Документ 11

РЕЗЮМЕТА НА НАУЧНИТЕ ТРУДОВЕ

на гл. ас. д-р Камелия Жечкова Братоева, д.м.

за участие в конкурс за заемане на академична длъжност „Доцент"
в област на висшето образование 7. Здравеопазване и спорт, професионално направление 7.1 Медицина, специалност „Патофизиология", обявен в Държавен вестник, бр. 36 от 27.04.2018 г.

За участие в конкурса са представени общо 63 научни труда, от които:

- Монография-1
- Автореферат на дисертационен труд - 1
- Пълнотекстови публикации – 28
- Учебни пособия - 1
- Участия в научни прояви - 32

Научните трудове имат общо 12 цитирания.

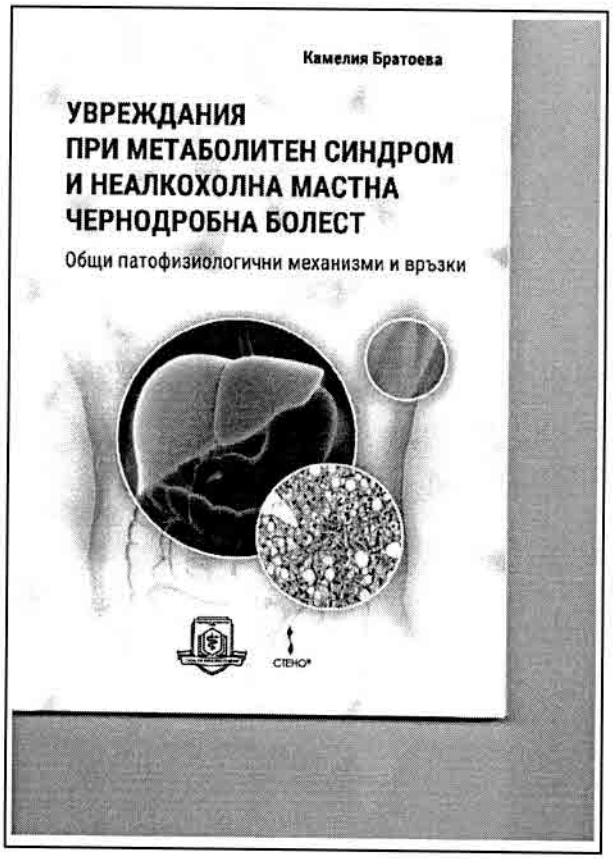
Общият импакт фактор на научните трудове е 22,463.

Научните трудове могат да бъдат тематично групирани в три основни направления:

- Експериментални проучвания при метаболитен синдром и неалкохолна мастна чернодробна болест
- Изследване на патогенетични механизми при системен лупус еритематодес и неговата остра клинична изява- лупусен нефрит
- Биомаркери на клетъчна смърт при солидни тумори и прояви на дистрес при онкологично болни пациенти.

1. РЕЗЮМЕ НА ПУБЛИКУВАН МОНОГРАФИЧЕН (ХАБИЛИТАЦИОНЕН) ТРУД

„ Увреждания при метаболитен синдром и неалкохолна мастна чернодробна болест-общи патофизиологични механизми и връзки". Камелия Братоева, СТЕНО/ МУ- Варна, 2018, ISBN 978-619-221-134-9, ISBN 978-954-449-961-7



Монографичният труд е представен в обем от 126 страници, разпределен в общо 14 глави, включващи въведение, заключение и следните глави: Въведение; Метаболитен синдром; Неалкохолна мастна чернодробна болест; Увреждане на черния дроб при затлъстяване и инсулинова резистентност; Роля на етиологичните фактори в патогенезата на затлъстяването; Инсулинова резистентност; Адипоцитна дисфункция при затлъстяване; Адипоцитокини – връзка между мастна тъкан и черен дроб; Нискостепенно възпаление-връзка между затлъстяване, инсулинова резистентност, стеатоза и сърдечносъдови заболявания; Чернодробна стеатоза – липиди на погрешното място; Дисбаланс в антиокси-

дантната защита при неалкохолна чернодробна стеатоза; Роля на клетъчната смърт в патогенезата на неалкохолната мастна чернодробна болест; Неалкохолната мастна чернодробна болест като мултиситемно заболяване; Заключение; Библиография.

Монографията е онагледена е с 19 фигури и 5 таблици. Библиографията включва 377 литературни източника, повечето от които публикувани през последните 10 години.

Монографията разглежда проучени основни патофизиологични механизми на чернодробни увреждания свързани със затлъстяването и инсулиновата резистентност, които съществуват едновременно в патогенезата на метаболитния синдром и неалкохолната мастна чернодробна болест. Отделено е внимание на съвременните схващания за ролята на етиологичните фактори, оксидативния стрес, възпалението, нарушенията в липидния метаболизъм и видовете клетъчна смърт, сложните взаимодействия между тях, хранителните навици и гените, които играят важна роля в патобиологията на метаболитния синдром и свързаните с това многосистемни увреждания.

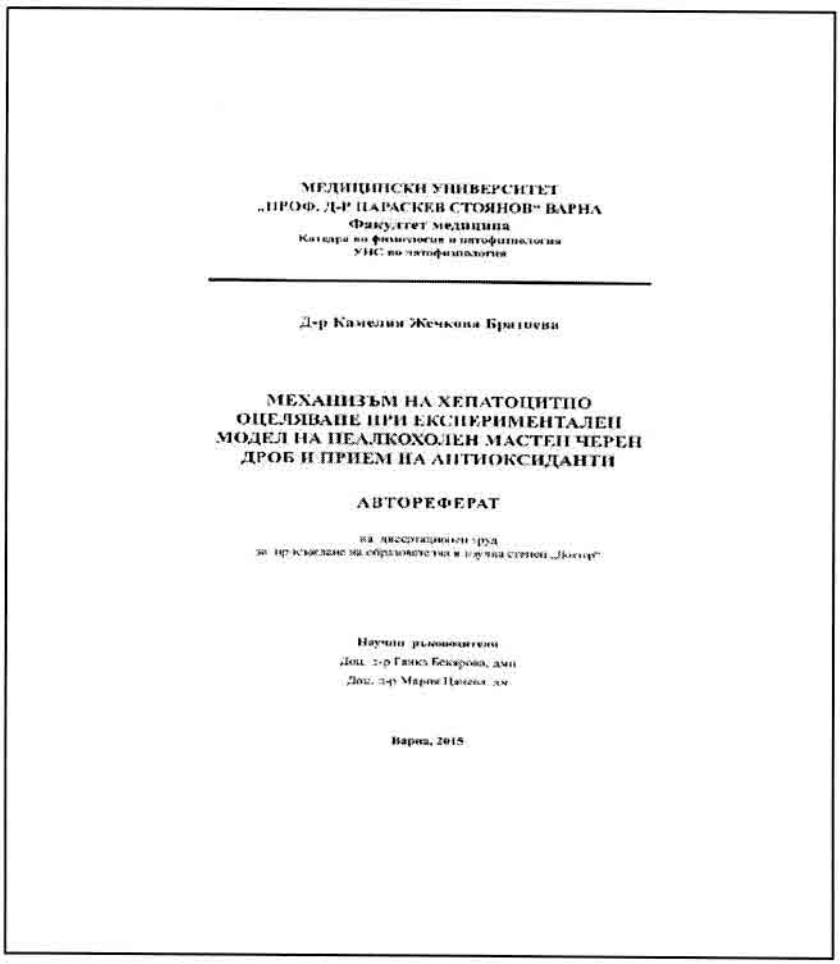
Към настоящия момент, систематичният преглед на литературата показва, че наличието на НМЧБ е тясно свързано с метаболитния синдром. Това се дължи на световната епидемия от метаболитен синдром и прогресивното разпространение на инсулинова резистентност и затлъстяване, които се проявяват със захарен диабет тип 2, артериална хипертензия, дислипидемия, хиперурикемия и чернодробна стеатоза. НМЧБ с нейните 3 клинично ясно различими стадия- чернодробна стеатоза, неалкохолен стеатохепатит и чернодробна цироза, е най-бързо и най-стръмно увеличаващата се кохорта на болните-кандидати за чернодробна трансплантация. Една от причините за тази тенденция е, че механизмите на увреждане, както и преходът на различните форми в НМЧБ все още не са добре установени и по тази причина липсват критерии и ранни неинвазивни биомаркери за диагноза.

Става все по-очевидно, че различните хистологични модели на НМЧБ, а именно обикновената стеатоза и неалкохолния стеатохепатит, не само имат различен риск от прогресия, но и могат да отразяват различни молекулярни взаимнопреплитащи се патогенетични механизми. Взаимодействието на различните фактори и начина, по който те влияят през етапите на НМЧБ показват тясната функционална връзка между това заболяване и различните аспекти на метаболитния синдром. В това отношение са изложени многобройни факти и патогенетични връзки, подкрепящи значението на адипоцитната дисфункция, системен оксидативен стрес, нарушеният митохондриален редокс баланс, различните модели на клетъчна смърт и нискостепенно възпаление в патогенезата и прогресията на НМЧБ при пациенти с метаболитен синдром.

В настоящата монография е акцентуирано и на схващането, че НМЧБ е силна детерминанта за бъдещото развитие на извънчернодробни заболявания. Триадата чернодробна стеатоза-инсулинова резистентност-метаболитен синдром увеличава риска от развитие на захарен диабет тип 2, сърдечно-съдови заболявания, хронично бъбречно заболяване, ментални разтройства и много други. Проучването на общите механизми и патогенетични връзки между тези събития е необходимо, защото ни дава нова преспектива за идентифицирането на атрактивни биомаркери за ранното диагностициране на НМЧБ и терапевтични подходи за намаляване на заболеваемостта и рискът от бъдещо развитие на многосистемни усложнения.

2. РЕЗЮМЕ НА ДИСЕРТАЦИОНЕН ТРУД

„Механизъм на хепатоцитно оцеляване при експериментален модел на неалкохолен мастен черен дроб и прием на антиоксиданти". Камелия Братоева, 2015, МУ-Варна.



МЕДИЦИНСКИ УНИВЕРСИТЕТ
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МЕХАНИЗЪМ НА ХЕПАТОЦИТНО ОЦЕЛЯВАНЕ ПРИ ЕКСПЕРИМЕНТАЛЕН МОДЕЛ НА НЕАЛКОХОЛЕН МАСТЕН ЧЕРЕН ДРОБ И ПРИЕМ НА АНТИОКСИДАНТИ

АВТОРЕФЕРАТ

на дисертационен труд
за придобиване на образователната и научна степен „Доктор"

Научни ръководители
Доц. д-р Ганка Бекярова, дмн
Доц. д-р Мария Цанкова, дм

Варна, 2015

Дисертационният труд е обсъден, приет и насочен към защита пред научно жури от разширен Катедрен съвет при Катедрата по физиология и патофизиология на Медицински университет „Проф. д-р Параскев Стоянов"-Варна на 05. 11. 2015 г.

Дисертационният труд съдържа общо 145 страници, онагледен е с 47 фигури и 6 таблици. Книгописът включва 319 заглавия, от които 1 на кирилица и 318 на латиница.

В настоящата разработка за първи път са проучени основни патофизиологични механизми на чернодробни увреждания свързани със затлъстяването, оксидативния стрес, възпалението и апоптоза при високо-фруктозна диета на плъхове и прием на S-AMe и Алопуринол.

Неалкохолната мастна чернодробна болест (НМЧБ) е най-разпространеното хронично чернодробно заболяване, свързана с нарастващата честота от затлъстяване, захарен диабет тип2, кардиометаболитни заболявания и се смята за неразделна част от метаболитния синдром. НМЧБ е клинико-патологичен синдром със широк спектър от увреждания, които варират от обикновена стеатоза до стеатохепатит, напреднала фиброза и цироза при липса на алкохолна злоупотреба. Неалкохолния стеатохепатит (НСХ) е напреднала форма НМЧБ, който е с необратими по характер увреждания и е най-бързо и стръмно увеличаващата се кохорта на болните-кандидати за чернодробна трансплантация. Конкретните причини и отличителни механизми за прогресията на обикновената стеатоза в НСХ и последващи увреждания не са достатъчно изяснени. Липсват разработени биомаркери, които да регистрират най-ранните алтеративни промени на хепатоцитите при липсващи макроскопски промени за чернодробни увреждания.

Важно място в комплекса от рискови фактори заемат ниската двигателна активност, приема на високо-калорични храни и напитки, както и свръхпотреблението на диетична фруктоза. Предполага се, че инсулиновата резистентност, повишената продукция на провъзпалителни цитокини, свободни мастни киселини(СМК) и активни форми на кислорода(АФК) от хипертрофиралата мастната тъкан при затлъстяване, намалението на чернодробната антиоксидантна защита стимулира апоптозата на хепатоцитите и последващи чернодробни увреждания.

На базата на тези данни ние създадохме хипотезата, че чернодробната апоптоза е ключов фактор в развитието и прогресирането на НСХ, а оксидативния стрес и възпалението в стеатозния черен дроб се очертават като основни звена в патофизиологичните механизми на клетъчните увреждания и смърт. Поставената цел бе, да се проучат някои възможни механизми на оцеляване на хепатоцитите при експериментален модел на метаболитен синдром с високо-фруктозна диета (ВФД) и прием

1. Да се изследват метаболитните промени при ВФД: телесно тегло и тегло на ретроперитонеална мастна тъкан и черен дроб; нива на серумна глюкоза и липиден профил;
нива и съотношение на наситени и ненаситени СМК в чернодробен хомогенат.
2. Да се изследват морфологичните (стеатоза) промени и някои биохимични показатели на чернодробна дисфункция.
3. Да се проучи ролята на оксидативния стрес (нива на МДА-малондиалдехида, редуциран глутатион и глутатион пероксидаза в серуми и чернодробен хомогенат и нискостепенното възпаление (изследване нивата на CRP, TNFa и пикочна киселина) за чернодробното увреждане.
4. Да се изследват апоптотични маркери (серумни- CK18 и тъканни- Bax, Bcl2 и CK18 протеини) при увреждане на черния дроб.
5. Да се проучат корелационни зависимостти между някои показатели на метаболитни нарушения, оксидативен стрес, възпаление и чернодробни увреждания.
6. Да се проучи хепатопротективния ефект на S-AMe(S-аденозилметионин) и Алопуринол при ВФД.

За изпълнението на поставените задачи сме изследвали 4 групи мъжки плъхове (линия Wistar): на стандартна диета и 3 групи с ВФД (35% високо-фруктозен царевичен сироп, поставен като разтвор за пиене). 3-тата и 4 тата група са приемали съответно S-AMe и Алопуринол. Използвали сме имонологични, имунохистохимични, хроматографски и биохимични методи на изследване. Статистическата обработка на резултатите в проучването включва дескриптивни методи и аналитични методи (T –test ($p \leq 0.05$); вариационен анализ (ANOVA, $p<0.05$); корелационен анализ ($r > 0.5$); Регресионен анализ.

Резултатите от проучването доказват, че ВФД предизвиква метаболитни нарушения като затлъстяване, хипергликемия, дислипидемия и хиперурикемия, съответстващи на критериите за диагностика на метаболитния синдром при хора. Това от една страна определя съществената роля на високо-фруктозната диета за спонтанната проява на синдрома (за разлика от генетичните модели) и характеризира значението на хранителните фактори за развитието на затлъстяване и инсулинова резистентност, както и последващи метаболитни аномалии-дислипидемии, оксидативен стрес, нискостепенно възпаление, мастна дегенерация на черния дроб и апоптоза. От друга страна, резултатите подсказват установената от други автори роля на храненето за епигенетичните промени включени в регулацията на чернодробният глюкозен и липиден метаболизъм, някои от които са замесени в развитието и прогресирането на НМЧБ.

