UNIVERZITET U BEOGRADU

BIOLOŠKI FAKULTET

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Uticaj insulinu sličnog faktora rasta 1 na ekspresiju i aktivnost natrijum-kalijumove pumpe u srcu gojaznih pacova

Doktorska disertacija

Beograd, 2026.

UNIVERSITY OF BELGRADE

FACULTY OF BIOLOGY

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The effects of insulin like growth factor 1 on the expression and activity of the sodium-potassium pump in the heart of obese rats

Doctoral dissertation

Belgrade, 2026.

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Datum i mesto javne odbrane: _____

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SAŽETAK

Cilj istraživanja ove doktorske disertacije bio je izučavanje molekulskih mehanizma kojima insulinu sličan faktor rasta 1 (IGF-1) u *in vivo* uslovima deluje na ekspresiju, aktivnost i interakciju natrijum-kalijum adenozin trifosfataze (Na^+/K^+-ATPaze) sa drugim proteinima u srcu u fiziološkom i stanju gojaznosti, kao i da li promene u aktivnosti Na^+/K^+-ATP-aze mogu da se dovedu u vezu sa smanjenjem hipertrofije srca izazvane gojaznošću. Dobijeni rezultati pokazuju da davanje IGF-1 *in vivo* normalno uhranjenim pacovima dovodi do povećanja relativne ekspresije gena za α_1 subjedinicu Na^+/K^+-ATPaze, nivoa proteina α_1 i α_2 subjedinice, stepena fosforilacije α subjedinice i aktivnosti Na^+/K^+-ATPaze, kao i povećanja stepena fosforilacije supstrata receptora za insulin-1 (IRS-1), fosfoinozitid-zavisne kinaze 1 (PDK1), protein kinaze B (Akt), ciljnog molekula za rapamicin kod sisara (mTOR) i kinaze ribozomalnog proteina S6 (S6K) u srcu. Davanje IGF-1 normalno uhranjenim pacovima dovodi do smanjenja interakcije između α_1 subjedinice Na^+/K^+-ATPaze i beklina-1 i fosforilacije adenozinmonofosfat-aktivirane protein kinaze (AMPK), odnosno povećanja fosforilacije β subjednica hibridnog receptora za IGF-1 i insulin (IGF-1Rβ/IRβ) i FOXO1 proteina u srcu. Tretman gojaznih pacova IGF-1 dovodi do smanjenja mase srca, fosforilacije mTOR, S6K i nivoa AT_1R u srcu, kao i nivoa Ang II i jona K^+ u serumu, odnosno do značajnog povećanja nivoa α_1 subjedinice i fosforilacije α subjedinice Na^+/K^+-ATPaze, aktivnosti Na^+/K^+-ATPaze, fosforilacije IGF-1Rβ/IRβ, IRS-1, Akt i nivoa angiotenzin II (Ang II) receptora tipa 2 (AT_2R), relativne ekspresije gena za α-teške lance miozina (MHC), kao i odnosa između α-MHC i β-MHC. Rezultati dobijeni u okviru ove doktorske disertacije ukazuju da IGF-1 *in vivo* u fiziološkim uslovima povećava ekspresiju i aktivnost Na^+/K^+-ATPaze, molekulskim mehanizmom koji uključuje učešće IRS-1/PDK-1/Akt/mTOR/S6K signalnog puta u srcu pacova. Takođe, pokazano je da IGF-1 smanjuje autozu tako što smanjuje interakciju Na^+/K^+-ATPaze i beklin-1, uz učešće FOXO1 i AMPK signalnih molekula u srcu normalno uhranjenih pacova. Davanje IGF-1 gojaznim pacovima povećava ekspresiju i aktivnost Na^+/K^+-ATPaze tako što stimuliše IGF-1Rβ/IRβ/IRS-1/Akt signalni put, dok istovremeno smanjuje aktivaciju mTOR/S6K signalnog puta, što se može dovesti u vezu sa smanjenjem hipertrofije srca izazvane gojaznošću. Dobijeni rezultati ne samo da ukazuju na potencijalnu ulogu IGF-1 u lečenju disfunkcije srca povezane sa gojaznošću, već predstavljaju dobru osnovu za dalja istraživanja i kliničku primenu IGF-1 u lečenju srčanih bolesti.

Ključne reči: IGF-1, Na^+/K^+-ATPaza, IRS-1/Akt, mTOR/S6K, gojaznost, hipertrofija srca
Naučna oblast: Biologija
Uža naučna oblast: Molekularna endokrinologija
UDK broj:

The effects of insulin like growth factor 1 on the expression and activity of the sodium-potassium pump in the heart of obese rats

ABSTRACT

The aim of this doctoral dissertation was to explore the molecular mechanism by which insulin like growth factor 1 (IGF-1), under *in vivo* conditions, affects expression, activity of sodium/potassium adenosine triphosphatase (Na^+/K^+-ATPase) and its interaction with other proteins in heart in physiological conditions and in obesity, as well as if modulation of Na^+/K^+-ATPase activity could be associated with the reduction of obesity-induced heart hypertrophy. The obtained results have shown that *in vivo* administration of IGF-1 leads to elevation in relative gene expression of α_1 subunit of Na^+/K^+-ATPase, protein levels of α_1 and α_2 subunits, phosphorylation of α subunit and Na^+/K^+-ATPase activity. Furthermore, IGF-1 treatment led to an increase in phosphorylation of insulin receptor substrate-1 (IRS-1), phosphoinositide-dependent kinase-1 (PDK-1), protein kinase B (Akt), mammalian target of rapamycin (mTOR) and ribosomal protein S6 kinase (S6K) in normal rats' heart. In addition, IGF-1 administration in normal rats reduced the interaction between α_1 subunit of Na^+/K^+-ATPase and beclin-1, as well as phosphorylation of adenosine monophosphate-activated protein kinase (AMPK), while it increased the phosphorylation of β subunits of hybrid receptor for IGF-1 and insulin (IGF-1Rβ/IRβ) and FOXO1 protein in heart. In obesity, the IGF-1 treatment resulted in a significant elevation of α_1 subunit protein level and phosphorylation of α subunit and Na^+/K^+-ATPase activity, phosphorylation of IGF-1Rβ/IRβ, IRS-1, Akt, protein level of angiotensin II (Ang II) receptor type 2 (AT_2R), relative gene expression of α-myosin heavy chain (MHC), as well as the α-MHC/β-MHC ratio. Conversely, the administration of IGF-1 in obese rats reduced the cardiac mass, phosphorylation of mTOR, S6K and the protein level of Ang II receptor type 1 (AT_1R) in heart, as well as the levels of Ang II and K^+ in serum. The obtained results of this doctoral dissertation indicate that IGF-1 *in vivo* in physiological conditions increases Na^+/K^+-ATPase expression and activity through molecular mechanism involving IRS-1/PDK-1/Akt/mTOR/S6K signaling pathway in rat heart. Additionally, IGF-1 reduces autosis by decreasing the interaction between α_1 subunit of Na^+/K^+-ATPase and beclin-1, with the involvement of FOXO1 and AMPK signaling molecules in normal rats. Administration of IGF-1 increases Na^+/K^+-ATPase expression and activity through IGF-1Rβ/IRβ/IRS-1/Akt signaling pathway, while decreases mTOR/S6K signaling pathway activation in obese rats, which could be associated with the reduction of obesity-induced heart hypertrophy. These results not only suggest potential role of IGF-1 in the treatment of obesity- associated cardiac dysfunction, but provides solid basis for further research and clinical application of IGF-1 in the treatment of cardiovascular disease.

Key words: IGF-1, Na^+/K^+-ATPase, IRS-1/Akt, mTOR/S6K, obesity, heart hypertrophy
Scientific Group: Biology
Specific Area Within A Group: Molecular Endocrinology
UDK number:

Eksperimentalan deo ove doktorske disertacije je urađen u Laboratoriji na radiobiologiju i molekularnu genetiku, Instituta za nuklearne nauke „Vinča“ – Instituta od nacionalnog značaja za Republiku Srbiju – Univerziteta u Beogradu.

Posebno se zahvaljujem dr Milanu Obradoviću, neposrednom mentoru, na ukazanom strpljenju, poverenju i razumevanju, podršci i velikodušnoj pomoći od samog početka izrade ove doktorske disertacije. Njegov trud, ogromno zalaganje i spremnost da u svakom trenutku pruži pomoć i usmerenje bili su od neprocenjivog značaja za uspešno završavanje ove teze.

Zahvaljujem se mentoru, prof. dr Tanji Jevđović, na stručnoj pomoći i trudu pri pregledu i oceni ove doktorske disertacije, kao i na konstruktivnim sugestijama čime je doprinela konačnom uobličavanju ove teze.

Zahvaljujem se prof. dr Esmi R. Isenović, članu komisije, na ukazanom poverenju, velikodušnoj i stručnoj pomoći i korisnim savetima tokom izrade ove doktorske disertacije. Posebno se zahvaljujem na pruženoj šansi da postanem deo njenog naučno-istraživačkog tima.

Veliku zahvalnost dugujem dragoj koleginici i članu komisije, dr Sonji Zafirović, na podršci, prijateljstvu, pozitivnoj energiji, vremenu i trudu koji je uložila u svim koracima izrade ove doktorske disertacije.

Zahvaljujem se prof. dr. Ivi Lakić, članu komisije, na izdvojenom vremenu i trudu koje je uložila pri pregledu i oceni ove doktorske disertacije, kao i nesebičnoj pomoći i angažovanju tokom završne faze izrade teze.

Hvala mojim koleginicama dr Anastasiji Pajčin, dr Julijani Stojanović i dr Jeleni Radovanović na prijateljstvu, savetima, pozitivnoj energiji, nesebičnoj pomoći tokom izrade ove doktorske disertacije. Njihovo razumevanje, ohrabrivanje i ljubav učinili su ovaj put mnogo lakšim i lepšim.

Zahvaljujem se koleginicama dr Emini Sudar Milovanović, dr Božidarki Zarić, MSc Nataši Đorđević i dr Sanji Soskić na prijatnoj radnoj atmosferi, kolegijalnosti i korisnim savetima.

Najiskrenije se zahvaljujem mojim bliskim prijateljima na podršci i razumevanju koje su mi pružili. Njihova prisutnost, ohrabrivanje i ljubav su pomogli da prevaziđem najteže trenutke i istrajem do samog kraja izrade teze.

Sa najvećom ljubavlju, zahvaljujem se mojim roditeljima i bratu Aleksi, bez čije ljubavi, podrške i vere ne bih nikada postigla ovo što jesam.

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SKRAĆENICE

Akt	protein-kinaza B (*engl. protein kinase B, PKB*)
AMP	adenozin monofosfat (*engl. adenosine monophosphate*)
AMPK	AMP-aktivirana protein-kinaza (*engl. AMP-activated protein kinase*)
Ang II	angiotenzin II
AT_1R	Ang II receptor tip 1
AT_2R	Ang II receptor tip 2
ATP	adenozin-trifosfat (*engl. adenosine triphosphate*)
BMI	indeks telesne mase (*engl. body mass index*)
cAMP	ciklični adenozinmonofosfat
Ca^{2+}	jon kalcijuma
cGMP	ciklični guanozinmonofosfat
CTS	kardiotonični steroidi
DMT2	dijabetes mellitus tipa 2
DNK	dezoksiribonukleinska kiselina
eNOS	endotelna azot monoksid sintaza
ERK1/2	ekstracelularnim signalom regulisana kinaza 1 i 2 (*engl. extracellular signal-regulated kinase 1/2*)
FOXO1	*engl. forkhead box protein O1*
GRB2	protein 2 vezan za receptor faktora rasta
HF	ishrana obogaćena mastima (*engl. high-fat*)
HUVEC	humane endotelne ćelije pupčane vene (*engl. human umbilical vein endothehal cells*)
IGF-1	insulinu sličan faktor rasta 1 (*engl. insulin-like growth factor 1*)
IGF-1R	receptor za IGF-1
IGF-1R/IR	hibridni receptor za IGF-1 i insulin
IGFBP	vezujući protein insulinu sličnog faktora rasta-1 (*engl. insulin-like growth factor binding protein-1*)
IGT	poremećena tolerancija glukoze (*engl. impaired glucose tolerance)*
iNOS	inducibilna NOS (*engl. inducible NOS*)
IP3	inozitoltrifosfat
IR	receptor za insulin
IRS-1	supstrata receptora za insulin-1 (IRS-1, *engl. insulin receptor supstrate-1)*
JNK	c-Jun N-terminalna kinaza
K^+	jon kalijuma
KVB	kardiovaskularne bolesti
MAPK	mitogen-aktivirana protein-kinaza (*engl. mitogen-activated protein kinase)*
MHC	teški lanaci miozina (*engl. myosine heavy chain*)
miRNK	mikro-ribonukleinska kiselina
mTOR	ciljni molekul rapamicina kod sisara (*engl. mammalian target of rapamycin*)
mTORC1	kompleks ciljni molekul za rapamicin kod sisara
Na^+	joni natrijuma
Na^+/K^+-ATPaza	natrijum/kalijum-adenozintrifosfataza
NO	azot-monoksid

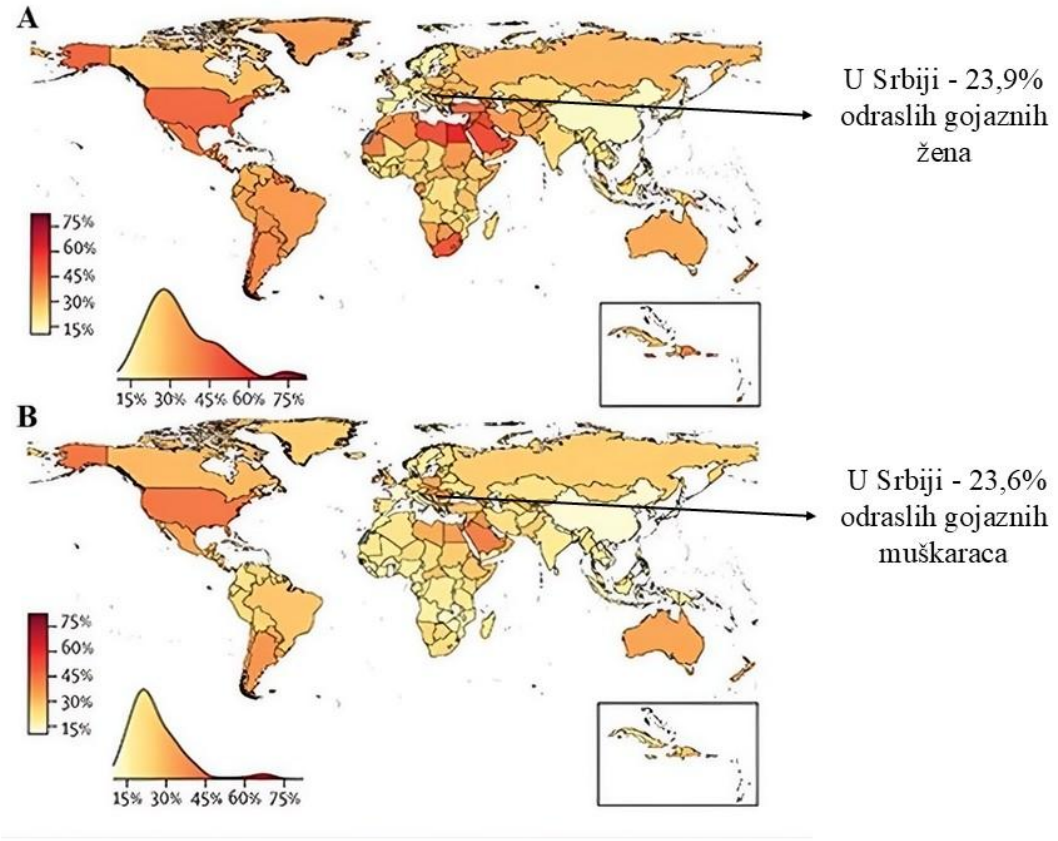
PDK1	fosfoinozitid-zavisna protein-kinaza 1 (*engl. phosphoinositide-dependent protein kinase 1*)
PI3K	fosfoinozitid-3-kinaza (*engl. phosphoinositide 3-kinase*)
PIP2	fosfatidilinozitol-4,5-bisfosfat
PIP3	fosfatidilinozitol-3,4,5-trifosfat
PKC	protein kinaza C
PKG	fosfoglicerat kinaza
PLC	fosfolipaza C
RAEC	endotelne ćelije izolovane iz aorte pacova (*engl. rat aortic endotelial cells*)
RIAEC	endotelne ćelije interlobarnih arterija bubrega pacova (*engl. rat renal interlobar artery endothelial cells*)
ROS	reaktivne vrste kiseonika (*engl. reactive oxygen species*)
S6K1	kinaza ribozomalnog proteina S6
Ser	serin (*engl. serine*)
SERCA	Ca^{2+}-ATPaza sarkoplazmatskog retikuluma (*engl. sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase*),
Shc	Shc proteini (*engl. Src homology 2 domain-containing*)
SHR	spontano hipertenzivni pacovi (*engl.spontaneously hypertensive rats*)
SMK	slobodne masne kiseline
SOS	(*engl. Son of Sevenless protein*)
Thr	treonin (*engl. threonine*)
Tyr	tirozin (*engl. tyrosine*)
VSMC	glatke mišićne ćelije krvnih sudova (*engl. vascular smooth muscle cells*)
WKY	*engl. Wistar Kyoto rats*

SADRŽAJ

1. UVOD

1.1. Gojaznost

Prekomerna telesna masa i gojaznost predstavljaju nakupljanje masnog tkiva u organizmu. Iako ne postoje opšteprihvaćene definicije za prekomernu telesnu masu i gojaznost, u svakodnevnoj i stručnoj praksi najčešće se za te svrhe primenjuje indeksa telesne mase (BMI; *engl. body mass index*). Shodno tome, prekomerna telesna masa se definiše sa vrednošću BMI $\geq$ 25 kg/m^2, dok se gojaznost karakteriše sa vrednošću BMI > 30 kg/m^2 (WHO 2025). Specifično posmatrano, gojaznost se može definisati kao hronično, složeno i progresivno oboljenje koje značajno utiče na zdravlje, kvalitet života i smrtnost (Lingvay i sar., 2024). Prema preporukama Američkog udruženja za medicinu od 2013. godine, gojaznost treba smatrati bolešću sa višestrukim patofiziološkim aspektima koja zahtevaju niz terapijskih intervencija u cilju unapređenja lečenja i prevencije gojaznosti (Kyle i sar., 2016). Prekomerna telesna masa i gojaznost su među glavnim faktorima za nastanak rezistencije na insulin, dijabetesa melitusa tipa 2 (DMT2) i kardiovaskularnih bolesti (Tian i sar., 2022). Najnoviji literaturni podaci pokazuju da više od 2,5 milijardi odraslih ljudi širom sveta ima prekomernu telesnu masu, dok je 890 miliona ljudi gojazno (Xavier 2024). Podaci ukazuju da je u Srbiji 23,9% odraslih žena i 23,6% odraslih muškaraca gojazno (Global nutrition report. S 2024). (**Slika 1.**). Takođe, skoro 60% osoba sa prekomernom telesnom masom i gojaznošću ima kardiometabolički poremećaj (Kivimäki i sar., 2017; Dash 2025). Imajući u vidu visoku učestalost gojaznih osoba širom sveta, naročito u razvijenim zemljama, smatra se da gojaznost ima karakteristike pandemije (Marija i sar., 2017).



Slika 1. Učestalost gojaznosti u svetu i Srbiji. A – žene, B – muškarci. Preuzeto i modifikovano iz (Phelps i sar., 2024).

1.1.1. Uzroci nastanka gojaznosti

Iako gojaznost u užem smislu nastaje kao posledica prekomernog unosa energije (kalorija) u odnosu na potrošnju energije (kroz metabolizam i fizičku aktivnost), etiologija gojaznosti je izuzetno kompleksna. Razvoj gojaznosti predstavlja složenu interakciju genetskih, fizioloških, psiholoških, socijalnih i ekonomskih faktora (Wright i Aronne 2012; Lin i Li 2021). Genetski uzroci obuhvataju monogenske i poligenske promene, kao i hromozomske aberacije (Prader-Vili sindrom) koje dovode do poremećaja u regulaciji energetskog balansa i apetita (Lin i Li 2021). Razvoj gojaznosti je takođe pod uticajem endokrinog sistema, koji je primarno regulisan neuroendokrinim centrima koji se nalaze u hipotalamusu i moždanom stablu (Skoracka i sar., 2025). Arkuatno jedro hipotalamusa predstavlja integracioni centar za regulaciju apetita i energetskog balansa. U ovom moždanom regionu se objedinjuju genetski uslovljene determinante sa perifernim signalima endokrinog sistema, među kojima su glavni hormoni leptin i grelin (Bombassaro i Araujo 2024; Skoracka i sar., 2025). Pored toga, razvoj gojaznosti može da bude uzrokovan i brojnim epigenetskim mehanizmima, kao što su metilacije dezoksiribonukleinske kiseline (DNK), modifikacije histona i regulacija posredovana mikro-ribonukleinskim kiselinama (miRNK), koji menjaju ekspresiju gena bez promene u nukleotidnoj sekvenci (Wu i Yin 2022). Pokazano je da gojaznost trudnice povećava verovatnoću razvoja gojaznosti kod deteta, što se prvenstveno dovodi u vezu sa epigenetičkim modifikacijama i u manjoj meri genskim mutacijama (Kong i sar., 2025). Endokrini poremećaji, kao što su hipotiroidizam, Kušingov sindrom, sindrom

policističnih jajnika, mogu da dovedu do razvoja gojaznosti (Meligi i sar., 2024). Danas je dobro poznato da su nastanak i razvoj gojaznosti u velikoj meri povezani sa načinom života. Fizička neaktivnost i nezdrava ishrana su među glavnim uzročnicima gojaznosti, dok manjak obrazovanja, loš kvalitet hrane i savremeni način života dodatno doprinose problemu (Sandoval-Bórquez i sar., 2024; Ahmed i Mohammed 2025).

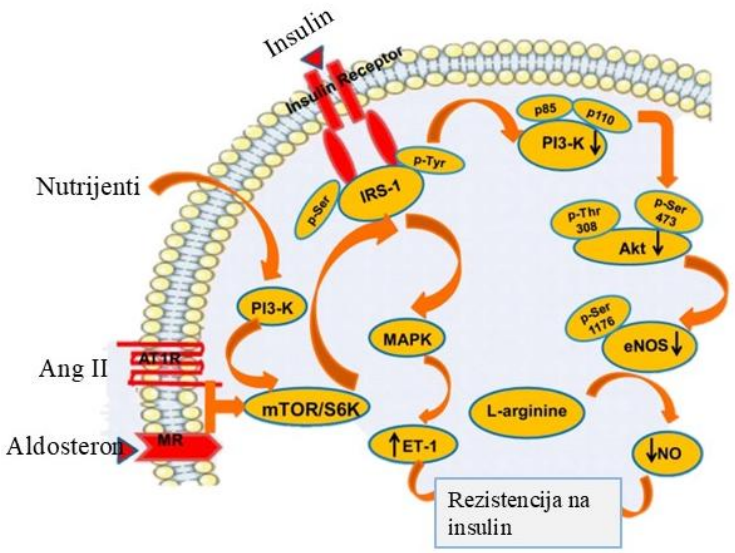
1.1.2. Patofiziologija gojaznosti

Gojaznost karakteriše povećavanje veličine (hipertrofija) i broja (hiperplazija) adipocita; osnovnih ćelija masnog tkiva (Sandoval-Bórquez i sar., 2024; Shimi i sar., 2024). U stanju gojaznosti, hipertrofija i hiperplazija adipocita dovode do razvoja lokalne hipoksije usled ograničene difuzije kiseonika, čime se aktiviraju hipoksijom-izazvani signalni putevi i podstiče razvoj zapaljenskih procesa u masnom tkivu (Sandoval-Bórquez i sar., 2024). Takođe, izmenjena funkcija adipocita dovodi do taloženja kolagena, remodelovanja ekstracelularog matriksa i hronične inflamacije (Ghaben i Scherer 2019). Pored uloge u skladištenju energije, belo masno tkivo ima izraženu endokrinu funkciju, sintetiše i luči brojne peptide i adipokine (leptin, adiponektin, angiotenzinogen, faktor nekroze tumora α, interleukin-6 i hemerini), steroide i masne kiseline. U gojaznosti sinteza i sekrecija ovih molekula se menja, što doprinosi patologiji gojaznosti (Sandoval-Bórquez i sar., 2024; Shimi i sar., 2024).

Gojaznost je praćena hroničnim inflamatornim stanjem niskog stepena, koje nastaje usled povećanog lučenja proinflamatornih citokina i adipokina, kao i pojačane infiltracije proinflamatornih ćelija u masno tkivo, kao što su makrofagi M1 fenotipa (Jin i sar., 2023). U stanju gojaznosti dolazi do povećane lipolize i oslobađanja slobodnih masnih kiselina (SMK) iz masnog tkiva. Slobodne masne kiseline iz visceralnog masnog tkiva se transportuju preko portne cirkulacije u jetru, dok se SMK iz subkutanog masnog tkiva oslobađaju u sistemsku cirkulaciju (Saponaro i sar., 2022). Povećana koncentracija SMK u cirkulaciji doprinosi ektopičnoj akumulaciji lipida u različitim organima uključujući i srce, dovodeći na taj način do razvoja različitih patomorfoloških i patofizioloških promena (Luo i sar., 2025).

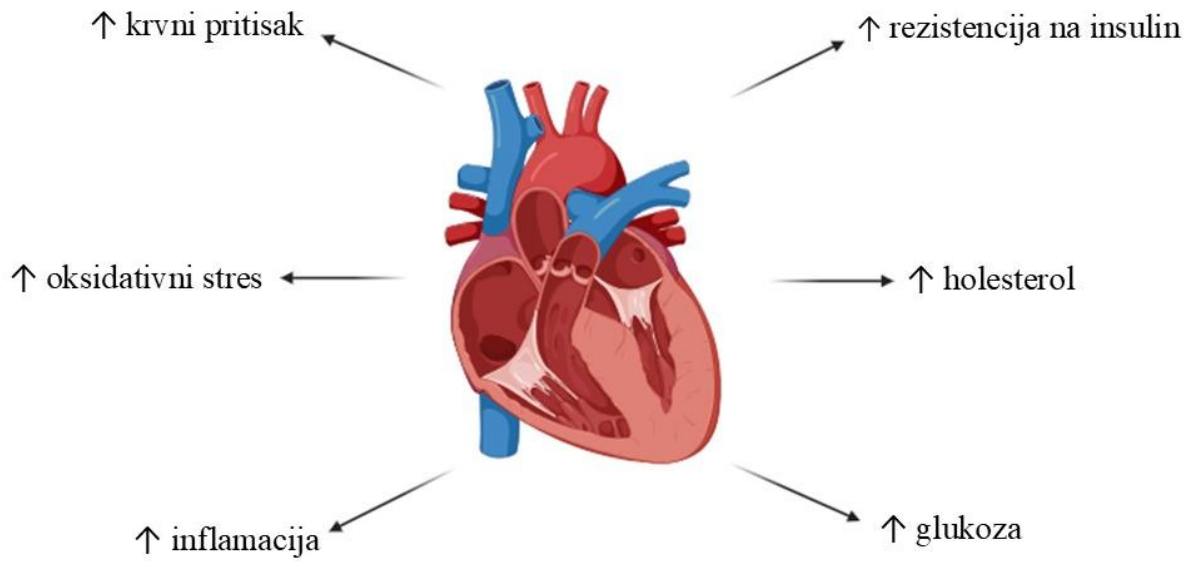
Povećan dotok SMK narušava normalnu funkciju jetre i dovodi do razvoja lokalne hepatične rezistencije na insulin, posredstvom aktivacije protein kinaze C ε koja se direktno vezuje i inhibira aktivaciju receptora za insulin (Samuel i Shulman 2016; Saponaro i sar., 2022). Takođe, povećana koncentracija SMK u kombinaciji sa pojačanim lučenjem adipokina štetno utiče na lučenje i delovanje insulina i time doprinosi razvoju sistemske rezistencije na insulin (Shimi i sar., 2024). Molekulska osnova razvoja rezistencije na insulin u stanju gojaznosti uključuje aktivaciju renin-angiotenzin sistema i hronično povećanje koncentracije angiotenzina II (Ang II) u cirkulaciji (**Slika 2.**). Tokom gojaznosti, hipertrofija masnog tkiva nije praćena odgovarajućom kompenzatornom angiogenezom što dovodi do razvoja hipoksije i oksidativnog stresa. Kao odgovor na ove promene dolazi do povećane sinteze Ang II čime se pojačava inflamatorni odgovor i narušava insulinska signalizacija (Stanciu i Jinga 2024).

Vezivanjem za receptor Ang II tipa 1 (AT_1R), Ang II fosforiliše ciljni molekul za rapamicin kod sisara (mTOR, *engl. the mechanistic target of rapamycin*) i kinazu ribozomalnog proteina S6 (S6K1) koji potom dovode do inhibicije aktivnosti supstrata insulinskog receptora 1 (IRS-1, *engl. insulin receptor supstarte-1*). Fosforilacija IRS-1 na serinu^{307} (Ser^{307}) sprečava aktivaciju signalnog puta fosfatidilinozitol-3 kinaze/protein kinaze B (PI3K/Akt), čime dolazi do poremećaja insulinske signalizacije i razvoja rezistencije na insulin (Pulakat i sar., 2011; Stanciu i Jinga 2024).



Slika 2. Uticaj gojaznosti na razvoj rezistencije na insulin. Ang II – Angiotenzin II, AT_1R – Ang II receptora tip 1, Akt – protein kinaza B, ET-1 – endotelin-1, IRS-1 – supstrat receptora za insulin-1, MAPK – mitogenim signalima aktivirana protein kinaza, mTOR – ciljni molekul za rapamicin kod sisara, NO – azot-monoksid, PI3K - fosfatidilinozitol-3-kinaze. Preuzeto i modifikovano sa (Jia i sar., 2014).

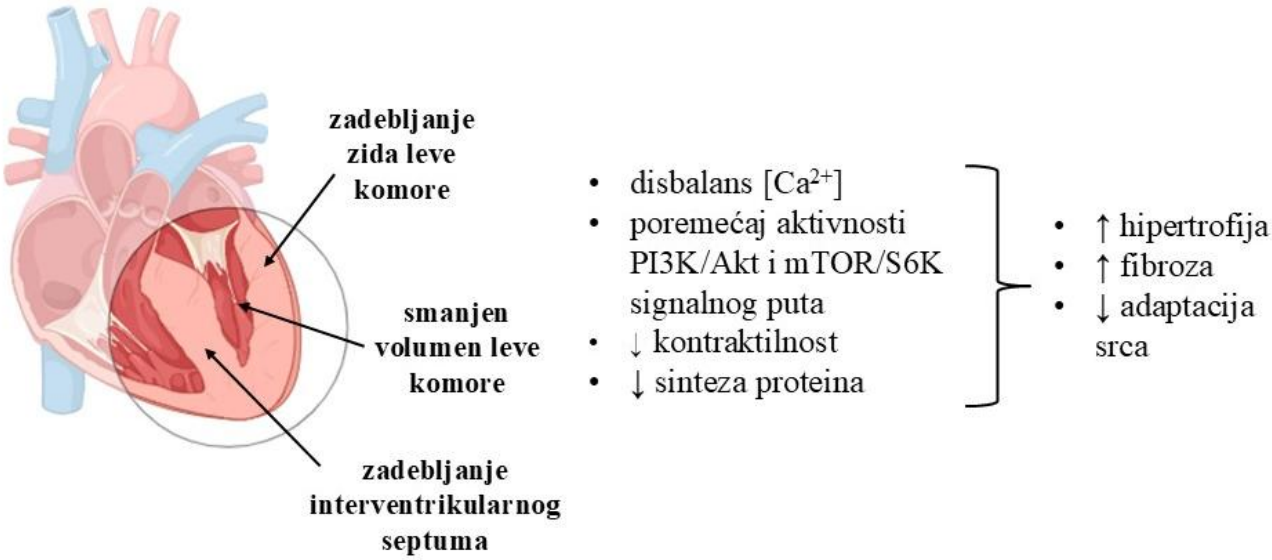
Visceralno masno tkivo luči pro-inflamatorne citokine koji remete ćelijsku homeostazu, pokreću inflamatorne procese i menjaju signalne puteve u kardiovaskularnom sistemu (Su i Peng 2020; Kirichenko i sar., 2022). Proinflamatorni citokini stimulišu sintezu C-reaktivnog proteina koji uzrokuje bolesti kardiovaskularnog sistema (Obradovic i sar., 2015; Carbone i sar., 2019; Stanimirovic i sar., 2022). Dugotrajna gojaznost značajno doprinosi razvoju DMT2, hipertenzije i dislipidemije, koji leže u osnovi nastanka metaboličkog sindroma (Ahmed i Mohammed 2025). Gojaznost je jedan od glavnih faktora nastanka kardiovaskularnih bolesti koje uključuju hipertenziju, aterosklerozu i srčanu insuficijenciju. Povišen krvni pritisak, oksidativni stres i hronična inflamacija dodatno narušavaju funkciju srca u stanju gojaznosti. Prisustvo rezistencije na insulin i dislipidemija dovode do povećavanja koncentracije glukoze i holesterola u krvi, što zajedno doprinosi oštećenju srca (Jin i sar., 2023; Welsh i sar., 2024). (**Slika 3.**).



Slika 3. Efekti gojaznosti na srce. ↑ - povećanje. Slika je izrađena korišćenjem BioRender.com softvera.

