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Heat preconditioning and aspirin treatment attenuate hepatic carbohydrate-related disturbances in diabetic rats

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ABSTRACT

Heat preconditioning (HP) is a powerful adaptive and protective phenomenon and induces moderation of diabetic alterations in glycogen metabolism of rats. Aspirin (acetylsalicylic acid, ASA), as a multifunctional drug has also been reported to exert hypoglycemic effects in the treatment of diabetes. We estimated the effect of HP (45 min/41 ± 0.5 °C/24 h recovery) and single dose aspirin (100 mg/kg b.w./i.p) treatment over carbohydrate-related enzymes and substrates in a time-dependent (2, 7 and 14 days) manner of duration of diabetes in the liver of rats.

Heat preconditioning resulted in lower liver glucose concentration, but higher HK activity and lower G6P-ase; very evident and significantly higher glycogen content and GPho-ase activity, as well as very evident and significantly lower F1,6BP-ase and higher PFK activity compared to control diabetic animals.

Aspirin pretreatment of HP-diabetic animals is manifested with significantly lower blood and liver glucose, higher G6P concentration, lower G6P-ase and HK activity as well as higher Glk content and GPho-ase activity, compared both to diabetic and HP-diabetic animals.

In conclusion, both HP and aspirin, as physiological and pharmacological inductors of HSP70, respectively, attenuate the carbohydrate-related disturbances in diabetic rats, with almost tendency to normalisation to the control values for most of the estimated parameters.

1. Introduction

It is known that organisms/cells previously exposed to sublethal heat stress (HS) display greater resistance to the effects of higher intensity stress, including hypoxia, ischemia–reperfusion (Joyeux et al., 1999), or a strong cytotoxic agent, such as the diabetogenic agent streptozotocin (STZ) (Najemnikova et al., 2007; Panchnadikar and Bhonde, 2003), a phenomenon known as a heat preconditioning (HP). Heat therapy, via hot tub immersion, improves diabetic glycemic control and diabetic neuropathy in patients with type 2 diabetes (Hooper, 1999) and significantly improves lipid profile, antioxidant capacity, insulin secretion, and serum HSP70 level in diabetic rats (Bathaie et al., 2010).

Heat preconditioning (41 ± 0.5 °C/1 h, with 1 h and 24 h recovery at room temperature) provided moderation of diabetes-induced alterations in carbohydrate-related parameters in liver of rats (Miova et al., 2014). In our previous work Dinevska- Kjovkarovska. et al. (2007) and Miova (2010) we also have found existence of cross-tolerance effect between heat acclimation (30 days to 35 ± 1 °C, as a moderate physiological stress) and streptozotocin-induced diabetes (such as intensive pharmacological stress), observed by moderation of the carbohydrate-related alterations caused by experimental diabetes. It was also found that whole body hyperthermia (WBH) may provide a new therapeutic or preventive modality against obesity-related diseases such as type 2 diabetes mellitus and metabolic or insulin resistance syndrome (Kokura et al., 2007).

Aspirin (acetylsalicyl acid – ASA), an anti-inflammatory drug (non-specific cyclooxygenase (COX) inhibitor), is also known to exert different pharmacological effects. Between them, the earlier findings include its hypoglycemic effect in diabetic rats through decrease in the

Abbreviations: HS, Heat stress; HSPs, heat-shock proteins; HP, heat preconditioning; ASA, acetylsalicyl acid; STZ, streptozotocin; G6P-ase, glucose-6-phosphatase; G6P, glucose-6-phosphate; F1,6BP-ase, fructose-1,6-bisphosphatase; HK, hexokinase; GPho-ase, glycogen phosphorylase a

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intestinal absorption of glucose (Arvanitakis et al., 1977), as well as reduction of liver and muscle glycogen (Balasubramanian and Ramakrishnan, 1980; Smith et al., 1952) and decreased hepatic gluconeogenesis (Winters and Morrill, 1955). Later on, Hundal et al. (2002), has confirmed reduction in the hepatic gluconeogenesis, hepatic glucose production and the decrease in the insulin clearance in diabetic patients.

