also to activated macrophages which infiltrate adipose tissue - a significant source of proinflammatory cytokines. Adipocytes express low levels of monocyte chemotactic protein 1 (MCP-1). With the occurrence of obesity and the accumulation of MCP-1, macrophage attraction and accumulation occur, they usually surround dead adipocytes forming a “crown-like structure”. At the beginning of the phylogenetic process, it was believed that the presence of macrophages in adipose tissue was necessary for defense against infection or injury, however, it is known today that macrophages actually have a role in removing necrotic adipocytes (12). If adipocyte necrosis is the initial event leading to the macrophage infiltration, it is likely that hypoxia is the leading cause of adipocyte necrosis. With obesity and progressive enlargement of adipocytes, their blood supply becomes reduced, hypoxia and necrosis ensue, followed by macrophage infiltration and onset of inflammation.

Enlargement of adipocytes and accumulation of triglycerides can be benign phenomena as they will prevent the accumulation of fat in the liver, muscles and other ectopic tissues. Inflammation will occur when the expansion of adipocytes is limited, either due to defective development of adipose tissue (PPAR-γ disorder), or any disturbance in the synthesis of fat, where free fatty acids will accumulate in the liver and muscles, which will be accompanied by insulin resistance. PPAR-γ agonists (thiazolidinediones) promote adipogenesis, increase the production of adiponectin and have a clear antinflamatory effect on adipose tissue resident macrophages, thus reducing insulin resistance.

Tumor necrosis factor- α

The pricipal cytokine of adipose tissue is tumor necrosis factor-α (TNF-α). Although TNF-α is secreted mainly by macrophages which infiltrate adipose tissue, a certain amount of mRNA for this cytokine is detected also in adipocytes - the more so if obesity is more pronounced (11). Circulating levels of TNF-α increase with obesity and correlate with the degree of insulin resistance.

TNF-α primarily reduces the activity of insulin receptors: by activatingits TNF-R2 receptors it reduces phosphorilation of tyrosine and induces insulin resistance. By stimulating its TNF-R1 receptor via nuclear factor kappa B (NF-kB), the activity of PPAR-γ is inhibited, which not only stops the differentiation of preadipocytes to adipocytes, but also causes reversal of mature adipocytes to less differentiated forms. The larger the adipocyte, the more TNF-α it produces, which in turn limits the size of adipocytes and induces apoptosis.

In this way - by inducing apoptosis of preadipocytes, blocking their differentiation and promoting regression of mature adipocytes to less differentiated forms, TNF-α actually allows deposition of fat in tissues other than the adipose tissue, thus promot-

ing insulin resistance and occurrence of the metabolic syndrome. Moreover, TNF-α induces lipolysis and increases circulating levels of FFA, in which way it encourages hepatic lipogenesis and further exacerbates insulin resistance.

Finally –the final constitution ofmarked insulin resistance in obesity in the presence of increased concentrations of TNF-α is a consequence of inflammation resulting from a decrease inproduction of antiinflammatory adiponectin and increased activation of NF-kB.

Interleukin-6

Interleukin-6 (IL-6), the second most important adipose tissue cytokine, monitors the behavior of TNF-α and leptin, thus in obesity its secretion is also increased. Unlike TNF-α which is mostly secreted by macrophages, IL-6 is synthesized predominantly by mature adipocytes. About 30% of the total serum IL-6 originates from adipose tissue. Circulating IL-6 is the most important factor that controls the synthesis of acute phase reactants. In addition to the synthesis of CRP, IL-6 controls the production of fibrinogen, causes a rise in the number and activity of platelets, as well as the expression of adhesion molecules on endothelial cells thereby increasing the overall risk for thrombus formation. In addition to effects on insulin resistance similar to TNF-α-, IL-6 also induces lipid abnormalities - by increasing the activity of HSL it mobilizes lipids from adipocytes and increases concentrations of FFA, which in conjunction with increased blood coagulability leads to the develoment of atherosclerosis. In the CNS it acts almost identically to leptin binding to the same anorexigenic neurons.

Insulin and glucocorticoids, as well as TNF-α increase the production of IL-6. Therefore, in the setting of obesity and pronounced hyperinsulinemia, an increase in circulating concentrations of these cytokines will occur. The development of inflammation and insulin resistance is aided by the inhibitory effect of TNF-α on the expression of adiponectin genes (13). Elevated triglycerides and FFA are potent inducers of TNF-αsynthesis.

