Hydrogen Sulfide-releasing anti-inflammatory drug ATB-340 treatment potentially reduces mesenteric metaflammation in the experimental age- and high fructose dietary-induced injury

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Metaflammation (MF) is associated with visceral adiposities involved in the metabolic, cardiovascular, and gastrointestinal disease pathogenesis while their target therapeutic strategy is still limited. The link between mesenteric white adipocytes (MA) and stromal-vascular cellular remodeling in advanced age and Western diet consumption which is the base of MF remain undiscovered. Hydrogen Sulfide (H_2S)-non-steroidal anti-inflammatory drugs (H_2S-NSAIDs) are a promising novel class of drugs regarding their cytoprotective, regulatory redox signaling, vasodilatory, and anti-inflammatory effects.

Aim. To study the effectiveness of novel H_2S-NSAIDs ATB-340, a hybrid compound of H_2S and aspirin (ASA) over conventional ASA, and combination of ASA and NaHS on mesenteric cellular adaptive changes in experimental age- and high fructose dietary (HFD)-induced injury.

Methods: Mesenteric subcellular adaptive responses of aged male rats on a standard diet (SD) or 4 weeks HFD that underwent acute water-immersion restraint stress (WIRS) were evaluated by electron microscopy. The effects of 9 days exogenous administration of ATB-340 (17.5 mg/kg/day), ASA (10 mg/kg/day) and sodium hydrosulfide (NaHS, 5.6 mg/kg/day) were investigated. Serum glucose level, thiobarbituric acid reactive substances (TBARS), and activities of cystathionine γ-lyase (CSE) and cystathionine β-synthase (CBS), thiosulfate-dithiol sulfurtransferase (TST), and sulfite oxidase (SO) were examined biochemically using spectrophotometry.

Results: In HFD groups exposed to WIRS treatment with ATB-340 protected MA, as well their mitochondria, microvascular endothelial, and sub-endothelial structures, fibroblasts were observed vs the ASA and H2S+ASA-treated groups that had signs of endothelial dysfunction, MA damage with dysfunctional mitochondria, and mitochondria with fat incorporation. In rats fed with HFD and ASA treatment, low activities of CSE, CBS, TST and the rise of TBARS level and SO activity were observed. Treatment with ASA+NaHS, ATB-340 of aged rats lowered TBARS and enhanced H2S enzyme activities in contrast to the vehicle-treated group ($p < 0.05$).

Conclusions. Mitochondrial alterations, endothelial damage, and redox disbalance are key factors for aged rat mesenteric adipose tissue remodeling during Western diet consumption. Our

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results contributing to identifying powerful intervention by effective compound H2S-ASA, novel H_2S-NSAIDs, which has the potential to modulate mesenteric metaflammation, vascular function by enhancement H_2S synthesis and redox regulatory and cytoprotective activities.

***Keywords**:* Mesentery; white adipocytes, mitochondria; endothelial dysfunction; Hydrogen Sulfide-releasing anti-inflammatory drug (ATB-340); Hydrogen Sulfide (H2S); Thiobarbituric acid reactive substances (TBARS); Cystathionine gamma-lyase (CSE); Cystathionine beta-synthase (CBS); Thiosulfate-dithiol sulfurtransferase (TST); Sulfide oxidase (SO).

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Фінансування. Проведено у межах науково-дослідницької роботи кафедри нормальної фізіології, Львівського національного медичного університету імені Данила Галицького (Львів, Україна) за темами: «Дослідження ролі системних та паракринних регуляторних механізмів у забезпеченні гомеостатування функціонально-метаболічних параметрів організму за умов адаптації до дії екстремальних чинників різної природи» (номер державної реєстрації: 0116U004510) та «Вивчення механізмів регуляції інтегративних систем організму в умовах норми, функціональних розладів та з'ясування шляхів їх корекції» (номер державної реєстрації: 0121U100164).

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H_2S-вивільнюючий нестероїдний протизапальний препарат ATB-340 знижує мезентеріального метазапалення за умов старіння та пошкоджувального впливу високофруктозної дієти

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Метазапалення (запалення, пов'язане з метаболічними розладами), що часто асоційоване з вісцеральним ожирінням, лежить в основі патогенезу метаболічних, серцево-судинних та гастроентерологічних захворювань, проте стратегія цільового терапевтичного впливу на процес сьогодні залишається нез'ясованою. Залишаються недостатньо дослідженим взаємов'язок між ремоделюванням мезентеріальних адипоцитів (МА) і стромально-судинних клітин та метазапаленням за умов старіння організму та пошкоджувального впливу «західної дієти», що пов'язана з високовуглеводним харчуванням. Гібридні H_2S-вивільнюючі нестероїдні протизапальні препарати (H_2S-НПЗП) представляють собою перспективний новий клас лікарських засобів з огляду на поєднання їх цитопротекторних, судинорозширювальних, протизапальних і регуляторних властивостей щодо окисно-відновлювальних процесів.

Мета. Дослідити ефективність нових H_2S-НПЗП, на прикладі ATB-340 (гібридна сполука — H_2S-аспірин) у порівняні до звичайного аспірину (ASA) і поєднання ASA та NaHS на адаптаційні зміни мезентеріальних клітин у старих щурів, що отримували високофруктозну дієту (HFD).

Методи. Мезентеріальні адаптаційні зміни на субклітинному рівні у старих самців щурів, що перебували на стан-

дартній дієті (SD) або 4-тижневій HFD, та індукції гострого водно-імобілізаційного стресу (WIRS), оцінювали за допомогою електронної мікроскопії. Для оцінювання ефективності АТВ-340 використовували дев'ятиденне в/о введення (17,5 мг/кг/день), ASA (10 мг/кг/день) і гідросульфіда натрію (NaHS, 5,6 мг/кг/день). Концентрацію глюкози в сироватці крові, рівні реактивних субстанції тіобарбітурової кислоти (TBARS) та активність цистатіонін-γ-ліази (CSE) та цистатіонін-β-синтази (CBS), тіосульфат-дитиолсульфуртрансферази (TST) та сульфітоксидази (SO) досліджували біохімічними методами з використанням спектрофотометрії.