Finally, both heat preconditioning (HP) and ASA treatment exert their effects as a physiological and pharmacological heat shock proteins (HSPs) inductors, respectively, both in liver and in pancreas in healty and diabetic rats (Dimitrovska et al., 2017). In rodents, heat treatment (41–41.5 °C) and overexpression of HSP72 have been shown to prevent insulin resistance induced by a high-fat diet (Chung et al., 2008; Gupte et al., 2009). In addition to physiological induction by HP, a pharmacological methods of inducing HSP production includes non-steroidal anti-inflammatory drugs (NSAIDs), such as sodium salicylate, aspirin, and indomethacin, which have been reported to modulate heat shock response in different organisms (Fawcett et al., 1997; Winegarden et al., 1996) and improve thermal response during hyperthermia and other toxic conditions through increased induction of HSPs (Batulan et al., 2005). Moreover, in the previous data of Jafarnejad et al. (2008), high-dose (100 mg/kg in drinking water) and long-term ASA (5 mounts) therapy of type-1 diabetic rats causes a decrease in the glucose level, nonsignificant increase in the serum insulin level and induced serum HSP70.

Taking into consideration the above, we hypothesized that both heat preconditioning and aspirin treatment improve carbohydrate-related disturbances in diabetic rats. Thus, we aimed to estimate the effects of HP and ASA-pretreatment over hepatic carbohydrate related enzymes and substrates in a time-dependent manner (2, 7 and 14 days) of duration of experimental diabetes in rats.

2. Material and methods

2.1. Animals and tissue procedures

This experimental study was performed with adult (3–4 months old) male Wistar rats (n = 80) with a body weight of 250–300 g, which were housed under a 12-h light regime (6 a.m. -6 p.m. light) and fed laboratory chow and water ad libitum. All experimental animals were anaesthetized with Na-thiopental narcosis (45 mg/kg) and sacrificed using a standard laparothomic procedure, always at the same time of the day (9–10 a.m.).

The isolated pancreas and liver were washed with cold saline solution and immersed in liquid nitrogen. The tissues were kept at −80 °C until analysis and were converted into tissue powder before analysis (at liquid N_2–temperature). The tissue powder was homogenized with an ultrasonic homogenizer (Cole-Parmer Instrument - 4710) in several 7–10 s cycles. The whole procedure was performed at 0–4 °C (on ice).

All protocols were approved by the Animal Ethics Committee within the University “Ss Cyril and Methodius”, Skopje, R. Macedonia, in accordance with the International Guiding Principles for Biomedical Research Involving Animals, as issued by the Council for International Organizations of Medical Sciences. Anesthetics were applied according to the standards given by the guide of the EC Directive 86/609/EEC.

2.2. Study design and treatments

2.2.1. Experimental groups

The animals were divided into two general groups: healthy animals and diabetic ones. The first group was divided into three subgroups: control animals (C), heat-preconditioned control animals (HC) and heat-preconditioned control animals pretreated with ASA (AHC). The diabetic group was divided into nine subgroups: control diabetic groups (D2, D7 and D14 - sacrificed 2, 7 or 14 days after STZ-administration, respectively); heat-preconditioned diabetic groups (HD2, HD7 and HD14 - sacrificed 2, 7 or 14 days after STZ-administration, respectively) and heat-preconditioned diabetic groups, pretreated with ASA 1 h before induction of HS (AHD2, AHD7 and AHD14 - sacrificed 2, 7 or 14 days after STZ-administration, respectively).

2.2.2. Heat preconditioning (HP)

Heat preconditioning (HP) was carried out by placing animals in special temperature-controlled chambers (45 min at 41 ± 0.5 °C), followed by 24 h recovery at room temperature (20 ± 2 °C).