1.1.3. Uticaj gojaznosti na morfologiju srca

Gojaznost združena sa rezistencijom na insulin predstavlja jedan od vodećih faktora rizika za nastanak koronarne arterijske bolesti, infarkta miokarda i srčane insuficijencije (Afshin i sar., 2017). Povećanje telesne mase u gojaznosti dovodi do porasta ukupnog volumena krvi, što rezultuje hroničnim sistolnim i dijastolnim zapreminskim opterećenjem srca i doprinosi razvoju povišenog arterijskog pritiska (hipertenzije) (El Meouchy i sar., 2022). Pored povećanja ukupnog volumena krvi, kao odgovor na metaboličke potrebe srca dolazi do povećavanja minutnog volumena (Alansari i Lazzara 2025). U ranim fazama, ovo povećanje se ostvaruje kroz blago povećavanje broja otkucaja srca (srčane frekvence), dok u kasnijim fazama dolazi do postepenog uvećanja komora srca, a zatim i povećanja pritiska na zidove komora i njihovog zadebljanja (Alpert i sar., 2016; Tomar i sar., 2025; Alansari i Lazzara 2025). Ove promene su dodatno pojačane aktivacijom renin-angiotenzin sistema i simpatičkog nervnog sistema, što dovodi do povećanja perifernog vaskularnog otpora i zadržavanja jona natrijuma (Na^+) i vode, što dodatno opterećuje rad srca (Shariq i McKenzie 2019). Hipertenzija povećava napetost zida leve komore i stimuliše razvoj njene hipertrofije (Lembo i sar., 2024) (**Slika 4.**). Međutim, hipertrofija leve komore nije posledica samo mišićne hipertrofije, već i infiltracije masnog tkiva (miokardijalne steatoze), vezivnog tkiva (intersticijalne fibroze) i infiltracije ćelija imunskog sistema (proinflamatornih makrofaga, neutrofila i T-limfocita) (Cifarelli i sar., 2022; Zhao i sar., 2023; Dona i sar., 2023; Oneglia i sar., 2024). Ove promene dovode do smanjenja elastičnosti miokarda, poremećaja relaksacije leve komore i razvoju dijastolne disfunkcije i srčane insuficijencije sa očuvanom ejekcionom frakcijom (Lembo i sar., 2024).



Slika 4. Uticaj gojaznosti na srce. Akt – protein kinaza B, [Ca^{2+}] – koncentracija jona kalcijuma, mTOR –ciljni molekul za rapamicin kod sisara, PI3K – fosfatidilinozitol-3-kinaze, S6K – kinaza ribozomalnog proteina S6, ↑ – povećanje, ↓ smanjenje. Slika je izrađena korišćenjem BioRender.com softvera.

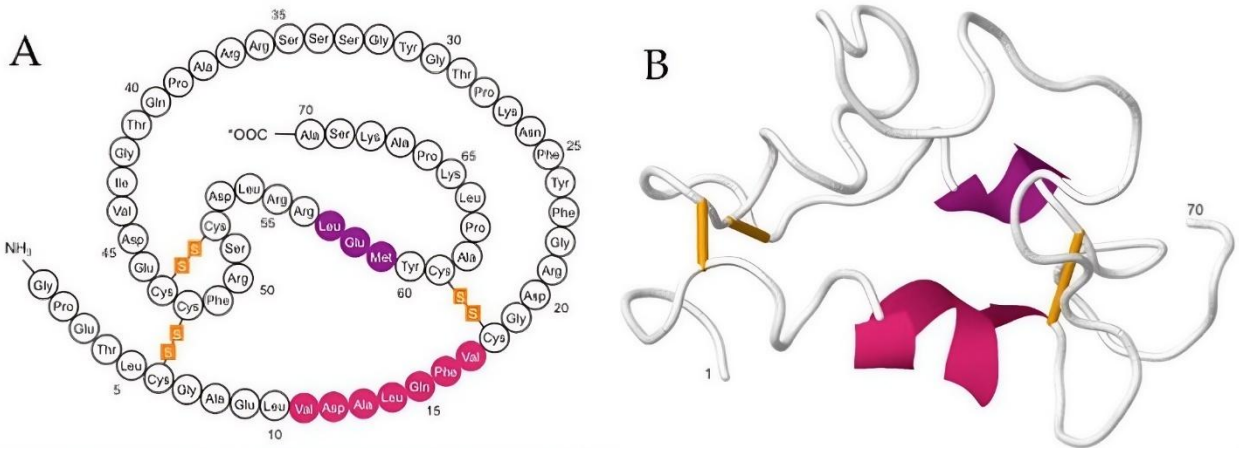
Gojaznost dovodi i do promena u ekspresiji gena u kardiomicima. Jedna od ključnih promena odnosi se na ekspresiju teških lanaca miozina (MHC, *engl. myosine heavy chain*). U srcu odraslih sisara eksprimiraju se dve izoforme MHC, koje se razlikuju po konktraktilnim osobinama. α-MHC se odlikuje većom brzinom kontrakcije, usled veće ATP-azne aktivnosti, dok druga izoforma β-MHC pokazuje sporiju kontraktilnost (Lu i sar., 2022). U fiziološkim uslovima, α-MHC dominantno je eksprimirana forma, dok se u patofiziološkim stanjima ekspresija α-MHC smanjuje, a ekspresija β-MHC povećava (Walklate i sar., 2021). Smanjenje odnosa α-MHC/β-MHC jedna je od karakteristika hipertrofije srca u gojaznosti, koja jasno ukazuje na adaptivni odgovor srca praćen smanjenjem njegove kontraktilne aktivnosti (Walklate i sar., 2021).

Gojaznost u kombinaciji sa hroničnom inflamacijom, oksidativnim stresom i rezistencijom na insulin dovodi do poremećaja autofagije u srcu, što može uzrokovati smrt kardiomicita (Sun i sar., 2019). Novija istraživanja ukazuju na specifičan tip ćelijske smrti uzrokovan izmenjenom autofagijom koji se zove autoza (Liu i sar., 2013; Nah i sar., 2020; Yang i sar., 2025). Autozu karakterišu specifične morfološke i biohemijske promene, uključujući izraženu vakuolizaciju citoplazme, promene na nivou mitohondrija i strukture jedarne membrane (Nah i sar., 2020). Glavnu ulogu u ovom procesu ima interakcija α_1 subjedinice Na^+/K^+-ATPaze i proteina autofagije beklin-1 (Liu i sar., 2013; Bai i sar., 2023). Istraživanja pokazuju da inhibicija autoze predstavlja potencijalno efikasan način smanjenja oštećenja miokarda nakon ishemije u fazi reperfuzije (Nah i sar., 2020; Nah i sar., 2022). Dosadašnja istraživanja su bila usmerena na opisivanje autoze, koja je zavisna od interakcije α_1 subjedinice Na^+/K^+-ATPaze i proteina autofagije beklin-1, uz doprinos mitohondrijalne disfunkcije i oksidativnog stresa tokom gladovanja, vežbanja, ishemije i reperfuzije (Liu i sar., 2013; Fernández Á i sar., 2020; Nah i sar., 2020; Nah i sar., 2022), dok su molekularni mehanizmi nastanka autoze u srcu i dalje nedovoljno izučeni.

1.2. Insulinu sličan faktor rasta 1 (IGF-1)

1.2.1. Građa i sinteza IGF-1

Insulinu sličan faktor rasta 1 (IGF-1) je polipeptidni hormon (~70 aminokiselina), koji učestvuje u regulaciji diferencijacije, sazrevanja, rastu i proliferaciji ćelija u različitim tkivima (Werner 2023). Sastoji se iz dva lanca (A i B) koja su povezana sa tri disulfidna mosta. IGF-1 pokazuje 40% strukturne sličnosti sa proinsulinom, ali za razliku od insulina aktivna forma IGF-1 poseduje C-domen, koji povezuje A i B lance (Werner 2023) (**Slika 5.**). IGF-1 se dominantno se sintetiše u jetri pod uticajem hormona rasta, a potom se izlučuje u krvotok. Periferna tkiva takođe imaju sposobnost da proizvode IGF-1, koji u tim tkivima može da ima parakrino i autokrino dejstvo (Lee i sar., 2024). Lokalno sintetisan IGF-1 omogućava precizniju regulaciju ćelijskih procesa u specifičnim organima (Lee i sar., 2024). Koncentracija IGF-1 molekula u krvi direktno je zavisna od IGF-1 vezujućih (IGFB, engl. *IGF binding*) proteina. Nakon sinteze, IGF-1 se vezuju za IGFB proteine čime se reguliše njegova stabilnost i biodostupnost u cirkulaciji. Poluživot IGF-1 u cirkulaciji može da varira od nekoliko minuta do više sati (Werner 2023; Khan i sar., 2025). Iako postoji šest izoformi IGFB proteina, kod ljudi se kroz cirkulaciju 80% IGF-1 prenosi vezan za IGFB-3 protein (Werner 2023; Khan i sar., 2025). Pored uloge u transportu IGF-1 molekula, IGFB proteini značajno utiču na biodostupnost IGF-1, jer različitim afinitetom vezivanja modulišu njegovu interakciju sa IGF-1 receptor (IGF-1R), u pericelularnom okruženju. Takođe, IGFB proteini mogu da modulišu signalizaciju IGF-1 kroz post-translacione modifikacije kao što je fosforilacija, interakcija sa integrinima i proteoglikanima ili kroz ograničenu proteolizu IGFB proteina (Baxter 2023).



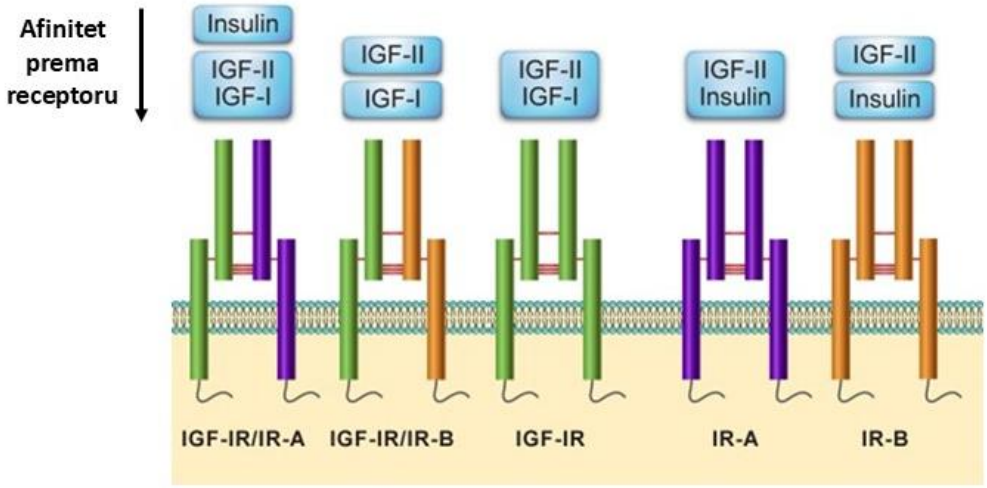
Slika 5. Građa IGF-1. A. Primarna struktura IGF-1 sa disulfidnim mostovima, B. 3D struktura IGF-1. Preuzeto iz (Bailes i Soloviev 2021).

1.2.2. Uloga i mehanizam delovanja IGF-1

Funkcionalno IGF-1 ostvaruje dvojako dejstvo, stimuliše sinteze proteina, rast i proliferaciju ćelija, a takođe utiče i na metabolizam glukoze i masti u perifernim tkivima (Werner 2023). Delovanje IGF-1 se ostvaruje prvenstveno preko IGF-1R. S obzirom da je IGF-1 strukturno sličan insulinu, može da se veže za receptor za insulin (IR), ali sa značajno manjim afinitetom u odnosu na insulin (Werner 2023; Khan i sar., 2025). Oba receptora, IGF-1R i IR

pripadaju porodici transmembranskih tirozin-kinaznih receptora. Izgrađeni su od po dve strukturno specifične α i dve β subjedinice. Zanimljivo je da subjedinice IGF-1R i IR mogu da se kombinuju i formiraju hibridni receptor (IGF-1R/IR) (Kiernan i sar., 2024; Khan i sar., 2025). Hibridni receptor se pojačano eksprimira u patološkim stanjima kao što je rezistencija na insulin, ali njegova uloga nije u potpunosti razjašnjena. Pretpostavlja se da ovaj hibridni recetor ima ulogu u modulaciji tkivno specifičnih efekata IGF-1 (Kiernan i sar., 2024; Khan i sar., 2025) (**Slika 6.**).

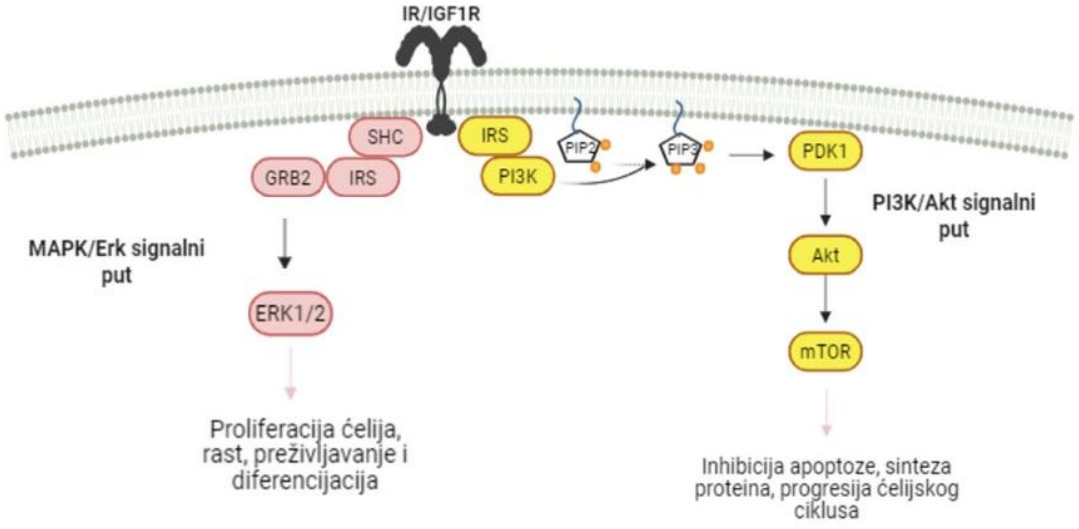
Pored brojnih pozitivnih efekta koje ostvaruje u organizmu, povišen i/ili snižen nivo IGF-1 utiče na pojavu različitih patofizioloških stanja. Tako su starenje, kardiovaskularne bolesti i metabolički poremećaji povezani sa smanjenim koncentracijama IGF-1 u krvi (Mukama i sar., 2023; Chu i sar., 2025). Sa druge strane, karcinomi i druge bolesti za koje je karakteristična proliferacija ćelija su povezani sa povećanim koncentracijama IGF-1 u krvi (AsghariHanjani i Vafa 2019). Takođe, pokazano je da gojaznost udružena sa rezistencijom na insulin značajno utiče na sintezu i bioaktivnost IGF-1 (Lee i sar., 2024).



Slika 6. Različiti tipovi receptora za IGF-1. IGF-1/2 – Insulinu sličan faktor rasta 1/2, IGF1R – Receptor za IGF-1, IR-A – Receptor za insulin-A, IR-B – Receptor za insulin-B. Preuzeto i modifikovano iz (Vishwamitra i sar., 2017).

1.2.3. Signalni putevi delovanja IGF-1

Vezivanjem za svoj receptor, IGF-1 pokreće aktivaciju unutarćelijskih signalnih kaskada. Glavni signalni putevi kojim IGF-1 ostvaruje svoje efekte su PI3K/Akt signalni put i mitogenom aktivirana protein kinaza (MAPK, *engl. mitogen-activated protein kinase*) (**Slika 7.**). Aktivacija PI3K/Akt signalnog puta dovodi do antiapoptotskog i mitogenog delovanja IGF-1, dok aktivacija MAPK signalnog puta podstiče proliferaciju i diferencijaciju ćelija (Kiernan i sar., 2024; Khan i sar., 2025).



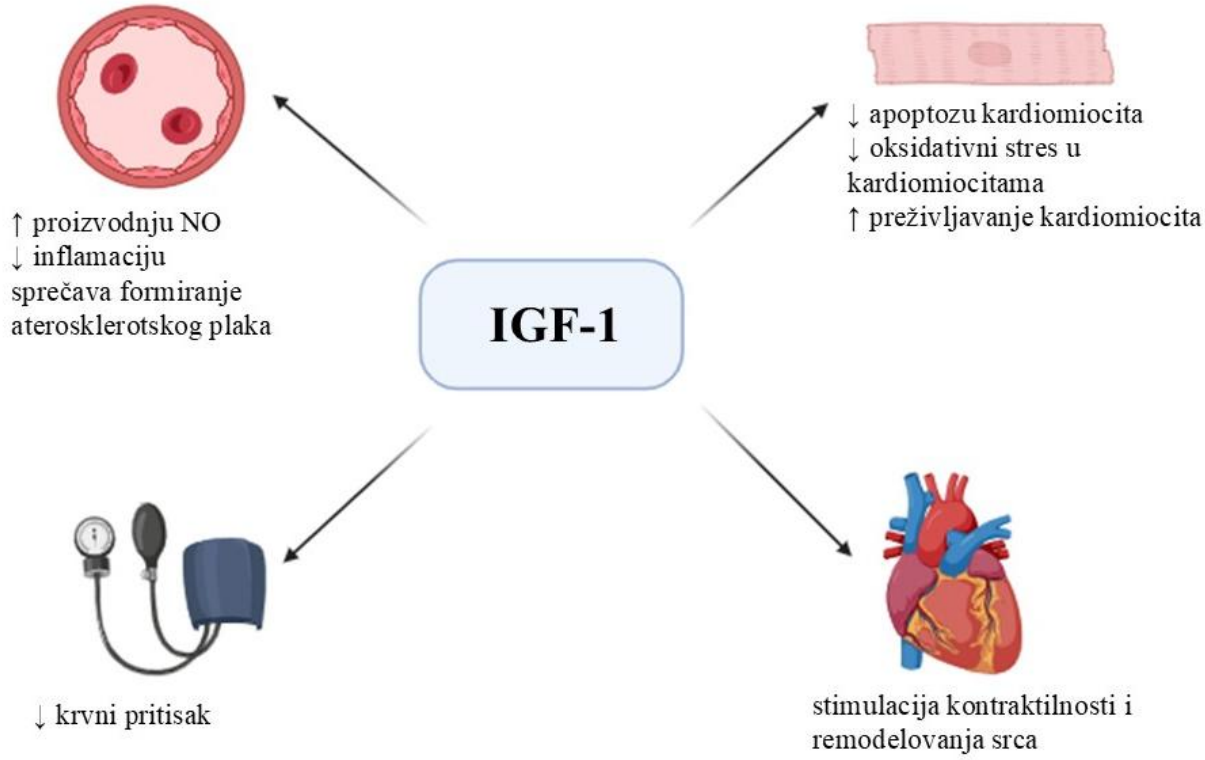
Slika 7. Mehanizam delovanja IGF-1 u ćelijama. Akt – protein kinaza B, IGF -1 – insulinu sličan faktor rasta 1, IGF -1R – IGF-1 receptor, IRS – supstrat receptora za insulin, ERK – vanćelijskim signalima regulisana kinaza, GRB2 – protein 2 vezan za receptor faktora rasta, mTOR – ciljni molekul za rapamicin kod sisara, PDK1 – 3-fosfoinozitid-zavisna kinaza 1, PIP2 – fosfatidilinozitol-4,5-bisfosfat, PIP3 – fosfatidilinozitol-3,4,5-trifosfat, Shc - *Src homology 2 domain-containing*. Slika je izrađena korišćenjem BioRender.com softvera.

Nakon vezivanja IGF-1 za spoljašnji domen receptora, dolazi do autofosforilacije β subjedinica receptora čime se aktiviraju mesta vezivanja adaptornih molekula za receptor (Werner 2023; Khan i sar., 2025). Ključni adaptorni proteini uključeni u ovaj proces su IRS i Shc proteini (*engl. Src homology 2 domain-containing*). Fosforilacija IRS-1 na aminokiselinskom ostatku tirozina^{1222} (Tyr^{1222}), dovodi do aktivacije PI3K molekula, koji omogućava strukturnu transformaciju fosfatidilinozitol-4,5-bisfosfat (PIP2, *engl. phosphatidylinositol 4,5-bisphosphate*) u fosfatidilinozitol-3,4,5-trifosfat (PIP3, *engl. phosphatidylinositol 3,4,5-trisphosphate*) i na ovaj način stvara uslove za aktiviranje Akt molekula (Werner 2023; Khan i sar., 2025), koji se prvo delimično aktivira fosforilacijom na aminokiselinskom ostatku treonina^{308} (Thr^{308}) posredstvom fosfoinozitid-zavisne kinaze 1 (PDK1, *engl.* phosphoinositide-dependent kinase-1), dok je za potpunu aktivaciju potrebna dodatna fosforilacija na aminokiselinskom ostatku Ser^{473} koja je posredovana kompleksom 2 mTOR (Khan i sar., 2025). Aktiviran Akt dalje fosforiliše nishodne proteine koji učestvuju u inhibiciji proapototskih proteina i stimulaciji kompleksa 1 mTOR čime se podstiče sinteza proteina, proliferacija i rast ćelija (Werner 2023; Khan i sar., 2025). Fosforilacija Shc proteina podstiče aktivaciju MAPK signalne kaskade koja dalje utiče na ekspresiju gena uključenih u regulaciju proliferacije, diferencijacije i rasta ćelija (Werner 2023; Khan i sar., 2025).

1.2.4. Efekti IGF-1 u kardiovaskularnom sistemu u fiziološkim i patofiziološkim stanjima

U kardiovaskularnom sistemu, IGF-1 ostvaruje mnogobrojne pozitivne efekte tako što deluje na homeostazu ćelija, odnosno metabolizam nutrijenata, različite faktore uključene u održavanje vaskularnog tonusa, autofagiju i apoptozu (**Slika 8.**) Takođe, IGF-1 ima značajnu

ulogu u antioksidativnom i antiinflamatornom odgovoru u kardiovaskularnom sistemu (Wang i sar., 2024).



Slika 8. Efekti IGF-1 na kardiovaskularni sistem. Slika je izrađena korišćenjem BioRender.com softvera.

Povećanjem ekspresije antioksidativnih enzima i smanjenjem proizvodnje reaktivnih vrsta kiseonika (ROS, *engl. reactive oxygen species*) IGF-1 utiče na smanjenje oksidativnog stresa u kardiomiocitama. Takođe, IGF-1 sprečava formiranje i progresiju aterosklerotskog plaka tako što poboljšava endotelnu funkciju i smanjuje adheziju inflamatornih ćelija na endotel (Lee i sar., 2024). IGF-1 stimuliše sintezu azot-monoksida (NO) povećavajući ekspresiju i aktivnost endotelne NO sintaze (eNOS) i utičući na vazodilataciju i poboljšanje funkcionisanja kardiovaskularnog sistema (Nyul-Toth i sar., 2025). Pozitivan uticaj IGF-1 na vaskularni tonus ostvaruje se i aktivacijom PI3K/Akt signalnog puta i inhibicijom proapoptotskih molekula, što je naročito izraženo u stanjima ishemije i reperfuzije (Liao i sar., 2019). Pokazano je da je smanjen nivo IGF-1 povezan sa aktivacijom proinflamatornih makrofaga, povećavanjem akumulacije lipida u zidu krvnih sudova, ubrzavanjem vaskularnog starenja, nastankom ateroskleroze i koronarne bolesti (Higashi i sar., 2016; Aguirre i sar., 2016).

U tabeli 1. prikazani su rezultati studija u kojima je izučavan efekat IGF-1 u kardiovaskularnom sistemu u eksperimentalnim modelima na ćelijama i kod životinja (**Tabela 1.**)

Tabela 1. Efekti IGF-1 u kardiovaskularnim patologijama (eksperimentalni modeli)

Eksperimentalni model	Efekti IGF-1	Referenca
Miševi sa deficitom endotelnog IGF-1R	Odsustvo IGF-1 rezultira smanjenjem vazorelaksacije i funkcije malih krvnih sudova	(Nyul-Toth i sar., 2025)
Miševi sa deficitom endotelnog IGF-1R	Odsustvo IGF-1 rezultira povećanjem broja senescentnih endotelnih ćelija i povećanjem kumulativne permeabilnosti mikrovaskulature	(Gulej i sar., 2024)
IGF-1 deficijentni miševi	Odsustvo IGF-1 rezultira promenama u vanćelijskom matriksu i glatkim mišićnim ćelijam, što doprinosi nastanku vaskularnih bolesti povezanih sa starenjem	(Tarantini i sar., 2016)
WKY i SHR pacovi	*In situ* tretman isečaka aorte IGF-1 dovodi do vazorelaksacije posredovane NO kod SHR pacova	(Yang i sar., 2010)
Miševi (normalno uhranjeni i gojazni) tretirani sa IGF-1	*In vivo* tretman IGF-1 povećava ekspresiju/aktivnost eNOS i vazorelaksaciju zavisnu od NO u fiziološkim uslovima u velikim krvnim sudovima	(Imrie i sar., 2009)
IGF-1 heterozigotni transgeni miševi (mladi i stari)	Smanjen uticaj starenja na unutarćelijsku koncentraciju Ca^{2+}, oštećenje proteina i apoptozu kardiomiocita	(Li i Ren 2007)
Normalno uhranjeni i gojazni Zucker pacovi	*In situ* tretman isečaka aorte IGF-1 dovodi do povećanja vazorelaksacije	(Yang i sar., 2007)
WKY i SHR pacovi	*In situ* tretman isečaka aorte IGF-1 smanjuje vazokonstrikciju izazvanu sa fenilefrinom i endotelinom-1 kod WKY pacova	(McCallum i sar., 2005)
VSMC	*In vitro* tretman IGF-1 povećava aktivnost Na^+/K^+-ATPazu preko PI3K/Akt signalnog puta	(Isenovic i sar., 2004)
RAEC	*In vitro* tretman IGF-1 povećava ekspresiju eNOS i dovodi do vazorelaksacije preko PI3K/Akt signalnog puta	(Isenovic i sar., 2003)
IGF-1 nokaut miševi	Odsustvo IGF-1 rezultira hipertenzijom i hipertrofijom leve komore	(Tivesten i sar., 2002)
RAEC	*In vitro* tretman IGF-1 povećava ekspresiju eNOS i dovodi do vazorelaksacije preko PI3K/Akt signalnog puta	(Isenovic i sar., 2001)
Mlade svinje	*In situ* tretman epikardijalnih arterija IGF-1 smanjuje vazokonstrokciju posredovanu endotelin-1	(Hasdai i sar., 1998)
Wistar pacovi tretirani sa IGF-1	*In vivo* Povećana vazodilatacija i smanjen srednji arterijski pritisak i glukoze u plazmi.	(Pete i sar., 1996)
HUVEC i RIAEC	*In vitro* tretman IGF-1 povećava u dozno-zavisnom pogledu produkciju NO aktivacijom eNOS	(Tsukahara i sar., 1994).

eNOS – endotelna azot-monoksida sintaza (*engl. endothelial nitric oxide synthase*), IGF-1 – insulinu-slični faktor rasta-1 (*engl. insulin-like growth factor-1*), Na^+/K^+-ATPaza – natrijum-kalijum adenozintrifosfataza (*engl. sodium-potassium adenosinethiphosphatase*), PI3K/Akt – fosfoinozitol-3 kinaza / protein kinaza B (*engl.phosphatidylinositol 3-kinase / protein kinase B*), RAEC – endotelne ćelije aorte pacova (*engl. rat aortic endotelial cells*), RIAEC – endotelne ćelije interlobarnih arterija bubrega pacova (*engl. rat renal interlobar artery endothelial cells*), HUVEC – humane endotelne ćelije pupčane vene (*engl. human umbilical vein endothehal cells*), SHR – spontano hipertenzivni pacovi (*engl.spontaneously hypertensive rats*), VSMC – glatke mišićne ćelije krvnih sudova (*engl. vascular smooth muscle cells*), WKY - (*engl. Wistar Kyoto rats*).

Mnogobrojne humane studije ukazuju da je snižena ili povišena koncentracija IGF-1 u serumu povezana sa povećanim rizikom od nastanka kardiovaskularnih bolesti. U tabeli 2, dat je prikaz rezultata kliničkih studija koje su pokazale značajnu povezanost između nivoa IGF-1 u cirkulaciji i kardiovaskularnih promena (**Tabela 2.**)

Tabela 2. Efekti IGF-1 u kardiovaskularnim patologijama (humane studije)

Ispitanici	Nivo IGF-1 u cirkulaciji	Efekat	Referenca
394 082 ispitanika	Snižen ili povišen	Povećan rizik od nastanka KVB	(Lin i sar., 2023)
22,995 ispitanika	Povišen	Smanjen rizik od nastanka KVB	(Li i sar., 2022)
2046 žena	Povišen	Smanjena učestalost arterijske hipertenzije kod žena koje nemaju dijabetes	(Zhang i sar., 2011)
1482 volontera (746 muškaraca i 736 žena)	Snižen	Oštećena endotelna funkcija kod muškaraca	(Empen i sar., 2010)
3977 ispitanika (46% muškarci, 54% žene)	Snižen	Povećan metabolički rizik	(Lam i sar., 2010)
100 nelečenih pacijenta sa hipertenzijom	Snižen	Smanjena endotelna funkcija	(Perticone i sar., 2008)
54 IGT, 98 DMT2, 357 kontrola	Snižen	Smanjena osetljivost na insulin	(Sesti i sar., 2005)
174 ispitanika (92 žene i 82 muškaraci)	Snižen	Povećan rizik od nastanka KVB i ateroskleroze	(Colao i sar., 2005)
403 starija muškarca	Snižen	Povećan rizik od nastanka KVB	(van den Beld i sar., 2003)

KVB – kardiovaskularne bolesti, DMT2 – dijabetes melitus tip 2 (*engl. diabetes mellitus type 2*), IGF-1 – insulinu slični faktor rasta-1 (*engl. insulin-like growth factor-1)*, IGT – poremećena tolerancija glukoze (*engl. impaired glucose tolerance)*.

1.2.5. Uticaj IGF-1 na srce

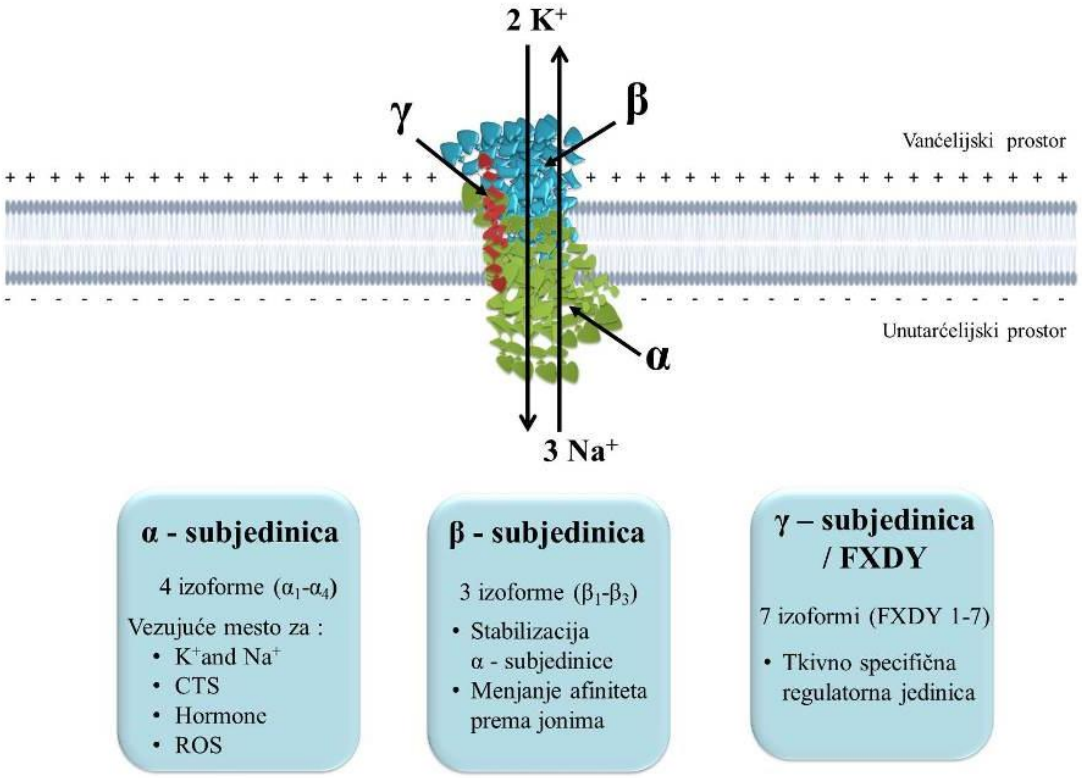
Srce je organ kardiovaskularnog sistema koje je longitudinalno je podeljeno septumom na desnu i levu polovinu, a transverzalno na komore i pretkomore. Desna polovina srca pumpa krv u pluća, gde se krv obogaćuje kiseonikom, a oslobađa ugljen-dioksida. Leva polovina srca pumpa oksigenisanu krv preko aorte u sistemsku cirkulaciju i ishranjuje sva tkiva i organe (Chaudhry i sar., 2022). Srce je zaštićeno perikardom, koji se sastoji iz spoljašnjeg fibroznog sloja i unutrašnjeg seroznog sloja. Unutrašnji serozni sloj je podeljen na parijetalni i visceralni list, između kojih je prostor ispunjen perikardijalnom tečnošću. Visceralni list seroznog sloja ujedno predstavlja epikard srca (Al-Sakini 2022). Na poprečnom preseku srca razlikuju se tri sloja: spoljašnji epikard, miokard i endokard. Miokard predstavlja mišični sloj srca izgrađen od aktinskih i miozinskih filamenata organizovanih u sarkomere, kao i krvnih sudova, vezivnog tkiva i nervnih vlakana. Najveći deo miokarda čine kardiomiociti koji su međusobno povezanih interkalarnim diskovima, koji olakšavaju prenošenje električnog impulsa sa jedne ćelije na drugu. Pored kardiomicita, u sastav miokarda ulaze i fibroblasti, endotelne ćelije, glatke mišične ćelije, ćelije imunskog sistema i periciti (Dewing i sar., 2022). Endokard predstavlja unutrašnji sloj izgrađen od jednoslojnog skvamoznog epitela, koji oblaže šupljine srca i učestvuje u izgradnji zalistaka (Ripa i sar., 2023). Srce se koronarnom cirkulacijom snadbeva krvlju, kiseonikom i nutrijentima. Primarni izvor energije za kontrakciju miokarda predstavljaju masne kiseline, ali srce može da koristi i ugljene hidrate, ketonska tela i aminokiseline (Karwi i sar., 2018).