Результати. У групах старих щурів, які перебували HFD та отримували АТВ-340, виявлено менше пошкодження мезентеріальних адипоцитів, їхніх мітохондрій, ендотеліальних та субендотеліальних структур гемокапілярів і фібробластів порівняно до груп, що отримували ASA та NaHS+ASA де встановлено ознаки ендотеліальної дисфункції, пошкодження МА та їхніх мітохондрій. У групах старих щурів з HFD встановлено низьку активність CSE, CBS, TST, збільшенням вмісту TBARS і активності SO. Введення препаратів NaHS, АТВ-340 у старих щурів знижувало рівень TBARS та посилювало активність ензимів, що відповідають за синтез H_2S порівняно до контролю ($p < 0,05$).

Висновки. Мітохондріальні зміни, ендотеліальна дисфункція та окисно-відновний дисбаланс виступають вирішальними факторами ремоделювання мезентеріальної жирової тканини у старих щурів під час пошкоджень викликаних HFD. H_2S-ASA (АТВ-340), як представник H_2S-НПЗП має потенціал зменшувати мезентеріальне метазапалення за рахунок посилення синтезу H_2S та регулювання окисно-відновлювальниз процесів і цитопротекторної активності.

Ключові слова: Брижа; адипоцити; мітохондрії; ендотеліальна дисфункція; гібридний H_2S-асоційований аспірин (АТВ-340); гідроген сульфід (H_2S); реактивні субстанції тіобарбітурової кислоти (TBARS); цистатіон гамма-ліаза (CSE); цистатіон бета-синтаза (CBS); тіосульфат-дитиолсульфуртрансфераза (TST); сульфіт-оксидаза (SO).

Metaflammation (low-grade «sterile» inflammation related to metabolic processes) is a defining characteristic of the early event of changes in metabolic physiology which play a crucial role in the development of numerous metabolic disorders associated with obesity, type 2 diabetes (DMT2), non-alcoholic fatty liver, cardiovascular diseases as well as cancer [1-4]. Chronic overnutrition and Western diet consumption are the key links to metaflammation and insulin resistance while its implication to accelerated aging, as a basis for «inflamm-aging», still lacks evidence [5, 6, 7]. Since the recent coronavirus disease (COVID-19) pandemic outbreak has shown the dangerous outcomes of a combination of COVID-19 comorbidities related to obesity and DMT2 associated with the increased global rate of mortality among aged patients, the pathogenetic-based therapy for metabolic disorders is urgently needed [8, 9]. Anatomical and functional changes in white adipocyte tissue during aging have been noted some time ago [10], but recently its contribution to low low-grade inflammation, mesenteric white adipocytes (MA) remodeling, and local diminished blood flow could result in various pathologies, including Crohn's disease, transmural inflammation, fibrosis [11]. Over the last 20 years, the potential of hydrogen sulfide (H_2S) for anti-inflammatory, antiradical, cytoprotective, and metabolic effects was demonstrated [12]. A growing body of evidence indicates that H_2S is unique in its ability to integrate different types of intercellular inputs and to translate them into intracellular signaling pathways responsible for pleiotropic processes [13]. However, the effect of H_2S enzymatic activities of Cystathionine γ-lyase (CSE, EC 4.4.1.1) and Cystathionine β-synthase (CBS, EC 4.2.1.22), which act within the «transsulfuration pathway», Sulfite oxidase (SO, EC 1.8.3.1) and Thiosulfate-dithiol sulfurtransferase (TST, EC 2.8.1.5) pathways that act within the sulfide oxidation pathway and could reprogram mitochondrial bioenergetics during metaflammation is

still lacking. Since the experimental evidence has shown that mitochondrial dysfunction is a prominent characteristic of several metabolic pathways [14], we hypothesized that donors of H_2S synthesis could preserve mesenteric tissue and result in the improvement of experimentally-induced metaflammation in age- and dietary fructose-induced animal models associated with metabolic alteration related to mitochondrial dysfunction. Accordingly, recent reports in both preclinical and clinical studies, have a new pharmacological approach associated with promising effects of exogenous H_2S treatment and hybrid H_2S-releasing non-steroidal anti-inflammatory drugs (H_2S-NSAIDs) for preventing oxidative stress, vasodilatation, anti-inflammatory potency for their gastrointestinal safety [15, 16, 17]. Despite numerous reports on the effect of metabolic toxemia during the Western diet with an overload of glycemic carbohydrates on white adipocyte tissue, there are no studies of H_2S-NSAIDs efficiency in case of age-related changes and the effect of high fructose diet on the gradual loss of physiological integrity of MA and mesenteric stromal-vascular cells as in the initiation and development of metaflammation [18]. Previously discussed promising results formed the basis of this study aimed at investigating the treatment by H_2S-aspirin (H_2S-ASA) vs conventional aspirin (acetylsalicylic acid, ASA) effects on mesenteric adipocytes and stromal-vascular cells of aged rats in the context of metaflammation process related to high fructose diet (HFD).

Materials and methods

All experiments were performed on male aged Wistar rats (age = 42-46 weeks, N=60) in compliance with the norms of laboratory animals under the ARRIVE guidelines and the EU Directive 2010/63/EU for animal experiments and the local animal care committee at the Danylo Halytsky Lviv National Medical University's Ethics Committee (protocol 23/04/2018 № 4). All efforts were made to minimize animal suffering and reduce the number of animals used.