Leptin

Leptin (from the Greek “Leptos”, thin) is a peptide of subcutaneous adipose tissue with 167 amino acids encoded by “OB” genes on the long arm of the seventh chromosome. Its secretion is proportional to the size of adipose tissue. The main role of this lipostatic hormone is to reduce appetite and increase energy consumption, thus by means of the negative feedback mechanism “communicate” with higher nerve centers and maintain body weight unaltered. Leptin, therefore, informs the hypothalamus about the size of energy depotsin peripheral tissues, binding to its receptors, class 1 cytokine receptors, at the level of the hematoencephalic barrier, and later in the nucleus

arcuatus. OBR genes, which encode theactivity of leptin receptors are located on the short arm of the 1stchromosome. Leptin secretion is pulsative, stimulating anorexigenic and inhibiting orexigenic neurons (14). In peripheral tissues, leptin shows a strong inhibitory effect on ghrelin orexigenic molecule - a decrease in ghrelin is not a result of direct action of leptin, but represents a physiological adaptation to conditions of positive energy balance associated with obesity.

The quantity of leptin is strongly dependent on the amount of white adipose tissue and triglyceride content, as well as acute changes in caloric intake (starvation or overeating). During starvationthe "level of adiposity" is reduced, as well as the concentration of leptin in the blood, which stimulates appetite and reduces energy consumption by preventing further loss of weight - within a couple of hours of fasting leptin concentrations decline rapidly in the blood. Conversely, increased food intake by means of "increasing adiposity" increases leptin concentrations in the blood which reduces appetite and increases energy consumption, thereby preventing further obesity (negative feedback). The longer the period that elapses from the last meal, the greater the speed and intensity of the drop in serum leptin concentrations -this reduction actually plays a role in the transition from a state of satiety to the feeling of hunger (15). In other words, low leptin is the signal for hunger,while high leptin is a sign for satiety .

At physiological concentrations, leptin easily transverses the hematoencephalic barrier and binds to anorexigenic receptors in the hypothalamus, above all those in proopiomelanocortine and cocaine-amphetamine neurons while inhibiting centers that stimulate appetite - one in neuropeptide Y and Agouti related peptide. Leptin also increases energy consumption in the periphery: through activation of corticotropin-ereleasing hormone (CRH) and via sympathetic nervous system-induced lipolysis, but also through up regulation of uncoupling protein 3 (UCP3) in charge of regulating the level of energy consumption in the mitochondria of peripheral tissues. Leptin, therefore, effectively reduces both appetite and body weight.

However, in obesity these effects of leptin are absent and resistance to leptin develops. Such resistance can be caused by a defect in the leptin receptor as well as disrupted transport of leptin through the hematoencephalic barrier. With constant food intake, due to reduced sensitivity of hypothalamic satiety receptors ("down" regulation), there is a constant and progressive increasein the production of leptin, that is resistance to leptin develops (16). In addition, leptin concentrations in cerebrospinal fluid compared to serum are much lower in the obese than in the lean. This is due to the fact that in obesity exists an overproduction of CRP by adipocytes, this CRP binds to leptin thereby inhibiting its transport across the hematoencephalic barrier to the satiety center. In obesity of anyetiology, existing leptin resistance prevents stimulation of proopiomelanocortine and cocaine-amphetamine neurons (and hence the secretion of those peptides that reduce appetite such as proopiomelanocortin, CRF and α-MSH) permitting dominance of the orexigenic hypothalamic influences, primarily neuropeptide Y (17).

A very special relationship exists between the two main “obesity hormones” leptin and insulin, in peripheral tissues, and at the level of the CNS. Insulin stimulates leptin production, probably via its trophic effect on adipocytes. In physiological conditions, leptin inhibits insulin secretion (and insulin mediated peripheral uptake of glucose, glycogen synthesis and lipogenesis), however, it can mediate an independent increasein uptake of glucose by skeletal muscle, brown adipose tissue, brain and heart (18). These effects are absent in cases of leptin resistance - functionally inactive hyperleptinemia that exists in obesity. Unlike insulin, leptin in skeletal muscle, increases the degree of oxidation of FFA. It is estimated that here leptin can attenuate 50% of the antioxidant and lipogenetic effect of insulin. In summary, insulin is an anabolic and attempts to mobilize energy (glucose, free fatty acid) in muscles – leptin is a catabolic, mobilizes triglycerides and oxidizes free fatty acids increasing energy consumption.