2.2.3. Experimental diabetes mellitus

Experimental diabetes was induced by a single intraperitoneal injection of streptozotocin (STZ, 55 mg/kg body weight) and was freshly dissolved in 0.1 M citrate buffer, pH = 4.5. All animals with clear diabetic symptoms (fasting glycemia levels higher than 15 mmol/L) 24–48 h after the induction of the experimental diabetes were included in the experiment. The duration of diabetes in our experiment was defined by the following criteria: 1. Minimum period for manifestation of the effect of administrated STZ (48 h) and optimal period for the development of diabetic complications (14 days); 2. Abundant HSP70 protein level in the first 24–48 h after the single HS, and further decrement 72 h after the HS [both in cells (Miova et al., 2015) and in rats (Horowitz, 2003). So, the purpose was to induce diabetes in a condition of high HSP70 level and in a condition of low HSP70 level. We choose 7 days as an intermediate period between 2 and 7 days, in order to have continuation of the experimental changes.

Aspirin (acetylsalicylic acid, A5376 Sigma) was freshly dissolved in water and administrated to the animals in a concentration of 100 mg/kg b.w. Subsequently, sodium carbonate was slowly added until the ASA crystals had dissolved (the pH of the solution remained just below 7.0 (Kelton et al., 1978) and was administrated intraperitoneally in a 0.5-mL volume (Fawcett et al., 1997; Locke and Atance, 2000), one hour before exposure to heat stress. Animals received single ASA treatment, 1 h before HS, both in control and diabetic animals.

2.3. Analytical methods

For determining enzyme activity, liver homogenates in appropriate medium of homogenization were prepared. For the interpretation of the activity as a specific enzyme activity in the tissue extracts by using bovine serum albumin as a standard, the total quantity of the proteins was determined by Lowry et al. (1951). Enzyme activity was expressed as nmol Pi/min/mg prot. The amount of released inorganic phosphate was determined by Fiske and Subbarow (1978).

Glucose-6-phospatase (E.C. 3.1.3.9) was assayed by the method of Hers (1959) and the substrate mixture contained G-6-P (100 mM) and EDTA (2 mM), pH 6,5.

Substrate mixture for **fructose-1,6-bisphospahtase** (E.C. 3.1.3.11) was prepared from 5 mM fructose-1,6- bisphosphate, 2,5 mM MgSO4, 5 mM MnSO4, 30 mM cysteine and 20 mM serine (Freedland and Harper, 1959).

The activity of liver **glycogen phosphorylase *a*** (E.C. 2.4.1.1) was determined by Stalmans et al. (1974). Substrate mixture contained 50 mM glucose-1- phosphate, 1% glycogen, 2,5 mM EDTA, 0,15 M NaF and 0.5 mM caffeine (pH = 6,0).

For determination of **hexokinase** (Bontemps et al., 1978) and PFK (Bergmeyer, Michal, 1974) we measured the production of NADH at 340 nm.

Liver glycogen, glucose and glucose-6-phosphate concentrations (Keppler and Decker, 1974) were determined in perchlorate homogenates, neutralized with 5 M K_2CO_3. We measured the production of NADPH at 340 nm in a reaction catalyzed by glucose-6-phoshate dehydrogenase. The substrate concentration was expressed as μmol/g tissue.

2.4. Statistics

Results are presented as means ± SD. To examine the statistical differences between each group, we used one-way ANOVA with Neuman–Keuls post hoc test analyses form the Statistica 7 and Statgraph 3 as statistical packets. In all tests, a probability level of $p < 0050$ was used as a significant difference.