Experimental Protocol

Animals were kept under standard environmental conditions and randomly assigned to ten experimental groups (n = 6). Figure 1 shows the design of the study.

Rats in control groups had free access to water and were fed based on a standard diet (SD), others received a high fructose diet (HFD) for 28 days [19]. The acute stress by water-immersion restraint stress (WIRS) model was induced on the 29th day of the study to investigate adaptive reactions to acute injury [20]. The rats' initial and the final body weights were recorded by an RN 10C13U, 100 g-10 kg, ±5 g (Vaga, Kyiv, Ukraine). Rat blood glucose concentrations were measured daily after 15 h of fasting (18:00 — 9:00) using a glucometer (Achtung TD-4207, Munich, Germany) on a blood sample taken from the tail vein. At the end of the experiments, all rats were deprived of food for 12 h. For euthanasia, rats were deeply anesthetized with an intramuscular injection of ketamine (60 mg/kg; Biovet, Bila Tserkva, Ukraine). Then, the blood was collected from the sacrificed animals and samples of the mesenterium tissue associated with the small intestine were resected.

Animals were subdivided into a control group with SD, and experimental groups receiving hypercaloric HFD for 28 days, with and without acute WIRS. To evaluate the efficiency of novel

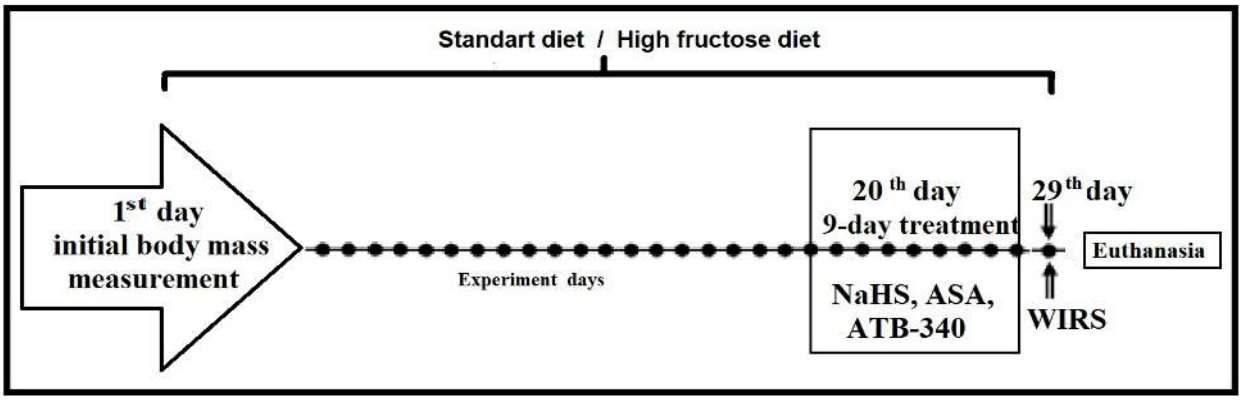


Fig.1. Study design

H_2S-NSAID substances considered as H_2S, donors were used from the 19th day of the experiment for rat treatment (during 9-days — intragastrically): NaHS at a dose of 5.6 mg/kg/day; ASA 10.0 mg/kg/day and NaHS, 5.6 mg/kg/day; ATB-340 (H_2S-NSAIDs from Antibe's drug platform, Canada) 17.5 mg/kg/day. The administration of ASA, NaHS and ATB-340 was performed in the previously tested doses [21].

The cellular and subcellular investigation via electron microscopy

For the cellular and subcellular analysis, the mesenteric material associated with the small intestine was fixed with a 2% solution of osmium oxide (OsO_4) eV 0.10 mol/L phosphate buffer. Subsequently, mesenteric material was processed according to generally accepted methods. Ultrathin sections (30-60 nm) were made using an ultramicrotome UTMTP-3M (Sumy Electron Optics PKF, Sumy, Ukraine) and after Reynolds staining, they were photographed and examined using an electron microscope «UEMV-100K» (Sumy Electron Optics PKF, Sumy, Ukraine) for magnification by 4,000, 6,000 and 10,000 times. Histologic microphotos were examined by two experienced researchers who were unaware of the treatment. About 15 different cells in each sample were analyzed per rat.

Determining TBARS levels and CBS, CSE, SO, and TST activities

Plasma TBARS levels were evaluated by assaying a reaction with thiobarbituric acid. Lipid peroxidation products from a red-stained complex which is extracted with butanol were received. Test tubes containing serum were cooled at room temperature and maximum light absorbance was measured at 535 nm using a UV-visible spectrophotometer (Apel PD-303, Saitama, Japan) [19, 20]. The resected mesenteric material was washed with cold 1.15% potassium chloride solution, after which the mucous membrane was separated and homogenized in a medium of 1.15% potassium chloride in a ratio of 1:4. The mesenteric homogenates were centrifuged at 600 g and 40° C for 30 minutes to obtain a post-nuclear fraction. The samples of mesenteric homogenates were evaluated for catalytic activities of CBS, CSE, SO, and TST (nmol/min*1 mg of protein), using a modified version of the method by Stipanuk, M.H. and Beck, P.W. [22, 23].

Statistical analysis

All results were evaluated using Statistical Analysis System and visualization program *Statistica 7.0* (StatSoft, Informer Technologies, Inc.) and expressed as mean ± standard deviation for a series of experiments. A paired Mann–Whitney U-test was used for comparisons of paired treatments between two groups, and one-way ANOVA using Dunnett's test was performed to compare different experimental groups with a control group. Statistical significance was set to p values ≤ 0.05.