Leptin also shows dominanat effects on the processes of angiogenesis, proliferation and migration of vascular smooth muscle cells, platelet agregation and arterial thrombosis.

TNF-α and IL-6 increase expression of leptin genes and levels of circulating leptin.

Adiponectin

Adiponectin is a cytokine produced also by adipocytes by means of unique regulatory principle: circulating adiponectin levels are inversely correlated with body fat and proportional to the degree of insulin sensitivity. In contrast to leptin, in obesity production of adiponectin declines due to a reduction in adiponectin gene expression, but also due to hyperinsulinemia, given the fact that insulin inhibits the release of adiponectin.

Its receptors are located in muscles and the liver. In the liver, by potentiating the inhibitory effects of insulin on gluconeogenesis, it improves insulin sensitivity, and thus the peripheral uptake of glucose. In muscles it has lipolytic actions - through AMP-activated protein kinases it stimulates β-oxidation of fatty acids which reduces the amount of triglycerides primarily in skeletal muscles thereby reducing insulin resistance (19). By Increasing the expression of PPAR-γ, adiponectin improves insulin sensitivity by increasing energy consumption. Similar to PPAR-γ agonists, adiponectin improves metabolic profile allowing expansion of adipose tissue,thus reducing the distinct infiltration of adipose tissue by macrophages. The influence of the thiazolidinediones on insulin sensitivity is significantly reduced in the absence of adiponectin suggesting a key role of adiponectin in reducing lipotoxicity and inflammation associated with obesity (20). Its decreased activity is a possible etiologic factor of the metabolic syndrome.

This important adipocyte hormone has a clear antiinflammatory role, since it is a powerful inhibitor of TNF-α production and stimulates IL-10 synthesis. Adiponectin also inhibits the activity of NF-kB and IL-2 stimulated NK cells. It is therefore logical that levels of adiponectin in diabetes and coronary artery disease are low - that is, in syndromes where inflammation may play a role in pathogenesis. Statistically proven peak incidence of diabetes in puberty can be explained by the fact that sexual maturation is accompanied by a reduction in adiponectin synthesis, being more common in boys due to the dominant inhibitory effect of testosterone (21). Low levels of adiponectin in prediabetes have a high predictive value, allowing the maintenance of inflammation of "low degree" and hint the onset of overt disease. The antidiabetic effect of adiponectin is represented by its effects on increasing insulin sensitivity, increasing FFA oxidation and reducting gluconeogenesis.

Apart for an antiinflammatory, adiponectin also possesses a vasoprotective and antiatherogenic effect via inhibition of expression of adhesion molecules, proliferation of blood vessel smooth muscle cells and transformation of macrophages into foam cells (22).

An increase in activity of transcription factor PPARγ, increasesserum levels of adiponectin. Oxidative stress, sympathetic nervous system, TNF-α and IL-6 suppress adiponectin expression.

Plasminogen activator inhibitor-1

Global fibrinolytic activity in obesity is often reduced. Plasminogen activator inhibitor-1 (PAI-1) is increased which reduces translation of plasminogen to plasmin. Although increased synthesis of PAI-1 in the liver may be due to permanent hyperinsulinemia, the largest amounts of PAI-1 are secreted by adipocytes under TNF-α stimulation. Additionally, there exists endothelial dysfunction – characterized by increased activity of endothelin-1, a marker of endothelial dysfunction and injury. Since endothelin-1 is a potent vasoconstrictive peptide, its increase is an early sign of abnormal vascular reactivity, which with subsequent dysfunction and dyslipidemia forms the basis for development of atherosclerosis. Thus, in obesity there is increased production of PAI-1 (23), this increase in production can serve as an excellent predictor of the development of type 2 diabetes.

Renin-angiotensin system

Mature adipocytes express all components of the renin-angiotensin system, including renin, angiotensinogen, converting enzyme - even the type 1 angiotensin receptor! Transformation of preadipocytes to mature adipocytesis accompanied by a rise in angiotensinogen expression and can be considered a late marker of adipocyte differ-

entiation. Angiotensinogen gene expression increases with obesity which is important in the regulation of blood flow through adipose tissue as well as for normal growth and adipocyte differentiation (24).