U srcu, IGF-1 reguliše proizvodnju energije neophodne za normalan rad srca, kroz stimuliaciju glikolize i oksidacije masnih kiselina (Lin i sar., 2023). Takođe, inhibicijom proapoptotskih i aktivacijom antiapoptotskih signalnih puteva IGF-1 podstiče preživljavanje kardiomiocita i regeneraciju miokarda nakon infarkta (Song i sar., 2016). Pored toga, antiinflamatornim delovanjem IGF-1 smanjuje ekspresiju proinflamatornih citokina i infiltraciju inflamatornih $Ly6C^+$ monocita i pomaže oporavak miokarda (Nederlof i sar., 2022). IGF-1 ima važnu ulogu u fiziološkoj hipertrofiji srca koju karakteriše povećanje srčane mase uz očuvanje dijastolne funkcije srca, a koja se javlja kao posledica pojačane fizičke aktivnosti (Abdellatif i sar., 2022). Pokazano je da u srcu normalno uhranjenih Zucker pacova IGF-1 povećava unutarćelijsku koncentraciju kalcijuma (Ca^{2+}), što dovodi do povećanja kontraktilnost miokarda (Ren i sar., 2000). Nasuprot tome, u miokardu gojaznih pacova, značajno je smanjena ekspresija gena za IGF-1R. Kod gojaznih pacova sa rezistencijom na insulin i izmenjenom funkcijom kardiomiocita tretman antagonistom IGF-1R dovodi do aktivacije signalnog puta IGF-1 čime se ostvaruju pozitivni efekti na rad srca (Hintz i Ren 2002). Nedostatak IGF-1 dovodi do značajnog smanjenja veličine srca i kardiomiocita, kao i smanjene kontraktilne funkcije srca, što je pokazano u studiji na miševima (Ren i Brown-Borg 2002).

Primena IGF-1 kod gojaznih dijabetičnih pacova dovodi do poboljšanja sistolne i dijastolne funkcije miokarda i homeostaze Ca^{2+} (Norby i sar., 2002). U eksperimentalnim modelima miševa sa infarktom miokarda, pokazano je da primena IGF-1 povećava stepen preživljavanja i proliferacije matičnih ćelija srca, smanjuje proinflamatorni odgovor imunskog sistema i značajno utiče na oporavak miokarda nakon infarkta (Urbanek i sar., 2005; Gallego-Colon i sar., 2015; Nederlof i sar., 2022). Takođe, brojne kliničke studije ukazuju na značaj IGF-1 u kardiovaskularnom sistemu. Snižene koncentracije IGF-1 u serumu su takođe izmerene kod pacijenata sa idiopatskom dilatativnom kardiomiopatijom, ishemijskom bolesti srca i srčanom insuficijencijom (Juul i sar., 2002; Laughlin i sar., 2004; Dong i sar., 2014; Naderi i sar., 2015; Liu i sar., 2024). U populacionoj studiji na starijim pacijentima, bez prethodnog infarkta miokarda, snižene koncentracije IGF-1 su bile povezane sa većim rizikom od kongestivnog srčanog zastoja (Vasan i sar., 2003). Slično tome, stepen srčane insuficijencije se dovodi u vezu sa koncetracijom IGFB-1 proteina, kao i odnosom IGF-1/IGFB-1 proteina u serumu (Guo i sar., 2021). Međutim, rezultati nekih studija nisu pokazali povezanost između koncetracije IGF-1 u serumu sa rizikom od koronarne bolesti srca (Kaplan i sar., 2007), čak su povišene koncentracija IGF-1 u serumu bile u korelaciji sa stepenom progresije koronarne arterijske bolesti (Yousefzadeh i sar., 2013). Ovi rezultati ukazuju da efekat IGF-1 na kardiovaskularni sistem zavisi od više faktora, prevashodno nivoa IGF-1, zatim nivoa IGFB proteina, ali i od stepena težine oboljenja.

1.3. Natrijum/kalijum adenozintrifosfataza - građa i funkcija

Primarna uloga natrijum-kalijum adenozin trifosfataze (Na^+/K^+-ATPaze) je transport jona Na^+ van ćelije i K^+ u ćeliju, uz potrošnju energije dobijene hidrolizom molekula adenozintrifosfata (ATP-a) (Skou 1957; Abe i sar., 2024) (**Slika 9.**). Ovaj transport omogućava održavanje stabilnog elektrohemijskog gradijenta ćelijske membrane, koji je ključan za razne procese u ćeliji, uključujući regulaciju ćelijskog volumena, ekscitabilnost i sekundarno aktivni transport drugih jona i molekula (glukoze, aminokiselinae, Ca^{2+}) (Contreras i sar., 2024). Ovaj integralni membranski enzim izgrađen je iz katalitičke α subjedinice, visoko glikozilovane β subjedinice i regulatorne γ subjedinice.



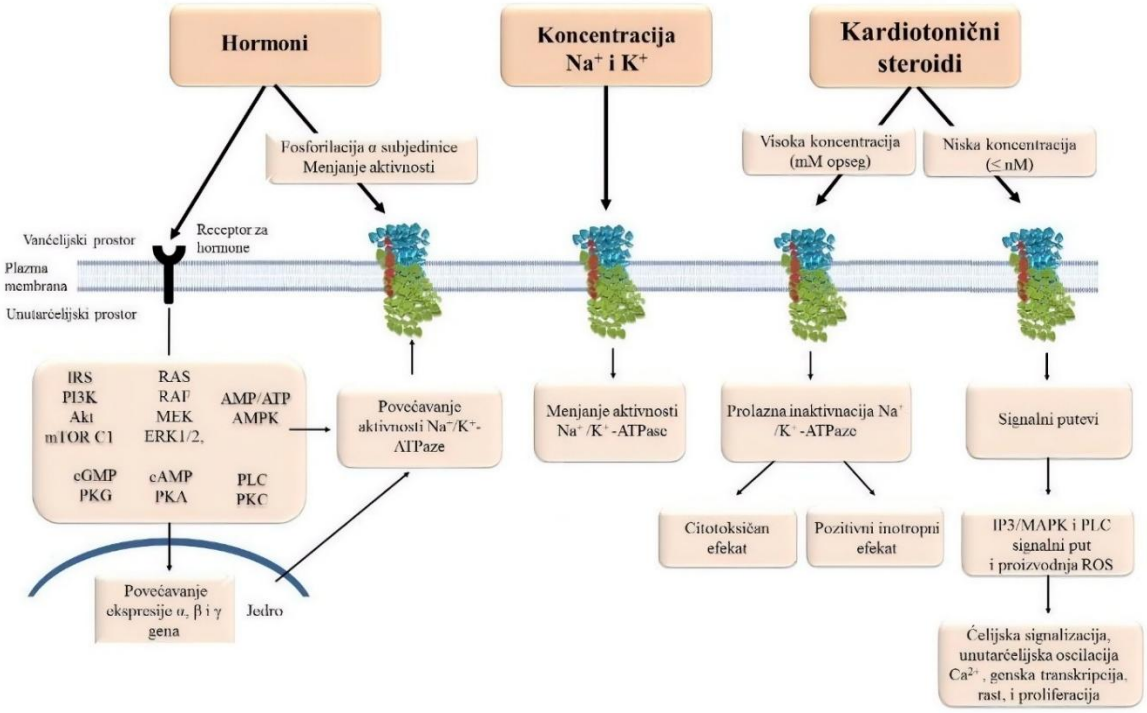
Slika 9. Građa i funckija Na^+/K^+-ATPaze. Strukturni raspored α subjedinice (zelena), β subjedinice (plava) i γ subjedinica ili FXYD (crvena); CTS – kardiotonični steroidi, Na^+ - natrijum, K^+ - kalijum, ROS – reaktivne vrste kiseonika. Preuzeto i modifikovano iz (Obradovic i sar., 2023).

Svaka subjedinica ima nekoliko izoformi koje su specifične za vrstu ili tkivo (Shull i sar., 1985; Abe i sar., 2024). Alfa subjedinica se eksprimira u četiri izoforme (α_1- α_4), dok se β subjedinica eksprimira u tri izoforme (β_1- β_3), što omogućava formiranje dvanaest različitih izoenzima Na^+/K^+-ATPaze sa različitim biofizičkim i biohemijskim osobinama (Sweadner 1989; Obradovic i sar., 2023; Contreras i sar., 2024). Regulatorna γ subjedinica, poznata i kao FXYD protein, eksprimirana je u sedam tkivno specifičnih izoformi (Kryvenko i sar., 2021). Katalitička α subjedinica ima tri domena; veliki unutarćelijski domen sa mestom za vezivanje ATP molekula i mestom fosforilacije, transmembranski domen od deset segmenata i vanćelijski domen sa mestom za vezivanje kardiotoničnih glikozida (Abe i sar., 2024). Glikozilovana β subjedinica ima ključnu ulogu u transportu α subjedinice iz unutarćelijskog prostora na membranu, kao i održavanju njene stabilnosti i menjanju afinitet prema jonima (Kryvenko i sar., 2021). Regulatorne γ subjedinice su tkivno specifične i utiču na afinitet Na^+/K^+-ATPaza prema jonima i ATP molekulima, a pored toga utiču i na stabilnost α i β kompleksa. Tkivno specifična γ subjedinica u srcu se zove fosfoleman i predstavlja bitan deo regulacije Na^+/K^+-ATPaze u kardiomicitama (Crambert i sar., 2002; Dhalla i sar., 2024). Usled fosforilacije γ subjedinice aktivnost Na^+/K^+-ATPaze se povećava, što se pretpostavlja da je rezultat prostorne konformacije celog enzima (Fedosova i sar., 2022). Mehanizam transporta jona kroz ćelijsku membranu je omogućen cikličnom promenom konformacije α subjedinice između dva konformaciona stanja – E1 i E2 (Campos i Beaugé 1994). U E1 konformaciji Na^+/K^+-ATPaza se otvara ka citoplazmatskoj strani i ima visok afinitet za unutarćelijske jone Na^+, dok se u E2 konformaciji Na^+/K^+-ATPaza otvara ka spoljašnjem matriksu i ima visok afinitet za vanćelijske jone K^+ (Nguyen i Deisl 2022; Contreras i sar., 2024). Celokupan proces promene konformacije enzima koristi energiju hidrolize ATP molekula, uz prisustvo jona

magnezijuma (Mg^{2+}), kao i odgovarajuću koncentraciju jona Na^+ i K^+ unutar i van ćelije (Contreras i sar., 2024).

1.3.1. Faktori regulacije aktivnosti Na^+/K^+-ATPaze

Regulacija Na^+/K^+-ATPaze se odvija na lokalnom i sistemskom nivou (**Slika 10.**). Faktori koji lokalno utiču na aktivnost Na^+/K^+-ATPaze su koncentracije jona Na^+ i K^+ unutar i van ćelije, zatim koncentracije ATP, ROS, NO, purina, pH vrednost i dostupnost kiseonika. Lokalna regulacija je odgovorna za kratkotrajne promene u funkcionisanju Na^+/K^+-ATPaze (Baloglu 2023; Abe i sar., 2024). Hipoksija, zajedno sa hiperkapnijom dovode do povećanja koncentracije jona Ca^{2+} nakon čega se aktivira adenozinmonofosfat-aktivirana protein kinaza (AMPK) koja podstiče translokaciju protein kinaze C (PKC) na plazma membranu gde direktno fosforiliše α subjedinicu Na^+/K^+-ATPaze i pokreće proces endocitotskog uklanjanja Na^+/K^+-ATPaze sa ćelijske membrane (Vadász i sar., 2008; Gusarova i sar., 2011; Lecuona i sar., 2013; Kryvenko i sar., 2021). Pored toga, AMPK i PKC aktiviraju i c-Jun N-terminalnu kinazu (JNK) koja podstiče reorganizaciju aktinskog citoskeleta dovodeći do endocitoze Na^+/K^+-ATPaze. Nivo cikličnog AMP takođe utiče na aktivnost Na^+/K^+-ATPaze preko protein kinaze A (Kryvenko i sar., 2021).



Slika 10. Uticaj različitih faktora na aktivnost Na^+/K^+-ATPaze. Akt – protein kinaza B, AMP/ATP – adenozinmonofosfat/adenozintrifosfat, AMPK –AMP-aktivirana protein kinaza, cAMP – ciklični adenozinmonofosfat, cGMP – ciklični guanozinmonofosfat, ERK1/2 – spoljašnjim signalima regulisana kinaza 1/2, IP3 – inozitoltrifosfat, IRS – supstrat receptora za insulin, MAPK – mitogen aktivnirana protein kinaza, mTORC1 – kompleks ciljni molekul za rapamicin kod sisara, PI3K – fosfatidilinozitol-3-kinaze, PKC – protein kinaza C, PKG – fosfoglicerat kinaza, PLC – fosfolipaza C. Preuzeto i modifikovano iz (Obradovic i sar., 2023).

Faktori koji na sistemskom nivou utiču na ekspresiju, aktivnost i stepen fosforilacije subjedinica Na^+/K^+-ATPaze, kao i na translokaciju α i β heterodimera iz unutarćelijskog prostora na plazma membranu uključuju hormone poput insulina, IGF-1, Ang II,

glukokortikoida, tioridnih hormona, estrogena, progesterona, kateholamina, dopamina, transformišućeg faktora rasta β i faktora rasta fibroblasta (Wen i Wan 2021; Obradovic i sar., 2023; Cordeiro i sar., 2024). Pored navedenih faktora, dodatan uticaj na aktivnost Na^+/K^+-ATPaze uključuje i tkivno specifična regulacija γ subjedinice, koja menja kinetičke osobine Na^+/K^+-ATPaze, njen afinitet za vezivanje jona i ATP, čime se ostvaruje promena aktivnosti Na^+/K^+-ATPaze speficična za metaboličke potrebe određenog tkiva (Cordeiro i sar., 2024). Takođe, Na^+/K^+-ATPaza može biti regulisana kardiotoničnim glikozidima i endogenim kardiotoničnim steroidima koji se kod sisara sintetišu u organizmu, a koji dovode do prolaznog smanjenja njene aktivnosti i pozitivnog inotropnog efekta (Orlov i sar., 2021). Inhibicija Na^+/K^+-ATPaze povećava koncentraciju Na^+ u ćeliji i time smanjuje gradijent potreban za sekundarno aktivni transport Na^+/Ca^{2+} izmenjivača, što za posledicu ima povećavanje koncentracije Ca^{2+} u ćeliji i povećanja kontraktilne funkcije srca (Orlov i sar., 2021). Povećana aktivnost Na^+/K^+-ATPaze u početnim fazama srčane insuficijencije predstavlja adaptivni mehanizam za održavanje jonske homeostaze, koja je povezana sa smanjenim unutarćelijskim koncentracijama Na^+ i Ca^{2+} (Abe i sar., 2024; Contreras i sar., 2024). Kardiotonični steroidi nisu poznati samo kao inhibitori pumpe, već i kao signalni molekuli koji mogu da se vezuju za Na^+/K^+-ATPazu i preko nje aktiviraju različite unutarćelijske signalne puteve (Gagnon i Delpire 2020; Cordeiro i sar., 2024).

1.3.2. Uticaj gojaznosti na Na^+/K^+-ATPazu

Gojaznost i metabolički sindrom mogu da dovedu do poremećaja nivoa ekspresije i aktivnosti Na^+/K^+-ATPaze, ključnog enzima za održavanje jonske homeostaze, membranskog potencijala i kontraktilnosti kardiomiocita (Rosta i sar., 2009; Obradovic i sar., 2015; Jovanovic i sar., 2017). Pokazano je da dijeta bogata mastima (HF, *engl. high-fat*) smanjuje ekspresiju, fosforilaciju i translokaciju α_1 subjednice Na^+/K^+-ATPaze u srčanom tkivu (Rosta i sar., 2009; Obradovic i sar., 2015; Jovanovic i sar., 2017). Takođe, gojaznost karakteriše povišen oksidativni stres u adipocitima, koji dovodi do aktivacije Na^+/K^+-ATPaze/Src signalne kaskade čime se uspostavlja amplifikaciona petlja koja dodatno povećava produkcija ROS (Nawab i sar., 2017). Hronični oksidativni stres podstiče aktivaciju proinflamatornih signalnih puteva i lučenje citokina koji dodatno doprinose produkciji ROS, što sve zajedno doprinosi nastanku rezistencije na insulin i dislipidemije (Pratt i sar., 2019). Izmenjena funkcija Na^+/K^+-ATPaze u gojaznosti je povezana sa nastankom hipertenzije, smrti kardiomiocita i izmenjenoj funkciji srca (Obradovic i sar., 2013; Yan i sar., 2019). Takođe, hronično smanjena aktivnost Na^+/K^+-ATPaze u gojaznosti dovodi do povećavanja unutarćelijske koncentracije Na^+, što za posledicu ima narušavanje kontraktilne funkcije srca (Shattock i sar., 2015; Deus i Vileigas 2019).

1.4. Uticaj IGF-1 na ekspresiju i aktivnost u srcu Na^+/K^+-ATPaze u stanju gojaznosti

Kod gojaznih životinja, kao i životinja sa dijabetesom primećeno je smanjene kontraktilnog odgovora miokarda u zavisnosti od nivoa IGF-1 (Ren i sar., 2000), što se može dovesti u vezu sa poremećajem funkcije Na^+/K^+-ATPaze. Takođe, Na^+/K^+-ATPaze ima važnu ulogu u relaksaciji krvnih sudova, a pokazano je da vazorelaksacija posredovana IGF-1 značajno smanjena kod spontano hipertenzivnih Wistar-Kyoto pacova (Yang i sar., 2010). Povećana ekspresija IGF-1 poboljšava kontraktilnost i elastičnost kardiomiocita (Kim i sar., 2008). Takođe, Yang i sar (Yang i sar., 2007) su pokazali da je vazorelaksacija zavisna od IGF-1 smanjena kod gojaznih Zucker pacova. Lokalno sintetisan IGF-1 uključen je u regulaciju vaskularnog tonusa, kroz povećavanje aktivnost i ekspresiju Na^+/K^+-ATPaze (Standley i sar., 1997). Takođe, IGF-1 podstiče preuzimanje glukoze u ćelije i samim tim povećava dostupnost

ATP molekula, čime se dodatno može stimulisati aktivnost Na^+/K^+-ATPaze (Kasprzak 2021). Jedan od glavnih signalnih puteva kojima IGF-1 ostvaruje svoje efekte u srcu, predstavlja PI3K/Akt signalna kaskada (Liao i sar., 2019). Pokazano je da aktivacijom PI3K/Akt signalnog puta se povećava ekspresija α i β subjedinica Na^+/K^+-ATPaze, modifikuje afinitet α subjednice za ATP i jone, kao i da se stimuliše translokacija Na^+/K^+-ATPaze iz citoplazme na membranu (Wu i sar., 2013; Jovanovic i sar., 2017; Li i sar., 2017). Rezultati *in vitro* studije na glatkim mišićnim ćelijama krvnih sudova pokazuju da IGF-1 utiče na aktivnost Na^+/K^+-ATPaze posredstvom PI3K/Akt signalnog puta (Isenovic i sar., 2004). Takođe, pokazano je da Ang II smanjuje IGF-1 zavisnu stimulaciju aktivnosti Na^+/K^+-ATPaze u glatkim mišićnim ćelijama krvnih sudova (Isenovic i sar., 2004). Ovi rezultati ukazuju na složene međuzavisne odnose hormona i njihovog delovanja na aktivnost Na^+/K^+-ATPaze u organizmu. Pored toga, IGF-1 podstiče sintezu NO u endotelnim ćelijama i glatkim mišićnim ćelijama krvnih sudova, dok NO pozitivno utiče na aktivnost Na^+/K^+-ATPaze (Juel 2016; Zafirovic i sar., 2026). Takođe, pokazano je da IGF-1 na indirektan način pozitivno deluje na aktivnost Na^+/K^+-ATPaze, tako što smanjuje oksidativni stres i proizvodnju ROS (Higashi i sar., 2010; Liu i sar., 2012).

Do sada su izučavani *in vitro* efekti IGF-1 na regulaciju Na^+/K^+-ATP-aze u različitim ćelijama i *in vivo* efekti IGF-1 u modelima infarkta kod pacova i skeletnim mišićima pacova (Dorup i Clausen 1995; Standley i sar., 1997; Isenovic i sar., 2004; Liao i sar., 2019), dok su *in vivo* efekti IGF-1 na ekspresiju i aktivnost Na^+/K^+-ATP-aze, kako u fiziološkom stanju tako i u stanju gojaznosti nedovoljno dokumentovani. Takođe, u literaturi nema podataka koji ukazuju na potencijalnu ulogu signalnih molekula IRS/Akt i mTOR/S6K1 u regulaciji aktivnosti Na^+/K^+-ATP-aze u srcu pod uticajem IGF-1, kao i *in vivo* efekte IGF-1 na strukturne promene srca.

2. CILJEVI I HIPOTEZA ISTRAŽIVANJA

2.1. Ciljevi

Ciljevi doktorske disertacije su:

- izučavanje *in vivo* efekata IGF-1 na ekspresiju i aktivnost Na^+/K^+-ATP-aze u srcu normalno uhranjenih pacova;
- izučavanje efekata ishrane bogate mastima na ekspresiju i aktivnost Na^+/K^+-ATP-aze u srcu pacova;
- izučavanje *in vivo* efekata IGF-1 na ekspresiju i aktivnost Na^+/K^+-ATP-aze u srcu gojaznih pacova;
- izučavanje učešća IRS/PI3K/Akt i mTOR/S6K posredovanih signalnih puteva u efektima IGF-1 na ekspresiju i aktivnost Na^+/K^+-ATP-aze u srcu normalno uhranjenih i gojaznih pacova.

2.2 Hipoteza

Imajući u vidu da IGF-1 ostvaruje brojne efekte u srcu, kao i ograničene literaturne podatke, koji se odnose na *in vivo* efekte IGF-1 na regulaciju Na^+/K^+-ATP-aze, cilj ove doktorske disertacije je da se izuči da li i na koji način IGF-1 utiče na ekspresiju i aktivnost Na^+/K^+-ATP-aze u srcu pacova sa eksperimentalno indukovanom gojaznošću. Pretpostavka je da IGF-1 *in vivo,* u fiziološkim uslovima, povećava ekspresiju i aktivnost Na^+/K^+-ATPaze u srcu putem IRS/PI3K/PDK/Akt/mTOR/S6K signalne kaskade. Takođe, pretpostavlja se da IGF-1 smanjuje interakciju Na^+/K^+-ATPaze i beklin-1 posredstvom AMPK/FOXO1 protein signalnog puta. U patofiziološkim uslovima, HF ishrana utiče na povećanu aktivnost Ang II koji stimulacijom mTOR/S6K1 signalnog puta povećava produkciju ROS i inhibira IRS/PI3K/Akt kaskadu, čime se narušava funkcija Na^+/K^+-ATPaze. Pretpostavlja se da bi u stanju gojaznosti *in vivo* tretman sa IGF-1 doveo do inhibicije mTOR/S6K1 i aktivacije IRS/PI3K/Akt signalnog puta, čime bi se normalizovala aktivnost Na^+/K^+-ATP-aze, što bi imalo pozitivne efekte na hipertrofiju srca izazvane gojaznošću.

S obzirom da u stanju gojaznosti dolazi do razvoja brojnih komplikacija u kardiovaskularnom sistemu, uključujući smanjenje ekspresije i aktivnosti $Na^{+/}K^+$-ATP-aze i hipertrofije srca, izučavanje efekata IGF-1 na regulaciju ekspresije i aktivnosti $Na^{+/}K^+$-ATP-aze u srcu ukazuje na značaj predloženih istraživanja. Izučavanje ovih procesa doprinelo bi boljem razumevanju patofiziologije gojaznosti i razvoju novih terapijskih pristupa u lečenju kardiovaskularnih komplikacija izazvanih gojaznošću.

3. RADOVI PROIZAŠLI IZ DOKTORSKE DISERTACIJE

Molecular Biology Reports (2024) 51:517
https://doi.org/10.1007/s11033-024-09451-3

ORIGINAL ARTICLE

The involvement of Akt, mTOR, and S6K in the in vivo effect of IGF-1 on the regulation of rat cardiac Na^+/K^+-ATPase

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Received: 1 December 2023 / Accepted: 15 March 2024

Abstract

Background We previously demonstrated that insulin-like growth factor-1 (IGF-1) regulates sodium/potassium adenosine triphosphatase (Na^+/K^+-ATPase) in vascular smooth muscle cells (VSMC) via phosphatidylinositol-3 kinase (PI3K). Taking into account that others' work show that IGF-1 activates the PI3K/protein kinase B (Akt) signaling pathway in many different cells, we here further questioned if the Akt/mammalian target of rapamycin (mTOR)/ribosomal protein p70 S6 kinase (S6K) pathway stimulates Na^+/K^+-ATPase, an essential protein for maintaining normal heart function.

Methods and results There were 14 adult male Wistar rats, half of whom received bolus injections of IGF-1 (50 µg/kg) for 24 h. We evaluated cardiac Na^+/K^+-ATPase expression, activity, and serum IGF-1 levels. Additionally, we examined the phosphorylated forms of the following proteins: insulin receptor substrate (IRS), phosphoinositide-dependent kinase-1 (PDK-1), Akt, mTOR, S6K, and α subunit of Na^+/K^+-ATPase. Additionally, the mRNA expression of the Na^+/K^+-ATPase α_1 subunit was evaluated. Treatment with IGF-1 increases levels of serum IGF-1 and stimulates Na^+/K^+-ATPase activity, phosphorylation of α subunit of Na^+/K^+-ATPase on Ser^{23}, and protein expression of α_2 subunit. Furthermore, IGF-1 treatment increased phosphorylation of IRS-1 on Tyr^{1222}, Akt on Ser^{473}, PDK-1 on Ser^{241}, mTOR on Ser^{2481} and Ser^{2448}, and S6K on Thr^{421}/Ser^{424}. The concentration of IGF-1 in serum positively correlates with Na^+/K^+-ATPase activity and the phosphorylated form of mTOR (Ser^{2448}), while Na^+/K^+-ATPase activity positively correlates with the phosphorylated form of IRS-1 (Tyr^{1222}) and mTOR (Ser^{2448}).

Conclusion These results indicate that the Akt/mTOR/S6K signalling pathway may be involved in the IGF-1 regulating cardiac Na^+/K^+-ATPase expression and activity.

Keywords IGF-1 · Na^+/K^+-ATPase · mTOR · S6K · Heart

Introduction

The insulin-like growth factor-1 (IGF-1) is a polypeptide growth factor sharing structural similarities with insulin, which regulates cell differentiation, maturation, development, and proliferation in nearly all organs [1]. Numerous studies proposed that IGF-1 plays a role in cardiovascular physiology homeostasis [1]. IGF-1, in particular, regulates vascular vasoconstriction/vasodilation, cardiac apoptosis, and autophagy [2, 3]. Also, IGF-1 exerts anti-inflammatory and anti-oxidant effects in the vasculature by lowering atherosclerotic plaque burden [4]. Furthermore, IGF-1 has angiogenic properties, as indicated by its capacity to regulate endothelial junction protein levels and stimulate angiogenesis in endothelial cells [5]. With the rising prevalence

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Published online: 15 April 2024

of cardiovascular diseases, research into IGF-1's role in cardiovascular system function is gaining attention.

The pivotal function of sodium/potassium adenosine triphosphatase (Na^+/K^+-ATPase) in cells is maintaining Na^+ and K^+ ions gradient across the membrane by utilizing the energy of hydroxylation of ATP molecule [6]. The Na^+/K^+-ATPase is required for rapid action potential upstroke and cardiomyocyte contractility by regulating ion exchange and cell membrane trafficking of many substances while maintaining proper cell function and cardiac output [7]. The Na^+/K^+-ATPase is a heterodimeric transmembrane protein with two essential subunits: a catalytic α subunit and a highly glycosylated β subunit. Additionally, α and β subunits can interact with the γ subunit belonging to the tissue-specific FXYD protein group [8]. The α subunit encompasses ten transmembrane segments responsible for ion transport, a significant intracellular domain with ATP-binding and phosphorylation sites, and an extracellular domain containing binding sites for cardiac glycosides [6]. The β subunit is essential for assembly and increases the stability of the α subunit and modulation of ions affinity [9]. Heart-specific γ subunit or phospholemman stabilizes the Na^+/K^+-ATPase and its kinetic and transport properties by modifying the affinity for ions and ATP [8].

The effects of IGF-1 are mediated by four types of tyrosine kinase membrane receptors [10]. IGF-1 acts primarily via its putative receptor (IGF-1R) and, with lower affinity, through the insulin receptor (IR), IGF-2 receptor and hybrid receptor with subunits of IGF-1 and IR (IGF-1R/IR) [11]. After interacting with its receptor, IGF-1 initiates autophosphorylation, which creates docking sites for recruiting and phosphorylating various adaptor proteins, including insulin receptor substrate (IRS) [1]. One of the major signalling pathways that are activated via IGF-1 receptors is phosphatidylinositol-3 kinase (PI3K)/protein kinase B (Akt)/mammalian target of rapamycin (mTOR) [1, 12, 13]. Multiple intracellular mediators, such as PI3K, phosphoinositide-dependent protein kinase-1 (PDK-1) and Akt, are involved in IGF-1 signalling across cell types. Despite its primary effect on PI3K, IGF-1 has also been associated with the activation of mTOR/ribosomal protein p70 S6 kinase (S6K) [14]. Another in vitro study showed the involvement of the S6K enzyme in regulating Na^+/K^+-ATPase protein expression using a model utilizing alveolar epithelial cells transfected with a dominant negative construct of p70S6K [15].

The IGF-1 causes vasorelaxation, which increases nitric oxide generation and Na^+/K^+-ATPase activation [16]. According to the literature, IGF-1 is a significant regulator of Na^+/K^+-ATPase in vitro, and its effects are mediated through the PI3K/Akt signalling cascade, which is associated with IGF-1-induced increased Na^+/K^+-ATPase messenger ribonucleic acid (mRNA) in vascular smooth muscle cells (VSMC) [12, 17, 18]. Through a signalling cascade involving IRS/PI3K/PDK/Akt/mTOR/S6K, this study sought to investigate the in vivo effect of IGF-1 on cardiac Na^+/K^+-ATPase expression/activity in rats. We hypothesize that a modification in the IRS/PI3K/PDK/Akt/mTOR/S6K signalling pathway, which regulates Na^+/K^+-ATPase expression and activity, is responsible for IGF-1's enhanced capacity to activate cardiac Na^+/K^+-ATPase activity in vivo. An important consequence of this hypothesis is that IGF-1 in vivo increases expression and activity of Na^+/K^+-ATPase in the heart through a PI3K-dependent activation of the Akt/mTOR cascade, which in turn amplifies mTOR/S6K signalling and, consequently, Na^+/K^+-ATPase expression and activity.

Materials and methods

Animals

The research was conducted on adult male Wistar rats that were nurtured at the Institute of Nuclear Sciences in Vinca, Belgrade. The animals were kept in cages in optimal laboratory conditions (22 ± 2 °C and 12-hour light-dark cycle) and supplied with food and water *ad libitum*. Animals were provided with a well-balanced meal specifically designed for laboratory rats, prepared by Veterinarski zavod Subotica in Serbia. The official Vinca Institute's Ethical Committee for Experimental Animals approved the experimental protocols under Veterinary Directorate No. 323-07-02710/2017-05.

Experimental treatment

A total of 14 animals were divided into 2 groups ($n = 7$), whereas 24 h before decapitation, the group of rats, labelled as "IGF-1", was treated intraperitoneally with a bolus injection of 50 µg/kg of IGF-1 (Sigma-Aldrich, I3769-50UG) dissolved in saline. This specific IGF-1 dosage was selected following previously published results [19]. At the same time, the other group of rats (labelled as "Control") were treated with saline. Animals were anaesthetized with a mixture of ketamine and xylazine (80 mg/kg of ketamine (VetViva Richter GmbH, Austria) and 12 mg/kg xylazine (VET-AGRO Multi trade Company Sp. z.o.o. Poland)), and sacrificed. The blood was collected through cardiac puncture and centrifuged, and obtained serum samples were stored at -80 °C until analysis. The hearts were removed, frozen in liquid nitrogen, and preserved at -80 °C.

Heart lysate preparation

Rat hearts were chopped and homogenized on ice using a homogenizer (Witeg Homogenizer HG-16D) in buffer (1 M

Tris, 1.5 M NaCl, 0.1 M EDTA; 10% glycerol, 1% Triton X-100, pH 7.4) using Complete ULTRA protease inhibitor cocktail tablets (Roche, Mannheim, Germany). Homogenates were incubated at 4 °C for 1 h with steady rotation before being ultracentrifuged for 20 min at 100,000 × g, and the resulting lysate was stored at -80 °C, as we previously described [20].

Protein extraction from the heart plasma membrane

Plasma membranes were prepared using the method described by Luiken et al. [21]. Rat hearts were cut on ice and incubated for 30 min in a high-salt solution (20 mM HEPES, 2 M NaCl, and 5 mM sodium azide, pH 7.4) at 4 °C. Following the incubation period, the suspension was centrifugation at 1,000 × g for 5 min. The pellet was homogenized on ice using a Witeg Homogenizer in a TES buffer containing 20 mM Tris, 250 mM sucrose, and 1 mM EDTA at pH 7.4, supplemented with complete ULTRA protease inhibitor cocktail tablets from Roche (Mannheim, Germany). After homogenization, the homogenate was centrifuged a few times, and the isolated plasma membrane fraction was stored at -80 °C, as we previously described [20].

Measurement of serum IGF-1 concentration

The IGF-1 concentration in serum was measured using the radioimmunoassay method with a commercially available kit and IGF-1 standards. The method involved IGF-1 labelled with ^{125}I and polyclonal rabbit antibodies to IGF-1, as was shown previously [22]. The results for IGF-1 were expressed in nM.

Na^+/K^+-ATPase assay

The Na^+/K^+-ATPase assay was done in two sets of epruvetes, whereas one set contained 50 µl of incubation mixture (2 M NaCl, 400 mM KCl, 100 mM $MgCl_2$, 1 M Tris pH 7.4 and ddH_2O), 105 µl of ddH_2O and 25 µl of the sample (1 µg/µl), while other set contained 50 µl of incubation mixture, 20 µl of ouabain, 85 µl of ddH_2O and 25 µl of the sample (1 µg/µl), as we previously described [20].

SDS-PAGE and Western blotting

Total protein lysates and plasma membrane protein extracts (40 µg per well) were separated using SDS-polyacrylamide gel electrophoresis and then transferred to polyvinylidene difluoride membranes. Membranes were further blocked in 5% bovine serum albumin. Heart lysate fractions were probed with antibodies (Cell Signaling) directed IRS, phospho-IRS (Ser^{307}) and phospho-IRS (Tyr^{1222}), PDK-1 and phospho-PDK-1 (Ser^{241}) Akt and phospho-Akt (Ser^{473}), mTOR, phospho-mTOR (Ser^{2481}) and phospho-mTOR (Ser^{2448}), S6K and phospho - S6K (Ser^{241}). Plasma membrane fractions were probed with antibodies against the phosphorylated (Ser^{23}) form of α subunit of Na^+/K^+-ATPase, α_1 and α_2 subunit of Na^+/K^+-ATPase (Santa Cruz Biotechnology). After the incubation, membranes were washed and incubated with the appropriate secondary HRP-conjugated anti-rabbit or anti-mouse secondary antibody (Santa Cruz Biotechnology). After 2 h of incubation with secondary antibodies, membranes were washed and used for subsequent detection using an ECL method. Membranes incubated with phosphorylated proteins were stripped and reblotted with antibodies directed against non-phosphorylated forms of the same proteins. All membranes were probed with mouse anti-actin monoclonal antibody (Santa Cruz Biotechnology) and HRP-conjugated secondary antibody as loading control. The signals were quantified using Image J 1.45s software (National Institutes of Health, USA).