Results

The general characteristic of all rats related to baseline and final body weights and fasting glucose levels are represented in Table 1. HFD induced changes in final body

Table 1

Changes of baseline and final body weights and fasting glucose levels under circumstances of administration ATB-340 and aspirin (ASA)-induced mesenteric injury in aged rats fed by high fructose diet (HFD) and control rats fed by standart rats (SD)

	SD +ASA (n=6)	SD +ASA +WIRS (n=6)	SD+ASA +NaSH +WIRS (n=6)	SD +NaSH +WIRS (n=6)	SD + ATB-340 (n=6)	HFD +ASA (n=6)	HFD +ASA +WIRS (n=6)	HFD+A-SA +NaSH +WIRS (n=6)	HFD +NaHS +WIRS (n=6)	HFD ATB-340 (n=6)
Baseline body weight (g)	252 ±28	253 ±25	256 ±26	256 ±22	256 ±2	254 ±30	254 ±23	257 ±23	256 ±27	258 ±25
Final body weight (g)	277 ±28	280 ±24	277 ±25	275 ±18	275 ±19	451 ±32	457 ±22	452 ±34	455 ±28	451 ±14
Baseline fasting glucose (nmol/L)	6,5 ±0,3	6,7 ±0,3	6,5 ±0,2	6,5 ±0,3	6,6 ±0,3	6,5 ±0,3	6,5 ±0,3	6,5 ±0,4	6,6 ±0,4	6,4 ±0,4
Final fasting glucose (nmol/L)	6,7 ±0,4	6,8 ±0,4	6,7 ±0,3	6,7 ±0,4	6,6 ±0,4	8,4 ±0,7	8,6 ±0,6	8,4 ±0,4	8,3 ±0,6	8,2 ±0,7

weight and final fasting glucose but we did not find significant differences vs animals fed with SD.

Electron Microscopy of mesenteric adipocytes and endothelial changes in aged rats fed with HFD and NaHS and ATB 340 treatment

Transmission electron microscopic studies of aged rat mesenteric material fed with SD demonstrated typical white monovacuolar adipocytes with microvessels, fibroblasts (Figure 2). The endothelial cell has an increased elongated nucleus with invaginations of the nuclear membrane, intact microvilli and blood cell in the lumen (Figure 2 A), and connective tissue of the mesentery fibroblast with collagen fibers (Figure 2 B). High-magnification electron micrographs of mesenteric adipocytes from rats fed with HFD and aspirin treatment showed partly degenerated hypertrophic fat drop and plenty of defective ring-like mitochondria in the cytoplasm (Figure 3 A). Rats fed with HFD showed stress with the treatment of aspirin white adipocytes were degenerative with small fat drops, defective ring-like mitochondria, and lipid-laden phagolysosomes in the peripheral cytoplasm (Figure 3 B). In rats fed with HFD exhibiting WIRS (Figure 4) with the treatment of aspirin mesenteric white adipocytes were hypertrophic and degenerative with plenty of smallest light electronic density fat droplets in peripheral cytoplasm (Figure 4 A), fibroblasts with vacuolation of the fibroblast's cytoplasm, and incorporated lipid droplets (Figure 4 B). High-magnification electron micrographs showed their capillaries exhibiting congestion (see deformed capillary lumens), associated with perivascular and inter-stromal edema (arrows) with many microvilli (Figure 4 C). After the administration of aspirin with NaHS, the donor of H2S synthesis was considered, the mesenteric white monovacuolar adipocyte had few lipid droplets at the periphery of the cellular cytoplasm and a large adipose vacuole devoid of the membrane (arrow). After treatment of ATB-340, the well-preserved nucleus between two white monovacuolar adipocytes showed the internalization of free lipid derived from the degenerating adipocytes, and the well-preserved capillary was between three white monovacuolar adipocytes with single lipid droplets.

Effect of NaHS, ATB 340 on TBARS levels and modulate activities of CBS, CSE, SO, and TST

Since age and HFD-induced metaflammation is characterized by the development of oxidative injury, we determine the relevance of our histological subcellular findings in mesenterial material by the investigation of TBARS (Figure 5).