3. Results

3.1. Rectal temperature (effects of HP, ASA and ASA + HP)

Our measurements showed a significant increase of rectal temperature of about 3.7 °C in HP-animals which returned to basal values after 24 h recovery at room temperature. Namely, in all experimental groups which were exposed to HS (HC, HD2, HD7 and HD14), we measured significant increase of rectal temperature from 37.0 ± 0.7 °C to 40.7 ± 0,7 °C just after the HS (HS +0'). Later on, 24 h after the HS, the measured RT was normalized to 36,9 ± 0,7 °C (HS +24 h).

It is important to note, that ASA by itself caused a rise of rectal temperature of about 1 °C one hour after the treatment (ASA + 1 h) and was significantly increase 36.9 ± 0,8°C to 37.7 ± 0,6°C). ASA in a combination to HS caused additional increase of RT of about 4.4 °C (increase to 41.4 ± 0.9°C, just after the HS (ASA + 1 h + HS + 0')). Finally, when animals had recovered for 24 h at room temperature (ASA + 1 h + HS + 24 h), their rectal temperature was returned to 37.1 ± 0.8°C (Table 1)

3.2. Body weight changes a function of heat stress, single ASA treatment and duration of diabetes

Body weight changes (g) in a function of heat stress and single ASA treatment was presented in Table 2. There was decrease of BW 1 h after the HS, regardless of ASA treatments, but in the following 24 h animals tended to normalize their BW.

Body weight changes (g) in a function of single ASA treatment, heat stress and duration of diabetes were presented in Table 3. Heat preconditioning of diabetic animals caused decrease of BW, almost like diabetic animals, but in HD14 these changes are lower than D14 group respectively. Finally, ASA pretreatment of HP-diabetic animals did not caused changes in the AHD2 and AHD7 groups, but only decreased BW measured in AHD14 groups. Nevertheless, these changes of 12.29% were lower than in the D14 groups (18.69%). In addition, we can say that ASA-treated diabetic animals did not manifest usual diabetic manifestation, overall look and behavior.

In addition, diabetic rats received ASA have a reduction of mortality rate in comparison to non-treated groups.

3.3. Blood glucose level

The obtained results showed that HS with or without ASA treatment did not cause significant changes in the blood glucose level (Fig. 1). As expected, experimental diabetes caused significant increase of blood glucose level. HP of diabetic animals did not resulted with significant changes compared to diabetic animals at room temperature. Finally, ASA pretreatment of HP-diabetic animals caused significant reduction of blood glucose level compared both to diabetic and HP-diabetic animals.

3.4. Hepatic glucose concentration and HK activity

Heat stress did not cause changes in the hepatic glucose concentration but caused significant decrease of HK activity (Fig. 2). ASA treated HS-animals have significantly higher HK activity compared to HS-animals, which is still significantly lower than control animals. This ASA treatment did not cause changes in liver glucose concentration.

Experimental diabetes caused significant increase of liver glucose concentration and significant decrease of HK activity.

HP of diabetic animals resulted in significant lower liver glucose concentration and higher HK activity compared to diabetic animals at room temperature. Moreover, there is a tendency of lowering the liver glucose concentration with a progression of duration of diabetes (7 and 14 days).

ASA pretreatment of HP-diabetic animals caused more evident reduction of blood glucose level and more evident increase of HK activity compared both to diabetic and HP-diabetic animals.

3.5. Hepatic glucose-6-phospahate concentration and glucose-6-phospahatase activity

Both G6P-ase activity and G6P concentration were significantly reduced by HS (Fig. 3). ASA pretreatment restored only the G6P-ase activity to the control values.

There was significant decrease of G6P concentration and significant increase of G6P-ase activity in diabetic animals, regardless of duration of diabetes.

HP of diabetic animals did not cause significant changes of G6P concentration, but caused significant reduction of G6P-ase activity compared to diabetic animals at room temperature. Moreover, there is a tendency of lowering the G6P-ase activity and increasing of G6P concentration with a progression of duration of diabetes (7 and 14 days).