Quantitative real-time PCR (qPCR)

According to the manufacturer's instructions, RNA was extracted from the heart tissue using a Trizol reagent (Life Technologies, USA). RNA purity and concentrations were measured using BioSpec-nano-Spectrophotometer (Shimazu, USA). RNA sample degradation was checked using 1.2% agarose electrophoresis. cDNK was synthesized with commercially available RevertAid H minus First Strand cDNK Synthesis Kit (Thermo Scientific, USA), using 1 µg of heart RNA and following the manufacturer's instructions. The quantitative polymerase chain reaction (qPCR) assay was conducted using the 7500 Real-Time PCR System (Applied Biosystems) in 96-well reaction plates (MicroAmp Optical, ABI Foster City, CA Each well contained 10 µl of reaction mix (Brilliant III Ultra-Fast SYBR Green) and appropriate sample volume and pairs of primers (forward and reverse) resupplied to total volume of 20 ml with demineralized, RNase-free water. The primers were designed using the Primer Express1 software v2.0 from Applied Biosystems. These are the forward primer 5′-CACGGCCTTCTTTGTCAGTA-3′ and the reverse primer 5′-TTGTTCTTCATTCCCTGCTG-3′ for the α_1 subunit of Na^+/K^+-ATPase (GenBank accession number: NM_031144). Furthermore, the PCR product length for the rat β-Actin gene (GenBank accession number: NM_031144) was 76 bp, and the forward primer was 5′-CCCTGGCTCCT AGCACCAT-3′, while the reverse primer was 5′-GAGCCAC CAATCCACACAGA-3′. The conditions for the α_1 subunit of Na^+/K^+-ATPase were 95 °C for 3 min, which was followed by 40 cycles run for 15 s at 95 °C and 32 s at 61 °C. Once the reaction ended, the $2^{-\Delta\Delta Ct}$ technique was used to analyze the relative quantification of mRNA expression, and the cycle

threshold values (Ct) were calculated. Next, the α_1 subunit of Na^+/K^+-ATPase 's expression level was adjusted relative to the β-Actin gene found in the same sample.

Statistical analysis

The values were presented as mean ± SEM. Student's t-test for independent samples and Pearson parametric correlation was used to assess the significance of differences and correlations between groups. Statistical analysis was performed using the SPSS program for Windows (SPSS, Chicago, IL, USA), with p-values < 0.05 considered significant.

Results

Anthropometric and biochemical parameters

The results of anthropometric and biochemical parameters are presented in Table 1. First, we assessed the effects of IGF-1 treatment on body mass, heart mass, body mass differential, and heart/body mass ratio. After bolus injection of IGF-1, there was no significant change in body and heart mass, body mass differential, or heart/body mass ratio as compared to control animals. Following the IGF-1 treatment, we assessed IGF-1 concentration in rat serum 24 h after the bolus injection. The results show that IGF-1 concentrations in the serum of IGF-1-treated animals were higher than in control rats ($p < 0.01$).

In vivo effects of IGF-1 on rat cardiac Na^+/K^+-ATPase activity and expression

To deepen our comprehension of the in vivo impact of IGF-1 on the Na^+/K^+-ATPase regulation, we evaluated the activity, protein phosphorylation, and protein expression of the cardiac Na^+/K^+-ATPase subunits. First, we looked at the dynamic characteristics of cardiac Na^+/K^+-ATPase: activity and subunit phosphorylation. IGF-1 treatment significantly increased Na^+/K^+-ATPase activity ($p < 0.05$) (Fig. 1a), and the ratio p-α Na^+/K^+-ATPase (Ser^{23})/total α_1 Na^+/K^+-ATPase ($p < 0.05$) (Fig. 1b). We examined Na^+/K^+-ATPase subunits α_1 and α_2 protein expression in the cardiac plasma membrane extract. IGF-1-treated rats had significantly higher protein expression of α_1 and α_2 Na^+/K^+-ATPase subunits ($p < 0.01$ and $p < 0.05$, respectively) in their hearts compared to control rats (Fig. 1c and d). To further understand how IGF-1 regulates Na^+/K^+-ATPase, we investigated the mRNA expression of the α_1 subunit in rat hearts. IGF-1 therapy significantly raised the expression of α_1 subunit mRNA in rat hearts compared to control rats by 5.6 fold ($p < 0.001$) (Fig. 1e).

Table 1 Anthropometric and biochemical parameters

	Experimental groups		Significance
Parameters	Control	IGF-1	p
Body mass before treatment [g]	512 ± 17	502 ± 15	n.s.
Body mass after treatment [g]	520 ± 16	514 ± 13	n.s.
Body mass difference [g]	8 ± 2	12 ± 4	n.s.
Heart mass [g]	1.46 ± 0.10	1.42 ± 0.04	n.s.
Heart/body mass ratio	0.0028 ± 0.0001	0.0027 ± 0.0001	n.s.
IGF-1 [nM] in serum	95 ± 4	118 ± 4	**< 0.01**

IGF-1 – Insulin-like growth factor 1, n - number of animals; n.s.- non-significant. The data shown represent mean ± SEM

In vivo effects of IGF-1 on IRS-1, PDK-1, and Akt phosphorylation in rat heart

The following experiments were conducted to evaluate the role of the IRS-1/PDK-1/Akt signalling pathway in IGF-1-regulated Na^+/K^+-ATPase. IGF-1 treatment resulted in enhanced IRS-1 phosphorylation on Tyr^{1222} ($p < 0.01$) and decreased IRS-1 phosphorylation on Ser^{307} ($p < 0.01$) (Fig. 2a and b). Additionally, IGF-1 treatment significantly elevated PDK-1 phosphorylation on Ser^{241} ($p < 0.05$) and Akt phosphorylation on Ser^{473} ($p < 0.05$) compared to the control group (Fig. 3a and b).

IGF-1 effects on mTOR and S6K phosphorylation in the rat heart

The study also investigated the mTOR involvement in Na^+/K^+-ATPase regulation by IGF-1 through the phosphorylation of mTOR on Ser^{2481} and Ser^{2448}. IGF-1 treatment significantly elevated mTOR phosphorylation on Ser^{2448} ($p < 0.01$) and Ser^{2481} ($p < 0.05$) (Fig. 4a and b). Furthermore, our study showed that IGF-1 increased the degree of S6K (Thr^{421}/Ser^{424}) phosphorylation ($p < 0.05$) in rat cardiac lysate compared to non-treated control rats (Fig. 4c).

Correlation between the concentration of IGF-1 in serum with Na^+/K^+-ATPase activity and phosphorylation of mTOR on Ser^{2448}

A correlation has been noted between the serum IGF-1 concentration and the Na^+/K^+-ATPase activity and phosphorylation of mTOR (Fig. 5). The Na^+/K^+-ATPase activity shows a significant ($p < 0.05$) correlation ($r = +0.607$) with the concentration of IGF-1 in serum (Fig. 5a). The concentration of IGF-1 and mTOR phosphorylation on Ser^{2448} show a significant positive correlation ($r = +0.646$, $p < 0.05$) (Fig. 5b).

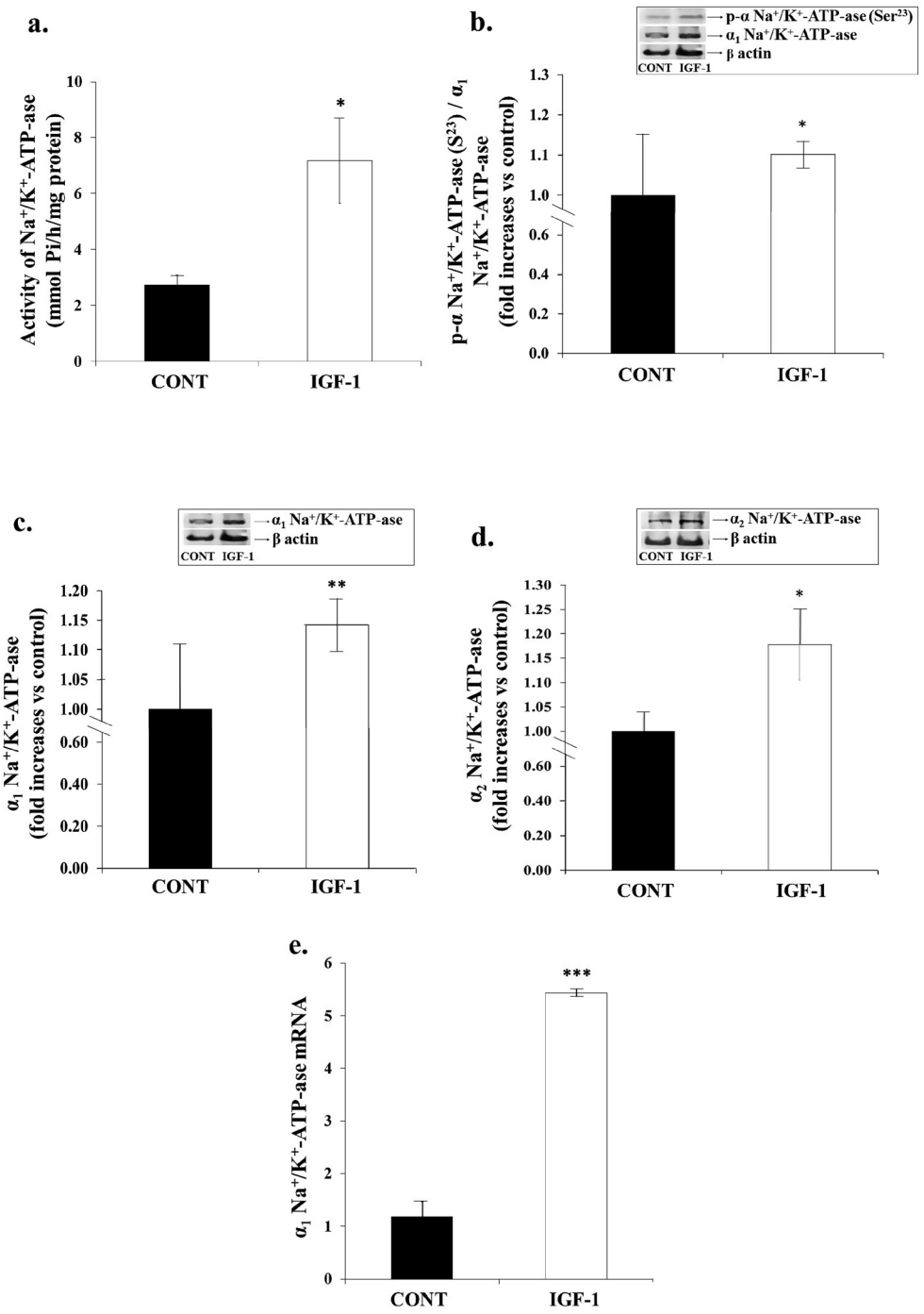

Fig. 1 Effects of IGF-1 on Na^+/K^+-ATPase activity and expression. (**a**) Specific activities of Na^+/K^+-ATPase are expressed in mmol P_i/h/mg of protein and represent mean ± SEM. (**b**) Western blot densitometry results (each bar is mean ± SEM). The y-axis represents Na^+/K^+-ATPase phosphorylated on Ser^{23} (p-α Na^+/K^+-ATPase) as fold changes vs. total α_1 Na^+/K^+-ATPase (CONT: arbitrarily set at 1), and the x-axis represents treatment. Inserts: representative western blots. (**c**) Western blot densitometry results (each bar is mean ± SEM). The y-axis represents α_1 Na^+/K^+-ATPase protein level as fold increase vs. control (CONT: arbitrarily net at 1), and the x-axis represents treatment. Inserts: representative western blots. (**d**) Western blot densitometry results (each bar is mean ± SEM). The y-axis represents α_2 Na^+/K^+-ATPase protein level as fold increase vs. control (CONT: arbitrarily set at 1), and the x-axis represents treatment. Inserts: representative western blots. (**e**) mRNA expression of α_1 subunit of Na^+/K^+-ATPase. The results for phosphorylation and expression are expressed as a percentage of the value obtained for the control. CONT-control group, IGF-1 - IGF-1-treated group, Na^+/K^+-ATP-ase - sodium/potassium adenosine triphosphatase, $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

Correlation between the activity of Na^+/K^+-ATPase with phosphorylation of IRS-1 and mTOR

A significant correlation ($r = +0.712$, $p < 0.05$) was detected between Na^+/K^+-ATPase activity and IRS-1 phosphorylation on Tyr^{1222} (Fig. 6a). Furthermore, we observed a significant ($p < 0.01$) positive correlation ($r = +0.841$) between Na^+/K^+-ATPase activity and phosphorylation of mTOR on Ser^{2448} (Fig. 6b).

Discussion

It is well documented that IGF-1, produced locally by cardiomyocytes, exerts a key physiological role in the heart via multiple processes that promote cardiomyocyte survival and proliferation [1]. One of the important actions of IGF-1 includes the simulative effects on Na^+/K^+-ATPase function that was established in vitro [12, 17, 18]. IGF-1 has been demonstrated to stimulate

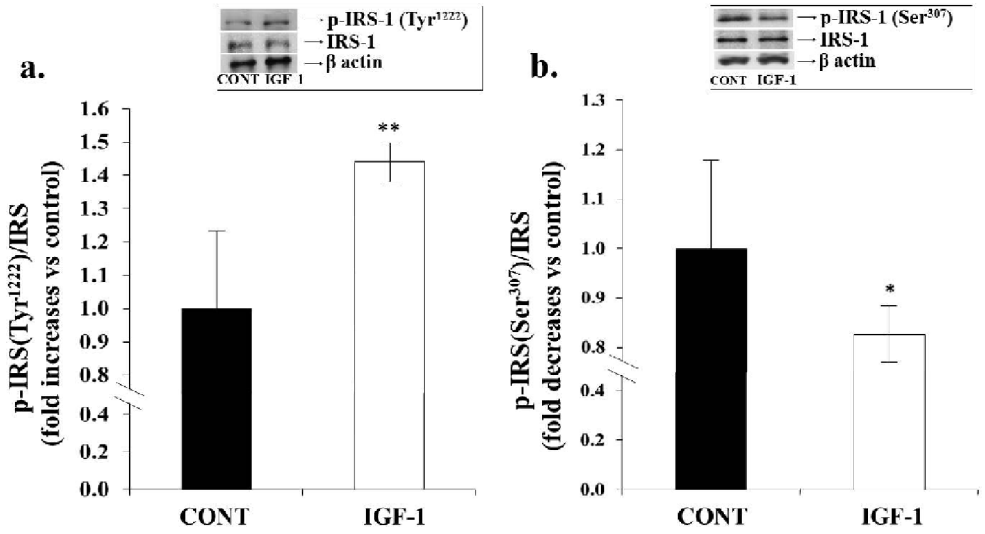

Fig. 2 Effects of IGF-1 on the phosphorylation of IRS-1 on Tyr^{1222} and Ser^{307} (**a**) Western blot densitometry results (each bar is mean ± SEM). The y-axis represents IRS-1 phosphorylated on Tyr^{1222} as fold changes vs. total IRS-1 (CONT: arbitrarily set at 1), and the x-axis represents treatment. Inserts: representative western blots. (**b**) Western blot densitometry results (each bar is mean ± SEM). The y-axis represents IRS-1 phosphorylated on Ser^{307} as fold changes vs. total IRS-1 (CONT: arbitrarily set at 1), and the x-axis represents treatment. Inserts: representative western blots. The results for phosphorylation and expression are expressed as a percentage of the value obtained for the control. CONT-control group, IGF-1 - Insulin-like growth factor 1-treated group, IRS-1 - Insulin receptor substrate – 1, Tyr-tyrosine, Ser-serine, $*p < 0.05$, $**p < 0.01$

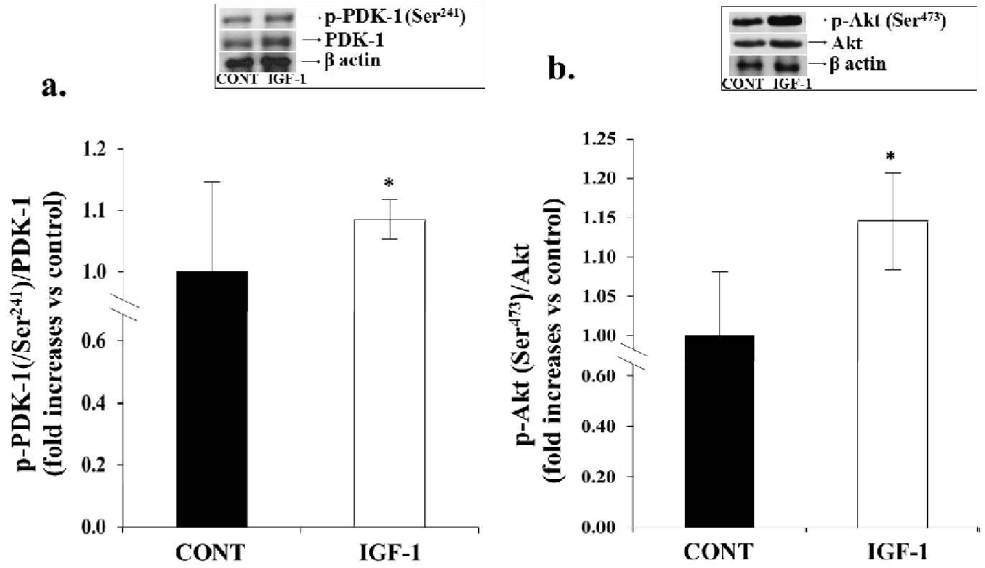

Fig. 3 Effects of IGF-1 on the phosphorylation of PDK1 on Thr^{421}/Ser^{241} and Akt on Ser^{473} in cell lysates of rat heart (**a**) Western blot densitometry results (each bar is mean ± SEM). The y-axis represents PDK-1 phosphorylated on Thr^{421}/Ser^{241} as fold changes vs. total PDK-1 (CONT: arbitrarily set at 1), and the x-axis represents treatment. Inserts: representative western blots. (**b**) Western blot densitometry results (each bar is mean ± SEM). The y-axis represents Akt phosphorylated on Ser^{473} as fold changes vs. total Akt (CONT: arbitrarily set at 1), and the x-axis represents treatment. Inserts: representative western blots. The results for phosphorylation and expression are expressed as a percentage of the value obtained for the control. CONT-control group, IGF-1 - Insulin-like growth factor 1-treated group, Akt – protein kinase B, Thr-threonine, Ser-serine, $*p < 0.05$

Na^+/K^+-ATPase activity in VSMC in vitro [18], implying that locally produced IGF-1 has a vasodilatory role via autocrine/paracrine activities [23]. Literature data indicate that IGF-1 enhances blood circulation by acting as a vasodilator [1, 23].

The IGF-1 produced locally can function as an autocrine and/or paracrine agent, promoting vasodilation through activation of Na^+/K^+-ATPase, which elevates the Na^+ gradient across the membrane, leading to Ca^{2+} efflux via Na^+/Ca^{2+} exchange [24, 25]. Alterations in the transcriptional and

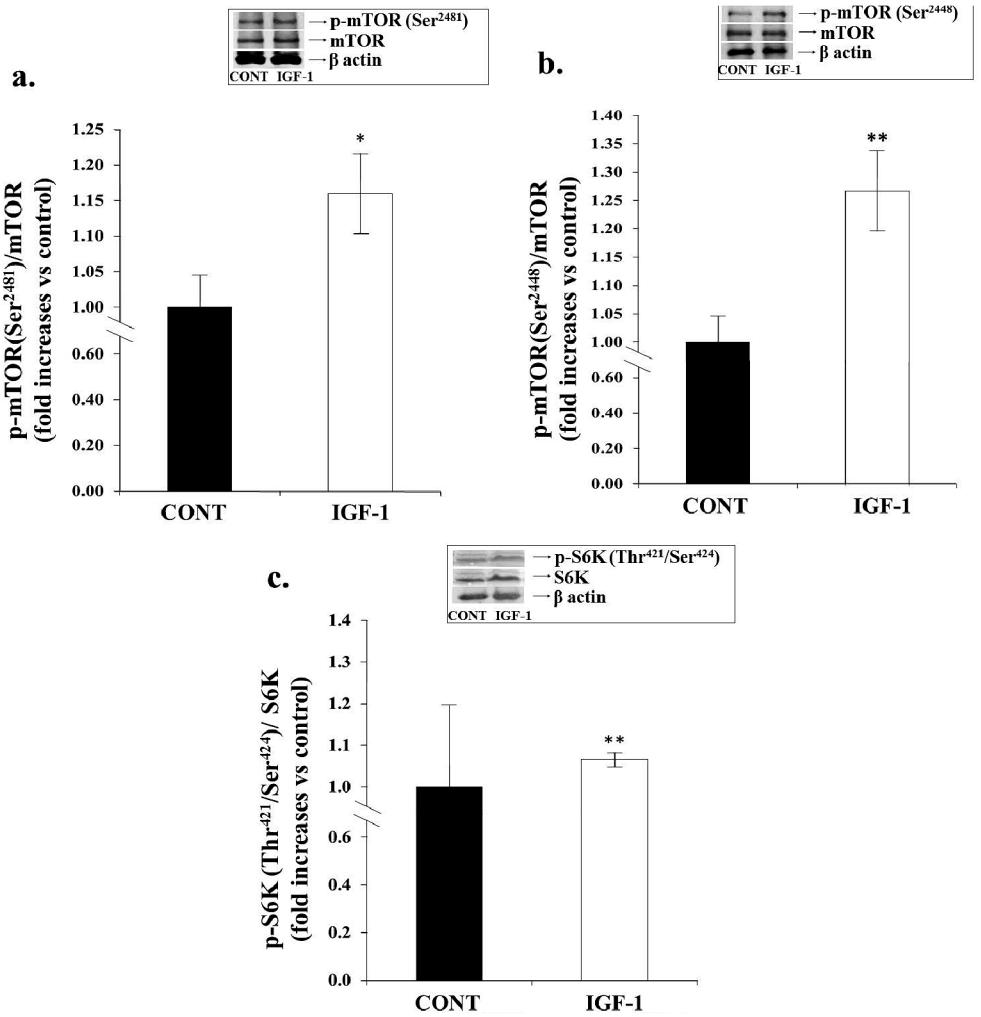

Fig. 4 Effects of IGF-1 on the phosphorylation of mTOR on $Ser^{2448}$and Ser^{2481} and S6K on Thr^{421}/Ser^{424}. (**a**) Western blot densitometry results (each bar is mean ± SEM). The y-axis represents mTOR phosphorylated on Ser^{2448} as fold changes vs. total mTOR (CONT: arbitrarily set at 1), and the x-axis represents treatment. Inserts: representative western blots. (**b**) Western blot densitometry results (each bar is mean ± SEM). The y-axis represents mTOR phosphorylated on Ser^{2481} as fold changes vs. total mTOR (CONT: arbitrarily set at 1), and the x-axis represents treatment. Inserts: representative western blots. (**c**) Western blot densitometry results (each bar is mean ± SEM). The y-axis represents S6K kinase phosphorylated on Thr^{421}/Ser^{424} as fold changes vs. total S6K (CONT: arbitrarily set at 1), and the x-axis represents treatment. Inserts: representative western blots. The results for phosphorylation and expression are expressed as a percentage of the value obtained for the control. CONT-control group, IGF-1 – Insulin-like growth factor 1-treated group, mTOR - Mammalian target of rapamycin, S6K - ribosomal protein p70 S6 kinase, Thr-threonine, Ser-serine, $*p < 0.05$, $**p < 0.01$

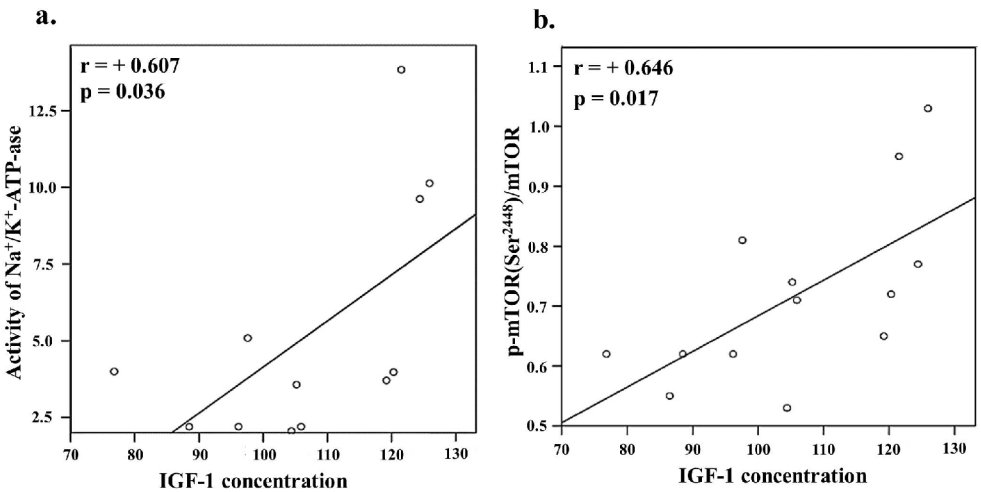

Fig. 5 Correlation between the concentration of IGF-1 in serum with Na^+/K^+-ATPase activity and phosphorylation of mTOR (**a**) Correlation between the concentration of IGF-1 in serum and Na^+/K^+-ATPase activity. (**b**) Correlation between the concentration of IGF-1 and changes in phosphorylation of mTOR on Ser^{2448}. IGF-1 – Insulin-like growth factor-1, Na^+/K^+-ATP-ase - sodium/potassium adenosine triphosphatase, mTOR - Mammalian target of rapamycin, Ser-serine

translational profiles of Na^+/K^+-ATPase isoforms have been documented in numerous tissues in response to diverse agonists and disease conditions [9, 26]. The IGF-1 was reported to selectively induce the α_1 isoform in astrocytes [27], an effect that tyrosine kinase inhibitors could block. Different hormones regulate Na^+/K^+-ATPase activity, stimulating cardiomyocytes' function [28]. The literature data regarding the effect of IGF-1 on different subunits' expression of the Na^+/K^+-ATPase in the heart is limited. Most studies mainly explore the α_1 subunit expression or

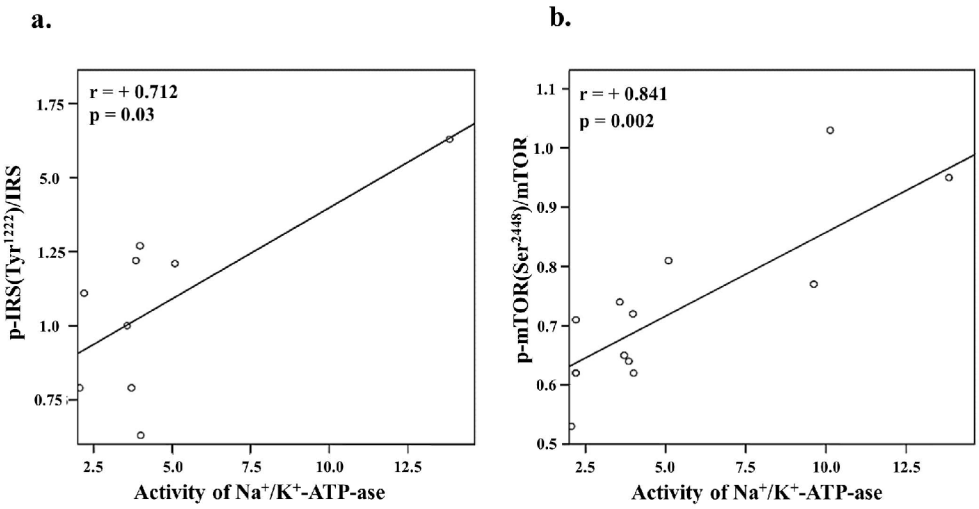

Fig. 6 Correlation between the activity of Na^+/K^+-ATPase with phosphorylation of IRS-1 and mTOR. (**a**) Correlation between the activity of Na^+/K^+-ATPase and phosphorylation of IRS-1 on Tyr^{1222}. (**b**) Correlation between the Na^+/K^+-ATPase activity and phosphorylation of mTOR on Ser^{2448}. IRS-1 - Insulin receptor substrate – 1, Na^+/K^+-ATP-ase - sodium/potassium adenosine triphosphatase, mTOR - Mammalian target of rapamycin, Tyr-tyrosine, Ser-serine

activity of Na^+/K^+-ATPase under IGF-1 treatment [12, 17, 18]. However, the current study detected a significant increase in α_1 protein and mRNA and α_2 protein expression in IGF-1-treated rats. In addition, we found that the concentration of IGF-1 in serum increases in IGF-1-treated rats, which positively correlated with Na^+/K^+-ATPase activity. Our results also showed that treating rats with IGF-1 increases cardiac Na^+/K^+-ATPase activity and phosphorylation of the α subunit of Na^+/K^+-ATPase on Ser^{23}. These results align with in vitro studies where IGF-1 positively affects the expression and activity of Na^+/K^+-ATPase [12, 17, 18]. In addition, oral administration of IGF-1 (3.5 mg/kg/day for 4 days) increases Na^+/K^+-ATPase activity in the enterocytes of pigs [29]. Our results indicate that IGF-1 stimulates not only Na^+/K^+-ATPase activity present on the plasma membrane but also increases the number of available Na^+/K^+-ATPase molecules since phosphorylation of α_1 subunit of Na^+/K^+-ATPase on Ser^{23} stimulates subunit trafficking from intracellular compartments to the plasma membrane [30]. Furthermore, two separate investigations examined the impact of IGF-1 on the regulation of Na^+/K^+-ATPase and observed stimulatory effects of IGF-1 on Na^+/K^+-ATPase activity in salmon gills [31, 32]. Additionally, Shimomura et al. discovered a positive correlation between serum IGF-1 levels and Na^+/K^+-ATPase activity in the gills of non-treated salmon [32], a similar correlation observed in our study.

The stimulative effects of IGF-1 in the heart are mediated via multiple signalling pathways, whereas IRS/PI3K/Akt is one of the significant pathways [1, 33]. The binding of IGF-1 to one of the IGF receptors at the plasma membrane leads to receptor autophosphorylation, which provides docking sites for IRS-1 molecules [11]. Further, phosphorylated IRS-1 initiates PI3K/Akt phosphorylation and activation of downstream signalling molecules mTOR/S6K [1]. The IRS molecules have multiple phosphorylation sites of serine, threonine, and tyrosine residues, that when phosphorylated lead to IRS activation or deactivation [34]. The IRS-1 phosphorylation on Ser^{307} leads to its inhibition and has an essential role in insulin resistance development [35], and IRS-1 phosphorylation on Tyr^{1222} provides its activation [36]. Our results showed decreased phosphorylation of IRS-1 on Ser^{307} and increased phosphorylation on Tyr^{1222} in the hearts of IGF-1-treated rats. In addition, our results show that the activity of Na^+/K^+-ATPase is positively correlated with stimulative phosphorylation sites of IRS-1 on Tyr^{1222}, implying the involvement of IRS-1 in IGF-1 regulation of the Na^+/K^+-ATPase activity.

Activated IRS-1 acts as a protein scaffold for the recruitment and activation of downstream proteins such as PI3K/Akt [37]. Considering its significant role, Akt is tightly regulated by several phosphorylation sites, whereas phosphorylation on Ser^{473} is required for its maximal activation [38, 39]. Kim and Park have reported that IGF-1 induces Akt phosphorylation in human neuroblastoma cells exposed to highly potent and selective PDK-1 inhibitors [40]. This aligns with our results showing that IGF-1 treatment increased PDK-1 phosphorylation on Ser^{241} in rat hearts. Also, according to Hart and Vogt's study [38], phosphorylation of Akt was increased in endothelial cells after stimulation with IGF-1 (50 ng/ml). Following treatment with IGF-1 at a concentration of 100 nM, our earlier research demonstrated that VSMC exhibited elevated levels of Akt phosphorylation on Ser^{473} and Na^+/K^+-ATPase activity [12]. Also, we have previously shown that IRS/PI3K/Akt signalling is involved in the up-regulation of cardiac Na^+/K^+-ATPase expression/activity of rats treated with estradiol [20]. A recent study showed that elevated levels of Akt phosphorylation lead to increased Na^+/K^+-ATPase α_1 and β_1 subunit protein expression in rat models of acute lung injury and alveolar epithelial cells (both in vivo and in vitro) after treatment with maresin

conjugates in tissue regeneration 1 [41]. Results of our study are consistent with these results as we showed that IGF-1 treatment increased phosphorylation of Akt on Ser^{473}, simultaneously with increased expression and activity of Na^+/K^+-ATPase.

The IGF-1-dependent Akt activation was shown to be involved in mTOR stimulation as a master regulator of many cell processes, including cell growth, proliferation and survival [42]. Thus, we further examined the participation of mTOR in IGF-1-induced stimulation of Na^+/K^+-ATPase. Phosphorylation of multiple sites on mTOR induces its activation under physiological conditions, with Ser^{2448} and Ser^{2481} being the most critical sites for kinase activity [43]. Additionally, the phosphorylation of Ser^{2448} is activated by Akt, while the phosphorylation of Ser^{2481} is considered an autocatalytic mTOR site [44]. The mTOR phosphorylated on Ser^{2448} is mainly involved in cell growth and proliferation, while the mTOR phosphorylated on Ser^{2481} acts as an Akt activator (Ser^{473} phosphorylation) [45]. Our results showed increased mTOR phosphorylation on both sites, Ser^{2448} and Ser^{2481}, in the hearts of IGF-1-treated rats, indicating IGF-1-induced stimulation of mTOR. Wang et al. showed that the treatment with L-Tryptophan activated mTOR and enhanced mRNA expression of α_1 subunit of Na^+/K^+-ATPase in intestinal epithelial cells, indicating the involvement of mTOR in Na^+/K^+-ATPase stimulation [46]. Results of correlation analysis show that the concentration of IGF-1 in serum positively correlates with phosphorylation of mTOR on Ser^{2448} and that the activity of Na^+/K^+-ATPase is positively correlated with simulative phosphorylation of mTOR on Ser^{2448}.