Това прави експерименталният модел с ВФД иновативен по отношение на епигенетичните механизми и предлага нови перспективи за изследване на патогенезата на чернодробните увреждания, откриване на диагностични биомаркери и терапевтични стратегии за НМЧБ.

Нашите данни представят убедителни доказателства, че при фруктозо-индуциран метаболитен синдром оксидативният стрес и нискостепенното системно възпаление са във функционална връзка с активираните апоптотични процеси (повишени съотношение на Bax/Bcl2 и CK18 протеин) в стеатозния черния дроб, което предполага, че апоптозата е доминиращ механизъм на клетъчна смърт при неалкохолен мастен черен дроб. Съотвествието на нивата на СК-18 с хистологичните и биохимични промени в черния дроб на експерименталните животни го определят като подходящ биомаркер за неинвазивна оценка на степента на чернодробните увреждания, тяхната прогресия и ефекта от прилагането на терапии при НМЧБ в експериментални и клинични условия.

Резултатите показват, че затлъстяването и дисфункцията на висцералната мастна тъкан са основен патологичен фактор за интрахепатално отлагане на липиди и оксидативен стрес. Увеличената висцерална мастна тъкан е основен източник на повишена продукция на TNFa и експорт на СМК към черния дроб, които стимулирайки чернодробната инсулинова резистентност, de novo липогенеза и изчерпване на антиоксидантния капацитет на хепатоцитите предизвикват тъканни увреждания.

В това проучване за първи път се установи въздействието на S-AMe и Алопуринол върху цитозащитата на хепатоцитите в условията на фруктозо-индуцираните метаболитни разстройства. Резултатите показват селективно действие на S-AMe и Алопуринол върху оксидативния стрес и производството на липиди, което протектира чернодробните клетки срещу клетъчна смърт и последващи увреждания. За разлика от други известни антиоксиданти, нашите данни показват, че S-AMe проявява антиоксидантно, антистеатозно и антиапоптотично действие. Благоприятното вляние върху липидния метаболизъм в черния дроб е значимо по-изразено от това на Алопуринола.

На базата на представените резултати може да се направи заключение, че апоптозата е съществен фактор в развитието и прогресирането на неалкохолния мастен черен дроб при ВФД, а оксидативния стрес и системното възпаление се очертават като основни звена в патофизиологичните механизми на клетъчни увреждания и смърт. Доказаният антиоксидантен, антилипидемичен, антистеатозен и хепатопртективен ефект на S-AMe и Алопуринол, разкрива нови възможности за терапевтично повлияване на свързаните със затлъстяването и инсулинова резистентност метаболитни нарушения.

3. РЕЗЮМЕТА НА ПУБЛИКУВАНИ ПЪЛНОТЕКСТОВИ ПУБЛИКАЦИИ

1. **Bratoeva K**, Bekyarova G, Kiselova Y, Ivanova D. Effect of Bulgarian herb extracts of polyphenols on metabolic disorders- induced by high-fructose diet. *Trakia Journal of Sciences 2010, 8(2), 56-60. ISSN 1313-7050*

Introduction: Recently, fructose consumption has been suggested to be one of the environmental factors contributing to the development of insulin resistance, obesity, dislipidemia and other abnormalities of the metabolic syndrome. **Aim:** Herbal extracts with established therapeutic efficiency in patients with obesity and diabetes have been traditionally used in Bulgarian ethnomedicine.This study investigated the protective effect of extract of selected Bulgarian herbs (high content of polyphenols- Herbal-Antiox 1) on high-fructose diet-induced metabolic disorders in rat liver. **Material and methods:** The animals were divided randomly into four groups (n=6); Control group rats- C; fructose-drinking rats- FRU (high-fructose corn syrup-12.5% fructose content); fructose- drinking rats treated with Herbal-Antiox-1 (HA-1)-FRU+HA1;control group rats treated with Herbal-Antiox-1 (HA-1)-C+HA1.Rats received 12.5% fructose solution in drinking water for 12 weeks, control rats were maintained on plain water. Phytochemical analysis and antioxidant capacity of the extract were determined. **Results:**We determine liver triglyceride (TG) concentration, body weight, liver weight, adipose tissue weight. In the FRU rat the levels of plasma glucose, liver TG as well liver and body weight were increased significantly. Herbal-Antiox-1 significantly reduced the hyperglicaemia, TG concentration and liver/body weight as well. In the control group Herbal-Antiox-1 had no effect on investigated parameters. **In conclusion:** Herbal-Antiox -1 limits the accumulation of TG in liver, adipose tissue and contributes to reduction of body weight in rats .

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2. **Bratoeva K**, Bekyarova G, Kiselova Y, Radanova M, Ivanova D. Metabolic changes in experimental model of metabolic syndrom- induced by high- fructose diet in rats. *Scripta Scientifica Medica, 2010;42 (4), 233-235. ISSN 0582-3250*

The global epidemic of metabolic syndrome (MS) correlates with changes in the environment, feeding, behavior and lifestyle, leading to obesity, glucose intolerans, dyslipidemià ànd elevated cardiovascular risk.

AIM: The aim of our study was to develop an experimental model of the MS in rat that imitate the investigated metabolic disorders using high-fructose diet.

METHODS: We used two groups: control group (C)- rats, maintained on plain water (n=6); fructose group (FRU)- rats received 12.5% high-fructose corn syrup in drinking water for 12 weeks (n=6). The main markers of metabolic abnormalities (glucose, total cholesterol, triglycerides, uric acid, body and organs weight), the markers of oxidative stress (malondialdehyde (MDA), total thiols) and C-reactive protein (CRP) - inflammatory marker were measured.

RESULTS: Our data showed hypercholesterolemia, hyperglycemia, hyperuricemia and significant elevated levels of CRP, MDA, body and organs weight, and inhibited antioxidant defense in fructose- drinking rats. **CONCLUSION:** The experimental model will support our studies associated with pathophysiology and pharmacology of MS.

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3. Бекярова Г, Христова М, **Братоева К**. Влияние на мелатонина върху оксидативния стрес и хемостазата при експериментална термична травма. *Сборник от IV Национална конференция по проблемите на термичната травма и пластична хирургия-Варна, 2010, I: 39-46. ISBN:978-954-579-854-2*

Целта на настоящата разработка е проучване ефекта на мелатонина върху нивото на липидната пероксидация, маркерите на възпалението и коагулацията при експериментална термична травма. Мелатонинът (10 мг/кг) беше въведен i.p.непосредствено на 24 час и след термичната травма. Използвахме малондиалдехидът (МДА) като маркер на липидна пероксидация, протромбиново време (аТРТ), С- реактивен протеин (CRP) и фибронеген като маркери на възпалението и коагулацията. Изгаряне на кожата (индуцирано с гореща вода 90 gr. за 10 сек.) предизвиква повишаване на нивото на МДА в плазмата, а също и РА, но не променя нивото на ТРТ на 24-ия час. Нивото на острофазовите белтъци като CRP и фибриноген нараства значително. Третирането с мелатонин нормализира стойностите на МДА, а също понижава повишената концентрация CRP и фибриноген, но не ги нормализира. Нивото на РА намалява, но това на ТРТ не се променя след въвеждането на мелатонина. В заключение, екзогенното приложение на мелатонин подиска активирането на липидната пероксидация и коагулация, индуцирани от термична травма.

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4. **Bratoeva K**, Bekyarova G. Effect of extract of selected Bulgarian herbs (HA-1) on high fructose diet- induced hepatic injury in rats. *Archives of the Balkan Medical Union 2011; 46 (4), 107-111. ISSN 0041-6940*

The herbal extracts a lot of Bulgarian plans are rich of polyphenols and exhibits antioxidant activity. The aim of this study was to investigate the possible relation between oxidative stress and triglyceride accumulation in liver as well the protective effect of extract of selected Bulgarian herbs (high content of polyphenols- Herbal-Antiox 1(HA1) on high fructose diet (HFD) -induced oxidative stress and metabolic disorders in rat liver.We used male Wistar rats breed divided into 4 groups: control (C); fructose fed (F); fructose fed treated with HA1(FRU+HA1); control group rats treated with HA1(HA1). For our experimental model, we used high-fructose corn syrup (12.5% fructose) that animal intake with drinking water for 12 weeks. They all received a standard diet and water ad libitum.

Phytochemical analysis and antioxidant capacity of the extract were determined. We determine ody and liver weight, triglycerides (TG) in liver as well malondialdehyde (MDA) and total thiols in liver (as markers of oxidative stress). Higher triglyceride levels in liver and malondialdehyde levels were found in rats fed the fructose diet, whereas thiol levels in liver were reduced. The extract restricted depletion of thiols and accumulation of triglycerides in liver of rats with high fructose diet. Our study demonstrates that HA-1 protects against fructose-induced metabolic pathology in liver. This effect may be associated with its possibility to restore the redox balance and to activate of endogenous antioxidant defense in liver.

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5. Ivanova D., Nashar M., Radanova M., **Bratoeva K.**, Bekyarova G., Kiselova Y. Modulatory effects of Agrimonia eupatoria L. on proinflammatory cytokines levels in experimental model of metabolic disturbances.*Trakia Journal of Sciences 2012;10 (1), 178-181. ISSN 1313-7050*

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MODULATORY EFFECTS OF *AGRIMONIA EUPATORIA* L. ON PROINFLAMMATORY CYTOKINES LEVELS IN EXPERIMENTAL MODEL OF METABOLIC DISTURBANCES

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ABSTRACT

Great amount of scientific studies report that fructose intake can cause metabolic disturbances and liver inflammatory injury. This study was designed to evaluate the effect of aqueous-alcoholic extract of *Agrimonia eupatoria* L. (agrimony) on liver proinflammatory cytokines in fructose-fed rats for 12 weeks. Rats on fructose diet exhibited significantly higher levels of liver cytokines then the control rats on standard diet. Dietary supplementation with agrimony extract significantly decreased levels of hepatic C-reactive protein in fructose-fed rats as well as in animals on standard diet. Decreased levels of interleukin 6 were estimated in animals on fructose diet supplemented with agrimony but in controls the herb itself stimulated the production of this cytokine. Our findings demonstrated that agrimony had an immunomodulatory potential and could be considered in support to the folk medicine usage of the herb as remedy in inflammatory processes and for prevention of inflammation.

Key words: agrimony, chronic inflammation, fructose, rats, liver, interleukin 6, C-reactive protein, tumor necrosis factor alpha

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6. **Братоева К.**, Ефтимов М., Вълчева-Кузманова С., Бекярова Г. Предварително проучване на протективният ефект на s- аденозилметионин върху метаболитните и поведенчески нарушения при плъхове на високо-фруктозна диета. *Известия на Съюза на учените,2012;17,(2),57-60. ISSN: 1310-6031*

High fructose consumption causes metabolic syndrome (MS). Authors reported that oxidative stress and other components of MS have an effect on mental health, compromising cognitive function and emotions. S- adenosylmethionine (SAM-e) is anutrient possessing a wide variety of effects including antioxidant and antidepressive. The aim of the present study was to in vestigate the effects of SAM-e on metabolic abnormalities and on rat behavior in a model of metabolic syndrome induced by high fructose intake. Male Wistar rats (n=21) were used in the experiment. They were divided into 3 groups: control, fructose fed (35 %, 16 weeks), fructose fed and treated with SAM-e (20 mg/kg b.w., 16 weeks). Glucose and triglycerides (TG) were measured as markers of metabolic abnormalities. Malondialdehyde (MDA) was the marker of oxidative stress. The working memory was inwestigated in the object recognition test (ORT). Our data showed that fructose feeding caused significant metabolic abnormalities and memory defecits. SAM-e reduced significantly plasma TG ($p<0.005$) and MDA ($p<0.001$). It prevented fructose-induced reduction of recognition index in the ORT. In conclusion, SAM-e prevented metabolic abnormalities and object recognition memory impairment in rats with high fructose-induced MS.

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7. **Камелия Братоева**, Иван Щерев, Мария Цанева, Ганка Бекярова.Оксидативен стрес и механизъм на хепатоцитно оцеляване при фруктозо-индуцирана чернодробна стеатоза. *Наука и младост- сборник научни съобщения от конкурсна сесия 2013 г.,1: 199-203. ISSN 1314-9229*

Хепатоцитната апоптоза и оксидативен стрес в стеатозния черен дроб са фактори за развитието на неалкохолна мастна чернодробна болест и прогресирането й до неалкохолен стеатохепатит, фиброза и цироза. Последни проучвания върху различни хранителни модели показват, че автофагията(процес свързан с оцеляването на клетките) е въвлечена в липидния метаболизъм и ограничаване на оксидативни увреждания в черния дроб. Ролята й при фруктозо- индуцирана чернодробна стеатоза е все още неизяснена. Целта на това изследване беше да се изследва връзката между апоптоза, оксидативен стрес и автофагия в мастен черен дроб при експериментален модел с фруктозно натоварване.
Използвани бяха бели плъхове Wistar, разделени в две групи(n=7): контролна на стандартна диета и група на фруктозна диета (35% във водата за пиене,16 седмици). Бяха измерени експресията на чернодробните Bax(проапоптотичен протеин) и Beclin1(иницииращ автофагията протеин) чрез метода на светлинна имунохистохимия; чрез съответния спектрофотометричен метод бяха определени нивата на малондиалдехида(МДА) и общи тиоли като маркери на оксидативен стрес.

д-р Камелия Братоева, д.м.

Чернодробна хистология за оценка на степента на стеатоза и възпалителни огнища беше определена чрез светлинна микроскопия. Бяха установени микровезикуларна стеатоза, повишени нива на МДА($p<0,01$), намалени общи тиоли($p<0,05$), повишена екапресия на Bax протеина ($p<0,005$) и намалена експресия на Beclin 1 ($p<0,005$) при фруктозно хранените плъхове в сравнение с контролната група.

В заключение нашите резултати показват, че в условия на фруктозо- индуциран оксидативен стрес автофагията и апоптозата вероятно са антагонистични процеси свързани с отлагането на излишни липиди в хепатоцитите. Кой от двата процеса ще бъде активиран има съществено значение за клетъчната функция и оцеляване на хепатоцитите и бъдещи чернодробни увреждания.

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8. Bekyarova G, **Bratoeva K**, Bekyarov N. Uric acid and vascular disorders in metabolic syndrome. *Cardiovascular Diseases 2013,44(1), 40-44. ISSN 0204-6865*

Summary. Increased consumption of high-calorie foods worldwide and fructose in particular, correlates positively with the alarming increase in the prevalence of obesity, dyslipidemia and metabolic syndrome and associated cardiovascular disease and type 2 diabetes mellitus. An increasing body of evidence suggests that high fructose intake can lead to an increased serum level of uric acid (hyperuricemia) in healthy subjects, which is even more substantial in adults with obesity and metabolic syndrome. Moreover, fructose-induced hyperuricemia causes increased free radical formation, which leads to decreased bioavailability of endothelial nitric oxide and endothelial dysfunction, the latter being a major factor in the pathophysiology of arterial hypertention, diabetes mellitus and atherosclerosis. FFData from experimental studies indicate that increased uric acid levels have proinfl ammatory, vasoconstrictive and prothrombotic effect and may play a role in vascular remodeling. Hyperuricemia is a predictor of arterial hypertension, diabetes mellitus and atherogenic triglyceridemia, all identifi ed as components of the metabolic syndrome. Therefore, monitoring of serum uric acid level along with infl ammatory and atherogenic markers is essential for assessing cardiovascular risk in patients with metabolic syndrome.