IMPACT OF OBESITY ON DIABETES ONSET

When adipocytes are filled with triglycerides, leptin secretion increases in an attempt to prevent the deposition of lipids in unprepared nonadiposetissues: skeletal muscle, liver, myocardium or beta cells. In obesity, when there is either leptin deficiency or resistance, functional lack of leptin will lead to generalized steatosis, lipotoxicity and lipoapoptosis. Lipotoxicity of beta cells, skeletal muscle or myocardium leads to type 2 diabetes, cardiomyopathy and insulin resistance, that is,to the greatest global problem of modern civilization - the metabolic syndrome (25)! After excessive accumulation of fat in nonadipose tissues, free fatty acids via increased production of nitric oxide cause apoptosis of myocardial or beta cells- hence an important role in the prevention of this syndrome belongs to restrictive diet and certain medications (thiazolidinediones).

Leptin leads to the reduction of expression of CD4+CD25+ cells - the so-called "regulatory T cells", which develop under the influence of IL-2 and transformational growth factor β (TGFβ), which are known to inhibit the immune response (26). In obesity due to overproduction of leptin and fall in activity of T regulatory cells, activation of Th1 response ensues, accompanied by the accumulation of macrophages in adipose tissue and increased production of interferon-γ (IFN-γ), IL-6 and TNF-α. The accumulation of these Th1 cytokines through a mechanism of oxidative stress and apoptosis may directly damage beta cells (TNF-ά) or modulate beta cell proliferation (IFN-γ). It has been proven that dieting and loss of body weight lowers leptin levels, which in turn reduces levels of IFN-γ. Inversely, obesity is accompanied not only by an increase in the level of IFN-γ but also an increase in the titer of antibodies to glutaminic acid decarboxylase (GAD) specific for type 1 diabetes.

There is no doubt that an increase in body fat is followed by mobilisation of macrophages and activation of Th1cytokine path and constitution of inflammation - inflammation of the "low degree" is hallmark of overnutrition and obesity (27)! It is likely that the two main types of diabetes occur according to the pathogenetic model of inflammation. In type 1,there exists a chronic inflammation within the pancreatic islets accompanied by autoimmune antibodies detectable in the periphery, whereas in type 2 diabetes we have a true model of systemic inflammation with acute phase reactants of inflammation in the circulation. Reliable markers of disease onset will include an increase in CRP, however determination of fibrinogen, plasminogen, PAI-1 and ceruloplasmin also has predictive significance. There is a strict correlation between CRP and BMI - body mass index is actually the best predictor of CRP levels in children. The

fact that there is also a high correlation between CRP levels and insulinemia during starvationsuggests not only a predictive, but also a causal relationship between CRP and insulin resistance.

The first evidence that systemic inflammation is the basis of diabetes datesfrom more than 130 years ago- Professor Ebsteins attempts to reduce glycosuria with high doses of aspirin were successful! Salicylates inhibit the activity of molecules that play a key role in the regulation of inflammation, immune response and apoptosis –transcription factor NF-kappaB. This factor is activated by diverse stimuli –from the "toll like" receptors (important in the pathogenesis of diabetes type 1) to free radicals and cytokines (important in the pathogenesis of both type 2 diabetes and type 1). Belongs to the group of ''fast acting" transcription factors which for their actions do not require the synthesis of new proteins, but in an effort to protect cells from harmful stimuli can directly activate several genes - a cytokine, chemokine, apoptotic, adhesion. Aberrant expression and activation of NF-kappaB may be responsible for the generation of systemic inflammation and the development of a range of autoimmune diseases - including diabetes type 1 (27). Also, by increasing the activity of serum-kinase, this pathological expression of NF-kB leads to increased phosphorilaion of serin and reduced phosphorilation of tyrosine which in turn inhibits the enzyme tyrosine-kinase which further reduces activity of insulin receptors and promotes insulin resistance-which forms the basis for development of type 2 diabetes.

In other words, obesity through pathological activation of NF-κB can be responsible for both the destruction of beta cells and development of insulin resistance, which confirms the link between the epidemiology of obesity in children and the youth and development of a new type of diabetes: both types 1 and 2 – ''double'' or ''hybrid'' (28).

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