To gain more insight into IGF-1 effects on Na^+/K^+-ATPase, we further analyzed S6K, one of the main mTOR and Akt effectors responsible for protein synthesis [47]. Bakker et al. showed that IGF-1 treatment (1, 10 and 100 ng/ml) dose-dependently increased Akt and S6K phosphorylation in osteocytes, while mTOR inhibitor rapamycin suppressed this IGF-1 stimulatory effects [48]. The same inhibitory effect of rapamycin was shown in a study by Pesce et al. [15], one of the rare studies exploring the involvement of S6K activation on Na^+/K^+-ATPase regulation. The same authors proposed that the long-term mechanism of regulation of Na^+/K^+-ATPase by β-adrenergic agonist isoproterenol is mediated via PI3K activation and subsequent downstream activation of mTOR/S6K in alveolar epithelial cells [15]. Results from our study show that IGF-1 treatment leads to increased phosphorylation of S6K in the hearts of rats, which may be at least partially involved in stimulating the expression of Na^+/K^+-ATPase subunits. The principal new finding of the present study is that the induction of Na^+/K^+-ATPase by IGF-1 in vivo is mediated by a mechanism that involves IRS/PI3K/PDK/Akt/mTOR/S6K signalling pathway. These data extend our previous finding that IGF-1 stimulate Na^+/K^+-ATPase activity in vitro in VSMC and that this stimulation is mediated through a pathway involving PI3K [12]. To illustrate our findings, we provide the following model of Na^+/K^+-ATPase regulation by IGF-1 in the heart in physiological conditions (Fig. 7).

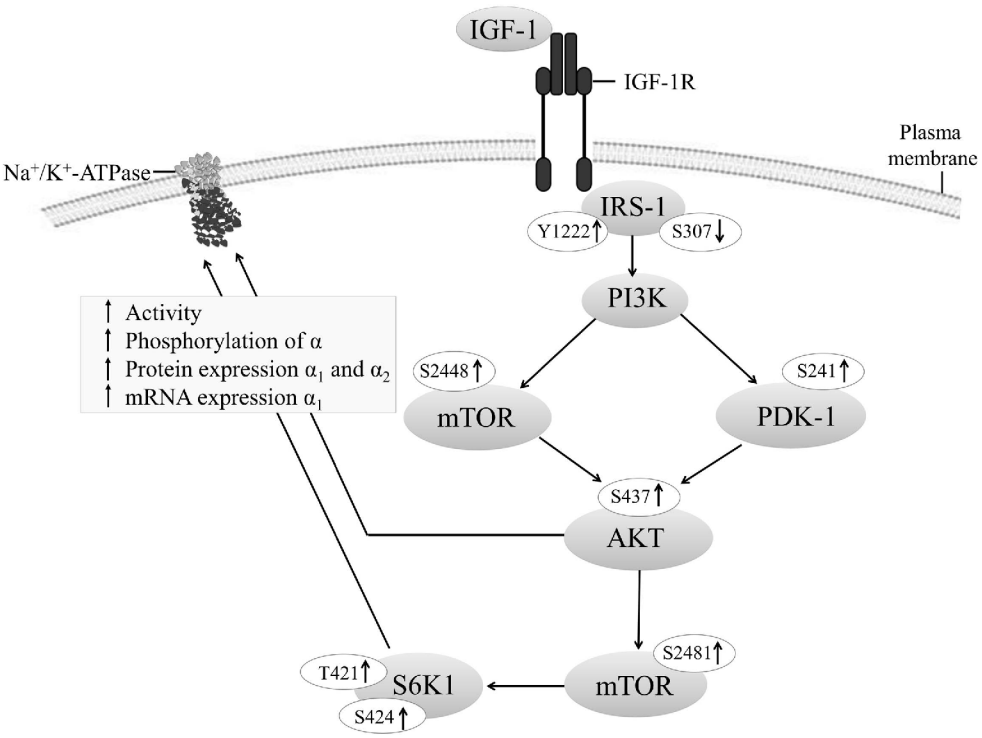

Fig. 7 Proposed mechanism of the in vivo effects of IGF-1 on Na^+/K^+-ATPase regulation in rat heart IGF-1 – Insulin-like growth factor-1, IGF-1R- IGF-1 receptor, IRS-1 - Insulin receptor substrate – 1, Y – Tyrosine amino acid, S – Serine amino acid, Na^+/K^+-ATP-ase - sodium/potassium adenosine triphosphates, PI3K - phosphatidylinositol-3 kinase, Akt - protein kinase B, mTOR - mammalian target of rapamycin, PDK – 1 - phosphoinositide-dependent kinase-1, S6K - ribosomal protein p70 S6 kinase, T – Threonine amino acid, ↑ increase

The IGF-1 binds to the receptor at the plasma membrane, which induces autophosphorylation and activation. Thus, the activated IGF receptor recruits and activates IRS-1 molecules, which further induces PI3K activation that stimulates downstream molecules PDK-1, Akt, mTOR and S6K, subsequently increasing Na^+/K^+-ATPase expression and activity in the heart.

Conclusion

The present study showed that in vivo treating rats with IGF-1 leads to increased Na^+/K^+-ATPase activity, phosphorylation of α subunit, and gene and protein expression of α subunits. Our results also indicate the involvement of IRS/PDK-1/Akt/mTOR/S6K pathway in cardiac Na^+/K^+-ATPase regulation under IGF-1 treatment. The results of this study represent the basis for further studies directed toward clarifying the molecular mechanisms by which IGF-1 affects cardiac Na^+/K^+-ATPase and for developing new therapeutics in cardiac diseases.

Abbreviations

Akt	protein kinase B
ATP	adenosine triphosphate
CVD	cardiovascular disease
IGF-1	insulin-like growth factor-1
IGF-1R	insulin-like growth factor-1 receptor
IR	insulin Receptor
IRS	insulin receptor substrate
mTOR	mammalian target of rapamycin
mRNA	messenger ribonucleic acid
Na^+/K^+ATPase	sodium/potassium adenosine triphosphatase
PCR	polymerase chain reaction
PDK-1	phosphoinositide-dependent kinase-1
PI3K	phosphatidylinositol-3 kinase
S6K	ribosomal protein p70 S6 kinase
VSMC	vascular smooth muscle cells

Acknowledgements This work is part of the collaboration between the Department of Radiobiology and Molecular Genetics, "VINČA" Institute of Nuclear Sciences - National Institute of the Republic of Serbia, University of Belgrade, Belgrade, Serbia and Computational Bioscience Research Center (CBRC), King Abdullah University of Science and Technology (KAUST), Thuwal 23955-6900, Kingdom of Saudi Arabia.

Author contributions Conception and supervision: M.O. and E.R.I.Performed the research: K.B., M.O., S.Z., and M.S.Interpretation or analysis of data: K.B., M.O., S.Z., Z.G. and E.R.I.Preparation of the manuscript: K.B., M.O., S.Z., M.E., Z.G., O.N. and E.R.I.All authors reviewed the manuscript.

Funding This work was funded by the Ministry of Science Technological Development and Innovation of the Republic of Serbia (Contract No#451-03-66/2024-03/200017) KAUST grant OSR#4129 (awarded to E.R.I).

Data availability The data supporting this study's findings are available from the corresponding author, [M.O.], upon reasonable request.

Declarations

Ethical approval The official Vinca Institute's Ethical Committee for Experimental Animals approved experimental protocols (Veterinary Directorate – No. 451-03-66/2024-03/ 200017).

Consent to participate Not applicable.

Competing interests The authors declare no competing interests.

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Insulin-like growth factor-1 reduces cardiac autosis through decreasing AMPK/FOXO1 signaling and Na^+/K^+-ATPase-Beclin-1 interaction

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Submitted: 12 October 2023; **Accepted:** 26 December 2023
Online publication: 12 January 2024

Arch Med Sci 2024; 20 (3): 1011–1015
DOI: https://doi/org/10.5114/aoms/177618

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Abstract

Introduction: Insulin-like growth factor-1 (IGF-1) promotes survival and inhibits cardiac autophagy disruption.
Methods: Male Wistar rats were treated with IGF-1 (50 µg/kg), and 24 h after injection hearts were excised. The level of interaction between Beclin-1 and the α_1 subunit of sodium/potassium-adenosine triphosphates (Na^+/K^+-ATPase), and phosphorylated forms of IGF-1 receptor/insulin receptor (IGF-1R/IR), forkhead box protein O1 (FOXO1) and AMP-activated protein kinase (AMPK) were measured.
Results: The results indicate that IGF-1 decreased Beclin-1's association with Na^+/K^+-ATPase ($p < 0.05$), increased IGF-1R/IR and FOXO1 phosphorylation ($p < 0.05$), and decreased AMPK phosphorylation ($p < 0.01$) in rats' hearts.
Conclusions: The new IGF-1 therapy may control autosis and minimize cardiomyocyte mortality.

Key words: Na^+/K^+-ATPase, association of Na^+/K^+-ATPase and Beclin-1, cardiac autosis, AMPK, FOXO1.

Understanding the correlation between autophagy rate and cardiomyocyte survival could be beneficial for preventing and treating cardiovascular diseases [1]. Uncontrolled autophagy can induce a type of cell death called autosis [2]. Different stress-induced conditions promote cardiomyocyte autosis and myocardial injury [3]. Some evidence points to the essential role of sodium/potassium-adenosine triphosphates (Na^+/K^+-ATPase) in autosis because, along with autophagy inhibitors, Na^+/K^+-ATPase antagonists prevent autosis [2, 4]. Moreover, Na^+/K^+-ATPase interacts with the autophagy protein Beclin-1 when autosis occurs [2, 4].

Insulin-like growth factor-1 (IGF-1) is one of the potent regulators of Na^+/K^+-ATPase activity, as demonstrated by us and other authors [5, 6]. The actions of IGF-1 are mediated through IGF receptors (IGFR), insulin receptor (IR), and a hybrid receptor composed of components from both the IGFR and the IR (IGFR/IR) [7]. The protein kinase B (Akt) pathway is recognized as one of the signaling pathways via which the effects of IGF-1 are mediated. The involvement of Akt in autophagy regulation is dominantly through the regulation of forkhead box O (FOXO) transcription factor [8]. Additionally, IGF-1 regulates nutrient-sensitive signaling molecules such

as AMP-activated protein kinase (AMPK), which is important in autophagy regulation [9]. FOXO1 and AMPK regulate Beclin-1, an essential autophagy molecule whose role depends on association with different proteins, enabling the cell response to diverse autophagy stimuli [10, 11]. Despite the ineffectiveness of therapeutic strategies targeting various types of cell death in treating myocardial injury, the suppression of autosis resulted in reduced infarct size, suggesting the importance of autosis regulation in stress-induced damage in the heart. Since signaling mechanisms involved in autosis are distinct from those governing apoptosis and necrosis, a comprehensive investigation of signaling pathways involved in autosis and the underlying mechanism by which IGF-1 influences autosis in cardiac tissue is of great importance. Furthermore, considering the autocrine/paracrine actions of locally produced IGF-1, examining the distinct signaling molecules involved in the IGF-1 transduction cascade, which are associated with the cardiomyocyte autosis, may yield valuable insights for developing novel therapeutic approaches.

This study hypothesized that IGF-1 might directly affect cardiac autosis by reducing Na^+/K^+-ATPase and Beclin-1 interaction by activating IGF-1R/IR and suppressing AMPK and FOXO1 signaling (Figure 1).

Methods. In order to test our hypothesis, we used the hearts of adult male Wistar rats, fed with a balanced diet for laboratory rats and water *ad libitum*. A total of 14 animals were divided into 2 groups (n = 7). Half of the rats were treated intraperitoneally with a bolus injection of 50 µg/kg of IGF-1 (Sigma-Aldrich) dissolved in saline (IGF-1 group), while another half of the rats were injected with saline (CONT group). After 24 h, animals were euthanized with 80 mg/kg of ketamine and 12 mg/kg xylazine. The hearts of the rats were excised and homogenized for further analysis. The Vinca Institute's official Ethics Committee for Experimental Animals approved the experimental treatment (Veterinary Directorate – No. 323-07-02710/2017-05).

The interaction of Beclin-1 with the α_1 subunit of $Na^{+/}K^+$-ATPase in the heart tissue of rats was measured by co-immunoprecipitation. Heart plasma membrane fractions (500 µg of protein) were incubated overnight at 4°C with 2 µg of antibody against Beclin-1 (Santa Cruz). Immunocomplexes were absorbed with protein A/G-Sepharose, incubated overnight at 4°C, and then recovered by centrifugation at maximum speed for 5 min. Pellets were centrifuged, washed three times with buffer, and separated on SDS-PAGE gel. After separation, immunocomplexes were transferred to the polyvinylidene difluoride (PVDF) membrane and probed with an antibody against the α_1 subunit of Na^+/K^+-ATPase (Santa Cruz). Membranes were subsequently washed, incubated with an appropriate secondary antibody and used for detection using an electrochemiluminescence method. Scanned films were quantified using Image J 1.45s software (National Institutes of Health, USA).

In order to obtain a deeper understanding of the mechanism via which IGF-1 regulates the interplay between Na^+/K^+-ATPase and Beclin-1 in the cardiac tissue of rats, we conducted an anal-

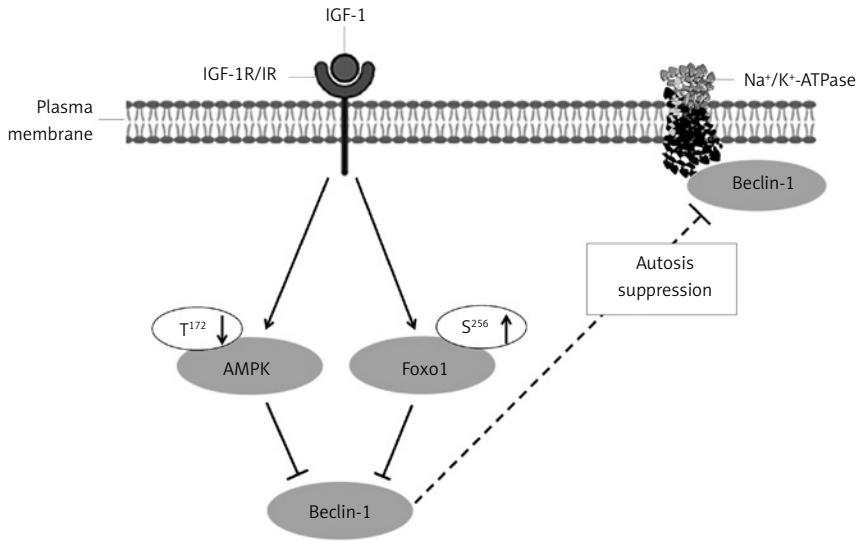

Figure 1. Potential mechanism for the *in vivo* effects of IGF-1 on the modulation of autosis in the rat heart. IGF-1 binds to the receptor at the plasma membrane, which induces autophosphorylation and activation. Activated receptor regulates downstream molecules such as AMPK and FOXO-1 and Beclin-1 and Na^+/K^+-ATPase interaction

AMPK – AMP-activated protein kinase, FOXO1 – forkhead box protein O1, IGF-1 – insulin-like growth factor-1, IGF-1R/IR – IGF-1 receptor β/insulin receptor β receptor, Na^+/K^+-ATP-ase – sodium/potassium adenosine triphosphatase, PI3K – phosphatidylinositol-3 kinase, S – serine, T – threonine, ↓ – decrease, ↑ – increase, ⊥ – inhibition.

ysis utilizing Western blotting to quantify the phosphorylation levels of IGF-1R/IR, FOXO1, and AMPK proteins. Total protein lysates and plasma membrane protein fractions were separated using SDS-PAGE and transferred to PVDF membranes. Membranes were probed with antibodies (Cell Signaling) directed against phospho-IGF-1Rβ (Tyr^{1131})/IR β (Tyr^{1146}), IR β, FOXO1 and phospho-FOXO1 (Ser^{256}), AMPK and phospho-AMPK (Thr^{172}). The blots were reprobed with mouse monoclonal anti-β-actin (Santa Cruz) antibody to ensure equal loading. The signals were quantified using Image J 1.45s software.

Statistical analysis. Results are presented as mean ± SEM. Statistical significance was evaluated by Student's *t*-test. The program SPSS for Windows (SPSS Inc., Chicago, IL, USA) was used for statistical analyses and $p < 0.05$ was considered significant.

Results. Autosis occurs in the heart in different physiological and pathophysiological conditions; therefore, suppression of autosis may reduce cardiomyocyte death and minimize myocardial injury. Although the molecular mechanism of autosis is currently under extensive research, the association of the autophagy protein Beclin-1 and Na^+/K^+-ATPase is required [2–4]. Our study was designed to explore the role of IGF-1 in autosis regulation and the potential mechanism of Na^+/K^+-ATPase engagement. Thus, we examined the *in vivo* effect of IGF-1 on the association of Beclin-1 with the α_1 subunit of Na^+/K^+-ATPase. The results showed that the association of Beclin-1 and the α_1 subunit of Na^+/K^+-ATPase was lower ($p < 0.05$) in IGF-1-treated rats compared to control rats (Figure 2 A). Since IGF-1 initiates a signaling pathway in the cell after binding to its receptor at the plasma membrane, some evidence suggests that IGF-1 binds to the IGF/IR hybrid receptor with high affinity, probably making it the dominant activator [7]. Thus, we investigated whether IGF-1 treatment affects the protein expression of p-IGF-1Rβ (Tyr^{1131})/IRβ (Tyr^{1146}). We found that the p-IGF-1R/IR / IR ratio was increased ($p < 0.05$) in IGF-1-treated rats compared to non-treated rats (Figure 2 B). Beclin-1 is a central factor of autophagy that forms complexes with different proteins and is regulated by various signaling molecules, including FOXO-1 and AMPK [12, 13]. We further examined the phosphorylation of FOXO1 and AMPK, important autophagy-related signaling molecules, in the heart of IGF-1-treated rats. The phosphorylation of FOXO1 on Ser^{256} leads to nuclear exclusion of the cytoplasm, resulting in the eventual inactivation of FOXO1. Our results indicate that the phosphorylation of FOXO1 on Ser^{256} was increased ($p < 0.01$) (Figure 2 C), while AMPK on Thr^{172} was decreased ($p < 0.01$) in IGF-1-treated rats compared to control rats (Figure 2 D).

Discussion. Following an injury, the heart has a limited potential for regeneration, increasing the risk of cardiomyocyte loss and decreased cardiac function. Due to the low regenerative capacity of cardiomyocytes, the important function of IGF-1 in promoting cell survival becomes evident in the heart, where diverse stresses can induce autosis and subsequent cardiomyocyte death [14]. Moreover, IGF-1 protects against the disruption of cardiac autophagy induced by various stressful conditions. Na^+/K^+-ATPase is a membrane protein that is required for normal cellular function. Cardiomyocyte function primarily relies on the activity of Na^+/K^+-ATPase. Additionally, Na^+/K^+-ATPase acts as a scaffolding and signaling molecule since exposing cells to Na^+/K^+-ATPase antagonists induces activation of various signaling pathways [15]. Tight regulation of Na^+/K^+-ATPase is significant in the heart since numerous pathologies correlate with aberrant Na^+/K^+-ATPase activity [15]. The literature data suggest that Na^+/K^+-ATPase plays a crucial role in the regulation of autophagy and is necessary for autosis to occur [3]. Autosis occurs in different stress-induced conditions, which subsequently lead to myocardial injury [3, 4]. In physiological conditions such as starvation or exercise, the interaction of Na^+/K^+-ATPase and the autophagy protein Beclin-1 was elevated, suggesting an increased autotic cell death rate. In addition, autosis occurs during the late phase of reperfusion following a period of ischemia, contributing to myocardial injury. Based on the results obtained from our study, it was observed that the interaction between Na^+/K^+-ATPase and Beclin-1 exhibited a reduction in the cardiac tissues of rats subjected to IGF-1 treatment. Considering that Na^+/K^+-ATPase and Beclin-1 association is essential for autosis, our results indicate that IGF-1 may be a potent antagonist for cardiac autosis.

In order to gain a better understanding of how IGF-1 regulates autosis, we conducted additional research into the potential mechanisms by which IGF-1 has this effect. Some data suggest that IGF-1 binds to IGF-1R/IR with high affinity, making it the primary activator of IGF-1 [7]. Our results indicate that exogenous IGF-1 recruits IGF-1R/IR by increasing its phosphorylation in the plasma membrane fraction of rat hearts. Because this particular form of the hybrid receptor is considered specific for IGF-1 [7], the influence on autosis in the hearts of IGF-1-treated rats is a consequence of this action. To gain more insight into the mechanism of autosis regulation by IGF-1, we further examined the involvement of FOXO1 and AMPK – key molecules involved in different autophagy stages – in IGF-1 regulation of autosis

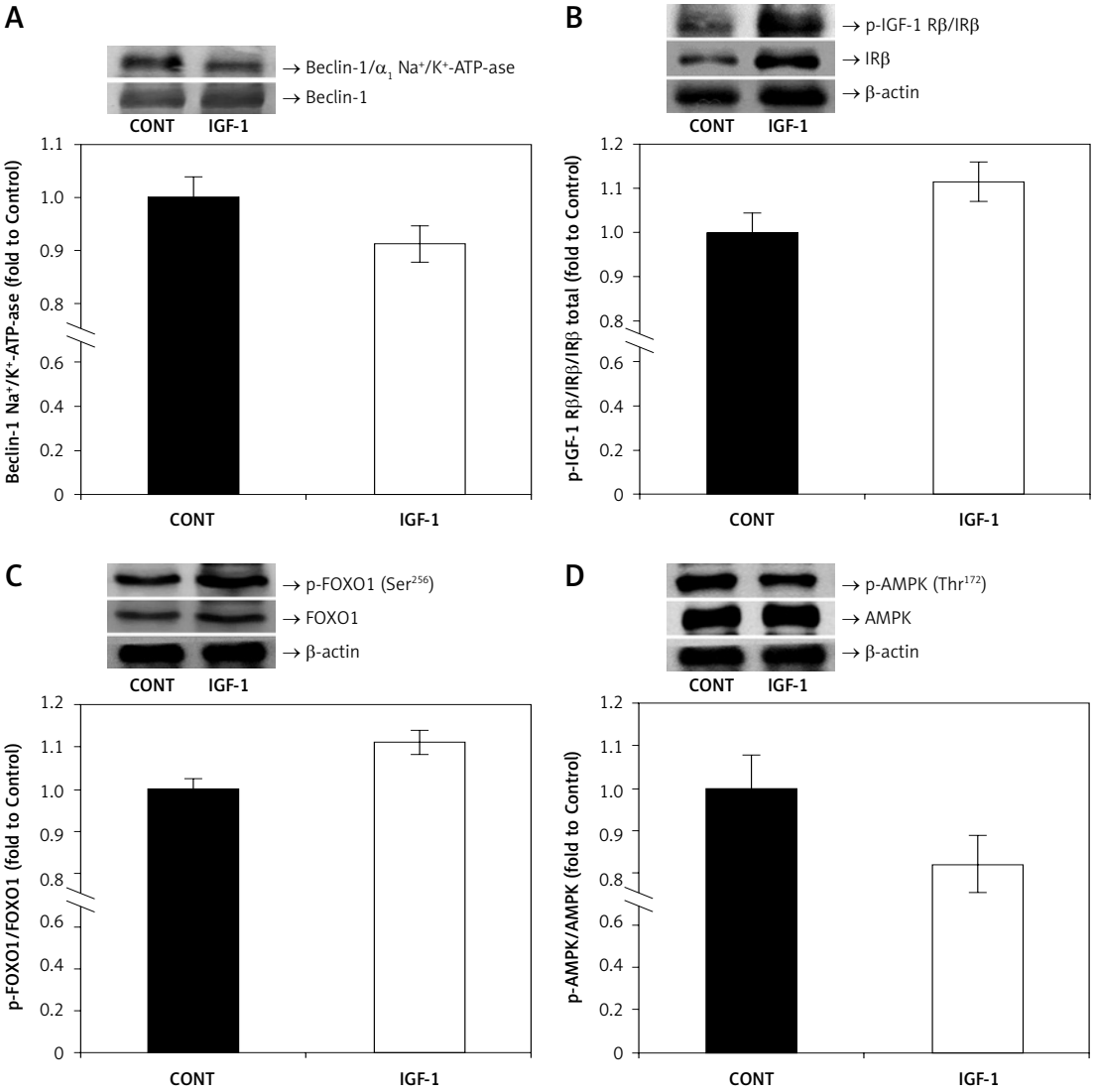

Figure 2. Effects of IGF-1 on the association of the α_1 subunit of Na^+/K^+-ATPase with Beclin-1 and phosphorylation of IGF-1R/IR, FOXO1 and AMPK in rat hearts. **A** – The *y*-axis represents the association level between Beclin-1 and Na^+/K^+-ATP-ase, and the x-axis represents treatment. Inserts: representative western blots. **B** – The *y*-axis represents p-IGF-Rβ/IRβ as fold changes vs. protein expression of IRβ, and the *x*-axis represents treatment. Inserts: representative western blots. **C** – The *y*-axis represents FOXO1 phosphorylated on Ser^{256} as fold changes vs. total FOXO1, and the *x*-axis represents treatment. Inserts: representative western blots. **D** – The y-axis represents AMPK phosphorylated on Thr^{172} as fold changes vs. total AMPK, and the *x*-axis represents treatment. Inserts: representative western blots. The results for phosphorylation and expression are expressed as a percentage of the value obtained for the control and represent mean ± SEM (CONT: arbitrarily set at 1)

*AMPK – AMP-activated protein kinase, CONT – control group, FOXO1 – forkhead box protein, IGF-1 – insulin-like growth factor-1 treated group, Na^+/K^+-ATP-ase – sodium/potassium adenosine triphosphatase, p-IGF-1Rβ/IRβ – phospho-IGF-I receptor β (Tyr^{1131})/insulin receptor β (Tyr^{1146}), *p < 0.05, **p < 0.01.*

[8–10]. Beclin-1 is a crucial component in autophagy; it forms complexes with various proteins, and its activity is controlled by several different signaling molecules, including AMPK and FOXO1 [10, 11]. In MDA-MB-231 cells treated with an autophagy stimulant (paclitaxel), knocking down FOXO1 expression decreased Beclin-1 gene expression and the autophagy rate [12]. According to these results, FOXO1 is vital in autophagy initiation and regulation of Beclin-1 expression and influences autosis. Based on our results showing that IGF-1 treatment decreased FOXO1 activity by phosphorylating it, we assume that this IGF-1 action also decreased Beclin-1's interaction with Na^+/K^+-ATPase, which affected autosis. According to other studies that confirmed the abolishing effect of IGF-1 on AMPK activity *in vitro* in diverse cells, the decreased AMPK activation in response to IGF-1 treatment shown in our study aligns with these findings [9, 13]. According to the current study's findings, the protective effect of IGF-1 in cardiomyocytes may be due to its ca-

pacity to reduce AMPK phosphorylation. This is significant because AMPK plays a vital role in regulating many physiological processes to maintain cardiomyocyte homeostasis, and its overexpression has been associated with heart failure [10]. Furthermore, a study with HEK293T cells, challenged with glucose starvation and pretreated with AMPK inhibitors, showed suppressed Beclin-1 activation [13]. These results suggest the importance of AMPK regulation of Beclin-1, and therefore in autosis, which can be related to our results showing that IGF-1 via decreasing AMPK activation reduced interaction of Na^+/K^+-ATPase with Beclin-1 and suppressed autosis [14].

Na^+/K^+-ATPase is considered as a promising therapeutic target [15]. Cardiotonic steroids are one of the most commonly used drugs that induce Na^+/K^+-ATPase inhibition and a subsequent positive inotropic effect. Cardiotonic steroids are used for treating various conditions such as persistent heart failure symptoms, heart rate management, hypertension, renal failure and cardiovascular disease. Among innovative drugs, the DRm217 antibody targets the DR extracellular region of the Na^+/K^+-ATPase α_1 subunit and enhances Na^+/K^+-ATPase activity, providing a protective effect against ischemic injury and cardiac remodeling. In addition, genetic silencing and pharmacological inhibition of Na^+/K^+-ATPase are deleterious for autosis *in vitro* [3].

Even though the requirement of Na^+/K^+-ATPase in autosis is established, additional investigation is necessary to unravel the molecular mechanisms of Na^+/K^+-ATPase involvement in autosis. Investigating the modulation of myocardial Na^+/K^+-ATPase activity and expression in the presence of different agents in animal models could contribute to better understanding of the molecular mechanisms underlying autosis associated with Na^+/K^+-ATPase. Since IGF-1 has positive effects on the cardiovascular system and is one of the most potent Na^+/K^+-ATPase stimulators, additional basic and human intervention studies are required in order to enhance understanding and elucidate the potential relevance and safety considerations of IGF-1 effects in autosis.

In conclusion, the current study is the first to investigate how exogenous IGF-1 impacts rat cardiac autosis by diminishing the association of Beclin-1 with the α_1 subunit of Na^+/K^+-ATPase in the rat heart. We propose that this outcome is achieved by activating IGF-1R/IR, a hybrid receptor, while simultaneously suppressing the signaling pathways of AMPK and FOXO-1. Thus, our results indicate that the administration of IGF-1 may help prevent autosis in cardiac tissue.

Funding

This work was funded by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contract No#451-03-47/2023-01/ 200017).

Ethical approval

Approval numer: 323-07-02710/2017-05.

Conflict of interest

The authors declare no conflict of interest.

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Peptides 190 (2025) 171418

Contents lists available at ScienceDirect

Peptides

journal homepage: www.elsevier.com/locate/peptides

IGF-1 contributes to cardiovascular protection in obesity by upregulating Na^+/K^+-ATPase activity and modulating key signaling pathways in rats on a high-fat diet

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ARTICLE INFO

Keywords:
Na^+/K^+-ATP-ase
HF diet
Obesity
IGF-1 treatment
Heart hypertrophy
Ang II

ABSTRACT

This study examined the ability of insulin-like growth factor-1 (IGF-1) to improve the expression and function of cardiac sodium/potassium adenosine triphosphatase (Na^+/K^+-ATPase) and reduce heart hypertrophy in obese rats. Adult male Wistar rats received a standard diet or a high-fat (HF) diet for 12 weeks. A bolus injection of IGF-1 (50 μg/kg, i.p.) was administered to half of the HF rats 24 hours before euthanasia. IGF-1 treatment increased: the activity of Na^+/K^+-ATPase and expression of phosphorylated and total Na^+/K^+-ATPase α_1 subunit, the phosphorylation of IGF-1 receptor β /insulin receptor β at Tyr^{1131}/Tyr^{1146}, insulin receptor substrate-1 (IRS-1) at Tyr^{1222}, mammalian target of rapamycin (mTOR) at Ser^{2481}, protein kinase B (Akt) at Ser^{473} and the expression of type-2 angiotensin II (AngII) receptor (AT_2R). Conversely, IGF-1 reduced the levels of IRS-1 phosphorylated at Ser^{307}, mTOR at Ser^{2448}, ribosomal protein p70 S6 kinase (S6K) at Thr^{421}/Ser^{424}, and the expression of type-1 Ang II receptor (AT_1R) in the heart, as well as the serum levels of Ang II in obese rats. IGF-1 treatment reduced cardiac mass and elevated mRNA expression of the α-myosin heavy chain (MHC), and the α/β MHC ratio in the hearts of obese rats. The results of this study suggest that the administration of IGF-1 to obese rats reduces the adverse effects of HF diet, potentially by lowering Ang II-mediated activation of mTOR/S6K and enhancing the IRS-1/Akt pathway, which promotes Na^+/K^+-ATPase activity in the heart and diminishes cardiac hypertrophy.

1. Introduction

Obesity is a chronic disease with numerous factors contributing to its development [1]. Excessive fat accumulation causes structural changes in the myocardium, leading to obesity-induced heart failure [2]. Increased body mass demands higher cardiac output and blood pressure, which enhances ventricular wall tension and leads to ventricular hypertrophy [2]. Overnutrition increases circulating angiotensin II (Ang II) levels, leading to systemic vasoconstriction and direct renal sodium and water retention, resulting in intravascular volume expansion and hypertension [3]. Besides these macro-pathological changes, obesity induces serious cardiac malfunctions on the molecular level, such as disturbed cardiomyocyte structure and metabolism [4]. Although the molecular mechanism of obesity-related cardiac disorders is not entirely clarified, some evidence points out the importance of sodium/potassium adenosine triphosphatase (Na^+/K^+-ATP-ase) alterations in this pathophysiology [5].

The Na^+/K^+-ATP-ase maintains the Na^+ and K^+ ion gradient across the plasma membrane, establishing plasma membrane potential, initiating action potentials, and regulating secondary transport in cardiomyocytes [6]. Numerous research groups, including ours, showed that activity and subunit expression of Na^+/K^+-ATP-ase are changed in the heart of different animal models of obesity, which eventually causes cardiovascular complications [7–9]. In addition, decreased

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https://doi.org/10.1016/j.peptides.2025.171418
Received 14 March 2025; Received in revised form 25 May 2025; Accepted 25 May 2025
Available online 27 May 2025

Na^+/K^+-ATP-ase activity was observed in various cell types isolated from obese patients [10,11]. Furthermore, reduced expression of Na^+/K^+-ATP-ase was found in endomyocardial biopsy specimens of patients with cardiac dysfunction, ischemic heart disease, and heart failure [12]. Contrary, the improvement of obesity-induced decreased Na^+/K^+-ATP-ase activity positively affects the cardiovascular system (CVS) [8,13].

Insulin-like growth factor-1 (IGF-1) is important and vital in CVS [14]. After IGF-1 binds to the extracellular domain of its receptor, it triggers its autophosphorylation. It initiates the activation of different signaling molecules, where the phosphatidylinositol-3 kinase/protein kinase B (PI3K/Akt) pathway is one of the main ones [15]. The protective effects of IGF-1 in the heart have been shown in spontaneously hypertensive rats, where the presence of IGF-1 improved cardiomyocyte contractility in hypertrophied hearts [16]. IGF-1 has a stimulatory effect on the survival of cardiac progenitor cells in obese mice, mitigating myocardial ischemia/reperfusion injury in rats via the PI3K/Akt signaling pathway [17,18]. Furthermore, earlier *in vitro* studies, including ours, showed that IGF-1 is a potent stimulator of Na^+/K^+-ATP-ase activity through the PI3K/Akt pathway in vascular smooth muscle cells (VSMC), implying that IGF-1 regulates Na^+/K^+-ATP-ase in an autocrine/paracrine manner in CVS *in vivo* [19,20]. Our recent study shows that IGF-1 *in vivo* stimulates cardiac Na^+/K^+-ATP-ase expression and activity through insulin receptor substrate-1 (IRS-1)/Akt/mammalian target of rapamycin (mTOR)/ribosomal protein p70 S6 kinase (S6K) signaling pathway in intact rats [21]. However, the regulation of cardiac Na^+/K^+-ATPase by IGF-1 in obesity remains unclear.