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9. **Kameliya Bratoeva**, Maria Radanova, Albena Merdzhanova Effect of allopurinol on oxidative stress in obesity and liver content of free fatty acids. *J. BioSci. Biotechnol. 2015, SE/ONLINE: 91-96. ISSN: 1314-6446*

Oxidative stress appears as the key feature associated with dysfunction in adipose tissue and a major factor in the mechanisms of altered lipid metabolism in obesity. Cellular response of adipocytes in the conditions of oxidative stress results in maintaining systemic pro-inflammatory state, insulin resistance and increased accumulation ofvery long-chain saturated fatty acids (VLCSFAs) to the liver, which are lipotoxic and lead to further injury. Therefore, the therapeutic purposes of lowering the production of ROS, may have beneficial effects on obesity and its associated complications. The aim of the study was to determine the influence of allopurinol (xanthine oxidase inhibitors) on oxidative stress in adipose tissue and liver saturated fatty acids content in a model of fructoseinduced obesity. We used a model of high-fructose diet (HFD) in male rats Wistar (16 weeks, 35% glucose-fructose corn syrup), divided into three groups: control; HFD; HFD and allopurinol administration (150 mg/kg in drinking water for 16 week). Analysis of fatty acids was performed by Gas Chromatograph with MS detector. Serum levels of glucose and uric acid (UA); weight, markers of oxidative stress- MDA (malondialdehyde), glutathione (GSH) and glutathione peroxidase (Gpx) in the retroperitoneal tissue were investigated. The results showed significantly elevated of VLCSFAs, retroperitoneal tissue/ body weight ratio, MDA, Gpx, glucose and UA levels in serum and decreased levels of glutathione in HFD rats compared to the control group. In the group treated with allopurinol the retroperitoneal tissue/ body weight ratio, the levels of MDA, Gpx, VLCSFAs, UA and glucose levels in serum were significantly reduced while glutathione levels were elevated in comparison with HFD rats. The inhibition of xanthine oxidase and UA by allopurinol prevents the development of oxidative changes in adipose tissue. This effect probably suppresses inflammation in adipose tissue, improves insulin sensitivity, reduce VLCSFAs levels and thereby prevent the further lipotoxicliver damage.

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10. **Братоева К.**, Г. Стоянов, Г. Бекярова, М. Раданова. S-аденозилметионин в превенцията на оксидативни увреждания при затлъстяване. *Известия на съюза на учените – Варна, 2015; 2(XX): 64-68. ISSN: 1310-6031*

Връзката между затлъстяването и метаболитните нарушения е неясна и е обект на интензивни проучвания. Оксидативният стрес се проявява като ключов фактор отговорен за дисфункцията в мастната тъкан и механизмите на променения липиден метаболизъм при затлъстяване. Клетъчният отговор на адипоцитите в условията на оксидативен стрес води до поддържане на системно възпаление, инсулинова резистентност и повишено натрупване на извънматочни липиди, водещи до развитието на метаболитен синдром. S-аденозилметионин (S-AMe) е нутриент, притежаващ установени антиоксидантни и

противовъзпалителни ефекти. Целта на изследването е да се определи влиянието на S-AMe върху оксидативния стрес в мастната тъкан при модел на фруктозо-индуцирано затлъстяване.

Използвахме мъжки плъхове Wistar (N=21), разделени в три групи: контролна на стандартна храна; на ВФД (16 седмици, 35% глюкозо-фруктозен сироп) и на ВФД с прием на SAM-e. Изследвахме промени в съотношението тегло на ретроперитонеална мастна тъкан/ телесно тегло; като маркери на оксидативен стрес: нива на редуциран глутатион (клетъчен антиоксидант) и МДА (малондиалдехид, продукт на липидната пероксидация) в ретроперитонеалната мастна тъкан, както и серумни нива на редуциран глутатион. Установени бяха статистически значимо увеличено съотношение тегло на ретроперитонеалната мастна тъкан/ телесното тегло и МДА, както и понижени тъканни и серумни нива на редуциран глутатион при плъховете на ВФД спрямо контролната група. В групата на ВФД с прием на SAM-e, установихме значително понижено съотношение тегло на ретроперитонеалната мастна тъкан/ телесното тегло и МДА, а повишени тъканни и серумни нива на редуциран глутатион спрямо групата на ВФД.

Нашите резултати показват, развитието на оксидативен стрес в хипертрофиралата висцерална мастна тъкан при фруктозо-индуцирано затлъстяване. Администрация на S-AMe подобрява антиоксидантната защита на адипоцитите, оксидативните увреждания и прекомерното натрупване на липиди във висцералната мастна тъкан и последващи метаболитни нарушения.

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11. Radanova M., **Bratoeva K**, Vasilev V, Deliyska B, Ikonomov V, Argirova T Association between anti-c1q and anti-dsdna antibodies in patients with lupus nephritis. *Science & Technologies. 2015;Medicine; V;(1),55-60. ISSN 1314-4111*

Lupus nephritis (LN) is severe organ manifestation of the systemic lupus erythematosus (SLE). LN usually occurs within 5 years of the onset of disease, but can occur any time throughout the course of the disease. Anti-nuclear antibodies (ANA) and anti-double stranded DNA (anti-dsDNA Abs) antibodies are aiding tools to the kidney biopsy findings in early diagnosis. The aim of this study was to assess the role of anti-C1q Abs, in predicting of renal involvement in comparison with the „gold standard" for SLE anti-dsDNA Abs. Thirty patients 23 (77%) women and 7 (23%) men with biopsy-proven LN were studied. Sera were tested for anti-C1q Abs, anti-dsDNA Abs and complement proteins – C1q, C3 and C4. Association of parameters of renal disease activity and the presence of anti-C1q Abs and anti dsDNA Abs were further evaluated.

All 30 patients were distributed in three groups according to the degree of the clinical activity of LN: 1) patients with active LN (AN); 2) patients in partial remission (PR) and 3) patients in complete remission (CR). We found significant differences between frequencies of anti-C1q Abs in AN patients and in CR patients ($p = 0.007$) and between levels of anti-C1q Abs in AN patients and those with PR ($p = 0.026$). Positive for anti-dsDNA Abs were 56.67% of patients. We found that positive for anti-dsDNA Abs had higher level of anti-C1q Abs in comparison to negative for anti-dsDNA Abs ($p = 0.005$). 67% of patients with both anti-C1q Abs and anti-dsDNA Abs had high clinical LN activity and all had Class IV LN. Patients who were seropositive for anti-C1q Abs and negative for anti-dsDNA Abs had lower serum C1q, C3 and C4 levels compared with the other patients.

The presence of anti-C1q Abs in serum closely correlated with LN activity. This study indicates the potential superior utility of anti-C1q Abs over anti-dsDNA Abs to track renal disease activity. Systematic detection of anti-C1q and anti-dsDNA Abs should be used in combination to monitor the renal involvement.

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12. Раданова М., **К. Братоева**, В. Василев, В. Икономов, Д. Иванова. Пациенти с дифузен пролиферативен лупусен нефрит са позитивни за антитела срещу глобуларния фрагмент на C1q. *Известия на съюза на учените – Варна, 2015; 2(XX): 52-57. ISSN: 1310-6031.*

Приблизително от 20% до 50% от пациентите със Системен лупус еритематодес (СЛЕ) са серопозитивни за анти-C1q автоантитела. Високите нива на тези антитела корелират с развитие на дифузен лупусен нефрит (клас IV) и се откриват в активната фаза на заболяването. Неотдавна в пациенти с лупусен нефрит доказахме антитела, насочени към глобуларния фрагмент на C1q – анти-gC1q антитела. С цел да проучим тяхната патогенетична роля при лупусната нефропатия ние изследвахме връзката на тези антитела с хистоморфологичните характеристики в бъбречните биопсии и с активността на лупусния нефрит.

Изследвани бяха 62 пациенти със СЛЕ, като 46 от тях бяха с биопсично доказан лупусен нефрит. Контролната група включваше 196 здрави доброволци. Нивата на анти-C1q и анти-gC1q антитела бяха определяни чрез ELISA. За експресията на A-, B- и C-веригите на C1q използвахме *E.coli* BL21. Получените резултати бяха обработени със статистическия пакет GraphPad Prism 5.0. Установени бяха високи нива на анти-C1q антителата при дифузен лупусен нефрит в сравнение с другите класове лупусни нефрити, без статистически значима разлика между нивата на анти-C1q антителата в пациенти с клас IV

и клас II лупусен нефрит. Пациентите с клас IV лупусен нефрит бяха също позитивни и за анти-gC1q антитела. Тези антитела бяха високи и при пациентите в активна фаза на заболяването.
Намерена беше връзка на анти-gC1q автоантитела с активна и тежка лупусна нефропатия. Получените резултати допълват наличната информация за анти-gC1q автоантитела и поставят нови въпроси за механизма на тяхното патогенетично действие.

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13. Assia Konsoulova, Ivan Donev, Nikolay Conev, Sonya Draganova, Nadezhda Petrova, Eleonora Dimitrova, Hristo Popov, **Kameliya Bratoeva**, Petar Ghenev. First line 5-fu-based chemotherapy with/ without bevacizumab for metastatic colorectal cancer: tissue biomarker candidates. *J of IMAB. 2016,22;(1): 1038-1044. ISSN: 1312-773X*

Purpose: Colorectal cancer is the second leading cause of cancer mortality in the USA. According to Bulgarian National Statistics Institute, 2370 colon and 1664 rectal cancer cases were diagnosed in 2012 with total number of patients 29995. Adding bevacizumab to chemotherapy in patients with metastatic disease improves progression-free survival (PFS) but no predictive markers have been proven in the clinical practice. In our study we examined two tissue biomarkers that may correlate with response to bevacizumab-containing chemotherapy in patients with metastatic colorectal cancer.

Patients and Methods: 54 patients with metastatic colorectal cancer were assigned to first line 5-Fu-based chemotherapy with/without bevacizumab. The primary end point was PFS, with additional determination of response and toxicity. Paraffin-embedded samples from primary tumors were collected from all 54 patients. Expression levels of two tumor biomarkers VEGFR-2 and Neuropilin 1 (NP-1) were evaluated with immunohistochemistry.

Results: The median PFS for the group treated with CT/Bev was 8.8 months, compared with 5.4 months for the group with chemotherapy alone (95% CI, log-rank test P =0.003). The corresponding overall response rates were 19.3% and 10.2% respectively ($P < 0.05$ for CT/Bev vs CT). Patients with low NP-1 had statistically significant prolongation of PFS as compared to those with high NP-1 (95% CI, log rank test p= 0.017). Patients with low NP-1 appeared to experience a larger bevacizumab treatment effect in terms of PFS (p=0,049,HR 0.333, 95% CI, 0.111 to 0.995) than patients with high NP-1.**Conclusion:** The addition of bevacizumab to 5-Fu based chemotherapy improves PFS for patients with metastatic colorectal cancer. Expression of tumor NP-1 is a potential biomarker candidate for prediction of clinical outcome in patients with metastatic colorectal cancer, treated with first line chemotherapy plus bevacizumab.

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14. Radanova M, Stoyanova V, **Bratoeva K**, Vasilev V, Ivanova D. Antibodies recognizing the globular domain of C1q - view on association between lupus nephritis activitty and anti-gC1q autoantibodies. *Scripta Scientifica Medica, 2016; 48(1): 13- 21. ISSN: 1314-6408*

Introduction: Lupus nephritis (LN) is a serious complication of the systemic lupus erythematosus (SLE). Anti-C1q antibodies correlate with the occurrence and high clinical activity of LN, especially proliferative LN. The first reported anti-C1q antibodies recognized autoepitopes within collagen-like region (CLR) of C1q. Recently we have found autoantibodies against globular C1q domain (gC1q antibodies) in LN patients. **The aim** of the present study was to evaluate the potential pathological consequences of the presence of anti-gC1q antibodies in LN. **Material and Methods**: The recombinant globular head region of the three chains of C1q - A, -B and -C were expressed in E. coli BL21 and purified. Anti-C1q, anti-gC1q autoantibodies, complement proteins - C1q, C4, C3 and IgG-, IgM-CICs levels were screened by ELISA in 53 sera from LN patients. Sera from 196 normal controls served as controls. **Results**: We found that patients positive for anti-B-gC1q antibodies presented with significantly lower serum C4 levels than patients positive for anti-A and anti-C-gC1q antibodies ($p = 0.014$) and with significantly lower levels of C3 than patients positive for anti-A and anti-C-gC1q antibodies and patients without anti-C1q antibodies ($p = 0.005$; $p = 0.018$). Significant correlations to IgG CICs were detected for anti-C1q ($r = 0.371$, $p = 0.001$) and anti-B-gC1q antibodies ($r = 0.431$, $p = 0.003$). **Conclusions**: These findings suggest that the binding of anti-B-gC1q autoantibodies with C1q may possibly trigger mechanical stress and induce a structural change within the CLR domain of C1q, compatible with C1r-C1s complement activation in the fluid phase.

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15. Maria Atanasova Radanova, **Kamelia Bratoeva**, Neshe Nazifova-Tasinova, Miglena Todorova, Vasil Vasilev, Diana Ivanova. The GG rs292001 genotype prevails in seronegative for ANA and anti-dsDNA antibodies patients with lupus nephritis. *Scripta Scientifica Medica, vol. 48, No. 3, 2016, pp. 34-38. . ISSN: 1314-6408*

Rs292001 is single nucleotide polymorphism in non-coding regions of C1QA gene. C1q is subcomponent of the C1 first component of the classical pathway of complement activation. Rs292001 was investigated for association with some conventional immunological markers of lupus nephritis activity in SLE patients – levels of C1q, C3, C4, anti-C1q, anti-nuclear (ANA) and anti-dsDNA autoantibodies.Genomic DNA was isolated from peripheral blood of 18 patients with biopsy-proven lupus nephritis (LN). SNP genotyping for the presence of rs292001 was performed by quantitive real-time PCR method. Presence of complement C1q, C3 and C4 and anti-C1q autoantibodies was screened by ELISA.

ANA and anti-dsDNA antibodies were detected by indirect immunofluorescence.We found that GG rs292001 genotype prevailed in seronegative for ANA and anti-dsDNA antibodies LN patients (p=0.008; p<0.012). The AA rs292001 genotype showed a trend towards lower serum C1q levels.These results reaffirm a previously established probable protective role of G allele against the clinical activity of the SLE.

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16. Раданова М, **Братоева К**, Икономов В. Ролята на IgM анти-двДНК антитела като протективни маркери при лупусна нефропатия. *Варненски нефрологичен форум, 2016; 3(1): 29-35. ISSN: 1313-7662*

IgG anti-dsDNA antibodies are usually associated with active lupus disease, particularly lupus nephritis. We reviewed the studies showing negative correlation between IgM anti-dsDNA antibodies and glomerulonephritis. We discussed the possible mechanism of the protective effect of IgM anti-dsDNA antibodies against immune complex-mediated organ damage has been. The detection of IgM antidsDNA antibodies seems to improve our ability to refine SLE diagnosis and prognosis of lupus nephritis.