HP-diabetic animals pretreated with ASA have significant higher G6P concentration compared to diabetic and HP-diabetic animals, most evident in AHD7 and AHD14 group. On the other side, there is progressive decrease of G6P-ase activity which is significant only at AHD14 group.

Table 1
Organization of the experimental groups according the treatments timing.

Group	ASA (single dose)	Recovery	Heat stress (HS)	Recovery	Duration of diabetes	Sacrifice
C						Sacrifice
HC			45 min/41 ± 0.5 °C	24 h/ 20 ± 2 °C		Sacrifice
AHC	ASA	1 h/20 ± 2 °C	45 min/41 ± 0.5 °C	24 h/ 20 ± 2 °C		Sacrifice
D2					STZ + 2 days	Sacrifice
HD2			45 min/41 ± 0.5 °C	24 h/ 20 ± 2 °C	STZ + 2 days	Sacrifice
AHD2	ASA	1 h/20 ± 2 °C	45 min/41 ± 0.5 °C	24 h/ 20 ± 2 °C	STZ + 2 days	Sacrifice
D7					STZ + 7 days	Sacrifice
HD7			45 min/41 ± 0.5 °C	24 h/ 20 ± 2 °C	STZ + 7 days	Sacrifice
AHD7	ASA	1 h/20 ± 2 °C	45 min/ 41 ± 0.5 °C	24 h/ 20 ± 2 °C	STZ+ 7 days	Sacrifice
D14					STZ + 14 days	Sacrifice
HD14			45 min/41 ± 0.5 °C	24 h/ 20 ± 2 °C	STZ + 14 days	Sacrifice
AHD14	ASA	1 h/ 20 ± 2 °C	45 min/41 ± 0.5 °C	24 h/ 20 ± 2 °C	STZ+ 14 days	Sacrifice

Table 2
Body weight changes (g) in a function of heat stress and single ASA treatment.

Groups	Average BW (g) before HS	Average BW (g) HS + 0'	Average BW (g) HS + 24h
HC	245.0	235.7	241.7
AHC	Average BW (g) before ASA	Average BW (g) ASA + 1h + HS + 0'	Average BW (g) ASA + 1h + HS + 24h
	274.0	265.9	269.1

Table 3
Body weight changes (g) in a function of single ASA treatment, heat stress and duration of diabetes.

	Average BW (g) before STZ (x_1)	Average BW (g) 2, 7 or 14 days after STZ (x_2)	Differences in % x_1-x_2
D_2	329.0	317.0	− 3.65
HD_2	268.3	239.8	− 10.62
AHD_2	235.6	237.1	0.64
D_7	374.8	352.4	− 5.98
HD_7	251.5	226.7	− 9.86
AHD_7	279.0	280.5	0.54
D_{14}	367.0	298.4	− 18.69
HD_{14}	219.0	203.0	− 7.31
AHD_{14}	290.5	254.8	− 12.29

3.6. Hepatic glycogen content and glycogen phosphorylase activity

Heat stress, with or without ASA pretreatment caused significant reduction of both hepatic glycogen content and GPho-ase activity (Fig. 4).

There was significant decrease of both hepatic glycogen content and GPho-ase activity in diabetic animals, regardless of duration of diabetes.

We observed that HP-diabetic animals have significant higher liver glycogen content and GPho-ase activity (especially HD7 and HD14 groups) and these increments were more evident by ASA pretreatment of HP-diabetic animals.

3.7. Hepatic fructose – 1,6- bisphospatase activity and PFK activity

Fructose – 1,6- bisphospatase activity was significantly decreased by HS, but ASA pretreatment did not cause any significant changes compared to control animals (Fig. 5) On the other side, HS caused significant increase of PFK activity, and ASA pretreatment caused more evident elevation.

Experimental diabetes resulted with significant increase of F1,6BP-ase and significant decrease of PFK activity.

Compared to diabetic controls, HP-diabetic animals have significantly lower F1,6BP-ase and higher PFK activity. ASA pretreatment of HP-diabetic animals did not cause any significant changes compared HP-diabetic animals.