This study explored the ability of IGF-1 to improve the expression and activity of cardiac Na^+/K^+-ATPase and reduce obesity-induced heart hypertrophy. We hypothesized that IGF-1 may ameliorate Ang II-mediated activation of the mTOR/S6K pathway and suppress the IRS-1/Akt cascade in rats fed an HF diet. By upregulating Na^+/K^+-ATPase activity, IGF-1 might prevent ventricular hypertrophy, offering a valuable therapeutic approach to combat obesity-induced cardiac complications (Fig. 1).

2. Materials and methods

2.1. Rats and experimental procedure

Adult, 8-week-old, male Wistar rats were housed within cages at 22 ± 2 °C and in a 12-hour light-dark cycle. Tap water and food were available to rats *ad libitum*, whereas control rats (CONT n = 7) received a standard laboratory rat diet (made by Veterinarski zavod Subotica, Serbia). In comparison, HF rats (n = 14) were administered a HF diet supplemented with 42 % sunflower oil for 12 weeks (Table S1). This work utilized HF rats as an obesity model, corroborated by our prior research [22] and other studies [23,24] indicating that a HF diet effectively promotes obesity. Following the 12 weeks and 24 hours before the sacrifice, the HF group of rats was segregated into two subgroups: the initial group designated as HF + IGF-1 (n = 7) received an intraperitoneal bolus injection of IGF-1 (Sigma-Aldrich) in the dose of 50 μg/kg, dissolved in saline, while the second group identified as HF (n = 7) was administered saline. The specific dosage of IGF-1 was selected based on prior investigations [25]. After 24 hours following IGF-1 administration, animals were anaesthetized with a mixture of 80 mg/kg of ketamine (VetViva Richter GmbH, Austria) and 12 mg/kg of xylazine (VET-AGRO Multi trade Company Sp. z.o.o., Poland), and decapitated. The blood samples were collected through cardiocentesis. Serum was extracted from each blood sample and properly preserved before following experimental procedures. Furthermore, hearts were surgically extracted, quantified, cryopreserved, and properly stored. The experimental protocols have received approval from the Ethical Committee for Experimental Animals at the Vinca Institute (Veterinary Directorate – No. 323–07–02710/2017–05).

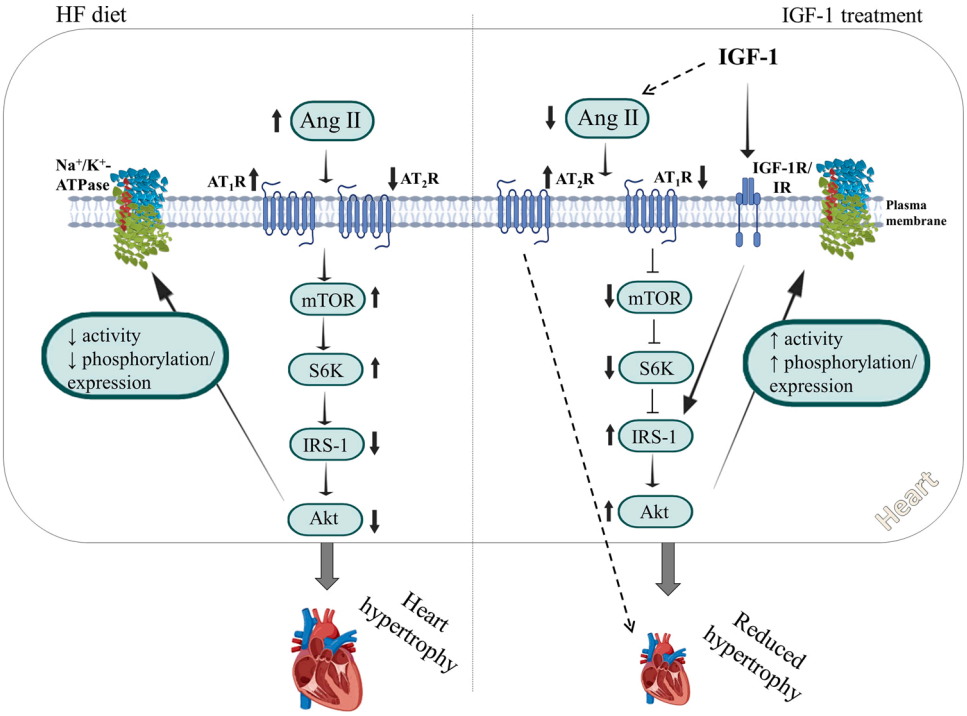

Fig. 1. A potential mechanism of the *in vivo* IGF-1 positive impact on obesity-induced cardiac complications. Obesity is accompanied by increased levels of Ang II, which, through the AT_1R and mTOR/S6K cascade, is involved in insulin resistance and heart hypertrophy development. However, exogenous IGF-1 binds to a receptor, activating and inducing IRS-1/Akt pathway activation. Simultaneously, IGF-1 attenuates Ang II-mediated stimulation of mTOR/S6K, improving Na^+/K^+-ATPase activity and reducing cardiac hypertrophy. Akt – Protein kinase B, Ang II – Angiotensin II, AT_1R - Type-1 angiotensin II receptor, HF – High fat, IGF-1 - Insulin-like growth factor-1, IGF-1R/IR - IGF-1 Receptor /Insulin Receptor receptor, IRS-1 – Insulin receptor substrate-1, Na^+/K^+-ATP-ase - Sodium/potassium adenosine triphosphatase, mTOR - Mammalian target of rapamycin, S6K - Ribosomal protein p70 S6 kinase, ↑ - increase, ⊥ - inhibition.

2.2. Preparation of heart tissue lysate

The hearts were cut and liquefied on ice with a Homogeniser HG-15D (Witeg) in a lysis buffer for protein extraction supplemented with protease inhibitors cOmplete ULTRA (Roche, Mannheim, Germany). The liquefied tissue samples were incubated for 1 hour under constant rotation at 4ºC and centrifuged for 20 min at 4ºC and 100,000 × g. Afterwards, the sample supernatants were collected, transferred to cooled tubes, and preserved at −80°C.

2.3. Extraction of heart plasma membrane proteins

The cardiac membrane proteins were extracted following the methodology of Luiken et al. [26]. Hearts were kept cold, dissected, and incubated for 30 min in a hypertonic solution at 4ºC. After incubation, samples were centrifuged for 5 min at 1000 × g. The pellet was subsequently liquefied with a Homogeniser HG-15D (Witeg) in TES buffer supplemented with protease inhibitors at 4ºC. The resulting sample was centrifuged for 5 min at 1000 × g, and supernatants were removed and saved. The pellets were again liquefied with a homogeniser in TES buffer at 4ºC and recombined with the saved supernatants. Samples are centrifuged for 10 min at 100 × g. Then, the pellets were resuspended in TES buffer, and the supernatants centrifuged for 10 min at 5000 × g. The resulting pellets were resuspended in TES buffer and properly stored at −80ºC. Protein concentrations from both lysates and membrane protein extractions were analyzed with the Lowry method to determine the protein concentrations [27].

2.4. Assessment of Ang II levels in serum

The serum concentrations of Ang II were quantified by the enzyme-linked immunosorbent assay, which utilizes the quantitative sandwich enzyme immunoassay methodology following the manufacturer's guidelines (Elabscience-E-EL-R1430). The assay demonstrated a sensitivity of 9.38 pg/ml, with a detection range of 15.63–1000 pg/ml, and acceptable intra-assay (CV: 4.7–6.0 %) and inter-assay (CV: 6.77–8.62 %) values across three sample concentrations. Ang II concentrations were quantified in pg/ml.

2.5. Assessment of serum glucose, Na^+, and K^+ concentrations

Glucose concentrations in serum were determined with a GLUC-PAP kit in the fasting state, specifically after a 12 h fast. As we previously demonstrated, the serum concentrations of Na^+ and K^+ were measured using the Synchron System (Beckman Coulter, UK) [8]. Concentrations of both ions and glucose were reported in mmol/l.

2.6. Sodium/Potassium-ATPase assay

The quantification of Na^+/K^+-ATPase activity was conducted using an ouabain-specific assay. The Na^+/K^+-ATPase assay was performed using two sets of tubes. Each tube in the first set contained 50 µl of incubation mixture (composed of 2 M NaCl, 400 mM KCl, 100 mM $MgCl_2$, 1 M Tris pH 7.4, and ddH_2O), 105 µl of ddH_2O, and 25 µl of the sample (1 µg/µl). In the second set, tubes contained 50 µl of the same incubation mixture, 20 µl of ouabain, 85 µl of ddH_2O, and 25 µl of the sample (1 µg/µl). After preparation, 20 µl of 20 mM ATP was added to initiate the reaction, followed by incubation at 37 °C for 15 minutes. The reaction was then terminated with the addition of 22 µl of ice-cold 3 M perchloric acid. A modified spectrophotometric method based on the stannous chloride procedure [28] was used to quantify the inorganic orthophosphate released from ATP hydrolysis. The reaction mixtures were diluted with 4.5 ml of deionized water and 200 µl of 0.2 M ammonium heptamolybdate in 30 % (w/v) sulfuric acid. A drop of $SnCl_2$ dissolved in glycerol was then added to develop color. After 15 minutes, absorbance was measured at 690 nm using a Lambda 35 UV/VIS spectrophotometer (PerkinElmer). The Na^+/K^+-ATPase activity was determined by calculating the difference in absorbance between samples with and without ouabain. The data were presented as mmol Pi/min/mg protein.

2.7. SDS-PAGE and Western Blot analysis

The protein fractions of lysate and plasma membrane were isolated via SDS-polyacrylamide gel electrophoresis. Subsequently, proteins were transferred from the gel onto a membrane, applying an electrical charge, and blocked adequately. Total cell lysate fractions were analyzed using antibodies (Cell Signalling) specific to IRS-1, phospho-IRS at Ser^{307}, phospho-IRS at Tyr^{1222}, Akt, phospho-Akt at Ser^{473}, mTOR, phospho-mTOR at Ser^{2481}, phospho-mTOR at Ser^{2448}, S6K, and phospho-S6K at Thr^{421}/Ser^{424} (Table S2). Additionally, the plasma membrane fractions were analyzed using antibodies specific to the phosphorylated form of IGF-1 receptor β /insulin receptor β (IGF-1Rβ/IRβ) (Tyr^{1131}/Tyr^{1146}) and IRβ, sourced from Cell Signalling, as well as antibodies targeting the phosphorylated α subunit of Na^+/K^+-ATPase (Ser^{23}), the α_1 subunit of Na^+/K^+-ATPase, type-1 angiotensin II receptor (AT_1R), and type-2 angiotensin II receptor (AT_2R) obtained from Santa Cruz Biotechnology. After incubation, membranes were thoroughly rinsed and exposed to HRP-conjugated or ALP-conjugated antibodies (Santa Cruz Biotechnology). Later, membranes were rinsed again in buffer and subsequently prepared for imaging utilizing electrochemiluminescence or BCIP/NBT techniques. To normalize the level of detected protein, we incubated all membranes with anti-β-actin monoclonal antibody and suitable HRP-conjugated secondary anti-mouse antibody (Santa Cruz Biotechnology). The obtained signals were assessed using Image J 1.45 s software (National Institutes of Health, USA).

2.8. Quantitative polymerase chain reaction (qPCR)

Total ribonucleic acid (RNA) was isolated from cardiac tissue using the TRIzol reagent (Life Technologies, USA), following the manufacturer's extraction protocol. The concentration of the isolated RNA was measured using a Nanodrop spectrophotometer (NanoDrop 1000 Spectrophotometer). Although we did not perform a formal integrity check for these groups of animals, we relied on the Nanodrop measurement for RNA quality assessment, since all the values obtained were in the range 1,9–2,1 (A260/A280). Complementary DNA was synthesized, and a quantitative polymerase chain reaction (qPCR) experiment was conducted as previously outlined [29]. The forward primers employed for the detection of α myosin heavy chain (α-MHC) were 5′-GCTGGAGCTGATGCACCTGT-3′, while the reverse primer was 5′-TCGGCATCTGCCAGGTTGTC-3′. Additionally, the primers employed for β-MHC consisted of the forward primer 5′-TCGGGAAGCAGTGCCAGAAC-3′ and the reverse primer 5′-AGGAGCAGGAAGGGTCGGTT-3′. The rat β-actin primers were 5′-CCCTGGCTCCTAGCACCAT-3 ′ (forward primer) and 5′-GAGCCACCAATCCACACAGA-3′ (reverse primer). The parameters for both α-MHC and β-MHC were 95°C for 3 minutes, followed by 40 cycles of 15 seconds at 95°C and 32 seconds at 57°C. Upon completion of the reaction, the cycle threshold values (Ct) were ascertained, and the $2^{-\Delta\Delta Ct}$ technique was employed to analyze the relative quantification of messenger RNA (mRNA) expression. The expression levels of α-MHC and β-MHC were normalized to the expression level of the β-Actin gene identified in the same sample.

2.9. Statistical analysis

The data were presented as mean ± SEM. The students' *t*-test was employed to evaluate the significant difference between groups. The statistical analysis was utilized using SPSS software (Chicago, USA), with $p < 0.05$ being significant.

3. Results

3.1. Variations in clinical parameters

The findings related to body mass revealed that both final body mass and final and starting body mass differences were unchanged following IGF-1 treatment in obese rats compared to their untreated counterparts. Given that obesity impairs carbohydrate metabolism, we assessed the glucose content in rats' serum. The serum glucose concentrations were elevated ($p < 0.05$) in obese rats compared to the control rats. However, the results demonstrate that IGF-1 treatment did not alter serum glucose concentrations in obese rats compared to untreated animals. The serum Ang II concentrations were slightly elevated, but nonsignificantly, in obese animals compared to control animals. Additionally, IGF-1 administration reduced Ang II levels ($p < 0.05$) in the serum of obese rats relative to untreated obese rats. We also examined whether IGF-1 treatment affected serum Na^+ and K^+ ion concentrations in obese rats. Obese animals showed decreased concentration of Na^+ ions ($p < 0.05$), while the concentration of K^+ ions was unchanged compared to control animals. Although both ion concentrations fell within reference ranges (K^+=5.2–7.8 mM, Na^+=141–150 mmol/l), our findings suggest that IGF-1 therapy did not alter serum Na^+ ion levels. In contrast, it significantly reduced K^+ serum concentration compared to their untreated counterparts ($p < 0.05$) (Table 1).

3.2. The activity and expression of Na^+/K^+-ATPase in the heart of IGF-1-treated HF rats

We assessed the impact of IGF-1 administration on Na^+/K^+-ATPase dynamics in the hearts of obese rats by measuring the activity of Na^+/K^+-ATPase and the level of phosphorylation of the α subunit. The results indicate that obesity decreased Na^+/K^+-ATPase activity ($p < 0.05$), the level of phosphorylation of Na^+/K^+-ATPase α_1 subunit ($p < 0.05$), as well as the protein level of the Na^+/K^+-ATPase α_1 subunit ($p < 0.05$) in hearts of obese rats in comparison with control rats. On the other hand, IGF-1 therapy enhanced Na^+/K^+-ATPase activity ($p < 0.05$), as well as the phosphorylation of α Na^+/K^+-ATPase on Ser^{23} (Figs. 2**a and 2b**) in IGF-1-treated obese rats relative to untreated obese rats. We also analyzed the protein level of the α_1 subunit of Na^+/K^+-ATPase in the cardiac plasma membrane fraction (Fig. 2**c**). The findings demonstrate that α_1 protein expression was elevated ($p < 0.001$) in IGF-1-treated obese rats compared to untreated obese rats.

3.3. The phosphorylation level of IGF-1Rβ/IRβ and protein expression of AT_1R and AT_2R in the heart of IGF-1-treated HF rats

Taking into account that IGF-1 is considered to be a dominant activator of IGF-1R/IR hybrid receptor in an obese state [30], we explored the impact of IGF-1 treatment on the activation of IGF-1R/IR through phosphorylation at a specific site on β subunits in the heart of obese rats. The cardiac p-IGF-1Rβ/IRβ (Tyr^{1131}/Tyr^{1146})/IRβ ratio was significantly decreased ($p < 0.05$) in obese animals compared to control animals. Following the IGF-1 administration, the cardiac p-IGF-1Rβ/IRβ (Tyr^{1131}/Tyr^{1146})/IRβ ratio was increased in IGF-1-treated obese rats compared to obese nontreated rats ($p < 0.05$) (Fig. 3**a**). Considering the strong relationship between obesity and Ang II-induced heart hypertrophy [31], we explored whether IGF-1 treatment influences protein expression of AT_1R and AT_2R in plasma membrane fractions of rat hearts (Figs. 3**b and** 3**c**). The protein level of AT_1R was elevated ($p < 0.001$), while the protein level of AT_2R was unchanged in the hearts of obese animals compared to control animals. The IGF-1 treatment reduced the protein level of AT_1R ($p < 0.05$) in obese rats, while the protein level of AT_2R was elevated ($p < 0.05$) in obese rats treated with IGF-1, matched with non-treated obese rats.

Table 1
Anthropometric and biochemical parameters in serum. The data shown represent mean ± SEM (n = 4–7; HF vs HF+IGF-1 *$p < 0.05$; HF, HF+IGF vs CONT $^{\#}p < 0.05$). Ang II - angiotensin II, K^+ - potassium ion, Na^+ - sodium ion.

Experimental groups			
Parameters	**CONT**	**HF**	**HF+IGF-1**
Initial body mass [g]	187 ± 4	189 ± 9	182 ± 7
Final body mass [g]	520 ± 16	594 ± 29$^{\#}$	590 ± 28$^{\#}$
Body mass difference [g]	333 ± 18	405 ± 27$^{\#}$	408 ± 27$^{\#}$
Heart/body mass ratio ($\times 10^{-3}$)	2.81 ± 0.14	2.81 ± 0.17	2.56 ± 0.05
Glucose [mmol/l]	9.57 ± 0.58	12.23 ± 0.85$^{\#}$	13.12 ± 1.25$^{\#}$
Ang II [pg/ml]	61.7 ± 7.3	67.9 ± 2.6	57.3 ± 3.4*
Na^+ [mmol/l]	143.5 ± 0.5	141.5 ± 0.3$^{\#}$	143.7 ± 1.3
K^+ [mmol/l]	7.20 ± 0.09	7.38 ± 0.19	6.62 ± 0.20$^{\#}$ *

3.4. The phosphorylation level of IRS-1 and Akt in the heart of IGF-1-treated HF rats

Obesity is defined by diminished IRS-1/Akt signaling, which adversely impacts Na^+/K^+-ATPase activity and cardiac function [8,32]. Consequently, we investigated the involvement of IRS-1 and Akt pathway in modulating Na^+/K^+-ATPase in the heart tissue in rats treated with IGF-1. Initially, we investigated IRS-1 phosphorylation level at its inhibition (Ser^{307}) and activation (Tyr^{1222}) sites [33]. The obtained results showed that the level of Ser^{307} phosphorylated form of IRS-1 was elevated ($p < 0.05$), while the level of Tyr^{1222} phosphorylated form of IRS-1 was unchanged in hearts of obese rats in comparison with control rats. However, the level of phosphorylation of IRS-1 at Ser^{307} was reduced ($p < 0.05$), while at the same time, was enhanced at Tyr^{1222} ($p < 0.05$) in the hearts of obese rats treated with IGF-1 relative to untreated obese rats (Figs. 4**a and** 4**b**). Activated IRS-1 triggers a signaling cascade that activates Akt, a crucial protein implicated in various regulatory processes within the heart, encompassing cardiac development and the Na^+/K^+-ATPase activity [34]. The cardiac phosphorylation of Akt at Ser^{473} was shown to be decreased ($p < 0.001$) in obese rats compared to control rats. Contrarily, our findings demonstrate that the phosphorylation level of Akt at Ser^{473} was elevated ($p < 0.05$) in the hearts of IGF-1-treated obese rats compared to untreated obese rats (Fig. 4**c**).

3.5. The phosphorylation level of mTOR and S6K in the heart of IGF-1-treated HF rats

We conducted a further examination of the phosphorylation level of mTOR and S6K under the influence of IGF-1, as the overexpression of mTOR/S6K contributes to obesity-induced insulin resistance by down-regulation of IRS-1 and PI3K/Akt signaling pathways [35]. The results indicate reduced ($p < 0.05$) phosphorylation of mTOR at Ser^{2481} and elevated ($p < 0.05$) phosphorylation of mTOR at Ser^{2448} in the hearts of obese rats compared to control rats. Contrary, IGF-1 administration in obese rats enhanced the phosphorylation level of mTOR at Ser^{2481} ($p < 0.05$) while it reduced the phosphorylation at Ser^{2448} ($p < 0.05$) in the heart, compared to untreated obese rats (Figs. 5**a and** 5**b**). The phosphorylation of cardiac S6K at Thr^{421}/Ser^{424} was elevated ($p < 0.05$) in obese animals compared to control animals. In addition, the phosphorylation level of S6K (Thr^{421}/Ser^{424}) was decreased ($p < 0.01$) in the hearts of obese rats administered IGF-1 compared to untreated obese rats (Fig. 5**c**).

3.6. Impact of IGF-1 on heart mass and α-MHC and β-MHC mRNA expression in the heart of HF rats

Obesity causes alterations in the heart, ultimately resulting in

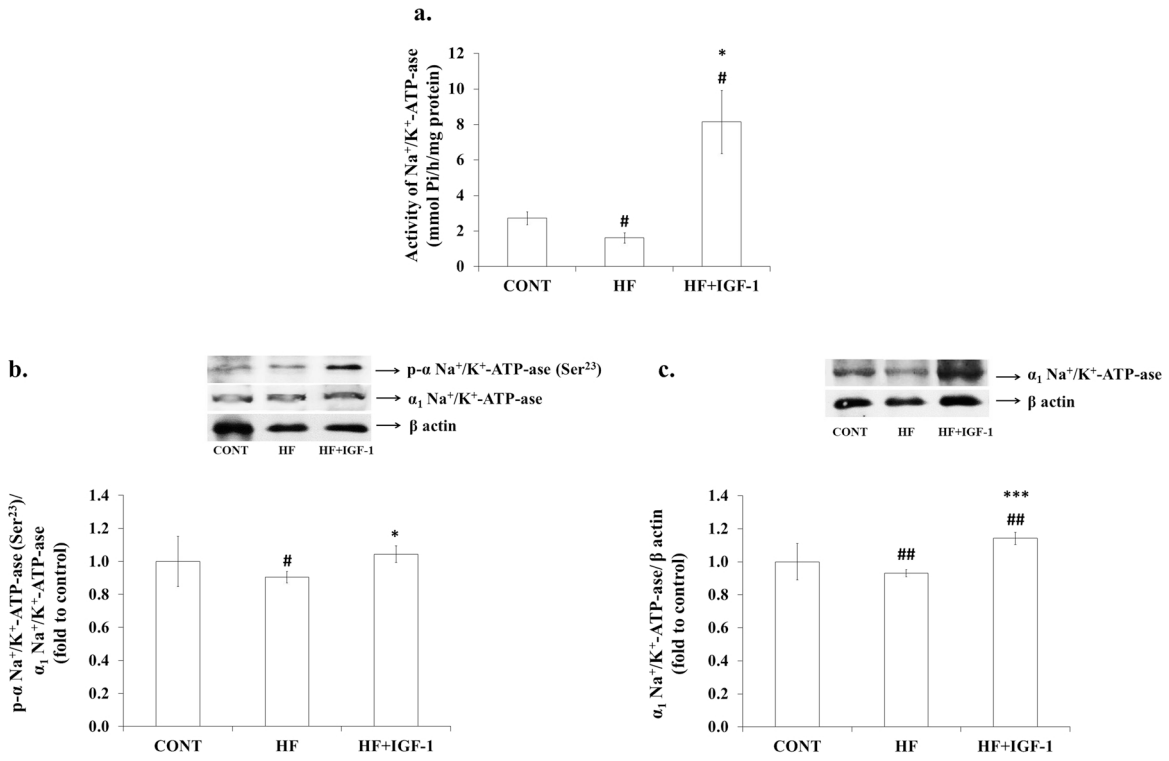

Fig. 2. *In vivo* effects of IGF-1 on cardiac Na^+/K^+-ATPase activity and expression in obese rats. a) Specific activities of Na^+/K^+-ATPase are expressed in mmol Pi/h/mg of protein and represent mean ± SEM (n = 6). b) The results obtained for the ratio of α Na^+/K^+-ATPase phosphorylated on Ser^{23} (p-α Na^+/K^+-ATPase) and total protein expression of α_1 Na^+/K^+-ATPase are presented as a percentage relative to the control value (n = 6). c) The Total protein expression of α1 Na^+/K^+-ATPase is a percentage relative to the control value (n = 6–7). The results for phosphorylation and expression represent mean ± SEM (HF+IGF-1 vs HF $^{*}p < 0.05$, $^{***}p < 0.001$; HF, HF+IGF vs CONT $^{\#}p < 0.05$, $^{\#\#}p < 0.01$). Inserts: representative Western blots. Na^+/K^+-ATP-ase - Sodium/potassium adenosine triphosphatase, Ser – serine.

ventricular enlargement and heart failure [22]. Utilizing the same model of obese rats, we have previously demonstrated that an HF diet induces cardiac hypertrophy, characterized by an increased transverse diameter of cardiomyocytes [22]. The current study examined the impact of IGF-1 on ventricular hypertrophy generated by obesity at the molecular level to further enhance understanding. Consequently, we assessed heart mass and α-MHC and β-MHC mRNA expression, which are significant indicators of cardiac hypertrophy [36]. The heart mass in obese animals was elevated ($p < 0.01$) compared to control animals, while IGF-1 reduced the cardiac mass of obese rats compared to untreated obese rats ($p < 0.05$) (Fig. 6**a**). Although cardiac α-MHC and β-MHC mRNA expression were changed but non-significant, the cardiac α-MHC/β-MHC ratio decreased ($p < 0.05$) in obese animals compared to control animals. Contrary, IGF-1 administration in obese rats elevated α-MHC mRNA expression (Fig. 6**b**) and the α-MHC/β-MHC ratio ($p < 0.05$) (Fig. 6**d**), but β-MHC mRNA expression remained statistically unchanged (Fig. 6**c**) in comparison to untreated obese rats.

4. Discussion

Obesity-induced heart dysfunction and hypertrophy are major contributors to the pathogenesis of cardiovascular diseases [2], with altered metabolic and signaling pathways playing a critical role [4]. While IGF-1 is widely known for its cardioprotective effect [16,18,37], our study examines how IGF-1 regulates cardiac Na^+/K^+-ATPase in obese rats, a novel aspect of obesity-induced heart dysfunction. Furthermore, this study examines the impact of IGF-1 on the IRS-1/Akt and mTOR/S6K signaling pathways, particularly on cardiac dysfunction induced by obesity. We conducted our research in male rats to minimize hormonal variability, providing a more controlled model to assess IGF-1 effects. We acknowledge, however, that sex differences are highly relevant, as female physiology, shaped by fluctuating estrogen levels and distinct inflammatory and metabolic profiles, may alter IGF-1 responses. The synergistic interaction between estradiol and IGF-1 in cardioprotective pathways suggests that females might exhibit enhanced or different outcomes [38–40]. Future studies must fully elucidate sex-specific effects of IGF-1 in cardiac remodeling.

In our study, we have shown that the body mass and serum glucose of the IGF-1-treated rats remained unchanged compared to untreated obese animals, a result indicating that a limited treatment with IGF-1 does not interact with those parameters, which fully concurred with previous findings reporting the inefficiency of immediate effect IGF-1 application both on body weight or disturbances in glucose metabolism of models involving obesity [41]. However, although IGF-1 did not directly affect changing body mass or glucose levels, it had a modulatory role in other metabolic and cardiovascular parameters. Such results suggest that the therapeutic effectiveness of IGF-1 may occur independently of significant changes in body weight or blood glucose concentrations via more specific and localized effects within the heart and its associated signaling pathways.

We noticed a significant decrease in the levels of serum Ang II in IGF-1-treated obese rats, supporting that IGF-1 may, by lowering Ang II levels, decrease its pro-hypertrophic effects on the heart [42]. These findings align with studies indicating that IGF-1 can modify cardiovascular health by affecting vascular tone and endothelial characteristics, which may, in turn, lower the injurious effects of Ang II in obesity [43,44]. Furthermore, IGF-1 treatment affected the protein expression levels of Ang II receptors in cardiac tissue. Specifically, IGF-1 lowered AT_1R expression while increasing AT_2R expression. The AT_1R promotes hypertrophy and inflammation [42], whereas the AT_2R promotes vasodilation and anti-fibrotic responses while preventing cardiac hypertrophy [42,45]. A reduction of HF diet-induced vascular pathologies was found when an AT_1R antagonist was employed [46]. IGF-1 regulates the renin-angiotensin system (RAS), an important pathway for blood

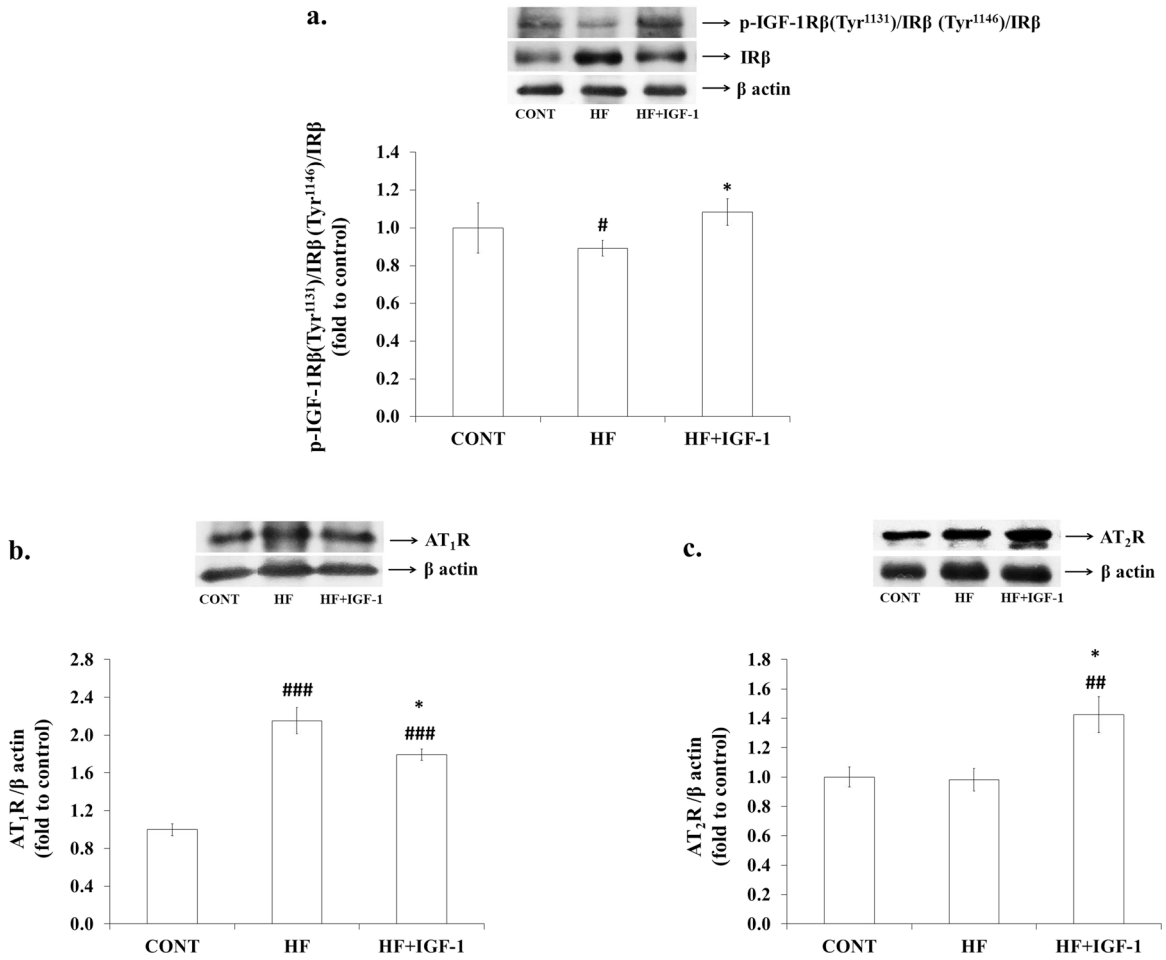

Fig. 3. *In vivo* effects of IGF-1 on the phosphorylation of cardiac IGF-1Rβ/IRβ hybrid receptor, AT1R, and AT2R in obese rats. a) The results obtained for the ratio of IGF-1Rβ/IRβ phosphorylated on Tyr^{1131}/Ty^{r1146} (p-IGF-1Rβ/IRβ) and total protein expression of IRβ are presented as a percentage relative to the control value (n = 7). b) Total protein expression of AT_1R is presented as a percentage relative to the control value (n = 5–6). c) Total protein expression of AT_2R is presented as a percentage relative to the control value (n = 5). The results for phosphorylation and expression represent mean ± SEM (HF+IGF-1 vs HF $^{*}p < 0.05$; HF, HF+IGF vs CONT $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$). Inserts: representative Western blots. AT_1R - Type-1 angiotensin II receptor, AT_2R - Type-2 angiotensin II receptor, IGF-1Rβ/IRβ - IGF-1 Receptor β/Insulin Receptor β, Tyr – tyrosine.

pressure and cardiac function regulation. It reduces renin secretion from the kidneys and thus inhibits the production of Ang II [20,47]. Angiotensin-converting enzyme is widely recognized for its function in generating Ang II, a key vasoconstrictor [48]. Overnutrition elevates Ang II level, further activating mTOR/S6K signalling and inhibiting IRS-1-mediated Akt stimulation, subsequently leading to insulin resistance development [49,50]. This process was considered an adaptive mechanism to prevent organisms from overfeeding. In addition, it was recently shown that the cardioprotective effects of CGEN-856S are specifically through inhibition of Ang II-induced heart hypertrophy [51]. Our findings suggest that IGF-1 reduced Ang II level in circulation as well as the expression of AT_1R in the heart at least partially through a mechanism that involved activation of Akt signaling that further increased Na^+/K^+-ATPase activity that promotes vasodilatation and acceleration of metabolic processes, such as glucose consumption. In addition, IGF-1-induced vasorelaxation is mediated through increased NO production and decreased Ang II signalling [52]. Earlier studies showed that overexpression of IGF-1 reduced the level of Ang II and expression of AT_1R in the hearts of male transgenic FVB.*Igf*+/- mice and in this manner protect myocyte hypertrophy [47]. The cardioprotective role of IGF-1 was demonstrated through suppression of p53 function and Ang II production and thus AT_1R activation, by a decrease in myocyte death [53]. Locally produced IGF-1 in mouse cardiomyocytes was shown to reduce and prevent Ang II-induced hypertrophic response through SirT1 activation [54]. In addition, IGF-1 suppresses Ang II signalling and cardiac fibrosis through the Akt signalling pathway and the suppression of Rho-associated coiled-coil containing kinases 2-mediated α-smooth muscle actin expression [43]. The IGF-1 increases nitric oxide production by stimulating endothelial nitric oxide synthase, thereby antagonizing Ang II action and down-regulating AT_1R expression, most commonly in the heart [55]. Besides, IGF-1 inhibits RAS activity in dopaminergic neurons and glial cells, subsequently inhibiting Ang II signalling [56]. Proposed mechanisms suggest that IGF-1 may contribute to cardiac protection against myocardial hypertrophy, while Ang II and AT1R signalling contribute to the progression of the pathological process.