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17. **Bratoeva K**, Stoyanow S. G, Merdzhanova A., Radanova M. Manifestations of Renal Impairment in Fructose-induced Metabolic Syndrome. *CUREUS, 2017;9(11):e1826. ISSN 2168-8184*

Introduction: International studies show an increased incidence of chronic kidney disease (CKD) in patients with metabolic syndrome (MS). It is assumed that the major components of MS - obesity, insulin resistance, dyslipidemia, and hypertension - are linked to renal damage through the systemic release of several pro-inflammatory mediators, such as uric acid (UA), C-reactive protein (CRP), and generalized oxidative stress. The aim of the present study was to investigate the extent of kidney impairment and manifestations of dysfunction in rats with fructoseinduced MS. **Methods:** We used a model of high-fructose diet in male Wistar rats with 35% glucose-fructose corn syrup in drinking water over a duration of 16 weeks. The experimental animals were divided into two groups: control and high-fructose drinking (HFD). Serum samples were obtained from both groups for laboratory study, and the kidneys were extracted for observation via light microscopy examination.
Results: All HFD rats developed obesity, hyperglycemia, hypertriglyceridemia, increased levels of CRP and UA (when compared to the control group), and oxidative stress with high levels of malondialdehyde and low levels of reduced glutathione.

The kidneys of the HFD group revealed a significant increase in kidney weight in the absence of evidence of renal dysfunction and electrolyte disturbances. Under light microscopy, the kidneys of the HFD group revealed amyloid deposits in Kimmelstiel-Wilson-like nodules and the walls of the large caliber blood vessels, early-stage atherosclerosis with visible ruptures and scarring, hydropic change (vacuolar degeneration) in the epithelial cells covering the proximal tubules, and increased eosinophilia in the distant tubules when compared to the control group. **Conclusion:** Under the conditions of a fructose-induced metabolic syndrome, high serum UA and CRP correlate to the development of early renal disorders without a clinical manifestation of renal dysfunction. These phenomena are of particular importance for assessing the risk of developing future CKD.

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18. **Kameliya Bratoeva**, Eleonora Dimitrova, Assia Konsoulova, Chavdar Bachvarov, Georgi Todorov, Kalin Kalchev, Nadezhda Stefanova, Ivan Donev. Beclin 1 - The Autophagy Regulatory Protein. *Варненски медицински форум, 2017; 6(2): 114-123. ISSN 2367-5519*

The complex role of different types of cell death in cancer is very complicated and continues to be revealed. The observations show that all three processes - apoptosis, autophagy and necrosis may exist in a tumor, and their relative involvement dictates the trajectory of tumor growth, regression and response to anti-tumor therapy. Cellular signaling analysis may reveal and provide new biomarkers reflecting the functional activity of these processes that will support individualized therapy in cancer patients. In this review we have accentuated of some major roads and regulators associated with cell death and survival, which provide the metabolic stability in tumors, leading to resistance to chemotherapy and unsuccessful cancer treatment.

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19. **Kameliya Bratoeva**, Eleonora Dimitrova, Nikolay Conev, Georgi Todorov, Kalin Kalchev, Mariya Radanova, Ivan Donev. Regulation of cell death in cancer diseases - importance and therapeutic effect. *Варненски медицински форум, 2017; 6(2): 92-98. ISSN 2367-5519*

The complex role of different types of cell death in cancer is very complicated and continues to be revealed. The observations show that all three processes - apoptosis, autophagy and necrosis may exist in a tumor, and their relative involvement dictates the trajectory of tumor growth, regression and response to anti-tumor therapy. Cellular signaling analysis may reveal and provide new biomarkers reflecting the functional activity of these processes that will support individualized therapy in cancer patients. In this review we have accentuated of some major roads and regulators associated with cell death and survival, which provide the metabolic stability in tumors, leading to resistance to chemotherapy and unsuccessful cancer treatment.

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20. Eleonora G Dimitrova, Borislav G Chaushev, Nikolay V Conev, Javor K Kashlov, Aleksandar K Zlatarov, Dilyan P Petrov, Hristo B Popov, Nadezhda T Stefanova, Anelia D Klisarova, **Kameliya Z Bratoeva**, Ivan S Donev. Role of the pretreatment 18F-fluorodeoxyglucose positron emission tomography maximal standardized uptake value in predicting outcomes of colon liver metastases and that value's association with Beclin-1 expression. *BioScience Trends. 2017; 11(2):221-228. ISSN : 1881-7823,* ***IF=1,545***

The current study sought to evaluate the predictive and prognostic performance of the maximum standardized uptake value (SUVmax) prior to treatment in 43 patients with colon cancer and unresectable liver metastases. Patients with colon cancer who underwent (18)F-FDG-PET/computed tomography (CT) scans for staging before the start of first-line 5-fluorouracil-based chemotherapy were retrospectively analyzed. Expression of Beclin-1 in cancer cells was evaluated in primary tumors using immunohistochemical staining. The pretreatment SUVmax for liver metastases was not able to predict progression-free survival but was significantly associated with poorer overall survival, with a hazard ratio of 2.05 (95 % CI, 1.016-4.155). Moreover, a negative correlation was noted between SUVmax and expression of a marker of autophagy - Beclin-1 (rho = -0.42, p = 0.006). This suggests that the pretreatment SUVmax in (18)F-FDG PET/CT is a useful tool to help predict survival outcome in patients with colon cancer and unresectable liver metastases and may significantly distinguish between patients with low and high levels of Beclin-1 expression (AUC = 0.809, 95% CI: 0.670-0.948, p = 0.001).

21. **Kameliya Bratoeva**, Mariya Radanova, Albena Merdzhanova, Ivan Donev. Protective Role of S-Adenosylmethionine Against Fructose-Induced Oxidative Damage in Obesity. *Journal of Mind and Medical Sciences. 2017; 4(2): 163-171. ISSN: 2392-7674*

Introduction. It has been shown that S-adenosylmethionine (S-AMe) stimulates glutathione synthesis and increases cell resistance to the cytotoxic action of free radicals and pro inflammatory cytokines. The aim of this study was to determine the effect of Sadenosylmethionine on the oxidative stress in adipose tissue in a model of fructose-induced obesity. **Methods.** The study was performed on male Wistar rats divided into 3 groups: control, fructose fed (HFD) (35%, 16 weeks), and HFD + S-AMe (20 mg/kg). We examined the changes in the ratio of retroperitoneal adipose tissue weight / body weight; levels of reduced glutathione (GSH) and malondialdehyde (MDA) in the retroperitoneal adipose tissue, and serum levels of GSH and TNF-α. **Results.** Significant increases in the retroperitoneal adipose tissue, MDA, and serum TNF-α were identified, as well as decreased tissue and serum levels of GSH in rats fed with a high-fructose diet as compared with the control group.

In the group fed with HFD and SAMe, we found significant reduction in the retroperitoneal adipose tissue and decreased levels of MDA and serum TNF-α, as well as increased tissue and serum levels of GSH as compared with the group only on HFD.

In conclusion, our results show that fructose-induced obesity causes oxidative stress in hypertrophic visceral adipose tissue. The administration of S-AMe improves the antioxidative protection of adipocytes, and reduces oxidative damage and excessive accumulation of lipids and inflammation.

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22. G Bekyarova, M Tzaneva, **K Bratoeva**, I Kotzev. Heme-oxygenase-1 upregulated by s-adenosylmethionine. potential protection against non-alcoholic fatty liver induced by high fructose diet. *FARMACIA, 2017, 65(2):262-267. ISSN: 2065-0019,* ***IF=1,348***

Excessive dietary fructose intake may have an important role in the current epidemics of fatty liver disease, obesity and diabetes- features of metabolic syndrome. We evaluated the relationship between lipid peroxidation and other oxidative stress biomarkers with changes in expression of heme oxygenase-1 (HO-1) in rat fatty liver, induced by high fructose diet (HFD) and the effect of S-adenosylmethionine (SAMe). Twenty-one male rats were randomly assigned to three groups of seven animals each: HFD (35% fructose in drinking water for 16 week) group, HFD + SAMe (20 mg/kg in drinking water for 16 week) group and control group. HO-1 expression, MDA (marker of lipid peroxidation), triglycerides (TG), SH group levels and histological (H&E) studies were assayed in liver. HFD group showed microvesicular steatosis without inflammation and fibrosis. In HFD+SAM group microvesicular steatosis was not established. The HO-1 expression was significantly increased in HFD rats. SEMe augmented the increase in expression of HO-1. The levels of MDA and TG were elevated in HFD group. In HFD rats with lower levels of SH exhibited higher expression of HO-1. SAMe inhibited the elevation in lipid peroxidation and TG levels and prevented the decrease in SH levels. In conclusion SAMe has hepatoprotective effect and its protection likely exerted by increased expression of the antioxidant enzyme HO-1 to prevent the development of fatty liver.

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23. М. Иванова, А. Янчев, Н. Цонев, И. Донев, Е. Димитрова, Д. Стоянов, Я. Кашлов, **К. Братоева**, С. Пенева. Скрининг за дистрес при онкологично болни. *Studia Oncologica, 2018;8(1):32-39. ISSN:1313-7115*

The interest about distress screening in oncology patients is increasing rapidly. However there are also proves for difficulties in applying and understanding the screening problems. The nurse working in the oncology field also plays role in the process of screening.

Nowadays the screening mechanism and the part of the different participants (medical care providers) is still developing and needs more discussions. The screening depends on several factors – the health problem (in our case the oncology disease), the screening type and the healthcare system.

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24. Асен Янчев, Мартина Иванова, Иван Щерев, **Камелия Братоева** и др. Скрининг за дистрес при онкологично болни пациенти и фактори, повлияващи нивото му. *Списание на Българско Онкологично Научно Дружество, 2018;2(1):82-90. ISSN 1312-6601*

Screening for distress, in order to improve patient outcome, is recommended by many organizations, such as The National Comprehensive Cancer Network (NCCN). It defines distress as a multifactorial unpleasant emotional experience that may interfere with the ability to cope effectively with cancer. The aim of our study is to measure distress before the start of treatment among patients of the Medical Oncology Clinic at UMHAT "St. Marina" Varna, Bulgaria and the relationship with some demographical and clinicopathological factors. For 1 year a total of 225 oncological patients took participation in the study. 123 of them had metastatic disease. We used the NCCN Distress Thermometer at the beginning of treatment to determine the levels of distress. It measures distress on a scale from 1 to 10. The Mann-Whitney U-test shows significant difference between the mean distress level score of men (3.2±2.8) and women (4.3±3.2) (p=0.014). The same difference was detected among patients with Eastern Cooperative Oncology Group (ECOG) performance status 0 (3.4±3.2) and 1 (4.4±3.1) (p=0.029). Surprisingly we couldn't find significant difference between distress levels score in non-metastatic (3.6±3.1) and metastatic patients (3.9±3.2) (p>0.05). Multiple regression analysis shows that lung cancer and breast cancer are independent predictors for high distress level among patients OR 6.7, 95% CI 2.5-18.3, p=0.001 и OR 3.4, 95% CI 1.16-10.4, p=0.02. Our study indicates that women and patients with poor ECOG performance status experience higher levels of distress. The diagnosis with cancer causes the same distress levels among patients with metastatic and non-metastatic disease.

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25. Цонев Н, Щерев И, Манев Р, Димитрова Е, **Братоева К** и др. Невротоксичност на противотуморни медикаменти. *Варненски медицински форум, 2018; 7(2): 13-19. ISSN 2367-5519*

Невротоксичните ефекти на химиотерапията се появяват относително често и са причина за модификация на дозата на медикаментите – дозолимитираща токсичност. Рискът от развитие на невротоксичност се увеличава с повишаване на приложената доза и за разлика

от миелотоксичността (основния ограничаващ фактор при повечето химиотерапевтични режими), която може да бъде преодоляна с растежни фактори или трансплантация на костен мозък, няма стандартно поведение, което да я ограничи. Противотуморните препарати водят до два типа токсичност - периферна невротоксичност, състояща се основно от периферна невропатия и централна невротоксичност, която включва от незначителни когнитивни увреждания и дефицити до енцефалопатия с деменция или дори кома. Не съществуват утвърдени алгоритми за поведения и профилактика на невротоксичността, причинена от противотуморните препарати. Поведението основно се свежда до редукция на дозата или отлагане във времето на приложението, особено при пациенти, които са с по-висок риск от развитие на невротоксични странични ефекти. На този етап не съществуват невропротективни агенти, които се препоръчват за стандартна употреба при развитие на невротоксичност.

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26. George S Stoyanov, Galina Naskovska, Emran Lyutfi, Rumiana Kirneva, **Kameliya Bratoeva**. In Search of the Ninth Discipline: *The History of Pathophysiology, with an Emphasis on Pathophysiology in Varna, Bulgaria—Celebrating 100 Years of Pathophysiology in Bulgaria. Cureus. 2018 Apr; 10(4): e2404. ISSN 2168-8184*

Pathophysiology is a medical science whose subject is the change in regulatory mechanisms related to the onset, development, and outcome of diseases. The first lectures on pathophysiology were held in 1790 at the University of Erfurt, Germany, by Professor Augustus Hecker, who in 1791 also published the first work on the discipline-"Grundriss der Physiologia pathologica" in 770 pages. The teaching of pathophysiology as an independent discipline was introduced by academician Viktor Pashutin at the University of Kazan, Russia in 1874. Academician Pashutin called this new discipline "Pathological Physiology and Experimental Medicine." Despite the persuasiveness of Pashutin that pathological anatomy and pathophysiology are inseparable parts of a whole, his students, academician Nikolay Anichkov and Prof. Semyon Khalatov, implemented the so-called "divorce" due to the different, though complementary, approaches and methodologies of the two ideological fields. By Royal Decree on November 29, 1917, in the Bulgarian State Gazette, amendments were published in the law on the national education, which introduced new university "disciplines and departments". Under number nine in the law is the discipline of "Pathological Physiology and Experimental Medicine". Due to various factors, the Pathological Physiology and Experimental Medicine department was the only one of the first 25 departments not to be established. The beginning of the training for pathophysiology in Bulgaria was laid by Prof. Vassil Mollov and Assoc. Prof. Minko Dobrev, however due to their untimely deaths, the course lasted only three years (1936-1939) and was not continued in the next academic year. At the beginning of the academic year 1946/47, two assistants in pathophysiology were enrolled in the Department of Pathological Anatomy at Sofia University.

The following year a separate department was formed at the newly founded Plovdiv University and shortly after at Sofia University. For the 100 years since its legislative establishment, 82 years since its unofficial start and 71 years since its academic establishment pathophysiology in Bulgaria has distinguished itself by scientific, administrative and clinical contributions. In its 57 years in Varna, Bulgaria pathophysiology has widely carried out that tradition with immense contributions.