3.8. Hepatic and pancreatic HSP70 protein level

We presented the changes in hepatic and pancreatic HSP70 protein level in Table 4. The obtained results showed that HP led to a significant 10-fold increase in **hepatic HSP70 protein level**. Pretreatment with ASA caused an additional increase in HSP70 level compared to HP-animals.

Our results showed that STZ-diabetes led to a significant reduction in hepatic HSP70, but HP of diabetic rats resulted in a significant increase in HSP70 level. Treatment with ASA led to an additional increase of HSP70 in both 2d- and 14d-diabetic animals. In diabetic animals, time-dependent changes were manifested only with respect to HSP70 level - significantly lower HSP70 in 14d-diabetic animals versus 2d-diabetic animals, regardless of whether or not there was aspirin treatment.

Concerning **pancreatic HSP70 protein level**, we observed that twenty-four hours after HS there was a significant increase in the level of pancreatic HSP70 level. Pretreatment of HP - control animals with ASA resulted in an additional increase of HSP70.

Our results show that there was a significant increase in pancreatic HSP70 level both 2d- and 14d after STZ administration. Heat preconditioning of diabetic animals led to a significant increase in HSP70 level in 2d-diabetic animals and a significant decrease in HSP70 level in 14d-diabetic animals. Moreover, pretreatment of HP- diabetic animals with ASA resulted in a further increase in HSP70 level (only in 2d-diabetic animals).

4. Discussion

4.1. Acute heat stress and 24 h recovery

Evidence from many species suggests that carbohydrate metabolism is altered during heat stress (Streffer, 1988). Our results showed that acute HS with a recovery period of 24 h at room temperature did not cause significant changes in blood and liver glucose, but resulted in significant decrease of G6P concentration. This was accompanied with a significant and intensive decrease of both HK and slight decrease of G6P-ase activity. There was also significant decrease of liver glycogen

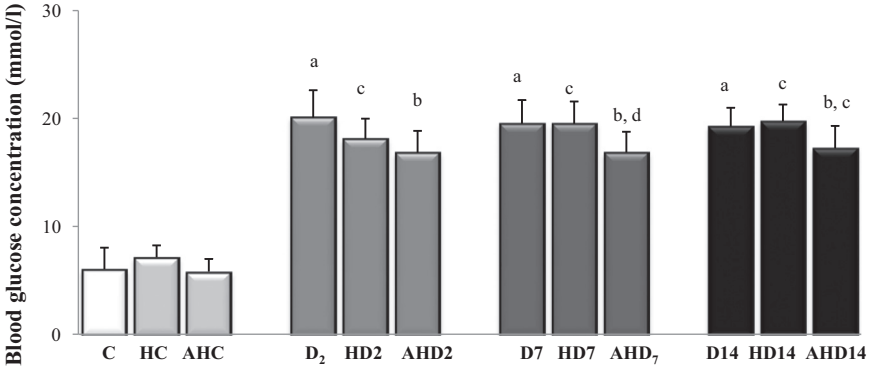

Fig. 1. Changes blood glucose level in heat-preconditioned intact and diabetic rats treated with aspirin. Legend: C- Control (intact) animals; HC - Control animals exposed to HS, allowed recovering 24 h at room temperature; AHC - Control animals treated with aspirin, 1 h before HS, allowed recovering 24 h at room temperature. D2, D7, D14 - Diabetic animals; HD2, HD7, HD14 - Diabetic animals exposed to HS, allowed to recover 24 h at room temperature before induction of diabetes; AHD2, AHD7, AHD14 - diabetic animals treated with aspirin, 1 h before HS, allowed to recover 24 h at room temperature before induction of diabetes. Significant difference ($p < 0.050$): a – relative to control animals (C: HC, C: D2, C: D7, C: D14); b - relative to diabetic animals (D2: HD2, D7: HD7, D14: HD14, respectively); c - relative to control animals exposed to HS (HC: AHC, HC: HD2, HC: HD7, HC: HD14). d - relative to diabetic animals exposed to HS (HD2: AHD2, HD7: AHD7, HD14: AHD14, respectively).