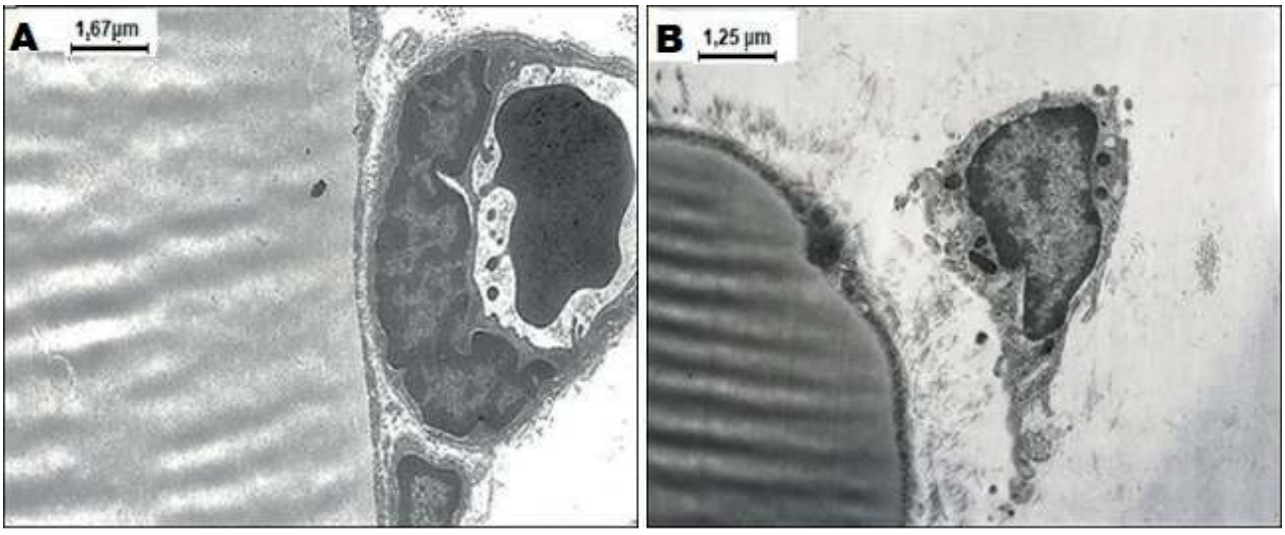


Fig. 2. Representative pictures of ultrastructural examination of mesenteric material harvested from the area associated with the rat's small intestine of aged animals on a standard diet and aspirin treatment. A: The fragment of the typical white monovacuolar adipocyte with the connective tissue of the mesentery with the presence of belonged hemocapillary with the endothelial cells with increased elongated nucleus with invaginations of the nuclear membrane and red blood cell in its lumen (original magnification ×6000). B: A fibroblast with collagen fibers belonged to adipocyte (original magnification ×8000)

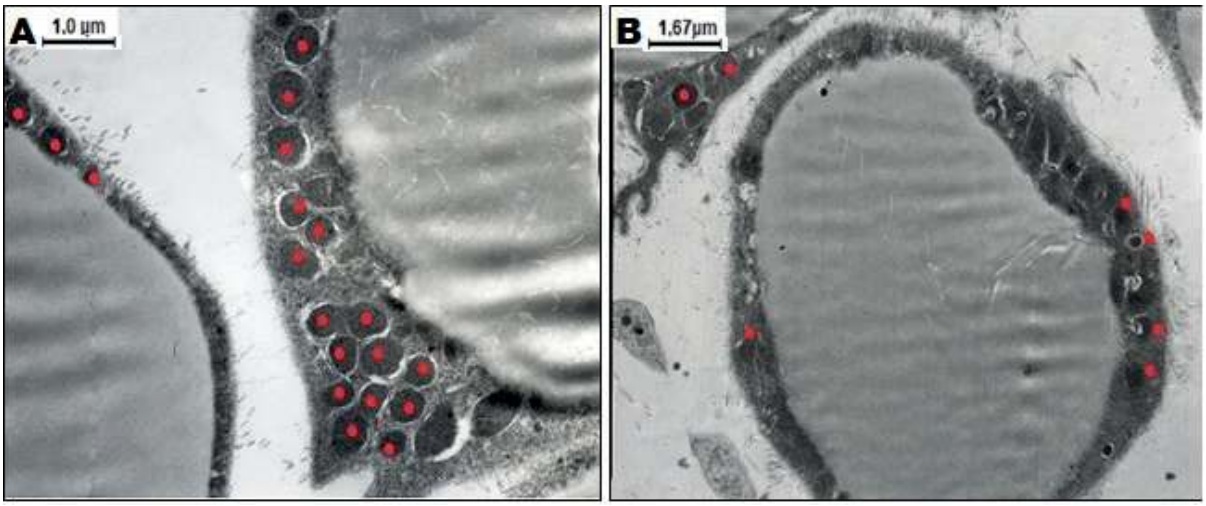
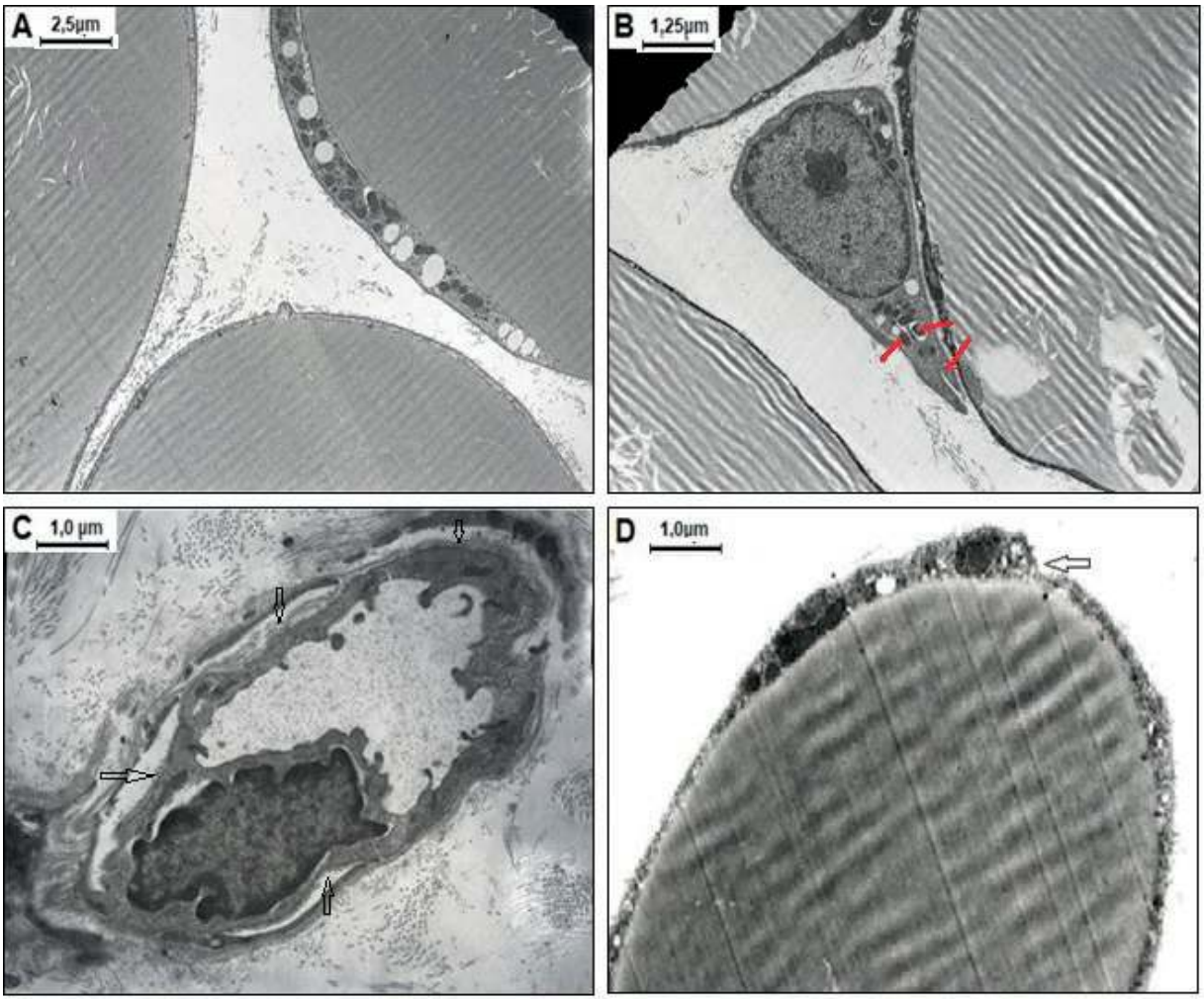