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27. **Bratoeva K**, Nikolova S, Merdzhanova A, Stoyanov G, Dimitrova E, Kashlov J, Conev N, Radanova M. Association between serum ck-18 levels and the degree of liver damage in fructose-induced metabolic syndrome. *Journal of Metabolic Syndrome and Related Disorders, 2018(in press). ISSN: 1557-8518,* ***IF=1,932***

Background: The pathogenesis of non-alcoholic fatty liver disease as a component of metabolic syndrome (MetS) involves the activation of apoptosis in steatotic hepatocytes. Caspase-generated fragments such as cytokeratin-18 (CK-18) in patients with various hepatic impairments are investigated as markers for diagnosis and assessment of disease severity. The goal of the study was to capture early biomarkers of apoptosis and elucidate their role in assessing the presence and extent of hepatic damage in a MetS model. **Methods**: We used male Wistar rats, divided into two groups (n=7): control and high-fructose drinking (HFD) (35% fructose corn syrup for 16 weeks). Metabolic disorders and liver damage were studied by histochemistry (H&E), immunohistochemical, immunological and biochemical testing. **Results:** Our results showed significant increase in liver and serum levels of CK-18 and pro/anti-apoptotic Bax/Bcl2 ratio, and decreased levels of HMGB1 (marker of necrosis) in the HFD group as compared to the control. All HFD rats developed obesity, hyperglycemia, hepatomegaly, microvesicular steatosis, an imbalance in hepatic anti-oxidative defense (by measuring malondialdehyde and sulfhydryl groups (SH) with no inflammation and fibrosis, elevated serum levels of triglycerides, tumor necrosis factor alpha (TNF-α) and C-reactive protein without changes in serum aminotransferase levels relative to the control group. As a result of the applied regression analysis, we have determined that the variables TNF-α (0.92) and SH (0.659) have a strong complex effect on hepatic CK18 levels with predicted value of the model R = 0.9. **Conclusion:** The elevated CK-18 serum levels in the HFD group and their association with the histological changes in the liver and biochemical indicators demonstrate the key role of apoptosis in the pathogenesis of HFD-induced liver damage as well as the reliability of CK-18 as a biomarker for non-invasive assessment of liver damages in MetS.

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28. **Bratoeva K**, Dimitrova E, Conev N, Kashlov J, Panajotova E, Stoyanov G, Radanova M, Donev I. Expression of hepatic HMGB1 levels in fructose- induced fatty liver. *Варненски медицински форум, 2018; 7(2): 151-157. ISSN 2367-5519*

The study of the processes leading to hepatocellular cell death is important for clinical practice to assess the severity of hepatic impairment as well as the application of effective interventions to prevent it. Observations show that both cell death processes - apoptosis and necrosis are activated at certain stages of the progression of non-alcoholic fatty liver disease from hepatic steatosis, steatohepatitis and cirrhosis. Currently, the most promising non-invasive specific method for detecting necrotic cell death is non-histone DNA binding protein with high mobility Group B1 (HMGB1). The aim of this study was to investigate the levels of HMGB1 expression and their relationship to hepatic injury and apoptosis activity in rat liver with fructose-induced metabolic syndrome. The results showed data for metabolic syndrome, microvascular steatosis, statistically reduced levels of HMGB1, an increased ratio of Bax / Bcl2 apoptotic proteins in fructose fed rats.

4. УЧЕБНИ ПОСОБИЯ

Сборник по патофизиология с тестови задачи и клинични случаи Бекярова Г, Радев Р, Маринов М, Христова М, **Братоева К**, Христов К, Варна 2014, 122 стр ISBN 978-619-7137-11-8, МУ-Варна.

Учебното пособие е предназначено за подготовка по патофизиология на студенти от специалностите „Медицина“. Съдържа две глави: Първа- примерни тестови въпроси и отговори на въпросите, които подпомагат студентите при подготовката им за колоквиуми и изпита по патофизиология, а втората глава - клинични случаи, включени в проблем базираното обучение по патофизиология.

5. УЧАСТИЯ В НАУЧНИ ПРОЯВИ (ДОКЛАДИ, ПОСТЕРИ)

1. Kiselova-Kaneva Y, Ivanova D, **Bratoeva K**, Bekyarova G. **Protective effect of Agrimonia eupatoria L. extract on fructose-indused metabolic syndrome in rats.** *NuGOweek 2010, 7th Nutrigenomics Conference, 31st August- 3 rd September 2010, Glasgow, United Kingdom.*

Background: The worldwide epidemic of metabolic syndrome (MetS) has been recently linked to a marked increase in total fructose intake in the European diet (in the form of table sugar and high-fructose corn syrup). In turn, the MetS has been epidemiologically associated with a cluster of pathologies including obesity, hypertriglyceridemia, impaired glucose tolerance, insulin resistance. **Objectives:** Herbal extracts with established therapeutic efficacy in patients with obesity and diabetes have been traditionally used in Bulgarian folk medicine. We investigated the beneficial effect of a herbal extract from Agrimonia eupatoria L. on fructose-induced MetS on animal model. **Procedures:** The protective role of the extract on MetS markers in rats receiving a high-fructose diet was studied in a 12 weeks chronic experiment. Body weight and serum triglycerides, total cholesterol, and blood glucose were measured. Phytochemical analysis and antioxidant capacity of the extract were determined. Statistical analyses were performed using GraphPad Prism 4.00 statistical software. **Results:** The administration of the extract prophylactically prevented fructose-induced hyperglycemia (6.73mmol/l vs. 8.13mmol/l, P=0.02), hyperholesterolemia (1.05mmol/l vs. 1.29mmol/L P=0.13), hypertriglyceridemia (0.62mmol/l vs. 0.91mmol/l, PHX0004), and weight gain (228.5 vs. 238.7 g, P=0.44) at 12 week. The extract did not affect dietary intake of control diet in rats. Additionally treatment with the extract reduced significantly serum triglycerides and total cholesterol in rats receiving normal diet. **Conclusion:** Our study demonstrates that the Agrimonia extract protects rats from fructose-induced metabolic pathologies. This protection is likely mediated by its antioxidant capacity, which may account for the therapeutic efficacy in metabolic disturbances.

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2. **Bratoeva K**, Bekyarova G, Kiselova Y, Ivanova D. **Effect of extract of selected Bulgarian herbs (HA-1) on high fructose diet- induced metabolic disorders in liver.** 31 st Balkan Medical Week, 28-31 October 2010, Athens, Greece.

Introduction: The increased fructose intake in the Bulgarian diet (in the form of table sugar and high-fructose corn syrup) has been recently linked to the development of insulin resistance, obesity, dyslipidemia and other abnormalities of the metabolic syndrome.

In turn, the metabolic syndrome has been associated with liver metabolic disorders and injury.We investigated that high fructose diet (HFD) increased fat storage in the liver. It has been shown that oxidative stress contributes to metabolic disorders in liver. The herbal extracts a lot of Bulgarian plans are rich of polyphenols and exhibits antioxidant activity. **Aim:**The aim of this study was to investigate the possible relation between oxidative stress and triglyceride accumulation in liver as well the protective effect of extract of selected Bulgarian herbs (high content of polyphenols - Herbal-Antiox 1) on HFD-induced oxidative stress and metabolic disorders in liver. **Methods:** The animals were divided randomly into four groups (n=6); Control group rats-C; fructose-drinking rats-FRU (high-fructose corn syrup-12.5% fructose content); fructose-drinking rats treated with Herbal-Antiox 1 (HA1)- FRU+HA1;control group rats treated with Herbal-Antiox 1 (HA1)- HA1. Rats received 12.5% fructose solution in drinking water for 12 weeks, control rats were maintained on plain water. Phytochemical analysis and antioxidant capacity of the extract were determined. We determine body and liver weight, triglycerides in liver as well as plasma malondialdehyde and total thiols in liver (as markers of oxidative stress). **Conclusion:** HFD causes oxidative stress established by increased malondialdehyde, reduced levels of total thiols and triglyceride accumulation in the liver as well. Herbal-Antiox 1 restricts oxidative stress, depletion of thiols and TG- storage in liver of HFD- rats.

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3. Ivanova D, Kiselova-Kaneva Y, Bekyarova G, **Bratoeva K. Protection by Agrimonia eupatoria L. extract against metabolic oxidative stress in rats.** *31 st Bal-kan Medical Week, 28-31 October 2010, Athens, Greece.*

Introduction: Fat accumulation in obesity is not only associated with hypertriglyceridemia, hypercholesterolemia and impaired glucose tolerance, but it also correlates with systemic oxidative stress. Recently increased total fructose intake in the Western diet was linked to the metabolic abnormalities accompanying obesity. **Aims:** A number of plants used in Bulgarian folk medicine are having therapeutic effect mediated by their antioxidant properties. The effect of extract from Agrimonia eupatoria L. was studied with the aim to investigate its protective effect on fructose-induced metabolic abnormalities using animal model. **Methods:** The protective role of the extract on oxidative stress markers and antioxidant defense was studied in 12-week chronic experiment in rats on a normal diet and in rats receiving high-fructose diet. Total thiols as a measure of antioxidant potential and MDA as a measure of oxidative stress were determined in serum and adipose tissue of experimental animals. Triglycerides, total cholesterol and glucose were used as markers of metabolic abnormalities in the group on fructose diet.

Statistical analyses were performed using GraphPad Prism 4.00. **Results:** The administration of Agrimonia extract prevented fructose-induced hyperlipidemia and increased antioxidant potential of serum and of adipose tissue. Adipose tissue weight was used as an indicator of experimentally-induced obesity correlating with clinical indices for metabolic abnormalities. Additionally, treatment with the extract reduced significantly serum triglycerides and total cholesterol in rats receiving normal diet and increased serum antioxidant potential. **Conclusion:** Agrimonia extract protects rats from fructose-induced metabolic oxidative stress and improves serum indices of metabolic abnormalities in experimentally induced obesity.

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4. **Bratoeva K**, Bekyarova G, Kiselova Y, Ivanova D. **Effect of Herb expracts of polyphenols on metabolic disorders-induced фу high-fructose diet.** *Юбилейна научна конфереция с международно участие"15 години Тракийски Университет" Стара Загора, 21 май 2010.*

Introduction. Recently, fructose consumption has been suggested to be one of the environmental factors contributing to the development of insulin resistance, obesity, dislipidemia and other abnormalities of the metabolic syndrome.

Aim: Herbal extracts with established therapeutic efficiency in patients with obesity and diabetes have been traditionally used in Bulgarian ethnomedicine.This study investigated the protective effect of extract of selected Bulgarian herbs (high content of polyphenols- Herbal-Antiox 1) on high-fructose diet-induced metabolic disorders in rat liver.

Material and methods: The animals were divided randomly into four groups (n=6); Control group rats- C; fructose-drinking rats- FRU (high-fructose corn syrup-12.5% fructose content); fructosedrinking rats treated with Herbal-Antiox-1 (HA-1)- FRU+HA1;control group rats treated with Herbal-Antiox-1 (HA-1)-C+ HA1.Rats received 12.5% fructose solution in drinking water for 12 weeks, control rats were maintained on plain water. Phytochemical analysis and antioxidant capacity of the extract were determined.

Results: We determine liver triglyceride (TG) concentration, body weight, liver weight, adipose tissue weight. In the FRU rat the levels of plasma glucose, liver TG as well liver and body weight were increased significantly. Herbal-Antiox-1 significantly reduced the hyperglicaemia, TG concentration and liver/body weight as well. In the control group Herbal-Antiox1 had no effect on investigated parameters.

In conclusion Herbal-Antiox -1 limits the accumulation of TG in liver, adipose tissue and contributes to reduction of body weight in rats.

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5. **Bratoeva K.**, G. Bekyarova, Y. Kiselova, M. Radanova, D. Ivanova. **Uric Acid as Biomarker of Metabolic syndrome.** *9- ти Международен симпозиум по затлъстяване и съпътстващи заболявания, май 2011год., Албена, България.*

Summary. Increased consumption of high-calorie foods worldwide and fructose in particular, correlates positively with the alarming increase in the prevalence of obesity, dyslipidemia and metabolic syndrome and associated cardiovascular disease and type 2 diabetes mellitus. An increasing body of evidence suggests that high fructose intake can lead to an increased serum level of uric acid (hyperuricemia) in healthy subjects, which is even more substantial in adults with obesity and metabolic syndrome. Moreover, fructose-induced hyperuricemia causes increased free radical formation, which leads to decreased bioavailability of endothelial nitric oxide and endothelial dysfunction, the latter being a major factor in the pathophysiology of arterial hypertention, diabetes mellitus and atherosclerosis. FFData from experimental studies indicate that increased uric acid levels have proinfl ammatory, vasoconstrictive and prothrombotic effect and may play a role in vascular remodeling. Hyperuricemia is a predictor of arterial hypertension, diabetes mellitus and atherogenic triglyceridemia, all identifi ed as components of the metabolic syndrome. Therefore, monitoring of serum uric acid level along with infl ammatory and atherogenic markers is essential for assessing cardiovascular risk in patients with metabolic syndrome.

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6. **Bratoeva K**, Bekyarova G, Tzaneva M Radanova M. **Infalmmatory response and oxidative stress in development of nonalcoholic fatty liver disease.** *X National Congress of Bulgarian Society for Physiological Sciences, Varna 6-9 October 2011, Scr Sci Med 2011;43(3):201.*

Nonalcoholic fatty liver disease (NAFLD) is the most frequent liver disease worldwide, and is commonly associated with the metabolic syndrome.The development of NAFLD is mainly associated with unhealthy lifestyle and increased dietary fructose consumption. NAFLD may lead to nonalcoholic steatohepatitis (NASH) by oxidative stress and inflammation.

Aim: The aim of this study is investigated the role of oxidative stress and inflammation in development of NAFLD induced in high fructose fed (HFF) rats. **Methods:** We used male Wistar rats divided into two (equal) groups: control and fructose fed received 12.5% high-fructose corn syrup in drinking water for 12 weeks (n=6). Hepatic triglycerides (TG), markers of oxidative stress (malondialdehyde (MDA), total thiols) and inflammatory markers such as hcC-reactive protein (hcCRP), complement C3 (C3), tumor necrosis factor- α (TNF-α), interleukin (IL-6) were measured. Liver histopathology was observed on light microscopy.

Results: Our data showed increased levels of TG and microvesicular steatosis as well as oxidative stress, elevation in hcCRP and IL-6 while C3 and TNF-α levels did not change in HFF-rats. **In conclusion**, our data show that HFF causes intrahepatic lipid accumulation-first step in development of NAFLD. We suggest that NAFLD may progress to NASH in longer-fructose load.

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7. **Bratoeva K**, Shterev I, Bekyarova G, Tsaneva M. **The role of s-adenosylmethionine and allopurinol as protectors of fructose- induced metabolic abnormalities.** *32nd Balkan Medical Week,21-23. September 2012.Nis, Serbia.*

An increasing body evidences that fructose has a key role for increased cardiovascular metabolic risk. Our previous data showed that high fructose diet causes obesity, dyslipidemia impaired glucose tolerance which correlated with oxidative stress in animal model of metabolic synidrome (MS).

Aims. S-adenosylmethionine (SAM) (as a precursor of glutathione) and allopurinol (xanthine oxidase inhibitor) are having therapeutic effect mediated by their antioxidant properties. The aim of this study is to investigate of SAM and allopurinol effect on oxidative stress and metabolic disorders in fructoseinduced metabolic syndrome.

Methods. In our study, serum triglycerides (TG), very low-density lipoprotein (VLDL), high low-density lipoprotein (HDL), total cholesterol and glucose; body, visceral fat and liver weight; malondialdehyde (MDA) and thiols (markers of oxidative stress) in rats receiving high- fructose diet (16-week) and treatment with SAM and allopurinol were investigated.

Results. High fructose diet causes visceral and liver fat accumulation, elevated levels of MDA, TG, cholesterol and VLDL accompanied with decreased levels of HDL and thiols depletion ($p<0.05$). SAM and allopurinol cause a significant reduction of body, visceral fat and liver weight, decrease of TG, total cholesterol, VLDL,MDA levels, increase of HDL and restricted thiol depletion in fructose-fed rats ($p<0.05$).