This observation was probably the most interesting finding about the influence of IGF-1 on ion homeostasis: no change in Na^+ but a highly significant depression in K^+. Importantly, both ions remained within their reference ranges, indicating that IGF-1 may play a crucial role in maintaining optimal ionic balance, a key factor in supporting heart function [57]. The potential of IGF-1 to regulate K^+ levels, an often dysregulated factor in heart conditions contributing to arrhythmias and impaired cardiac function [58], is reassuring for its cardioprotective effects. In addition, IGF-1 was shown to stimulate potassium Kir4.1/5.1 channel activity, a major contributor to the macroscopic K^+ current and therefore facilitate Na^+ reabsorption in principal cortical collecting duct cells [59]. Furthermore, IGF-1 increased basal transepithelial Na^+

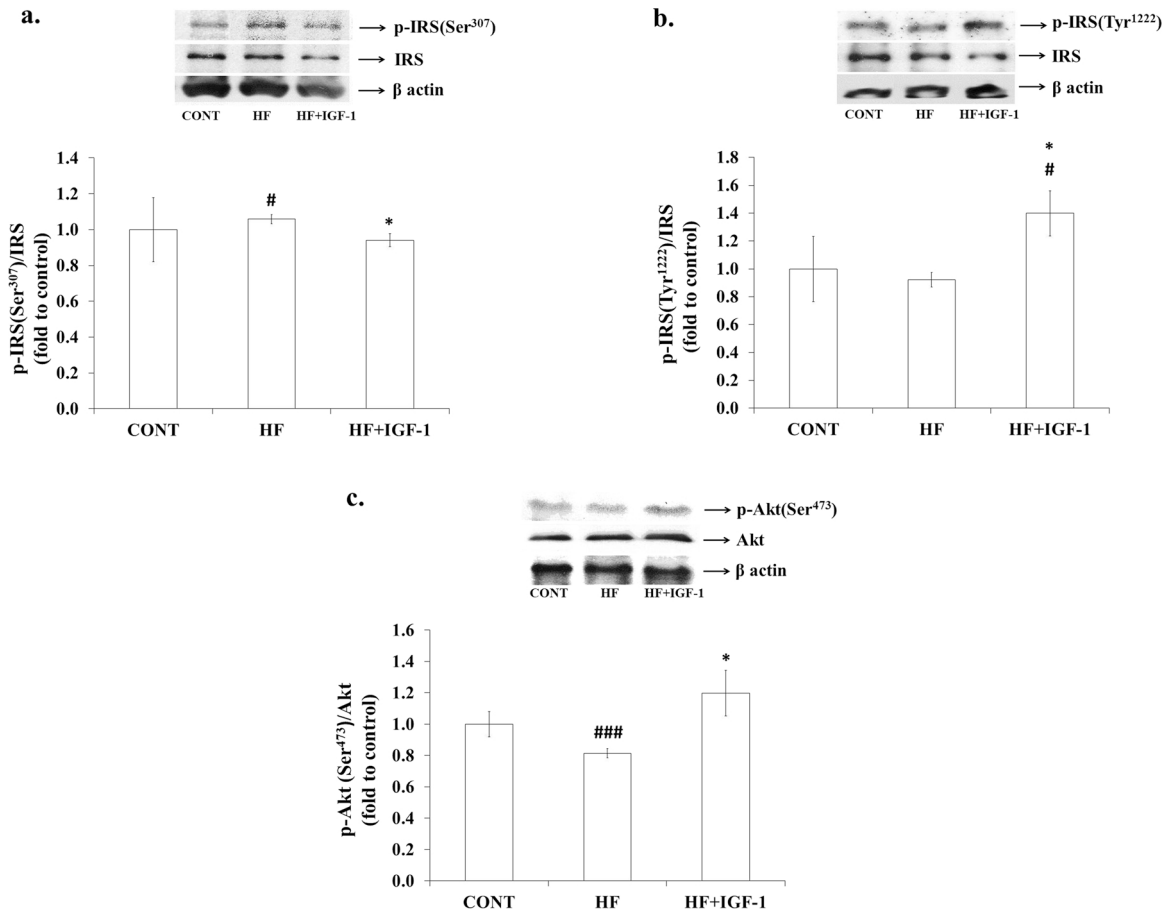

Fig. 4. *In vivo* effects of IGF-1 on IRS-1 and Akt phosphorylation in the heart of obese rats. a) The results obtained for the ratio of IRS-1 phosphorylated on Ser^{307} (p-IRS-1) and total protein expression of IRS-1 are presented as a percentage relative to the control value (n = 6). b) The results obtained for the ratio of IRS-1 phosphorylated on Tyr^{1221} (p-IRS-1) and total protein expression of IRS-1 are presented as a percentage relative to the control value (n = 4–5). c) The results obtained for the ratio of Akt phosphorylated on Ser^{473} (p-Akt) and total protein expression of Akt are presented as a percentage relative to the control value (n = 5–6). The results for phosphorylation and expression represent mean ± SEM (HF+IGF-1 vs HF *$p < 0.05$; HF, HF+IGF vs CONT $^{\#}p < 0.05$, $^{\#\#\#}p < 0.001$). Inserts: representative Western blots. Akt - Protein kinase B, IRS-1 - Insulin receptor substrate - 1, Ser - serine, Tyr – tyrosine.

transport via the PI3K [60].

Our study focused on the effects of IGF-1 on Na^+/K^+-ATPase, one of the significant pumps involved in maintaining cellular ionic gradient [6]. Metabolically, in obesity, this activity is impaired and results in abnormalities in the heart [8,12]. However, results from our study indicated significant activation of Na^+/K^+-ATPase function by IGF-1 treatment as evidenced by increases at Ser^{23} and phosphorylation of the α subunit. Thus, IGF-1 supports optimal ion transport and cellular function in the obese heart, highlighting the possible therapeutic implications of IGF-1 to cause an improvement in cell control in obesity. However, the effect of IGF-1 on Na^+/K^+-ATPase activity is much more pronounced than on α subunit expression and phosphorylation, suggesting other mechanisms involved in IGF-1 regulation of Na^+/K^+-ATPase. One of the ways may include its regulation of the third-γ Na^+/K^+-ATPase subunit and, in that manner, modulation of Na^+/K^+-ATPase affinity to Na^+ , K^+ , and ATP [61]. In addition, IGF-1 could also indirectly affect Na^+/K^+-ATPase activity through modulation of Na^+ and K^+ ion concentrations [59,60,62].

Our examination of the phosphorylation status of the IGF-1 receptor and insulin receptor in the hearts of obese rats revealed a notable increase in the phosphorylation of IGF-1Rβ/IRβ on their respective β-subunits after IGF-1 treatment. The IGF-1Rβ/IRβ could enhance downstream signaling pathways that activate pro-survival pathways [15]. The increased phosphorylation of these receptors in our study indicates that IGF-1 may activate pro-survival pathways, alleviate the effects of obesity-related cardiac malfunction.

The IRS-1/Akt signaling pathway, a crucial regulator of cellular processes, plays a significant role in maintaining normal cardiac function and overall heart health [63]. However, disturbances or disruptions in this vital pathway have been implicated in developing cardiac dysfunction arising from obesity [8,32,63]. Indeed, the phosphorylation of IRS-1 on Ser^{307} attenuates insulin signaling and promotes insulin resistance, while phosphorylation on Tyr^{1222} stimulates insulin signaling and glucose uptake [33]. Our results indicate that IGF-1 significantly enhances the activation of IRS-1. Moreover, this specific improvement was associated with increased phosphorylation of Akt at Ser^{473}, establishing a link between the observed cell function improvement and the crucial activity of Na^+/K^+-ATPase [8,20].

Overnutrition elevates the Ang-II level that activates mTOR/S6K signaling and inhibits IRS-1-mediated Akt stimulation, subsequently developing insulin resistance [50]. However, the administration of IGF-1 results in an increase in phosphorylation at Ser^{2481}, while at the same time, it decreases at Ser^{2448}. This suggests that IGF-1 may control mTOR signaling in a manner aimed at the favorable metabolic effects in the heart. Furthermore, IGF-1 reduced the phosphorylation of S6K, one of the primary targets of mTOR [64]. This result can also assist in avoiding harmful feedback mechanisms that interfere with the signaling between IRS-1/Akt in obese individuals. These findings imply that modifying the mTOR/S6K pathway could be an additional mechanism for protecting IGF-1 against the remodeling and malfunction of the heart caused by HF diet. Furthermore, IGF-1 signaling is suggested as a potential therapeutic target for cardiac fibrosis as it was shown to alleviate

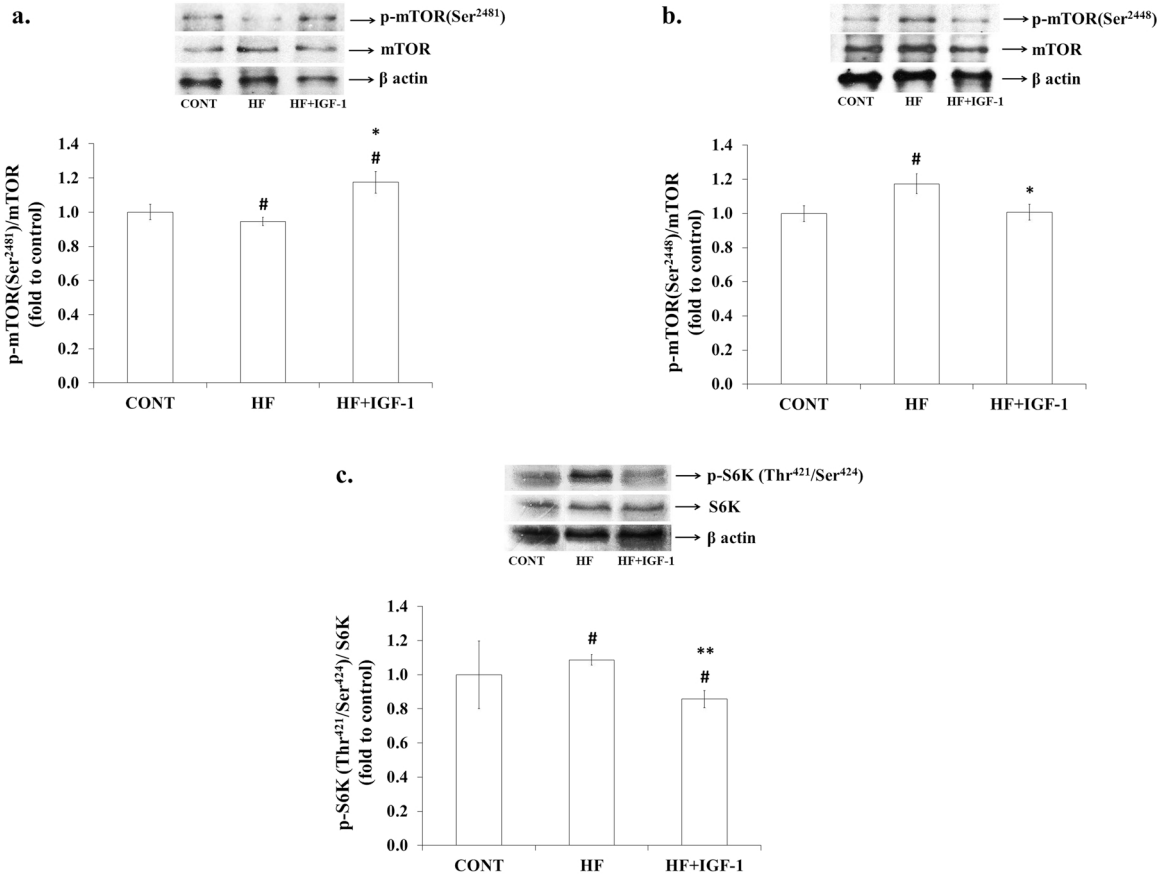

Fig. 5. *In vivo* effects of IGF-1 on mTOR and S6K phosphorylation in the heart of obese rats. a) The results obtained for the ratio of mTOR phosphorylated on Ser^{2481} (p-mTOR) and total protein expression of mTOR presented as a percentage relative to the control value (n = 5–7). b) The results obtained for the ratio of mTOR phosphorylated on Ser^{2448} (p-mTOR) and total protein expression of mTOR presented as a percentage relative to the control value (n = 6). c) The results obtained for ratio of S6K phosphorylated on Thr^{421}/Ser^{424} (p-S6K) and total protein expression of S6K presented as a percentage relative to the control value (n = 4). The results for phosphorylation and expression represent mean ± SEM (HF+IGF-1 vs HF $^{*}p < 0.05$, $^{**}p < 0.01$; HF, HF+IGF vs CONT $^{\#}p < 0.05$). Inserts: representative Western blots. mTOR - Mammalian target of rapamycin, S6K - Ribosomal protein p70 S6 kinase, Thr -threonine, Ser – serine.

the effects of Ang II through Akt activation [43]. The experimental model of Ang II/phenylephrine-infused myofibroblast-specific IGF-1R knockout mice showed signs of severe interstitial fibrosis [43]. However, even low doses of IGF-1 administration markedly attenuated Ang II-induced cardiac fibrosis through Akt signalling [43]. Contrarily, other study shows that IGF-1R signaling deficiency mitigates Ang II-induced cardiac fibrosis through the Akt/ERK/nuclear factor-κB pathway [65].

Cardiac hypertrophy is a hallmark feature of heart dysfunction associated with obesity [66]. The α-MHC/β-MHC ratio is a commonly used index to evaluate hypertrophic changes in the heart. Under normal, non-hypertrophic conditions, the α-MHC isoform predominates. In contrast, during pathological hypertrophy (such as that caused by pressure overload or heart failure), there is a shift towards β-MHC expression. Therefore, the α-MHC/β-MHC ratio is a reliable biomarker for studying heart diseases and hypertrophic remodeling [67,68]. Additionally, several studies have shown that the extent of change in α-MHC expression can be more pronounced than that of β-MHC [69–71]. One study showed that treatment with testosterone induced left ventricular hypertrophy by upregulation of α-MHC/β-MHC through a dose-dependent increase in α-MHC without changing the β-MHC expression. Interestingly, they assume that testosterone-induced changes are possibly through IGF-1 [69]. The IGF-1 was also shown to be involved in physiological hypertrophy development following chronic exercise [72]. It was demonstrated that α-MHC expression was increased without a consequent decrease in β-MHC expression after 1 week of exercise [70]. These results show that an early adaptation of the heart to increase the functional capacity was through the regulation of α-MHC dominantly. In addition, an anti-hypertrophic agent, trichostatin A, was shown to improve cardiac contractility dominantly through upregulation of α-MHC and tubulins without changing the level of β-MHC [71]. Our results show that IGF-1 administration leads to a significant decrease in heart mass, modification of the α-MHC to β-MHC ratio, and favors the expression of the α-MHC isoform. This change might suggest an induction of a more favorable myocardial phenotype by IGF-1, thus helping toward better cardiac function and a lesser severity of heart failure. The increased levels of α-MHC mRNA support the role of IGF-1 in enhancing myocardial contractility [68]. No change in β-MHC expression indicates that IGF-1 acts through the more functional α-MHC isoform in a specific way to blunt the adverse effects of obesity on heart function. We opted to follow a 24-hours post IGF-1 treatment for the effects on heart structure, allowing us to gain insights into the fast-acting mechanisms of IGF-1 without long-term changes. This noted the rapid early response that may have been missed under longer treatment. Previously published results mostly explain IGF-1 effects on signaling molecules and genes [54,73,74], while direct effects on cardiomyocyte size were recorded 48-hours post-IGF-1 treatment [73]. Our previous *in vivo* study showed that 24 hours after hormonal treatment of obese rats decreased heart mass and transverse diameter of cardiomyocytes [22]. Since the results presented in this study also demonstrate that IGF-1 decreased heart mass 24 hours after treatment, we assume that IGF-1 could have similar effects on heart morphology.

Although our results suggest that IGF-1-induced improvement of Na^{+}/K^{+}-ATPase phosphorylation and activity may contribute to its

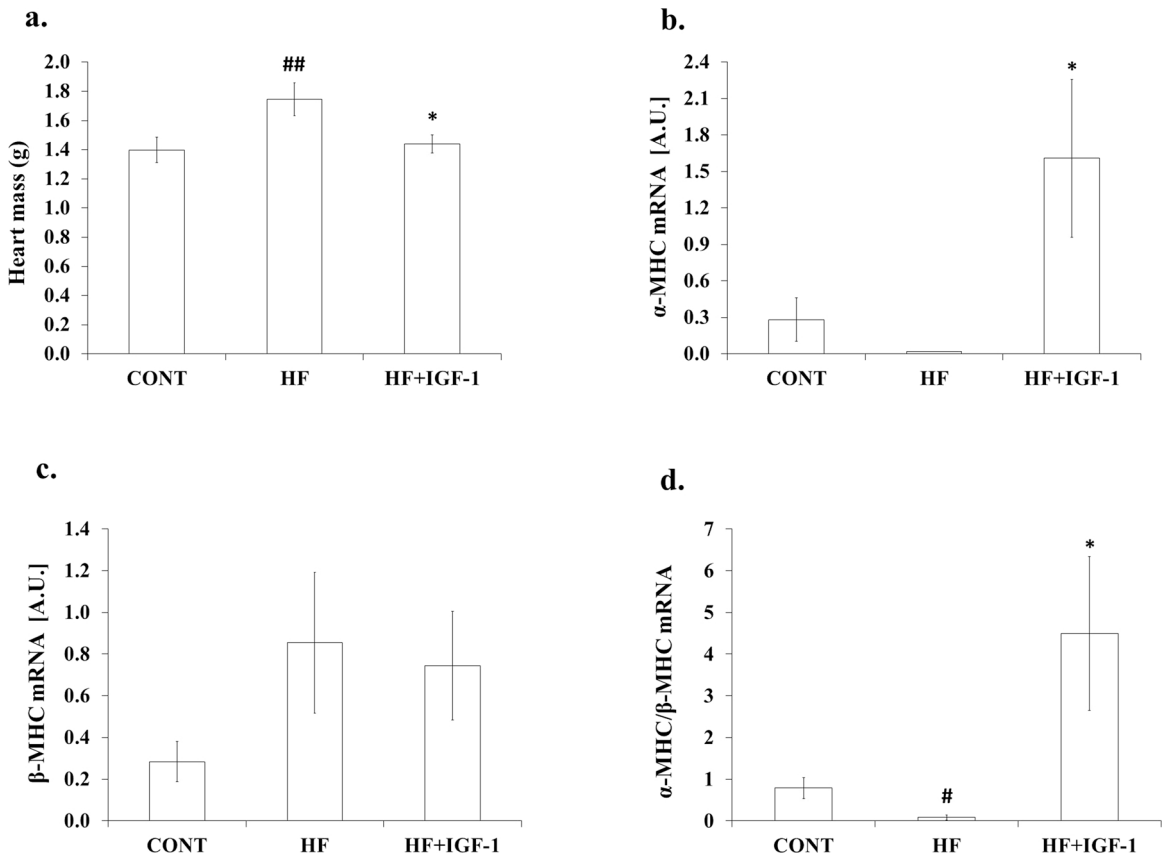

Fig. 6. *In vivo* effects of IGF-1 on heart mass and mRNA expression of α-MHC and β-MHC in obese rats. a) Heart mass is expressed in g and represents mean ± SEM (n = 5–6). b) mRNA expression of α-MHC (n = 5). c.) mRNA expression of β-MHC (n = 5). d.) The results were obtained by the ratio of α-MHC and β-MHC mRNA expression (n = 5). The mRNA results are expressed in A.U. (HF+IGF-1 vs HF *$p < 0.05$; HF, HF+IGF vs CONT $^{\#}p < 0.05$, $^{\#\#}p < 0.01$). MHC - Myosin heavy chain.

effects on cardiac hypertrophy, it is also possible that IGF-1 improves cardiomyocyte structure and function through other mechanisms. This alternative explanation warrants further investigation to clarify the causal relationship.

5. Conclusions

The present research provides a substantial advance in comprehending the molecular mechanisms underlying the pathology of obesity and the beneficial effects of IGF-1 in the heart. To the best of our knowledge, this is the first study to demonstrate that IGF-1 *in vivo* elevated Na^+/K^+-ATPase expression and activity in the heart and reduced heart hypertrophy in HF rats through a molecular mechanism that can involve the IRS-1/mTOR/Akt pathway. Also, treatment of obese rats with IGF-1 alleviates detrimental effects of HF diet on the heart, possibly through decreased Ang II level in circulation and in heart decreased AT_1R protein expression and increased AT_2R protein expression, which lead to reduced mTOR and S6K and increased IRS-1 and Akt phosphorylation, inducing amplification of Na^+/K^+-ATPase activity and reducing cardiac hypertrophy. These findings highlight IGF-1 as a potential therapeutic agent for treating obesity-induced cardiac disease. Moreover, the significant nature of these findings assumes clinically relevant considerations, particularly through the IGF-1 perspective. Reducing Ang II activation combined with protective pathways, such as IRS-1/Akt signaling, could provide innovative solutions toward cardiac impairment resulting from obesity and related disorders.

Our findings strongly suggest that IGF-1 promotes cardioprotection in obesity by regulating important signaling pathways that participate in heart function, hypertrophy, and ion homeostasis. Treatment with IGF-1 enhanced Na^+/K^+-ATPase activity in the heart of obese rats, changed the IGF-1R/IR and IRS-1/Akt cascades, and decreased cardiac hypertrophy. These results not only point to IGF-1 as a promising pharmacological drug for treating obesity-related heart dysfunction but also inspire further research and clinical applications in treating cardiac disease induced by obesity. They also emphasize that additional research is required regarding the long-term effects of IGF-1 and its clinical applications in treating cardiac disease induced by obesity.

Future perspectives

We are planning several experimental studies to broaden our findings and overcome this study's limitations. We aim to better understand IGF-1 treatment's advantages and disadvantages by investigating its long-term effects. Long-term treatment studies will determine if heart function and Na^+/K^+-ATPase activity improvements may be sustained and affect cardiac health. Future research will use dose-response analysis to find the optimal IGF-1 concentration for therapy. A practical and safe amount of IGF-1 can enhance cardiac function in obese patients without the side effects of a high dose. Echocardiography, electrocardiography, and pressure-volume loop analysis are needed to evaluate IGF-1's impact on heart function. In this latter approach, molecular and metabolic alterations would increase cardiac output, contractility, and well-being. Other IGF-1-modulated molecular pathways include oxidative stress, inflammation, and vascular function, which can be examined for all heart effects. Integrating IGF-1 with other signaling cascades may reveal its therapeutic potential for heart failure and metabolic diseases and improve treatment. Adding more sophisticated obesity-related cardiomyopathy models with insulin resistance, hypertension, and other comorbidities may better simulate human diseases. This would determine if IGF-1 effects are consistent across obesity and heart failure

stages and types. Further research on IGF-1 is important and could potentially revolutionize obesity treatment.

Ethics consideration

All procedures were performed in compliance with relevant laws and institutional guidelines and have been approved by the official Vinca Institute's Ethical Committee for Experimental Animals (Veterinary Directorate – No. 323–07–02710/2017–05).

Funding statement

This work was funded by the Ministry of Science, Technological Development, and Innovation of the Republic of Serbia (Contract No#451-03-136/2025-03/ 200017).

CRediT authorship contribution statement

Katarina Banjac: Investigation, Data curation, Writing – original draft. **Sonja Zafirovic:** Methodology, Validation. **Milan Obradovic:** Conceptualization, Supervision, Writing – original draft. **Esma R. Isenovic:** Supervision, Writing – review and editing.

Declaration of Competing Interest

The author(s) declared no potential conflicts of interest concerning this article's research, authorship, and/or publication.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.peptides.2025.171418.

Data availability

Data will be made available on request.

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4. DISKUSIJA

Gojaznost udružena sa rezistencijom na insulin i hipertenzijom doprinosi razvoja patološke hipertrofije srca, što posledično dovodi do ishemije i smrti kardiomiocita (Abel i sar., 2008; Afshin i sar., 2017). Imajući u vidu ograničen kapacitet regeneracije srčanog mišića, sprečavanje prevremene smrti kardiomiocita je od izuzetnog značaja za normalno funkcionisanje srca. Uprkos tome što danas postoje brojne studije u vezi sa uticajem IGF-1 na kardiovaskularni sistem, molekulski mehanizmi delovanja IGF-1 u gojaznosti i dalje nisu potpuno razjašnjeni.

U okviru ove doktorske disertacije prikazani rezultati su dobijeni istraživanjem *in vivo* efekta IGF-1 na ekspresiju i aktivnost Na^+/K^+-ATPaze u srcu normalno uhranjenih i gojaznih mužjaka pacova. Kako bi se ispitala polazna hipoteza u kojoj je pretpostavljeno da IGF-1 svoje zaštitne efekte u srcu ostvaruje tako što utiče na aktivnost Na^+/K^+-ATPaze, izučavan je uticaj IGF-1 na ekspresiju i aktivnost Na^+/K^+-ATPaze uz učešće IRS/PI3K/Akt i mTOR/S6K1 signalnih puteva u srcu normalno uhranjenih i gojaznih pacova. Takođe, izučavan je efekat IGF-1 na strukturne promena u srcu, kao i interakciju između Na^+/K^+-ATPaze i proteina autofagije beklin-1, kao ključnog događaja u procesu autoze.

U eksperimentalnim studijama kao model za izučavanje uticaja gojaznosti na strukturne i funkcionalne promene srca, često se primenjuje HF ishrana kod glodara. Pokazano je da HF dijeta dovodi do povećanja mase srca, zadebljavanja zidova leve komore srca, povećanja poprečnog preseka kardiomiocita, povećanja udela intersticijalnog kolagena, nakupljanja lipida u miokardu, povećavanja koncentracije proinflamatornih citokina (interleukin-6 i faktor nekroze tumora-α) i smanjene ekspresije Na^+/K^+-ATPaze, što se smatra ranim stadijumom disfunkcije srca (Nagarajan i sar., 2013; Obradovic i sar., 2015; Martins i sar., 2015; Obradovic i sar., 2015; Crisóstomo i sar., 2024). Imajući u vidu navedene literaturne podatke u ovoj doktorskoj disertaciji je ispitivan *in vivo* efekat IGF-1 na ekspresiju i aktivnost Na^+/K^+-ATPaze posredstvom IRS/Akt i mTOR/S6K signalnih puteva kod normalno uhranjenih i gojaznih pacova.

U okviru ove doktorske disertacije pacovi su tretirani sa IGF-1 u dozi od 50 μg/kg, koja se prema rezultatima drugih autora smatra optimalnom dozom za ispitivanje *in vivo* efekta IGF-1 (Honsho i sar., 2009; Kanno i sar., 2016; Wang i sar., 2021). Efekti IGF-1 na Na^+/K^+-ATPazu u srcu pacova ispitivani su tokom 24 sata, kako bi se izbegle dugoročne adaptivne promene koje mogu nastati usled hronične izloženosti ovom molekulu.

Podaci iz literature ukazuju na značajnu ulogu IGF-1 u regulaciji funkcije kardiovaskularnog sistema i njegov uticaj na vazodilataciju krvnih sudova i poboljšanje cirkulacije krvi (Sowers 1997; Higashi i sar., 2019). Takođe, pokazano je da autokrino i parakrino delovanje IGF-1 u kardiomicitima značajno doprinosi održavanju homeostaze srca tako što podstiče preživljavanje i optimalnu funkciju kardiomicita (Higashi i sar., 2019). Ranije *in vitro* studije su pokazale da IGF-1 povećava aktivnost Na^+/K^+-ATPaze u glatkim mišićnim ćelijama krvnih sudova, i tako pozitivno deluje na kardiovaskularni sistem (Sowers 1997; Standley i sar., 1997; Li i sar., 1999; Isenovic i sar., 2004). Prvi deo istraživanja u okviru ove doktorske disertacije bio je usmeren na ispitivanje *in vivo* efekta IGF-1 na regulaciju Na^+/K^+-ATP-aze. Nakon interperiotonealne aplikacije IGF-1 zabeleženo je povećanje koncentracije

IGF-1 u serumu tretiranih životinja u odnosu na kontrolnu grupu životinja tretiranih fiziološkim rastvorom. Rezultati pokazuju da nije došlo do značajnih promena u telesnoj masi, masi srca, kao i odnosu mase srca i telesne mase među eksperimentalnim grupama.

Uprkos brojnim studijama u kojima je ispitivan uticaj hormona, agonista i različitih patofizioloških stanja na ekspresiju i aktivnost Na^+/K^+-ATPaze u različitim tkivima, još uvek nema dovoljno podataka o uticaju IGF-1 na subjedinice Na^+/K^+-ATPaze u srcu (Therien i Blostein 2000; Pirkmajer i Chibalin 2019; Obradovic i sar., 2023). Veći broj ranijih studija je bilo usmereno na ispitivanje uticaja IGF-1 na ekspresiju α_1 subjedinice i aktivnost Na^+/K^+-ATPaze u *in vitro* uslovima (Standley i sar., 1997; Li i sar., 1999; Isenovic i sar., 2004). Rezultati ove doktorske disertacije su pokazali značajno povećanje nivoa genske ekspresije α_1 subjedinice, kao i povećanje nivoa proteina α_1 i α_2 subjedinica Na^+/K^+-ATPaze u frakciji plazma membrana homogenata srca pacova tretiranih IGF-1 u poređenju sa kontrolnim pacovima. Takođe, kod pacova tretiranih IGF-1 zabeležena je povećana aktivnost Na^+/K^+-ATPaze i povećana fosforilacija α subjedinice Na^+/K^+-ATPaze na mestu Ser^{23} u plazma membrani srca. Ovi rezultati su u saglasnosti sa rezultatima studije drugih autora koji su pokazali da fosforilacija α subjedinice na mestu Ser^{23} podstiče translokaciju Na^+/K^+-ATPaze iz unutarćelijskog prostora i povećavanje broja dostupnih molekula ovog enzima na membrani ćelija (Massey i sar., 2012). Dodatno, analiza korelacije pokazala je pozitivnu povezanost između koncentracije IGF-1 u serumu i aktivnosti Na^+/K^+-ATPaze u srcu pacova. Slični rezultati opisani su i u drugim modelima. U skladu sa ovim nalazom su rezultati prethodno objavljenih studija gde je pokazano da je oralna primena IGF-1 (3,5 mg/kg/dnevno tokom 4 dana) dovela do povećanja aktivnosti Na^+/K^+-ATPaze u enterocitima svinja (Alexander i Carey 2001). Zatim, u dva odvojena istraživanja koja su se bavila uticajem IGF-1 na regulaciju Na^+/K^+-ATPaze, uočen je pozitivan uticaj IGF-1 na aktivnost Na^+/K^+-ATPaze u škrgama lososa (McCormick 1996; Shimomura i sar., 2012). Na osnovu dobijenih rezultata grupe eksperimenata može se zaključiti da IGF-1 ne podstiče samo ekspresiju i aktivnost Na^+/K^+-ATPaze, već povećanjem fosforilacije α subjedinice povećava broj molekula Na^+/K^+-ATPaze na plazma membranama kardiomiocita.

Pokazano je da IGF-1 ostvaruje pozitivne efekte u srcu aktivacijom različitih signalnih puteva (Higashi i sar., 2019; Díaz del Moral i sar., 2022). Vezivanje IGF-1 za receptor na plazma membrani dovodi do njegove autofosforilacije čime se omogućava fosforilacija IRS-1 molekula (Hakuno i Takahashi 2018). Fosforilisan IRS-1 podstiče fosforilaciju PI3K/Akt i aktivaciju nishodnih proteina mTOR/S6K1 (Higashi i sar., 2019). Aktivacija i inhibicija IRS-1 zavisi od fosforilacije različitih Ser, Tyr i Thr aminokiselinskih ostataka (Peng i He 2018). Fosforilacija IRS-1 na mestu Ser^{307} dovodi do inhibicije njegove aktivnosti i ima značajnu ulogu u nastanku insulinske rezistencije (Liu i sar., 2015), dok fosforilacija IRS-1 na mestu Tyr^{1222} omogućava njegovu aktivaciju (Wu i sar., 2019).

U nastavku istraživanja ispitivana je uloga IRS-1/PDK-1/Akt signalnog puta u IGF-1 posredovanoj regulaciji Na^+/K^+-ATP-aze. Rezultati ove doktorske disertacije su pokazali da tretman IGF-1 dovodi do smanjenja fosforilacije IRS-1 na mestu Ser^{307}, i povećanja fosforilacije na mestu Tyr^{1222} u srcu pacova. Analiza korelacije pokazala je pozitivnu povezanost između fosforilacije IRS-1 na mestu Tyr^{1222} i aktivnosti Na^+/K^+-ATPaze u srcu pacova, ukazujući da je regulacija Na^+/K^+-ATPaze posredovana aktivacijom IRS-1 signalnog puta.