Fig. 3. Representative pictures of the effect of fructose diet and stress induction on ultrastructural examination of mesenteric material harvested from the area associated with the small intestine of aged rats with aspirin treatment (A) and exhibiting acute stress (B). A: Fragment of adipocyte with defective (ring-like) mitochondria (original magnification ×10000); B: Many smaller peripheral lipid droplets and lipid-laden phagolysosomes (arrows) are present in the marginal cytoplasm of adipocytes with defective mitochondria (original magnification ×6000). Red dots mark ring-like mitochondria



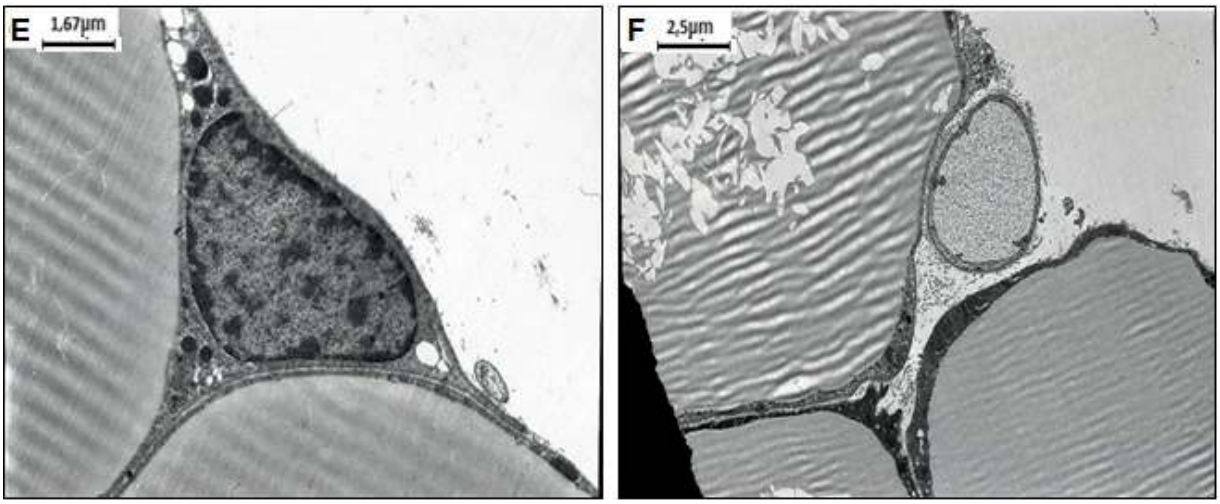


Fig. 4. Representative pictures of the effect of aspirin (A-C), the combination of aspirin and NaHS (D), and ATB-340 (G-H) on the ultrastructural changes of mesenteric material harvested from the area associated with the small intestine of aged rat on a high fructose diet and exposed to stress

A: The ultrastructure of fragments of three typical white adipocytes with evidence of fat fragmentation with the formation of several small fat drops and detective mitochondria in the cytoplasm (original magnification ×4000).
B: A fibroblast with vacuolation of the fibroblast cytoplasm and lipid droplets released from the degenerating adipocyte (original magnification ×8000).
C: The blood capillary belonged to adipocyte, the capillary basal membrane was uneven, fuzzy contoured, the endothelial cell had enlarged nucleus, the perivascular and inter-stromal edema is present (arrows), the collagen fibers in the perivascular space (original magnification ×10000).
D: A typical white monovacuolar adipocytes with lipid droplets at the periphery of the cell and a large adipose vacuole devoid of the membrane (arrow).
E: The well-preserved nucleus of white monovacuolar adipocytes with the internalization of free light electronic density fat droplets (original magnification ×6000).
F: The well-preserved capillary between three white monovacuolar adipocytes with single lipid droplets (original magnification ×4000)

In groups of aged rats fed with SD and treated by ASA, the TBARS content was 4.37±0.25 μM/L ($p < 0.01$). In HFD-fed rats treated by ASA, the levels of TBARS increased by 11.6% in aged groups (4.88±0.17 μM/L) in comparison to the results of SD-fed groups ($p < 0.05$). The effect of WIRS caused changes in the content of TBARS in rats treated ASA and fed HFD TBARS was significantly increased 56% (up to 7.64±0.26 μM/L). It was also found that administration of NaHS, ASA with NaHS, and ATB-340 in rats, exposed to stress significantly reduced TBARS. Quantitative measurement of effect of ATB-340 demonstrated decreased in 32% ($p < 0.05$) vs ASA+HFD fed group. It could be interpreted as an anti-oxidative effect of ATB-340.