Conclusion. SAM and allopurinol reduced visceral fat accumulation, metabolic oxidative stress and prevents dyslipidemia in experimental MS.

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8. **Братоева К.**, Ефтимов М., Вълчева-Кузманова С., Бекярова Г. **Предварително проучване на протективният ефект на s- аденозилметионин върху метаболитните и поведенчески нарушения при плъхове на високо-фруктозна диета.** *Научно заседание на секция "Медицина" към Съюз на учените - Варна 26.10.2012.*

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High fructose consumption causes met- abolic syndrome (MS). Authors reported that oxidative stress and other components of MS have an effect on mental health, compromising cognitive function and emotions. S- adenosylmethionine (SAM-e) is a nutrient possessing a wide variety of effects including antioxidant and antidepressive.

The aim of the present study was to investigate the effects of SAM-e on metabolic abnormalities and on rat behavior in a model of metabolic syndrome induced by high fructose intake. Male Wistar rats (n=21) were used in the experiment. They were divided into 3 groups: control, fructose fed (35 %, 16 weeks), fructose fed and treated with SAM-e (20 mg/kg b.w., 16 weeks). Glucose and triglycerides (TG) were measured as markers of metabolic abnormalities. Malondialdehyde (MDA) was the marker of oxidativestress. The working memory was inwestigated in the object recognition test (ORT).Our data showed that fructose feeding caused significant metabolic abnormalitiesand memory defecits. SAM-e reduced sig-nificantly plasma TG ($p<0.005$) and MDA ($p<0.001$). It prevented fructose-induced reduction of recognition index in the ORT. In conclusion, SAM-e prevented metabolic abnormalities and object recognition memory impairment in rats with high fructose-induced MS.

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9. **Bratoeva K.**,Shterev I.,Tsaneva M.,Bekyarova G. S-adenosylmethionine as a modulator of hepatocyte survival in non-alcoholic fatty liver disease in rats. *IUNS 20th International Congress of Nutrition Granada, Spain,15-20th September 2013.* ***Annals of Nutrition & Metabolism****, 63(s1),1297.* **IF=2.747**

Background and objectives: Chronic excessive fructose intake causes obesity, intrahepatic lipid accumulation, liver oxidative injury and Non-alcoholic fatty liver disease (NAFLD). Therapeutic approach to increase hepatic autophagy can prevent the progression of NAFLD from steatosis to steatohepatitis and liver diseases. Recent data suggest that pathologically activated apoptosis suppresses autophagy, which in an environment of oxidative stress and overfeeding should serve as a mechanism for hepatocyte survival. **The aim** of this study was to investigate the role of S-adenosylmethionine (SAM-e) (nutrient with antioxidant and antiapoptotic effects) in the mechanism of hepatocyte injury leading to cell death or survival. **Methods:** Male Wistar rats (n=21) were used in the experiment. They were divided into 3 groups: control, fructose fed (35 %,16 weeks), fructose fed and treated with SAM-e (20 mg/kg b.w.,16 weeks). The expression of hepatic Bax (proapoptotic protein) and Beclin 1(initiating autophagy protein) using light immunohistochemistry and malondialdehyde (MDA) as markers of oxidative stress were investigated. Liver histopathology was observed on light microscopy. **Results:** The results showed microvesicular steatosis, increase liver MDA levels ($p<0.05$), activation of Bax protein and diminished Beclin 1expression in fructose fed rats compared with control group.

In the group treated with SAM-e the expression of Beclin 1 was significantly higher while steatosis, MDA levels ($p < 0.001$) and Bax expression were reduced compared with fructose fed rats. **Conclusions:** Our data showed that high fructose diet induces oxidative injury, apoptosis and steatosis in the liver. The administration of SAM-e inhibited oxidative damage and apoptosis suppressed autophagy in hepatocytes and it may be critical in the elimination of lipid accumulation in hepatocytes and the prevention of high fructose-indused steatosis.

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10. Bekyarova, M. Tzaneva, **Bratoeva K.**, I. Kotzev. Heme oxygenase-1 levels and oxidative stress-related markers in fatty liver, induced by high fructose diet. G. *The International Liver Congress™ April 24-28, 2013, Amsterdam, Netherlands.* ***Journal of Hepatology*** *2013, 58, s513*, **IF= 10.401**

Background and Aims: Excessive dietary fructose intake may have an important role in the current epidemics of fatty liverdisease, obesity and diabetes – features of metabolic syndrome. The mechanisms associated with development of the fatty liver disease appear to involve multiple cellular adaptations to the oxidative stress occurring when fatty acid metabolism is altered. We evaluated the relationship between lipid peroxidation and other oxidative stress biomarkers with changes in expression of heme oxygenase-1 (HO-1) in rat fatty liver, induced by high fructose diet (HFD) and the effect of S-adenosylmethionine (SAMe).

Methods: Twenty-one male rats were randomly assigned to three groups of seven animals each: HFD (35% fructose in drinking water for 16 week) group, HFD + SAMe (20 mg/kg in drinking water for 16 week) group and control group. HO-1 expression, MDA (marker of lipid peroxidation), triglycerides (TG), glutathione (GSH) levels and histological (H&E) studies were assayed in liver.

Results: HFD group showed microvesicular steatosis without inflammation and fibrosis. In HFD+SAM group microvesicular steatosis was not established. The HO-1 expression was significantly increased in HFD rats ($p < 0.001$). SEMe augmented the increase in expression of HO-1 ($p < 0.01$). The levels of MDA and TG were elevated in HFD group ($p < 0.01$). In HFD rats with lower levels of GSH exhibited higher expression of HO-1. SAMe inhibited the elevation in lipid peroxidation and TG levels and prevented the decrease in GSH levels ($p < 0.05$). **Conclusions:** The induction of HO-1 is an adaptive response against oxidative damage elicited by lipid peroxidation and it may be critical in the pathogenesis of the high fructose-induced fatty liver in rats. SAMe has hepatoprotective effect and its protection likely exerted by increased expression of the antioxidant enzyme HO-1 to prevent the development of fatty liver.

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11. **Камелия Братоева**, Иван Щерев, Мария Цанева, Ганка Бекярова. **Оксидативен стрес и механизъм на хепатоцитно оцеляване при фруктозо-индуцирана чернодробна стеатоза**. *Конкурс „Наука и младост" 18-20 април 2013 г. МНД „ Асклепий" МУ- Пловдив.*
The hepatocytic apoptosis and the oxidative stress in the fatty liver are both factors for the development of nonalcoholic fatty liver disease and its progression to nonalcoholic steatohepatitis, fibrosis and cirrhosis. The latest studies of various dietary models show that autophagy (a cellular survival process) is involved in both the lipid metabolism and the limitation of the oxidative damages in the liver. Its role in the fructose-induced hepatic steatosis is still unclear. The aim of this study is to investigated the relationship between apoptosis, oxidative stress and autophagy in fatty liver in fructose-induced experimental model.
The study was performed on white rats Wistar rats divided into two groups (n=7): control group (on a standard diet), and fructose fed (35% in the drinking water, 16 weeks). The hepatic Bax expression (proapoptotic protein) and Beclin 1 (autophagy initiating protein) were measured by the light IHC method, while the MDA and the total thiol levels as oxidative stress markers were determined by using the respective spectrophotometric method. The hepatic histology for evaluation of the steatosis rate and the foci of inflammation was determined by the light microscopy. Microvesicular steatosis, elevated MDA levels ($p<0.01$), reduced total thiol levels ($p<0.05$), increased Bax protein expression ($p<0,005$) and decreased Beclin 1 expression ($p<0.005$) were established in the fructose-fed rats as compared to the control group.
In conclusion, our results show that under the condition of a fructose-induced oxidative stress the autophagy and the apoptosis are probably to be antagonistic processes connected with the deposition of excess lipids in the hepatocytes. The activation of the one process or the other plays a crucial role in the cellular function and the survival of the hepatocytes and the future hepatic damages.

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12. Stoyanov G., Mihaylova E., Stefanova T., Moneva K., **Bratoeva K. Kidney manifestations in experimental model of metabolic syndrome**. *Second Black Sea Symposium for Young Scientists in Biomedicine, Varna, Bulgaria, 2014.*
Purpose: Metabolic syndrome (MS) is a worldwide prevalence disease that affects more than 25% of the adult population in developed countries. The latest studies show an increased incidence of chronic kidney disease in patients with MS. It is assumed that the major components of MS - obesity, insulin resistance, dyslipidemia and hypertension are linked to renal damage through the systemic release of several pro-inflammatory mediators such as uric acid (UA) and C-reactive protein (CRP). **The aim** of the present study was to investigate the influence of UA and CRP on renal damage in an experimental model of MS.

Methods and Materials: Male Wistar rats were used in the experiment. They were divided into two groups (n=7): control group (on a standard diet), and fructose fed (35% fructose syrup in the drinking water for 16 weeks). Body and kidneys weight, serum glucose, triglycerides, UA and CRP were determined. Kidney
histopathology was observed via light microscopy. **Results:** All fructose-fed rats developed obesity, hyperglycemia and hypertriglyceridemia. In parallel with significantly increase kidneys weight($p<0.05$), CRP($p<0.05$) and UA($p<0.05$), the light microscopy showed amyloid deposits in the glomeruli, visible hydropic change (vacuolar degeneration) in the epithelial cells covering the proximal tubules and increased eosinophilia in the distant tubules in fructose-fed rats compared with control group. **Conclusion:** In conclusion, our results show that under the condition of a fructose-induced MS, UA and CRP probably induce tubulointerstitial injuries that are important for future development of chronic kidney disease.

13. **Bratoeva K**, Merdzhanova A. **Differential roles of free fatty acids in an experimental model of obesity.** *ECO 2014, European Congress on Obesity, May 28-31, Sofia, Bulgaria. Obesity Facts 2014;7(1), 157.* **IF=2.245**

Introduction: High-fructose diet causes obesity, metabolic and oxidative damage to hepatic steatosis in rodents and has been successfully used for the modeling of a fatty liver disease in humans. The latest studies of varioudietary models showed that autophagy is involved in both the lipid metabolism and the limitation of the oxidative damages in the liver. Different lipid classes may also have different effects on cells function. Given the importance of lipogenesis in liver injury, the aim of this study was to determine the effects of the free fatty acids on autophagy and oxidative stress in fatty liver in fructose-induced experimental model. **Methods:** Male Wistar rats were divided into two groups (n=7): control group (on a standard diet), and fructose fed (35%
in the drinking water, 16 weeks). Body and retroperitoneal fat weight were determined. Analysis of fatty acidswas performed by GasChromatography-Mass spectrometry. Liver injury was assessed biochemically and histologically together with hepatic Beclin1 (initiating autophagy protein) expression. **Results:** The results showed increase body ($p<0.05$) and retroperitoneal fat ($p<0.01$) weight, liver MDA levels ($p<0.05$), microvesicular steatosis, suppressed Beclin1 expression and an increased in the ratio of very long-chain saturated to unsaturated fatty acids-C20:0-C24:0 in fructose fed rats compared with control group. **Conclusion:** Fructose induces obesity and de novo lipogenesis in hepatocytes with threefold increased accumulation of very long-chain saturated fatty acids(VLCSFAs). Excess VLCSFAs coincided with suppressed autophagy, oxidative damage and steatosis, which identifies them as an important factor in liver injury in obesity.

14. **Bratoeva K**, Bekyarova G, Tsaneva M. **Influence of S- adenosylmethionine in fructose-induced hepatic injuries.** *ECO 2014, 21st European Congress on Obesity, May 28-31, Sofia, Bulgaria. Obesity Facts 2014;7(1), 173.* **IF=2.245**

Introduction Fructose is an important risk factor in the development of fatty liver. This is related to the de novo hepatic lipogenesis, excess production of free radicals and changes in redox balance. It is known that the overcoming of antioxidant protection in the cells leads to a change in the redox enzyme activity, mitochondrial dysfunction and cell death by apoptosis. **The aim** of this study was to investigate the influence of S- adenosylmethionine (SAM-e) administration in fructose-induced hepatic injuries. **Methods** The study was performed on male Wistar rats divided into 3 groups (n=7): control, fructose fed (35 %,16 weeks), fructose fed and treated with SAM-e (20 mg/kg b.w.,16 weeks). Liver injury was assessed biochemically and histologically together with hepatic Bcl-2 family proteins expression. **Results** The results showed microvesicular steatosis, increase liver MDA levels ($p < 0.05$), significantly elevated ratio Bax/BCL-2 by 92% ($p < 0.01$), reduced total thiol levels ($p<0,05$) in the fructose-fed rats compared to the control group. In the group treated with SAM-e steatosis, MDA levels ($p < 0.001$) the ratio Bax/BCL-2 were significantly reduced while total thiol levels ($p<0,05$) was elevated compared with fructose fed rats. **Conclusion** Our results showed that SAM-e administration inhibits fructose-induced hepatic injuries and apoptosis. As a precursor of glutathione SAM-e improves antioxidant defenses in hepatocytes. This effect likely modulates the expression of hepatic Bcl-2 family proteins by increasing anti-apoptotic Bcl-2 protein, suppressed apoptosis and protects against oxidative damage in the liver.

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15. **Bratoeva K**, Bekyarova G, Radanova M. **S-adenosylmethionine in the prevention of fructose-induced metabolic disorders.** *10th International symposium on obesity and related diseases, 1-3.06.2014, Albena, Bulgaria.*

Introduction: Increasing consumption of foods with high fructose content (drinks, juices, and many other foods of the confectionery industry) leads to obesity, diabetes and dyslipidemias. The development of these metabolic disorders especially in adolescents are with serious consequences for human health. They are associated with systemic oxidative stress and low-level inflammation, that lead to liver damage and a negative effect on mental health, affecting cognition and emotions. New approach to prevention and treatment is the use of functional foods and supplements with high antioxidant content. S-adenosylmethionine (S-AME) is the nutrient possessing a wide variety of proven effects as an antioxidant, hepatoprotective and antidepressant action. **The aim** of the study was to investigate the effects of S- AME in an experimental model of metabolic abnormalities induced by fructose fed rats.

We used male Wistar rats (n = 21), which were divided into three groups: control; fed fructose (35 %, 16 weeks); fructose -fed and treated with S-AMe (20 mg / kg body weight , 16 weeks). All fructose-fed rats developed obesity, hyperglycemia, hypertriglyceridemia, systemic inflammation and oxidative stress accompanied by the accumulation of triglycerides and increase expression of pro-apoptotic proteins in the liver, which positively correlated with deficits in working memory, tested in the object recognition test (ORT). Administration of S-AMe suppress these disturbances as a significant decrease ($p<0.05$) serum glucose, triglycerides, C-reactive protein, liver - malondialdehyde, ratio Bax/Bcl2, increased the expression of hepatic heme oxygenase – 1 levels and affect brain function improving working memory tested in ORT.