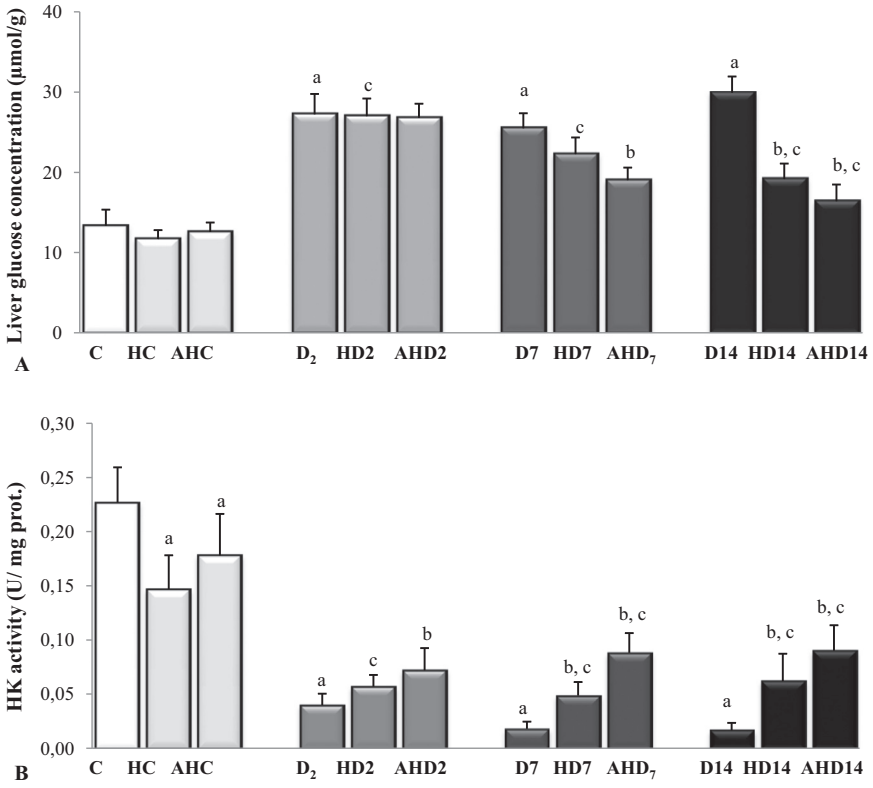

Fig. 2. Changes in hepatic glucose concentration (A) and HK activity (B) in heat-preconditioned intact and diabetic rats treated with aspirin. Legend as a Fig. 1.