To further understand the efficiency of ATB-340 in comparison to conventional aspirin or H_2S donor NaHS and their combination, in meta-flammation injury, the expressions of CBS, CSE TST and SO activities involved in endogenous

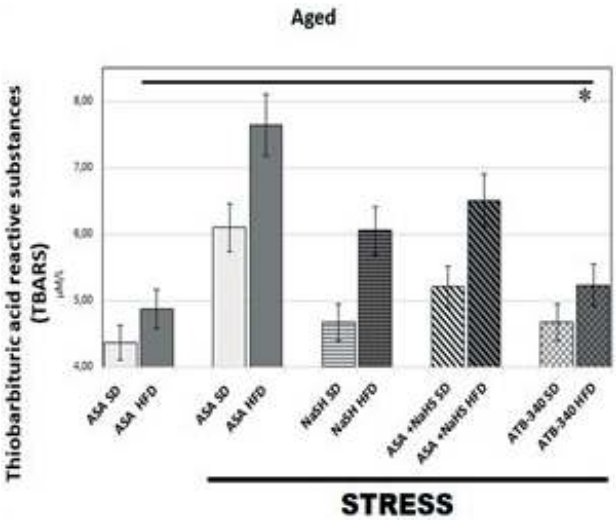


Fig. 5 TBARS levels in aged rats fed with a standard diet (SD) or high fructose diet (HFD) without and with H2S releasing therapy (NaHS, ASA and ATB-340) and induction of acute stress (n = 6); ($p < 0.05$) vs SD fed groups

H_2S signaling were investigated (Fig. 6 and 7). In aged rats on SD and ASA treatment, the activities of CBS, CSE, TST and SO were reaching 0.81±0.06 nmol/min×1 mg of protein, 1.1±0.07 nmol/min×1 mg of protein 1.0±0.04 nmol/min×1 mg of protein, and 3.61±0.1 nmol/min×1 mg of protein, respectively. In animals on ASA treatment and fed with HFD exposed to WRIS, enzyme activities were decreased (CBS, CSE, TST) and increase SO activity in rats compared to SD-fed rats. Aged rats on HFD had much lower activity of CBS — 53%, CSE — 55%, TST — 29%, and SO — 31% compared to SD group ($p < 0.05$). In contrast, the expression of CBS, CSE, and TST showed a tendency to decrease in the aged rats on HDF, they have lower enzyme activities of CBS, CSE, and TST versus aged rats on SD. Notably, the increased activities of all H_2S-related enzymes during induction WIRS were recorded, except SO, which decreased (Fig. 7 A, B). We found, that in rats exposed to stress on HFD, the efficiency of treatment by ATB-340 was similar to NaHS. These results indicate that ATB-340 has the potential for regulating redox dysbalance in rats during mesenteric injury induced by aging and HFD.

Discussion

In recent years, the importance of visceral white adipocyte tissue was revealed, with their functional roles depending on their location, cellular composition, humoral mediators (adipokines, cytokinins) [24]. The etiology of obesity is still not fully elucidated, but it involves a complex interaction between environmental, host genetic, metabolic and uncontrolled immune-derived factors that are responsible for metaflammation. Mesenteric white adipocyte remodeling has the potential to produce inflammatory mediators such as adipokines, cytokines TNF-α, IL-6, and IL-1β, hereby increasing tissue destruction and inducing inflammation even more in local tissues, but the whole understanding of the link of its remodeling and metaflammation is still incomplete [25, 26]. Studies *in vivo* rodent models based on high fructose diet have contributed significantly to our understanding of the molecular events involved in remodeling of white adipocytes and metaflammation and their outcomes [27]. We found that in aged rats fed with 28-day HFD of mesenteric adipocyte tissue remodeling demonstrated histological changes: degenerative adipocytes containing detective mitochondria, vessels with perivascular edema, and fibroblasts with incorporated fat drops. Recent advances in understanding the role of mitochondria in numerous physiological processes, including energy supply, excess of reactive oxygen species, oxidative stress, and H_2S signaling, indicate their important role in metaflammation [28, 29]. Decreased bioavailability of H_2S during less catalytic activities of CBS, CSE, and TST promotes changes in the endotheliocytes toward a vasoconstrictive and pro-in-