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16. Bekyarova G, **Bratoeva K**, Tzaneva M, Atanassova M. **Protection against nonalcoholic liver injury, induced by high fructose diet by attenuating oxidative stress, inflammatory response and apoptosis.** *33 Balkan Medical Week, September 2014. Bucuresti, Romania. Archives of the Balkan Medical Union 2014; 49 (Suppt I): A101.*

Introduction. Nonalcoholic fatty liver disease is the most common chronic liver disease and nonalcocholic steatohepatitis (NASH) is its advanced form. Oxidative stress and hepatocyte apoptosis may be involved in pathogenesis of NASH and particularly in progress of NASH to liver fibrosis and cirrhosis, which are initiated by the inflammation and which promotethe progress of the disease. The present investigation was designed to determine the effects of allopurinol on nonalcohol-fatty liver injury induced by high fructose diet (HFD). **Methods.** Twenty-one male rats were randomly assigned to three groups of seven animals each: HFD (35% fructose in drinking water for 16 week) group, HFD +Allopurinol (150 mg/kg in drinking water for 16 week) group and control group. Hepatic malondialdehyde (MDA) (marker of lipid peroxidation) and glutathione (GSH) levels, pro apoptotic Bax and antiapoptotic Bcl-2 proteins and plasma TNFα (inflammatory marker) and uric acid (UA), and histological (H&E) studies were assayed. **Results.** HFD group showed microvesicular steatosis and fibrosis, increased levels of hepatic MDA, plasma UA and TNF-α as well as pro-apoptotic Bax expression while and GSH and anti-apoptotic Bcl-2 protein decreased. Treatment with Allopurinol significantly reduced the severe extent of hepatic cell damage, steatosis, plasma UA and inflammatory cytokineslevels, tissue lipid peroxidation, inhibited the apoptosis of hepatocytes. **Conclusions.** These findings suggest that Allopurinol may represent a novel, protective strategy against nonalcoholic liver injury by attenuating oxidative stress, inflammatory response and apoptosis.

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17. Bekyarova G, **Bratoeva K**, M. Tzaneva. **Oxidative stress markers and hemeoxygenase-1 in fatty liver, inducesd by diet high in fructose.** *7 th National congress of Pharmacology, Pleven 17-19 October 2014, Journal of Biomedical and Clinical Research 2014 ;7(1) Suppl.1: 80.*

The dramatic rise in prevalence of fatty liver disease, obesity and diabetes - features of metabolic syndrome in developed countries is associated with overconsumption of dietary fructose. The pathophysiological mechanisms associated with development of fatty liver disease appear to involve multiple cellular adaptations to the oxidative stress occurringwhen fatty acid metabolism is altered. We explored the expression of heme-oxigenase-1 (HO-1) and its relationship with oxidative stress biomarkers in rat models of diet-inducible fatty liver and effect of allopurinol. Fatty liver was triggered in male rats with high fructose diet (HFD) (35% fructose in drinking water for 16 week) HFD +Allopurinol (150 g/ml in drinking water for 16 week) group and control group. HO-1expression, triglycerides (TG), uric acid (UA), malondialdehyde (MDA), glutathione (GSH) levels and histological studies were assayed in liver. HFD rat featured microvesicular steatosis. In HFD+All group microvesicular steatosis was not found. In HFD rats the expression of HO-1 was significantly increased ($p<0.01$) but GSH levels was decreased. Allopurinol augmented the increase of hepatic HO-1 expression ($p<0.01$). MDA, UA and TG levels were elevated in HFD ($p<0.05$). Allopurinol prevented the decrease in GSH ($p<0.05$) and inhibited the elevation in MDA, TG and UA levels. Fructose diet upregulates HO-1 expression, which correlates with the increased indicators of oxidative stress. Allopurinol shows hepatoprotective effect and its protection likely exerted by increased expression antioxidant enzyme HO-1 to restrict the development of fatty liver.

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18. Tsvetina Vasileva, Stanislav Morfov, Kamelia Bachvarova, Hristo Rankov, **Kamelia Bratoeva**. **Connection between lifestyle and pathophysiological mechanism of metabolic syndrome.** *Second Black Sea Symposium for Young Scientists in Biomedicine, Varna, Bulgaria, 2014.*

Aims/Objectives: Metabolic syndrome (MS) has emerged as a growing public health problem worldwide associated with changes in the environment, feeding, behavior and lifestyle. The increasing consumption of high calorie foods and those containing fructose leads to obesity, high fasting glucose, dyslipidemia and hypertension important components of MS. Several possible mechanisms explaining the relationship between lifestyle and the components of MS. The proinflammatory and prothrombotic states of metabolic syndrome derive largely from the secretory activity of adipose tissue, particularly intra-abdominal or visceral fat.

Contrary to the former understood concept of fat as an inert tissue mass, adipocytes are being increasingly recognized as secretory entities. Cytokines and other inflammatory markers or signaling molecules released by adipocytes termed "adipokines"-include leptin, TNF-alpha, interleukin-6, resistin and adiponectin. Adiponectin levels are inversely related to fasting plasma insulin and glucose levels. Weight loss by obese individuals has been associated with increased adiponectin levels. Several lifestyle behaviors may influence whether or not a person can maintain energy balance over a long term period. **Methods:** Meta-naliz bases on publications connected with lifestyle and pathophysiological progress of MS **Results:** Overall, the mean changes in lifestyle in the study population were small, the between-individual changes were large. In the NHS, for example, the difference between persons in the upper level of change and those in the lower level of change (95th percentile minus 5th percentile) was 3.1 servings per day of vegetable consumption, 25.3 metabolic equivalents (METs) per week for physical activity, and 0.66 drinks per day for alcohol consumption and low-fat dairy products. **Conclusions:** Identification and clinical management of this high-risk group is important. The lifestyle recommendations are based on the rules of a healthy diet, increased physical activity and reduction in harmful environmental factors.

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19. **Kameliya Bratoeva**, Mariya Radanova, Ganka Bekyarova, Mariya Tsaneva. **Apoptotic biomarkers in nonalcoholic fatty liver disease, induced by high-fructose diet.** *Юбилейна научна конференция „ Наука за здраве", 20-25 май 2015г. МУ-Пловдив. Folia Medica 2015; 57; (1), 35.*

Introduction: The pathogenesis of non-alcoholic fatty liver disease as a component of metabolic: syndrome (MS) is still unknown. Apoptosis may play an important role in pathophysiological mechanisms involved in liver damage and progression. Our aim was to detect early biomarkers of apoptosis in serum, as a cytokeratin-18 (CK-18) and its role in assessing the presence and severity of liver damage in high-fructose diet (HFD). **Methods:** The study was performed on male Wistar rats divided into 2 groups (n = 7): control, highfructose fed rats (35%, 16 weeks). Liver injury was evaluated immunohistochemically for presence of hepatic Bcl-2 family protein, biochemically for changes in aminotransferase levels and MDA (marker of lipid peroxidation) and histological (H&E) studies. Levels of CK-18 in hepatic and sera were measured by Western immunoblotting. **Results:** The results showed a signifi cant increase of hepatic CK-18 levels, pro/anti apoptotic Bax/BCL-2 ratio by 92% ($p < 0.01$), elevation of liver MDA levels ($p < 0.05$) in HFD compared to the control group.

HFD group showed microvesicular steatosis without changes in aminotransferase levels, infl ammation and fi brosis. CK-18 serum levels correlated with changes in Bax/ BCL-2 proteins ratio, and the oxidative and histological changes in both groups. **Conclusion:** Increased serum CK-18 levels in the HFD group and its correlation with histological and biochemical changes in liver. suggested the key role of apoptosis in pathogenesis of the HDFinduced fatty liver in rats. The data shows that CK-18 may be a useful biomarker for non-invasive assessment of the liver damage and progression in MS.

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20. Stanislav Morfov, Tsvetina Vasileva, Mila Radeva, Blagovesta Todorova, Kamelia Bratoeva. **Arterial pulmonary hypertension: What have we learned?** *Third Black Sea Symposium for Young Scientists in Biomedicine, March 26-29, 2015, Varna.Varna Medical Forum 2015,4; (1),14.*

Aims/Objectives: Pulmonary arterial hypertension is defined as a sustained elevation of pulmonary arterial pressure to more than 25 mm Hg at rest or to more than 30 mm Hg with exercise, with a mean pulmonary-capillary wedge pressure and left ventricular end-diastolic pressure of less than 15 mm Hg, leading to shortness of breath, dizziness, fainting, leg swelling and other symptoms. Pulmonary hypertension is usually classified as primary (idiopathic) or secondary. It is now clear, however, that there are conditions within the category of secondary pulmonary hypertension that resemble primary pulmonary hypertension in their histopathological features and their response to treatment. Better understanding them will assist identify therapeutic targets to improve this condition. **Methods:** Meta-naliz based on publications connected with arterial pulmonary hypertension **Results:** The cause of pulmonary arterial hypertension is heterogeneous and include specific heritable and environmental factors. Molecular genetic studies have shown that mutations in the gene encoding bone morphogenetic protein receptor type II (BMPR2) are present in approximately 70% of patients with familial pulmonary arterial hypertension, as well as in 10 to 25% of those with idiopathic pulmonary arterial hypertension. The main vascular changes in the lung vasculature are vasoconstriction, smooth-muscle cell and endothelial-cell proliferation, and thrombosis. These findings suggest the presence of perturbations in the normal relationships between vasodilators and vasoconstrictors, growth inhibitors and mitogenic factors, and antithrombotic and prothrombotic determinants, which are probably consequences of pulmonary endothelial-cell dysfunction or injury. Among the environmental factors associated with an increased risk of the development of pulmonary arterial hypertension, three hypoxia, anorexigens, and central nervous system stimulants have plausible mechanistic underpinnings.

Conclusions: We provide evidence that pulmonary arterial hypertension predisposing genes interact with the environment and influence the response to treatment relevant to disease prediction. There is no cure for pulmonary arterial hypertension. Treatment, however, has improved dramatically during the past decade, offering both relief from symptoms and prolonged survival. The mainstays of current medical therapy fall into several classes, including vasodilators, anticoagulants, antiplatelet agents, anti-inflammatory therapies, and vascular-remodeling therapies. Many of the most effective agents have pleiotropic effects. Lung transplantation cures pulmonary arterial hypertension, but leaves the patient with the complications of transplantation, and a postsurgical median

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21. Stoyanov G, Moneva T, Stefanova T, **Bratoeva K. Metabolic syndrome diabetes and kidney – the role of amyloid.** *Third Black Sea Symposium for Young Scientists in Biomedicine, March 26-29, 2015, Varna. Varna Medical Forum 2015,4; (1),62.*

Kidney involvement and kidney complications are amongst the leading causes of death in the developed world. Amongst the most common causes of chronic kidney disease are conditions caused by impairment of the metabolism of glucose - type 2 diabetes mellitus and the closely related metabolic syndrome. However the status quo of chronic kidney disease, associated with type 2 diabetes mellitus and metabolic syndrome, has not been put in to question despite new evidence regarding its starting point, development and progression.

The aim of this study is to point out the involvement of amyloid in type 2 diabetes mellitus and metabolic syndrome associated chronic kidney disease. Original research data from an established experimental animal model of metabolic syndrome, carried out with male Wistar rats for a duration of 16 weeks, was compared with the latest published papers, all using human biopsy material. All parties questioned the involvement of amyloid deposition in the genesis and progression of diabetic kidney disease. Although focused on two different types of amyloid – islet associated and serum reactive, all parties showed amyloid was present in a considerable amount of cases and was closely associated with severity and prognosis of the condition. One study showed a similar pattern of islet associated amyloid in obesity related glomerulopathy.

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22. Bekyarova G, P. Genev, **Bratoeva K. Protective effect of Allopurinol in the development of atherosclerosis in high fructose diet.** *The XI National Congress of Bulgarian Society for Physiological Sciences, Plovdiv 9-11 October 2015. Folia Medica, 2015, 57 suppl 3 :8.*

Strong epidemiologic evidence indicates a relationship between high fructose diet (HFD) and atherosclerotic heart disease, which results in a series of human and animal experiments in this regard. In this study we aimed to evaluated the relationship between the inflammatory and oxidative stress markers, and atherosclerotic lession development in high-fructose diet and the effect of treatment with Allopurinol. The study was performed on male Wistar rats divided into 3 groups (n = 7): control, fructose fed (35%,16 weeks), fructose fed and treated with Allopurinol (20 mg/kg b.w., 16 weeks). Thereafter, plasma glucose, triglycerides and uric acid (metabolic parameters), malondialdehyde (MDA)(oxidative stress), tumor necrosis factor (TNF)-α and C-reactive protein (inflammatory markers) and atheroma lesion presence were determined. HFD induced a marked increase in plasma glucose, uric and triglyceride levels and inflammatory response and release of inflammatory cytokines, appearance of the acute phase proteins and increased levels of lipid peroxidation products. Following 16-weeek HFD isolated foam cells were histologically detected in the thoracic aorta. Allopurinol inhibited the elevation in lipid peroxidation, metabolic marker, TNF-α and CRP levels ($p<0.05$). In HFD+allopurinol group foam cells was not established. In conclusion, these data show that high-fructose diet may induce a proinflammatory and prooxidant state and even to initiate early atherosclerotic changes. Allopurinol has protective effect and its protection likely exerts by decrease of oxidative stress, metabolic and inflammatory marker levels to prevent the development of atherosclerosis in high fructose diet.

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23. Bekyarova G, **Bratoeva K.**, M. Tzaneva. **Protection against fructose-induced apoptotic hepatic injury through activation of heme-oxygenase-1.** *XI National Congress of Bulgarian Society for Physiological Sciences, Plovdiv, 2015, Folia Medica 2015, 57 suppl.3*

Excessive dietary fructose intake may have an important role in the development of nonalcoholic fatty liver disease. The important role in pathogenesis of this liver disease play de novo hepatic lipogenesis, excessive production of free radicals, mitochondrial dysfunction and apoptosis. Recent studies indicate that antioxidant heme-oxygenase-1(HO-1), inhibits apoptosis and exerts hepatoprotective effect. In the present study, we aimed to investigate the mechanisms underlying the relationship between the expression of hepatic HO-1, lipid peroxidation and apoptosis and the effect of treatment with Sadenosylmethionine (SAMe) on high fructose diet (HFD)-induced liver injury.

The study was performed on male Wistar rats divided into 3 groups (n = 7): control, fructose fed (35%,16 weeks), fructose fed and treated with SAM-e (20 mg/kg b.w., 16 weeks). The levels of malondialdehyde (MDA) as marker of oxidative stress was quantified by thiobarbituric method. Hepatic HO-1, marker of antioxidant defense and apoptosis-related genes Bcl-2 and Bax was evaluated using light immunohistochemistry. The results showed microvesicular steatosis, increase liver MDA levels ($p < 0.05$), increased expression of HO-1, elevated ratio Bax/BCL-2 by 92% ($p < 0.01$), reduced total thiol levels ($p < 0,05$) in the fructose-fed rats compared to the control group. SAMe treatment augmented the increase in HO-1 expression, decreased both burn-induced peroxidative damage and apoptosis in liver as evidenced by reduced expression of Bax, enhanced expression of Bcl-2 protein. In conclusion, these results show that SEMe may attenuate fructose-induced apoptotic liver injury through HO-1 activation, suppression of lipid peroxidation and modification of Bax/Bcl-2 ratio.