Akt kinaza reguliše veliki broj procesa u ćeliji, a za njenu potpunu aktivaciju neophodna je fosforilacija na Ser^{473} aminokiselinskom ostatku (Partovian i Simons 2004; Hart i Vogt 2011). Korišćenjem specifičnog inhibitora PDK-1 molekula, Kim i Park (Kim i Park 2018) su pokazali da IGF-1 podstiče aktivaciju Akt molekula u ćelijama humanog neuroblastoma. Rezultati ove doktorske disertacije su pokazali da tretman IGF-1 dovodi do fosforilacije PDK-1 na mestu Ser^{241} u srcu pacova. Prethodno je pokazano da se i u endotelnim ćelijama tretiranim

sa IGF-1 u dozi od 50 ng/ml detektuju fosforilacije Akt na Ser^{473}, Thr^{308}, Thr^{405} (Hart i Vogt 2011). Primenom specifičnih inhibitora PI3K, pokazano je da IGF-1 povećava nivo fosforilacije Akt na mestu Ser^{473} kao i aktivnost Na^+/K^+-ATPaze u ćelijama glatkih mišićnih vlakana krvnih sudova (Isenovic i sar., 2004). Han i sar (2020) su pokazali da povećanje fosforilacije Akt na mestu Ser^{473} dovodi do povećavanja nivoa α_1 i β_1 subjednica Na^+/K^+-ATPaze u modelu pacova sa akutnim oštećenjem pluća kao i alveolarnim epitelnim ćelijama nakon tretmana sa konjugatom maresina koji je uključen u regeneraciju tkiva. Navedeni literaturni podaci su u saglasnosti sa rezultatima prikazanim u ovoj doktorskoj disertaciji, u kojoj je pokazana zavisnost povećanja fosforilacije Akt na mestu Ser^{473} i povećanja ekspresije i aktivnosti Na^+/K^+-ATPaze nakon IGF-1 tretmana.

Dalja istraživanja su bila usmerena na ispitivanje mTOR signalnog molekula u regulaciji Na^+/K^+-ATPaze u srcu pacova tretiranih IGF-1. Iako mTOR poseduje brojna mesta fosforilacije, aminokiselinski ostaci Ser^{2448} i Ser^{2481} prepoznati su kao ključni za njegovu kinaznu aktivnost. Fosforilacija na mestu Ser^{2448} je posredovana sa Akt, dok se fosforilacija na mestu Ser^{2481} dešava tokom autokatalitičke fosforilacije mTOR proteina (Fletcher i sar., 2013). Takođe, fosforilacija mTOR na mestu Ser^{2448} stimuliše procese rasta i proliferacije ćelija, dok fosforilacija mTOR na mestu Ser^{2481} dovodi do fosforilacije Akt na mestu Ser^{473} i njegove aktivacije (Wataya-Kaneda 2015). Rezultati ove doktorske disertacije su pokazali da tretman IGF-1 dovodi do povećanja nivoa fosforilacije oba aminokiselinska ostatka, Ser^{2448} i Ser^{2481}, u srcu pacova. Wang i sar. (2015) su pokazali da L-triptofan aktivira mTOR i povećava gensku ekspresiju α_1 sujedinice Na^+/K^+-ATPaze u intestinalnim epitelnim ćelijama, ukazujući na ulogu mTOR u regulaciji ovog enzima. Takođe, analizom korelacija ustanovljeno je da je koncentracija IGF-1 u serumu bila u pozitivnoj korelaciji sa fosforilacijom mTOR na mestu Ser^{2448}, kao i da je aktivnost Na^+/K^+-ATPaze bila u pozitivnoj korelaciji sa povećanjem fosforilacije mTOR na mestu Ser^{2448}. Rezultati ove doktorske disertacije zajedno sa podacima iz literature ukazuju da IGF-1 utiče na aktivaciju mTOR proteina.

Radi potpunijeg razumevanja efekata IGF-1 na regulaciju Na^+/K^+-ATPaze, u daljim istraživanjima ispitivan je uticaj IGF-1 na aktivaciju S6K1, kao jednog od ključnih efektornih molekula mTOR i Akt u procesu sinteze proteina (Morita i sar., 2015). Pokazano je da IGF-1 u dozama 1, 10 i 100 ng/ml povećava fosforilaciju Akt kao i S6K1 u osteocitima, dok primena rapamicina, inhibitora aktivnosti mTOR molekula, kao i efekte IGF-1 (Bakker i sar., 2016). Primena rapamicina je takođe pokazala da je S6K1 uključena u regulaciju aktivnosti Na^+/K^+-ATPaze (Pesce i sar., 2003). U istoj studiji je pokazano da izoproterenol, β-adrenergički agonist, povećava ekspresiju Na^+/K^+-ATPaze molekularnim mehanizmom koji uključuje aktivaciju PI3K i mTOR/S6K1 u alveolarnim epitelnim ćelijama (Pesce i sar., 2003). Ovi literaturni podaci su u saglasnosti sa rezultatima ove doktorske disertacije prema kojima je pokazano da tretman pacova IGF-1 dovodi do povećanja nivoa fosforilacije S6K1 u srcu, što je povezano sa povećanjem ekspresije i aktivnosti Na^+/K^+-ATPaze.

Rezultati novijih istraživanja ukazuju na direktnu uključenost Na^+/K^+-ATPaze u specifičnom tipu ćelijske smrti, odnosno autozi (Fernández Á i sar., 2020; Depierre i sar., 2024). Usled ograničenog regenerativnog potencijala srca i činjenicu da odrasli kardiomiociti nemaju sposobnost deobe (White i Chong 2020), istraživanja usmerena ka procesima koji doprinose preživljavanju kardiomiocita su od izuzetne važnosti. Iako su podaci iz literature o procesu autoze vrlo oskudni, *in vivo* efekat IGF-1 na ovaj tip ćelijske smrti do sada nisu ispitani. Uzimajući u obzir da IGF-1 predstavlja jedan od glavnih faktora koji utiče na aktivnost Na^+/K^+-ATPaze i ostvaruje pozitivne efekte u srcu u ovoj doktorskoj disertaciji je ispitivan *in vivo* efekat IGF-1 na interakciju Na^+/K^+-ATPaze i proteina autofagije beklin-1, ključnog regulatora inicijacije autofagije, koji učestvuje u formiranju autofagosoma i povezan je sa procesom autoze. Polazna hipoteza je bila da IGF-1 smanjenjem interakcije između Na^+/K^+-ATPaze i

beklin-1, može doprineti smanjenju autoze kardiomiocita, molekulskim mehanizmom koji uključuje aktiviranje hibridnog IGF-1R/IR receptora i AMPK/FOXO1 signalnog puta.

U cilju razumevanja mehanizma kojim IGF-1 *in vivo* ostvaruje efekat na interakciju između Na^+/K^+-ATPaze i beklin-1 u srcu u ovoj doktorskoj disertaciji su korišćene dve grupe normalno uhranjenih pacova, od kojih je jedna tretirana intraperitonealno jednom dozom IGF-1 24 sata pre žrtvovanja, dok je druga grupa tretirana istom dozom fiziološkog rastvora. Rezultati studija pokazali su da je ciljana inhibicija interakcije Na^+/K^+-ATPaze i beklin-1 smanjila oštećenje miokarda nakon infarkta, kao i veličinu zone infarkta (Fernández Á i sar., 2020; Jiang i sar., 2022). Rezultati ove doktorske disertacije su pokazali da je tretman pacova sa IGF-1 smanjio nivo interakcije Na^+/K^+-ATPaze i beklin-1. Imajući u vidu da je ova interakcija ključna za proces autoze (Nah i sar., 2020), dobijeni rezultati ukazuju na jedan od mogućih mehanizama kojim IGF-1 doprinosi preživljavanju kardiomiocita u srcu.

U nastavku istraživanja analiziran je potencijalni mehanizam kojim IGF-1 utiče na interakciju Na^+/K^+-ATPaze i beklin-1. Delovanje IGF-1 u ćeliji je posredovano različitim tipovima receptora, kao što su IGF-1R, IR i hibridni receptor koji je izgrađen od subjedinica IGF-1R i IR (Kiernan i sar., 2024; Khan i sar., 2025). Podaci iz literature pokazuju da se IGF-1 sa visokim afinitetom vezuje za IGF-1R/IR hibridni receptor, čija aktivacija pokreće unutarćelijske signalne puteve koji regulišu metabolizam, rast i proces autofagije (Xu i sar., 2022). Pretpostavlja se da hibridni receptor nastaje usled potrebe za specifičnim delovanjem IGF-1 i finom regulacijom u različitim tkivima (Kiernan i sar., 2024; Khan i sar., 2025). U skladu sa tim, rezultati ove doktorske disertacije su pokazali da je *in vivo* tretman pacova IGF-1 doveo do povećanja nivoa fosforilacije IGF-1R/IR na Tyr ostacima β subjedinica (Tyr^{1131}/Tyr^{1146}). Ovi rezultati ukazuje na jedan od mogućih mehanizama kojim IGF-1 ostvaruje svoj efekat na interakciju Na^+/K^+-ATPaze i beklin-1 u srcu posredstvom IGF-1R/IR hibridnog receptora.

Poznato je da beklin-1 ostvaruje interakcije sa različitim proteinima, formirajući komplekse koji učestvuju u regulaciji autofagije (Tran i Fairlie 2021). Među ključnim regulatornima aktivnosti beklin-1 izdvajaju se AMPK i transkripcioni faktor FOXO1 (Yue i sar., 2022; Park i sar., 2023). Pored toga, pokazano je da gensko utišavanje ekspresije FOXO1 smanjuje gensku ekspresiju beklin-1, kao i stepen autofagije u MDA-MB-231 ćelijama tretiranim stimulatorom autofagije (paclitaxel) (Xu i sar., 2022). Ovi rezultati ukazuju na značaj FOXO1 u regulaciji autofagije i ekspresije beklin-1, a samim tim i procesa autoze (Xu i sar., 2022). Radi potpunijeg razumevanja uticaja IGF-1 na interakciju između Na^+/K^+-ATPaze i beklin-1 u srcu, u narednim eksperimentima određivan je stepen fosforilacije FOXO1 i AMPK signalnih molekula. Rezultati ove doktorske disertacije pokazali su da je tretman IGF-1 doveo do smanjenja aktivacije FOXO1 usled povećane fosforilacije FOXO1 na mestu Ser^{256} u srcu tretiranih u poređenju sa kontrolnim pacovima. Takođe, rezultati su pokazali da je fosforilacija AMPK na mestu Thr^{172} smanjena u srcu pacova tretiranih IGF-1 u poređenju sa kontrolnim pacovima, što je u skladu sa podacima iz literature u kojima je pokazano da IGF-1 *in vitro* smanjuje aktivaciju AMPK u različitim tipovima ćelijama (Zhang i sar., 2016; Aghanoori i sar., 2019). Poznato je da AMPK ima ključnu ulogu u regulaciji brojnih fizioloških procesa koji pomažu održavanje homeostaze kardiomiocita, dok se njena prekomerna ekspresija dovodi u vezu sa srčanom insuficijencijom (Park i sar., 2023). Takođe, u studiji sprovedenoj na HEK293T ćelijama, koje su bile izložene restrikciji glukoze i prethodno tretirane sa inhibitorima za AMPK, uočena je smanjena aktivacija beklin-1 (Zhang i sar., 2016). Ovi rezultati ukazuju na značaj FOXO1 i AMPK u regulaciji beklin-1, što može da utiče i na proces autoze.

Dobijeni rezultati po prvi put pokazuju da IGF-1 *in vivo* utiče na smanjenje interakcije između Na^+/K^+-ATPaze i beklin-1 u srcu normalno uhranjenih pacova, posredstvom hibridnog IGF1R/IR receptora, molekulskim mehanizmom koji uključuje smanjenje interakcije Na^+/K^+-

ATPaze i beklin-1 i fosforilacije AMPK i FOXO1 signalnih molekula. Takođe, preliminarni rezultati dobijeni u okviru ove doktorske disertacije su pokazali da IGF-1 *in vivo* smanjuje nivo interakcije Na^+/K^+-ATPaze i beklin-1 u srcu gojaznih pacova.

Gojaznost doprinosi razvoju brojnih poremećaja srčane funkcije, uključujući funkcionalne i strukturne promene kardiomiocita (Csige i sar., 2018). Iako molekulski mehanizmi štetnog delovanja gojaznosti na srce nisu u potpunosti razjašnjeni, literaturni podaci ukazuju na značajnu ulogu poremećene funkcije Na^+/K^+-ATP-aze u razvoju patofizioloških promena srca (Obradovic i sar., 2015; Jovanovic i sar., 2017; Baloglu 2023; Dhalla i sar., 2024). U cilju izučavanja *in vivo* efekata IGF-1 na Na^+/K^+-ATP-azu u srcu u gojaznosti u ovoj doktorskoj disertaciji korišćeni su pacovi koji su hranjeni HF dijetom tokom 12 nedelja. Dobijeni rezultati su pokazali da su telesna masa pacova kao i koncentracije glukoze u serumu pacova ostale nepromenjene kod gojaznih pacova tretiranih IGF-1 u odnosu na gojazne netretirane pacove, što je u saglasnosti sa prethodnim istraživanjima u kojima je pokazano da IGF-1 ne utiče na telesnu masu i u različitim eksperimentalnim modelima gojaznosti (Hahn i sar., 2024). Međutim, izostanak efekta IGF-1 na koncentraciju glukoze u krvi može da bude posledica dužine perioda gladovanja, jer je pokazano da gladovanje duže od 6 sati predstavlja stres kod glodara (Hahn i sar., 2024).

Poznato je da prekomerni unos nutrijenata povećava koncentraciju Ang II u krvi i posledično dovodi do sistemske vazokonstrikcije i zadržavanja vode i Na^+, čime doprinosi nastanku hipertenzije (El Meouchy i sar., 2022). Pored toga, povišena koncentracija Ang II podstiče aktivaciju mTOR/S6K1 signalnog puta, koji potom inhibira IRS-1 i Akt, čime doprinosi nastanku rezistencije na insulin (Bodur i sar., 2022). Podaci iz literature ukazuju na pozitivan efekat IGF-1 na funkciju endotela održavanje normalnog tonusa vaskulature, tako što ublažava štetan efekat koji izaziva povišen nivo Ang II (Ock i sar., 2021; Zhong i sar., 2022). Rezultati ove doktorske disertacije su pokazali značajno smanjenje nivoa Ang II u serumu gojaznih pacova tretiranih sa IGF-1 u odnosu na netretirane gojazne pacove. Ang II svoje efekte ostvaruje posredstvom dva tipa receptora, AT_1R i AT_2R. Aktivacija AT_1R je povezana sa razvojem hipertrofije srca i inflamacije (Bhullar i Dhalla 2022), dok aktivacija AT_2R doprinosi vazodilataciji i sprečavanju razvoja hipertrofije srca (Dopona i sar., 2019; Bhullar i Dhalla 2022). Podaci iz literature ukazuju da korišćenje AT_1R antagonista smanjuje razvoj patofizioloških promena u kardiovaskularnom sistemu izazvanih HF dijetom (Krueger i sar., 2017). Rezultati ove doktorske disertacije su pokazali da je tretman gojaznih pacova IGF-1 smanjio nivo AT_1R i povećao nivo AT_2R u srcu, u poređenju sa netretiranim gojaznim pacovima. Promene na nivou ekspresije ovih receptora mogu da budu jedan od mehanizama kojim IGF-1 ostvaruje svoje efekte u smanjivanju hipertrofije srca modulacijom signalizacije posredovane sa Ang II.

Poremećaji srčane funkcije i hipertrofija srca izazvani gojaznošću u dužem vremenskom periodu dovode do razvoja ozbiljnih kardiovaskularnih komplikacija i smrti (Alpert i sar., 2018; Koliaki i sar., 2019). Gojaznost praćena hipertenzijom i rezistencijom na insulin negativno utiče na Na^+/K^+-ATPazu u srcu (Wu i Ballantyne 2020). Smanjena aktivnost Na^+/K^+-ATPaze u gojaznosti dovodi do poremećaja srčane funkcije, imajući u vidu značajnu ulogu Na^+/K^+-ATPaze u održavanju jonskog gradijenta i drugih procesa u kardiomiocitama (Obradovic i sar., 2015; Baloglu 2023). Dosadašnji literaturni podaci pokazuju rezultate *in vitro* efekata IGF-1 na aktivnost Na^+/K^+-ATPaze u ćelijama glatkih mišićnih vlakana krvnih sudova (Standley i sar., 1997; Isenovic i sar., 2004) i *in vivo* efekata IGF-1 na aktivnost Na^+/K^+-ATPazu u različitim tkivima u fiziološkim stanjima (Alexander i Carey 2001; Shimomura i sar., 2012). Međutim, podaci o uticaju IGF-1 na regulaciju Na^+/K^+-ATPaze u srcu u gojaznosti su nedovoljno dokumentovani u literaturi. Rezultati ove doktorske disertacije su pokazali značajno povećanje aktivnosti Na^+/K^+-ATPaze, kao i povećavanje fosforilacije α subjednice na mestu Ser^{23} kod gojaznih pacova tretiranih IGF-1 u poređenju sa netretiranim gojaznim

pacovima. Takođe, nivo α_1 subjedinice Na^+/K^+-ATPaze je bio značajno povećan nakon davanja IGF-1 gojaznim pacovima, u poređenju sa netretiranim gojaznim pacovima. U ovoj doktorskoj disertaciji su određivane i koncentracije Na^+ i K^+ u serumu pacova i dobijeni rezultati su pokazali da nije bilo značajnih razlika u koncentracijama ovih jona u serumu kod gojaznih pacova tretiranih sa IGF-1 u poređenju sa netretiranim gojaznim pacovima. Imajući u vidu ključnu ulogu Na^+/K^+-ATPaze u održavanju koncentracija K^+ i Na^+, ovi rezultati ukazuju da *in vivo* tretman sa IGF-1 iako je povećao aktivnost i ekspresiju Na^+/K^+-ATPaze, nije uticao na balans K^+ i Na^+ u cirkulaciji.

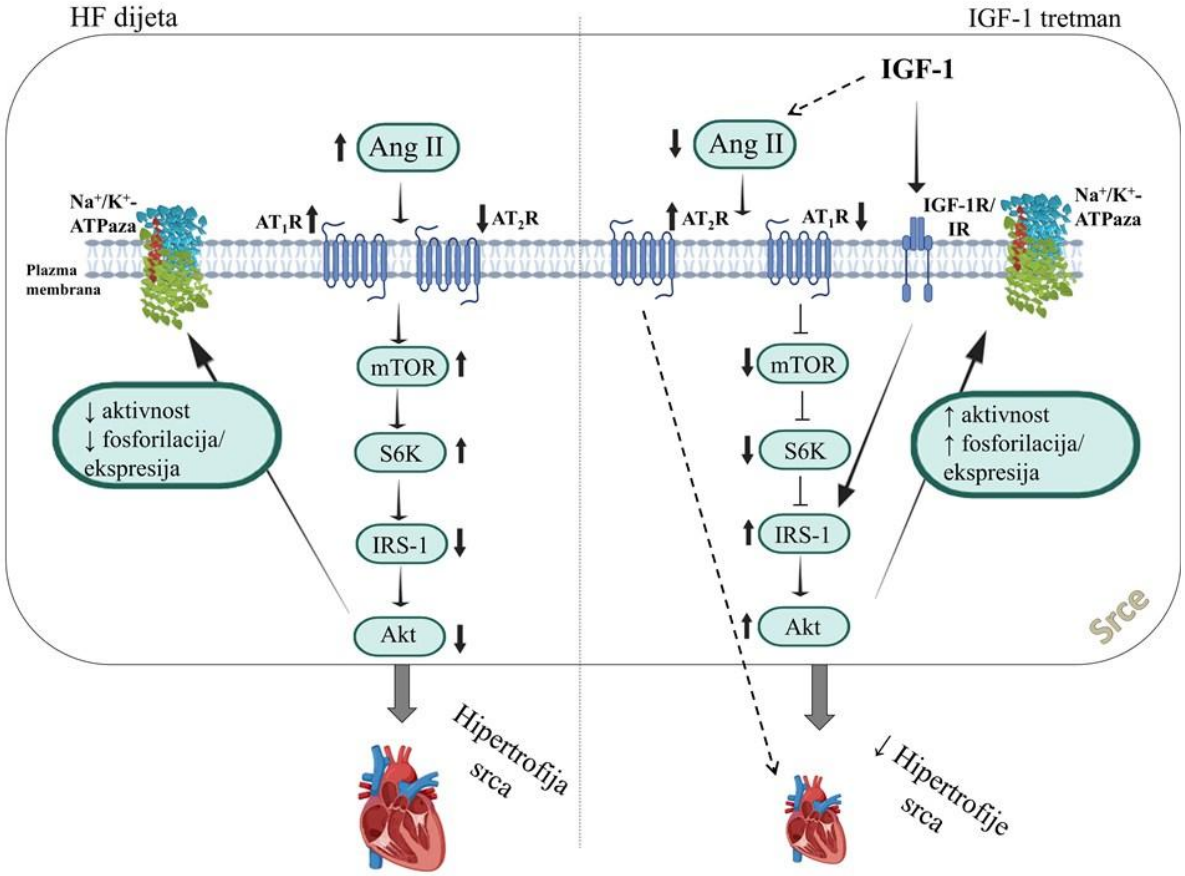
Podaci iz literature ukazuju da IGF-1 može da kompenzuje izostanak delovanja insulina u stanju gojaznosti združene sa rezistencijom na insulin, stimulacijom PI3K/Akt signalnog puta (Mughal i sar., 2019). Ovaj efekat IGF-1 verovatno ostvaruje preko IGF-1R/IR hibridnog receptora, koji predstavlja potentniji aktivator signalizacije u odnosu na insulin, pri čemu je povećana ekspresija ovog receptora u korelaciji sa koncentracijom IGF-1 u plazmi u gojaznosti (Slaaby 2015; Xu i sar., 2022). Rezultati ove doktorske disertacije ukazuju na povećanje nivoa fosforilacije hibridnog receptora IGF-1R/IR na Tyr ostacima β subjedinica (Tyr^{1131}/Tyr^{1146}). Takođe, pokazano je da ovaj receptor može da podstiče aktivaciju signalnih puteva uključenih u preživljavanje ćelija (Annunziata i sar., 2011). Stoga, povećana fosforilacija ovog receptora ukazuje da IGF-1 pozitivno deluje na preživljavanje ćelija srca i na taj način ublažava negativan uticaj HF dijete na srce.

U cilju boljeg razumevanja mehanizma kojima IGF-1 *in vivo* ostvaruje svoje efekte na regulaciju Na^+/K^+-ATPaze u srcu gojaznih pacova u daljem radu su izučavani IRS-1/Akt i mTOR/S6K1 signalni putevi. Signalni put IRS-1/Akt ima značajnu ulogu u održavanju normalne funkcije kardiovaskularnog sistema i srca (Riehle i Abel 2016). Poremećaji ovog signalnog puta izazvani gojaznošću su povezani sa nastankom različitih patoloških stanja srca (Obradovic i sar., 2015; Riehle i Abel 2016; Clausen i sar., 2017; Baloglu 2023). Rezultati ove doktorske disertacije pokazali su da je primena IGF-1 značajno povećala aktivaciju IRS-1, kroz povećavanje fosforilacije na mestu Tyr^{1222}, dok je smanjila inhibitornu fosforilaciju IRS-1 na mestu Ser^{307} u srcu gojaznih pacova u odnosu na netretirane gojazne pacove (Martínez Báez i sar., 2024). Takođe, pokazano je da IGF-1 dovodi do povećavanja fosforilacije Akt na mestu Ser^{473} kod gojaznih pacova u poređenju sa netretiranim gojaznim pacovima, što je u skladu sa rezultatima dobijenih posle tretmana IGF-1 kod normalno uhranjenih pacova. Rezultati ranijih *in vitro* i *in vivo* studija su pokazali da Akt pored uticaja na metabolizam glukoze i lipida i predstavlja jedan od ključnih signalnih molekul uključenih u regulaciju aktivnosti i ekspresije Na^+/K^+-ATPaze (Isenovic i sar., 2004; Obradovic i sar., 2015; Baloglu 2023).

Naredni eksperimenti u ovoj doktorskoj disertaciji bili su usmereni ka ispitivanju efekata IGF-1 na aktivaciju mTOR/S6K1 signalnog puta i njihove povezanosti sa regulacijom Na^+/K^+-ATPaze. Pokazano je da IGF-1 utiče na aktivaciju mTOR signalnog molekula (Aoyagi i sar., 2015), koji poseduje različita mesta fosforilacije, a koja direktno utiču na katalitičku aktivnost mTOR. Rezultati ove doktorske disertacije su pokazali da je IGF-1 povećao fosforilaciju mTOR molekula na mestu Ser^{2481} i smanjio fosforilaciju na mestu Ser^{2448} kod gojaznih pacova u poređenju sa netretiranim gojaznim pacovima. Takođe, pokazano je da je tretman gojaznih pacova IGF-1 doveo do smanjenja fosforilacije S6K1 na Thr^{421}/Ser^{424}, koji predstavlja jedan od glavnih nishodnih ciljanih molekula mTOR (Saxton i Sabatini 2017). Ovi rezultati ukazuju da IGF-1 smanjenjem mTOR/S6K1 signalnog i povećanjem IRS-1/Akt signalnog puta pozitivno deluje na ekspresiju i aktivnost Na^+/K^+-ATPaze u srcu gojaznih pacova.

Smanjena aktivnost Na^+/K^+-ATPaze dovodi se u vezu sa razvojem hipertrofije srca prisutne kod određenih kardiovaskularnih bolesti (Schwinger i sar., 2003; Liu i sar., 2012). Hipertrofija srca predstavlja jedno od glavnih oblika srčane disfunkcije povezane sa gojaznošću (Alpert i sar., 2016). Patološku hipertrofiju karakteriše povećavanje veličine kardiomiocita koje nije praćeno formiranjem novih kapilara, neophodnih za ishranu kardiomiocita, što

posledično dovodi do hipoksije i remodelovanja srca (Nakamura i Sadoshima 2018). Promene u ekspresiji α-MHC i β-MHC značajno utiču na kontraktilnost srca, budući da dovode do strukturnih promena u miokardu (Bai i sar., 2021). Tokom razvoja hipertrofije srca dolazi do povećanja ekspresije β-MHC, koja poseduje manju ATP-aznu aktivnost, kao i do smanjenja odnosa između α-MHC i β-MHC (Guo i sar., 2018; Bai i sar., 2021). Takođe, pokazano je da je inhibicija ekspresije β-MHC dovodi do smanjenja hipertrofije srca, aktivacijom PI3K/Akt signalnog puta (Meng i sar., 2019). Rezultati ove doktorske disertacije ukazuju da je IGF-1 doveo do značajnog smanjenja mase srca kod gojaznih pacova u odnosu na netretirane gojazne pacove. Takođe, pokazano je da IGF-1 povećava ekspresiju gena za α-MHC, kao i odnos između α-MHC i β-MHC. Povećanje ekspresije α-MHC dovodi se u vezu sa povoljnijim fenotipom miokarda što doprinosi boljoj srčanoj funkciji i smanjuje srčanu insuficijenciju (Bai i sar., 2021). Rezultati ove doktorske disertacije su pokazali da IGF-1 *in vivo* povećava ekspresiju i aktivnost Na^+/K^+-ATPaze i smanjuje hipertrofiju srca kod gojaznih pacova, molekularnim mehanizmom koji uključuje IRS-1/Akt i mTOR/S6K1 signalne puteve (**Slika 11.**).



Slika 11. Predloženi mehanizam kojim IGF-1 *in vivo* utiče na ekspresiju i aktivnost Na^+/K^+-ATPaze i smanjenje hipertrofije srca kod gojaznih pacova. Akt – protein kinaza B, Ang II – angiotenzin II, AT_1R – receptor tip 1 za Ang II, AT_2R – receptor tip 2 za Ang II, IGF-1 – insulinu sličan faktor rasta 1, IGF-1R/IR – hibridni receptor za IGF-1 i insulin, IRS-1 – supstrat receptora za insulin, mTOR - ciljni molekul za rapamicin kod sisara, Na^+/K^+-ATPaze – natrijum-kalijum adenozin trifosfataza, S6K1 - kinaze ribozomalnog proteina S6-1, ↓ - smanjenje, ↑ - povećanje.

4. ZAKLJUČCI

Na osnovu rezultata dobijenih u okviru ove doktorske disertacije mogu se izvesti sledeći zaključci:

Ispitivanje *in vivo* efekta IGF-1 na ekspresiju i aktivnost Na^+/K^+-ATPaze u srcu normalno uhranjenih pacova, pokazano je da IGF-1 u srcu dovodi do:

- pozitivne korelacije između koncentracije IGF-1 u serumu i aktivnosti Na^+/K^+-ATPaze, kao i između nivoa fosforilacije IRS-1 na mestu Tyr^{1222} i aktivnosti Na^+/K^+-ATPaze,
- povećanja nivoa fosforilacije IRS-1, PDK-1, Akt, mTOR i S6K1 signalnih molekula,
- povećanja nivoa fosforilacije IGF1R/IR i FOXO1, odnosno do smanjenja nivoa fosforilacije AMPK proteina,
- povećanja relativne ekspresije gena za α_1 subjedinicu Na^+/K^+-ATPaze, nivoa proteina α_1 i α_2 subjedinice Na^+/K^+-ATPaze, fosforilacije α subjedinice Na^+/K^+-ATPaze i aktivnosti Na^+/K^+-ATPaze,
- smanjenja interakcije između α_1 subjedinice Na^+/K^+-ATPaze i proteina autofagije beklin-1.

Ispitivanje *in vivo* efekata IGF-1 na ekspresiju i aktivnosti Na^+/K^+-ATP-aze u srcu gojaznih pacova kao i na strukturne promene u srcu, pokazano je da IGF-1 dovodi do:

- povećanja nivoa proteina α_1 subjedinice Na^+/K^+-ATPaze, nivoa fosforilacije α subjedinice Na^+/K^+-ATPaze i aktivnosti Na^+/K^+-ATPaze u srcu,
- smanjenja nivoa Ang II i jona K^+ u serumu,
- smanjenja nivoa AT_1R proteina i povećanja nivo AT_2R proteina u srcu
- povećanja nivoa fosforilacije IGF-1R/IR, IRS-1, Akt i smanjenja nivoa fosforilacije mTOR i S6K1 proteina u srcu,
- smanjenja mase srca i povećanja relativne ekspresije gena za α-MHC, kao i odnosa između α-MHC i β-MHC,
- smanjenja interakcije između α_1 subjedinice Na^+/K^+-ATPaze i proteina autofagije beklin-1.

U stanju gojaznosti dolazi do poremećaja u aktivnosti Na^+/K^+-ATPaze, ključnog enzima odgovornog za održavanje jonske homeostaze, kao i električne stabilnosti kardiomiocita. Istovremeno, smanjenje nivoa IGF-1 u gojaznosti dovodi do poremećaja IRS/Akt i mTOR/S6K signalnih puteva, što za posledicu ima smanjenu sintezu proteina i slabljenje kontraktilnosti srca, kao i smanjenju sposobnosti srca da odgovori na metabolički stres.

Detaljno razumevanje molekulskih mehanizama kojim IGF-1 reguliše Na^+/K^+-ATPaze u srcu u stanju gojaznosti omogućava razvoj novih pristupa u prevenciji i ublažavanju srčane disfunkcije povezane sa gojaznošću. Razumevanje ovih mehanizama otvara mogućnosti

razvoja i primenu ciljane terapije srčanih oboljenja, koje bi bile usmerene na obnovu IGF-1 signalizacije i stabilizaciju ekspresije i aktivnosti Na^+/K^+-ATPaze.

Naučni doprinos ove doktorske disertacije ogleda se u razumevanju molekulskih mehanizama delovanja IGF-1 u fiziološkim i patofiziološkim stanjima, kao što je gojaznost koja dovodi do poremećaja regulacije Na^+/K^+-ATPaze i hipertrofije srca. Dobijeni rezultati ukazuju da IGF-1 ostvaruje pozitivne efekte u srcu tako što povećava aktivnost Na^+/K^+-ATPaze i smanjuje interakciju Na^+/K^+-ATPaze sa beklinom-1, čime doprinosi preživljavanju ćelija srca. Pored toga, pokazano je da IGF-1 u gojaznosti povećanjem aktivnosti Na^+/K^+-ATPaze doprinosi smanjenju hipertrofije srca. Ovi rezultati ne samo da ukazuju na potencijalnu ulogu IGF-1 u lečenju disfunkcije srca povezane sa gojaznošću, već predstavlja dobru osnovu za dalja istraživanja i kliničku primenu IGF-1 u lečenju srčanih bolesti.

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BIOGRAFIJA

Katarina Banjac je rođena u Banjaluci 19.5.1996, godine, gde je završila osnovu školu i gimnaziju. Školske 2015/2016 godine je upisala Biološki fakultet, Univerziteta u Beogradu. Osnovne studije molekularne biologije i fiziologije je završila 2019. godine i stekla zvanje diplomiranog biologa. Iste godine upisala je master studije na studijskom programu molekularna biologija, na Biološkom fakultetu, Univerziteta u Beogradu, dok je eksperimentalni deo master rada uradila u Laboratoriji za radiobiologiju i molekularnu genetiku, Instituta za nuklearne nauke „Vinča" – Institut od nacionalnog značaja za Republiku Srbiju – Univerziteta u Beogradu. U septembru 2020. godine je odbranila master rad pod nazivom „ Prognostički značaj fosfolipida, slobodnih masnih kiselina i nitrita/nitrata u serumu i ispirku bioptata tiroidnih nodusa u dijagnostikovanju malignih nodusa štitaste žlezde" i stekla zvanje master molekularne biologije. U školskoj 2020/2021 je upisala doktorske studije na modulu animalna i humana fiziologija, studijskog programa biologija, pod rukovodstvom dr Milana Obradovića. Od decembra 2020. godine je zaposlena u Laboratoriji za radiobiologiju i molekularnu genetiku, Instituta za nuklearne nauke „Vinča" – Institut od nacionalnog značaja za Republiku Srbiju – Univerziteta u Beogradu i trenutno radi kao istraživač saradnik.

Iz naučno-istraživačke aktivnosti K. Banjac proistekli su rezultati koji su u autorstvu i koautorstvu objavljeni u 7 bibliografskih jedinica.

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Ментори:

1. др Милан Обрадовић, научни саветник, Универзитет у Београду – Институт за нуклеарне науке „Винча“ Институт од националног значаја за Републику Србију
2. Проф. др Тања Јевђовић, ванредни професор, Биолошки факултет – Универзитет у Београду

Изјављујем да је штампана верзија мог докторског рада истоветна електронској верзији коју сам предао/ла ради похрањивања у Дигиталном репозиторијуму Универзитета у Београду.

Дозвољавам да се објаве моји лични подаци везани за добијање академског назива доктора наука, као што су име и презиме, година и место рођења и датум одбране рада.

Ови лични подаци могу се објавити на мрежним страницама дигиталне библиотеке, у електронском каталогу и у публикацијама Универзитета у Београду.

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Изјава о коришћењу

Овлашћујем Универзитетску библиотеку „Светозар Марковић“ да у Дигитални репозиторијум Универзитета у Београду унесе моју докторску дисертацију под насловом:

Утицај инсулину сличног фактора раста 1 на експресију и активност натријум-калијумове пумпе у срцу гојазних пацова,

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