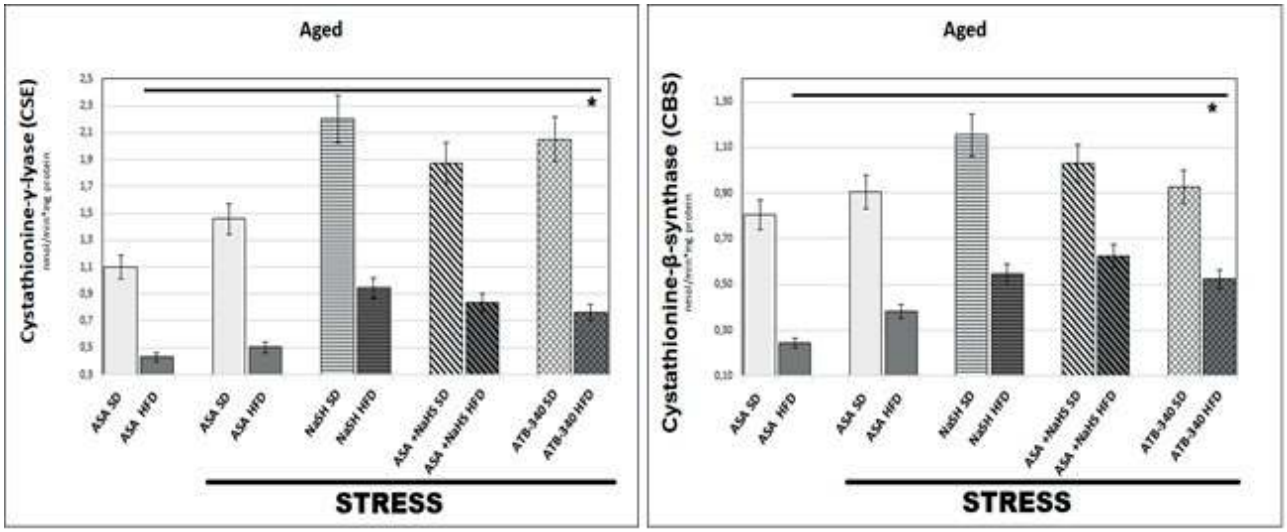


Fig. 6. Activities of Cystathionine-β-synthase (CBS), Cystathionine-γ-lyase (CSE) in aged rats (n = 6) fed with a standard diet (SD) or high fructose diet (HFD) without and with H_2S releasing molecule compound therapy (NaHS, ASA, and ATB-340) and induction of acute stress ($p < 0.05$) vs SD fed group

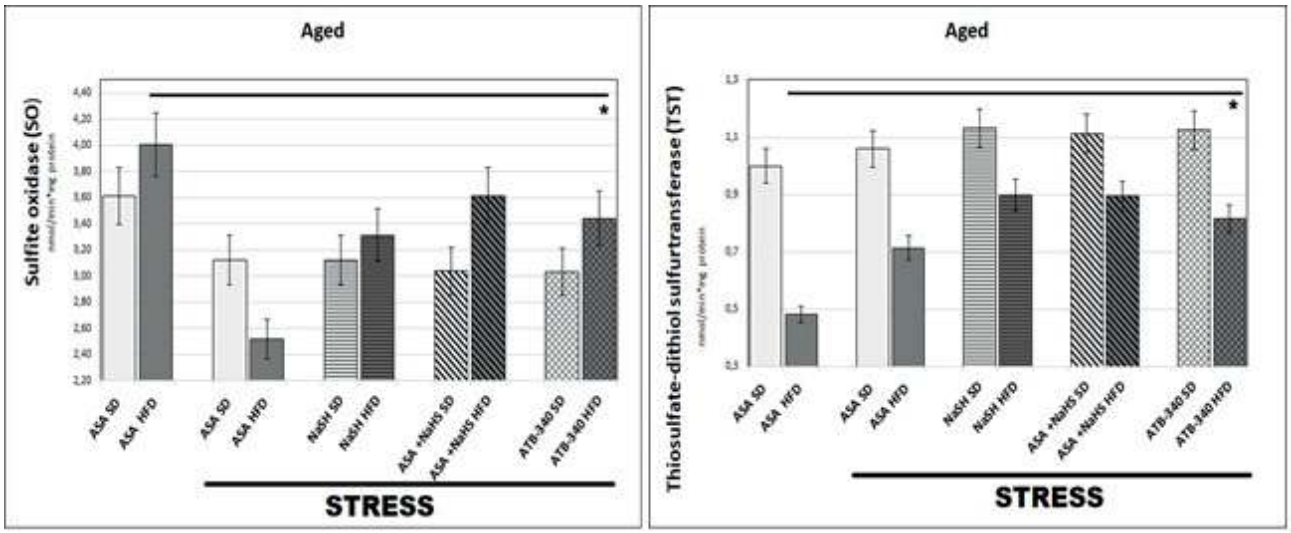


Fig. 7. Activities of Sulfide Oxidase (A, B) and Thiosulfate-dithiol sulfurtransferase (TST) (C, D) in adult (A, C) and aged rats (B, D) (n = 6) fed with a standard diet (SD) or high fructose diet (HFD) without and with H_2S releasing molecule compound therapy (NaHS, ASA and ATB-340) and induction of acute stress; ($p < 0.05$) vs SD fed groups

flammatory state. Several current clinical and experimental studies showed the prominent effects of H_2S-NSAIDs mostly based on potent anti-oxidant, anti-inflammatory, and regulatory redox system influences which are essential to maintain metabolic homeostasis and dysregulated visceral adipocytes [30, 31, 32]. These observations led us to speculate that novel H_2S-NSAIDs may induce cytoprotective and mitoprotective effects. Even though we found a significant difference in ultrastructural signs of MA mitochondrial injury in the experimental metaflammation model, during the administration of H_2S donors, the detective mitochondria in MA were lower. Our findings are in agreement with the latest studies in the pathogenesis of obesity, which have recently shown the impairment of endogenous H_2S signaling [33, 34]. Besides, our finding of novel H_2S-NSAIDs ATB-340 anti-oxidant effects related to decreased TBARS and modulation activities of H_2S enzymes will enhance understanding of their bioregulatory role on redox balance, underlying molecular mechanisms of metaflammation during aging and overload by high caloric fructose based diet [35], as well as will helpful for the discovery of novel therapeutic strategy able to ameliorate metaflammation. These findings were confirmed by transmission electron microscopic studies of efficiency ATB-340 on mesenteric adipocyte remodeling, as well as mitoprotective and cytoprotective effects on the mesenteric stromal-vascular cells and decreased metaflammation. To sum up, the present study is the first demonstration of the role of aging and overload of fructose as a driving force of mesenteric adipose tissue maladaptive responses which contribute to metaflammation and efficiency of H_2S-aspirin in its ameliorating. Mitochondrial alterations, endothelial and fibroblastic damage, and redox disbalance are key factors for aged rat mesenteric adipose tissue remodeling during HFD injury. H_2S-ASA, novel H_2S-NSAIDs have the potential to modulate mesenteric metaflammation by enhancement H_2S synthesis and redox regulatory and cytoprotective activities.

Future perspectives: These findings may contribute to a better understanding of the cellular/molecular mechanisms of mesenteric adipose remodeling pathogenesis, and provide new targets for the treatment of these disorders.

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