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24. **Bratoeva K**, Bekiarova G, Radanova M. **Effects of SAM-e on the adipose tissue function with a high-fructose diet.** *XI Congress of Bulgarian Society of Physio-logical Sciences with International Participation, October 9-10, 2015, Plovdiv. Folia Medica 2015; 57; (3), 54.*

Adipose tissue inflammation and oxidative stress in obesity lead to chronic remodeling of adipocytes, an increased secretion of pro-inflammatory adipokines and ectopic fat deposition. These changes are highly integrated processes in the pathogenesis of insulin resistance, metabolic syndrome and other metabolic complications. It has been shown *in vitro* that SAM-e (S-adenosylmethionine), directly inhibits the IKK-b kinase activity of pro-inflammatory IKK-b/ NF-kb pathway in 3T3-L1 adipocytes and thus TNF-a mediated-insulin resistance. The objective was to investigate the effects of SAM-e on the adipose tissue function *in vivo*. We used a model of high-fructose diet (HFD) in male rats Wistar (16 weeks, 30% glucose-fructose corn syrup), divided into three groups: control; HFD; HFD and SAM-e administration. Serum levels of TNF-a and glucose; weight, markers of oxidative stress- MDA (malondialdehyde) and glutathione in the retroperitoneal tissue were investigated. The results showed significantly elevated retroperitoneal tissue/ body weight ratio, MDA, TNF-a and glucose levels in serum and decreased levels of glutathione in HFD rats compared to the control group. In the group treated with SAM-e the retroperitoneal tissue/ body weight ratio, the levels of MDA, TNF-a and glucose levels in serum were significantly reduced while glutathione levels were elevated in comparison with HFD rats.

We propose that SAM-e provides antioxidant and anti-inflammatory activity *in vivo* by reducing the fructose-induced hypertrophy, oxidative and inflammatory damage to adipose tissue. These effects are probably crucial for function of adipocytes, since they reduce the risk of development of insulin resistance and therefore, the metabolic complications of obesity.

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25. Radanova M., **Bratoeva K**, Vasilev V, Deliyska B, Ikonomov V, Argirova T. **Association between anti-C1q and anti-dsDNA antibodies in patients with Lupus nephritis**. XXV International Sciense Conference, June 4-5, 2015, Stara Zagora.

Introduction: Lupus nephritis (LN) is severe organ manifestation of the systemic lupus erythematosus (SLE). LN usually occurs within 5 years of the onset of disease, but can occur any time throughout the course of the disease. Anti-nuclear antibodies (ANA) and anti-double stranded DNA (anti-ds DNA Abs) antibodies are aiding tools to the kidney biopsy findings in early diagnosis. The aim of this study was to assess the utility of anti-C1q Abs, in predicting of LN in comparison with the „gold standard" anti-dsDNA Abs. **Materials and Methods:** The group of LN patients included 23 (77%) women and 7 (23%) men. The levels of anti-C1q Abs levels were measured by ELISA. The presence of anti-ds DNA Abs was detected by indirect immunofluorescence. For comparison between groups, the two-tailed Student's t-test for unpaired samples with Welch's correction was used. **Results:** All 30 patients were distributed in three groups according to the degree of the clinical activity of LN: 1) patients with active LN (AN); 2) patients in partial remission (PR) and 3) patients in complete remission (CR). We found significant differences between frequencies of anti-C1q Abs in AN patients and in CR patients ($p = 0.007$) and between levels of anti-C1q Abs in AN patients and those with PR ($p = 0.026$). Positive for anti-ds DNA Abs were 56.67% of patients. We found that positive for anti-ds DNA Abs had higher level of anti-C1q Abs in comparison to negative for anti-ds DNA Abs ($p = 0.005$). **Conclusions:** The presence of anti-C1q Abs in serum closely correlated with LN activity. This study indicates the potential superior utility of anti-C1q Abs over anti-dsDNA Abs to track renal disease activity. Systematic detection of anti-C1q and anti-dsDNA aAbs should be used in combination to monitor the renal involvement.

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26. Ikonomov V, Radanova M, Ivanova I, **Bratoeva K**, Deliyska B, Vasilev V. **Can rs172378 SNP be used as a new marker for Lupus nephritis activity?** World Congress of Nephrology, March 13-14, 2015, Cape Town, South Africa.

Introduction: Systemic lupus erythematosus (SLE) is a remarkably complex and heterogeneous systemic autoimmune disease and one of its most severe manifestations is lupus nephritis (LN). We found that single nucleotide polymorphism (SNP) of the C1q gene cluster – rs172378 is associated with susceptibility to LN in Bulgarian population. In present study we investigated whether the carriage of a particular genotype or allele of rs172378 SNPs is associated with some immunological marker for Lupus nephritis diagnosis and activity.

Methods: Forty two patients with LN and 196 healthy controls, all Caucasians from Bulgaria, were genotyped for rs172378 C1q SNP, by quantitative real-time PCR methods. We also determined serum levels of C1q, C4, C3, anti-C1q autoantibodies, ANA antibodies, anti-dsDNA antibodies, IgG-, IgM-containing circulating immune complexes (CICs) and hemolitic activity of C1q in relation to the SNP genotypes.

Results: The GG rs172378 genotype was associated with susceptibility to class IV LN (OR=4.24, 95%CI: 1.33-13.59, p=0.015) in our cohort. We found that patients in complete remission at time of the investigation had predominantly GG genotype (OR=4.18, 95%CI: 1.18-14.80, p=0.018). In positive for the investigated markers patient GG genotype bound with low hemolytic activity of C1q (OR=5.43, 95%CI: (1.48 – 19.97), p=0.005) and high levels of IgG CICs (OR=3.09, 95%CI: (0.99 – 9.60), p=0.042). The SNP rs172378 showed a trend towards high anti-dsDNA antibodies in LN patients.

Conclusions: These results showed that the SNPs analysis for rs172378 could be used as new marker for evaluation of LN activity.

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27. **Kameliya Bratoeva**, Maria Radanova, Albena Merdzhanova. **Effect of allopurinol on oxidative stress in obesity and liver content of free fatty acids.** *Втора Национална конференция за млади учени "Биологически науки за по-добро бъдеще" 30–31·Октомври·2015, ПУ„Паисий Хилендарски".*

Oxidative stress appears as the key feature associated with dysfunction in adipose tissue and a major factor in the mechanisms of altered lipid metabolism in obesity. Cellular response of adipocytes in the conditions of oxidative stress results in maintaining systemic pro-inflammatory state, insulin resistance and increased accumulation of very long-chain saturated fatty acids (VLCSFAs) to the liver, which are lipotoxic and lead to further injury. Therefore, the therapeutic purposes of lowering the production of ROS, may have beneficial effects on obesity and its associated complications.

The aim of the study was to determine the influence of Allopurinol (xanthine oxidase inhibitors) on oxidative stress in adipose tissue and liver saturated fatty acids content in a model of fructose-induced obesity.

Methods: We used a model of high-fructose diet (HFD) in male rats Wistar (16 weeks, 35% glucose-fructose corn syrup), divided into three groups: control; HFD; HFD and Allopurinol administration. (150 mg/kg in drinking water for 16 week). Analysis of fatty acid was performed by Gas Chromatograph with MS detector. Serum levels of TNF-a and glucose; weight, markers of oxidative stress- MDA (malondialdehyde), glutathione(GSH) and glutathione peroxidase(Gpx) in the retroperitoneal tissue were investigated.

The results showed significantly elevated VLCSFAs, retroperitoneal tissue/ body weight ratio, MDA, Gpx ,TNF-a, glucose levels in serum and decreased levels of glutathione in HFD rats compared to the control group. In the group treated with Allopurinol the retroperitoneal tissue/ body weight ratio, the levels of MDA, Gpx , VLCSFAs , TNF-a and glucose levels in serum were significantly reduced while glutathione levels were elevated in comparison with HFD rats. The inhibition of xanthine oxidase by Allopurinol prevents the development of oxidative and inflammatory changes in adipose tissue. This effect probably improves insulin sensitivity, reduce VLCSFAs levels and thereby prevent the further lipotoxic liver damage.

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28. **Братоева К**, Стоянов Г, Бекярова Г, Раданова М. S-аденозилметионин в превенцията на оксидативни увреждания при затлъстяване *"Месец на науката-варна 2015" "науката в служба на обществото", Варна 30 октомври 2015.*

High fructose consumption causes metabolic syndrome (MS). Authors reported that oxidative stress and other components of MS have an effect on mental health, compromising cognitive function and emotions. S- adenosylmethionine (SAM-e) is a nutrient possessing a wide variety of effects including antioxidant and antidepressive. The aim of the present study was to investigate the effects of SAM-e on metabolic abnormalities and on rat behavior in a model of metabolic syndrome induced by high fructose intake. Male Wistar rats (n=21) were used in the experiment. They were divided into 3 groups: control, fructose fed (35 %, 16 weeks), fructose fed and treated with SAMe (20 mg/kg b.w., 16 weeks). Glucose and triglycerides (TG) were measured as markers of metabolic abnormalities. Malondialdehyde (MDA) was the marker of oxidative stress. The working memory was inwestigated in the object recognition test (ORT). Our data showed that fructose feeding caused significant metabolic abnormalities and memory defecits. SAM-e reduced significantly plasma TG ($p<0.005$) and MDA ($p<0.001$).

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It prevented fructose-induced reduction of recognition index in the ORT. In conclusion, SAM-e prevented metabolic abnormalities and object recognition memory impairment in rats with high fructose-induce.

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29. **Kameliya Bratoeva**, George Stoyanov, Klementina Moneva, Tanya Stefanova, Valentin Ikonomov, Mariya Radanova. **Amyloid deposition and oxidative stress mediated micro and macro vascular damage in glucose impaired kidneys.** *26-th Annual Assembly of International Medical Association Bulgaria (IMAB),12 - 15 May 2016,Varna.*

BACKGROUND AND OBJECTIVE The glucose impairment syndromes – metabolic syndrome and diabetes mellitus have recently been associated with elevated blood levels of serum amyloid A, with some of their complications being attributed to it. The aim of this study was to determine the involvement of an amyloid mediated micro and macro vascular injury to the kidneys in an experimental model of metabolic syndrome.

METHODS We used a model of high-fructose diet (HFD) in male rats Wistar (16 weeks, 35% glucose-fructose corn syrup), divided into two groups: control and HFD. The serum samples were obtained for laboratory study and the kidneys were extracted to be examined further via light microscopy.

RESULTS The sera revealed hyperglycemia and dyslipidemia in HFD compared to control group, confirming that the experimental model had been successful with all the serum measurable hallmarks of the condition developing. Were established an increase in the pro-inflammatory markers C-reactive protein and uric acid, confirming the chronic inflammatory status and high levels of malondialdehyde (marker of lipid peroxidation) and low levels of glutathione, indicating oxidative stress. Under the light microscope the kidneys revealed amyloid deposits in both the Kimmelstiel-Willson nodules and the walls of the large caliber blood vessels, early stage atherosclerosis, with visible ruptures and scarring around some of the vessels.

CONCLUSION Based on the findings of this and other similar studies, extravasal amyloid deposition play an important role in the pathogenesis of micro and macro vascular damage and therefore contributes in the development of the glucose impairment syndromes systemic complications

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30. **Kameliya Bratoeva**, Ganka. Bekyarova, Mariya. Radanova. **The link between hyperuricemia and nonalcoholic liver injury, induced by high fructose diet.** *26-th Annual Assembly of International Medical Association Bulgaria (IMAB),12 - 15 May 2016,Varna.*

BACKGROUND AND OBJECTIVE An increasing body evidences that fructose has a key role for higher cardiovascular and metabolic risk via the increased uric acid (UA) level. It is known that uric acid exhibit proinflammatory, prooxidant and prothrombotic effect on tissues that are reduce by administration of allopurinol (inhibitor of xanthine oxidase). But its role and the UA in the pathogenesis of nonalcoholic liver injury remains obscure. It is assumed that deposition of intrahepatic lipids, oxidative stress and proinflammatory factors are essential in the progression of liver injury in nonalcoholic fatty liver disease. The aim of this study was to establish the relationship between uric acid and liver injury in an experimental model of metabolic syndrome.
METHODS Twenty-one male rats were randomly assigned to three groups of seven animals each: HFD (35% fructose in drinking water for 16 week) group, HFD + Allopurinol (150 mg/kg in drinking water for 16 week) group and control group. The serum samples were obtained for laboratory study and hepatic homogenates and histological (H&E) studies were assayed.
RESULTS The results showed elevated serum levels of TNF ($p<0,05$), C-reactive protein ($p<0,05$), UA ($p<0,01$), glucose ($p<0,05$), triglycerides ($p<0,01$), and hepatic malondialdehyde ($p<0,05$) and histological evidence of microvesicular steatosis, which were significantly reduced in the group treated with allopurinol.
CONCLUSION Our data demonstrate that the fructose-induced hyperuricemia is closely related to oxidative stress, low-level inflammation, hyperglycemia and triglycerides production. Reduction of hyperuricemia with allopurinol is probably reliable new approach to reducing the severity of liver injury in metabolic syndrome.

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31. *РОЛЯ НА АВТОФАГИЯ ПРИ МЕТАБОЛИТЕН СИНДРОМ И РАК.* **Камелия Братоева**, гост-лектор на 27 Биомедицински форум, декември 2016, МУ-Варна.

Автофагията е еволюционно-консервативен и генетично регулиран адаптивен метаболитен процес, който удължава оцеляването на клетки, подложени на клетъчен стрес от липса на хранителни вещества, растежни фактори, хипоксия, свободни радикали и др. Реализира се чрез серия от процеси, които селективно разграждат, рециклират излишни или повредени вътреклетъчни компоненти, органели, липиди или нефункционални протеини и се прекратяват по време на клетъчния цикъл. Нарушенията в процесите на автофагия се свързват с пропатогенни ефекти, които предизвикват метаболитен стрес, геномни и дегенеративни увреждания, повишена туморогенеза или апоптоза и клетъчна смърт в засегнатите тъкани, както и резистентност към химиотерапия и оцеляване на ракови клетки. Двойствената роля на автофагията усложнява ефекта от прилаганите терапии и клиничното протичане на редица заболявания, което изисква по-доброто разбиране и проучване на молекулярните механизми, регулиращи тези процеси.

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32. Tsvetan Popov, Ivan Valkadinov, Martina Ivanova, **Kameliya Bratoeva**, Nikolay Conev, Ivan Donev. *DISTRESS IN BULGARIAN ONCOLOGY PATIENTS. International Biomedical Congress Sofia (IBCS) 17-19th November2017.*

Introduction: Screening for distress in order to improve patient outcome, is recommended by many organizations, such as The National Comprehensive Cancer Network (NCCN). It defines distress as a multifactorial unpleasant emotional experience that may interfere with the ability to cope effectively with cancer. The aim of the study is to measure distress before the start of treatment among patients of the Medical Oncology Clinic at UMHAT "St. Marina" Varna, Bulgaria. **Methods:** For 1 year a total of 227 oncological patients (99-male and 128-female) took participation in the study. 123 of them had metastatic disease. We used the NCCN Distress Thermometer at the beginning of treatment to determine the levels of distress. It measures distress on a scale from 1 to 10. Nonparametric Mann-Whitney U-test was used for statistical analysis. **Results** The Mann-Whitney U-test shows significant difference between the mean distress level score of men (3.2±2.8) and women (4.3±3.2) ($p=0.014$). Difference was detected among patients with Eastern Cooperative Oncology Group (ECOG) performance status 0 (3.4±3.2) and 1 (4.4±3.1) ($p=0.029$). Surprisingly we couldn't find significant difference between distress levels score in non-metastatic (3.6±3.1) and metastatic patients (3.9±3.2) ($p>0.05$). **Conclusion:** This study indicates that women and patients with poor ECOG performance status experience higher levels of distress. The diagnosis cancer causes the same distress levels among patients with metastatic and non-metastatic disease.

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21. 06. 2018 г.

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