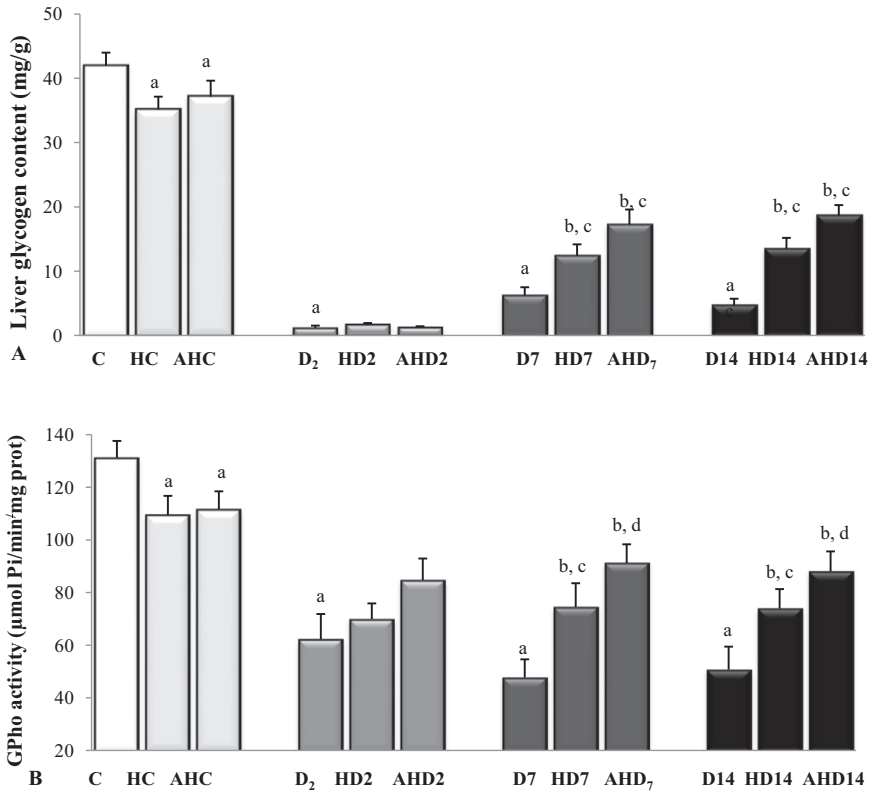

Fig. 3. Changes in hepatic glucose-6-phospahate concentration (A) and glucose-6-phospahatase activity (B) in heat-preconditioned intact and diabetic rats treated with aspirin. Legend as a Fig. 1.

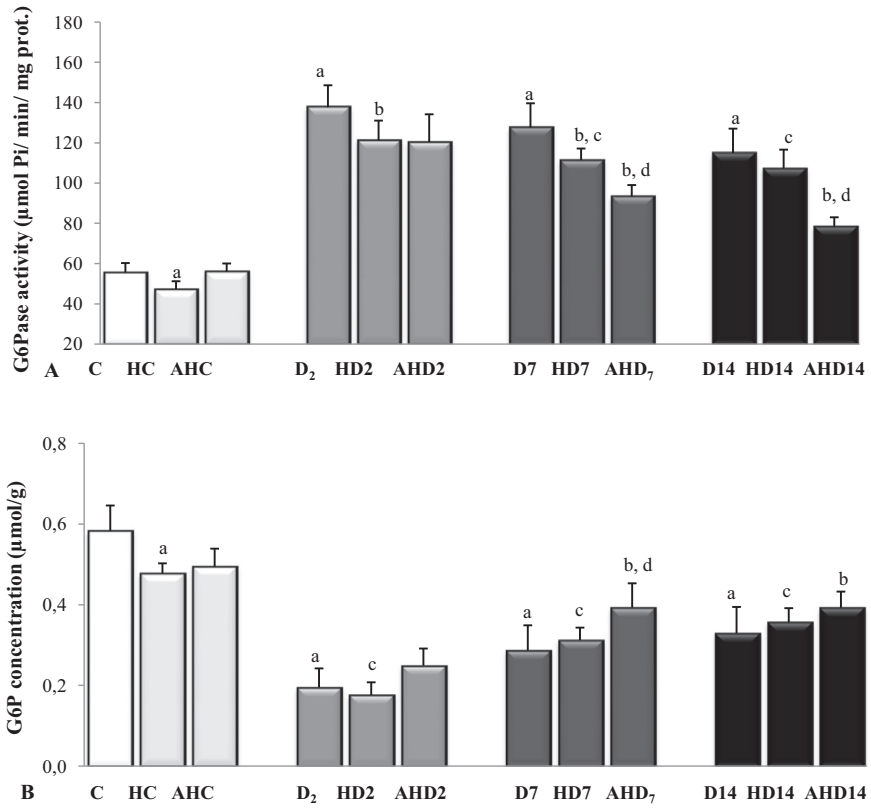

Fig. 4. Changes in hepatic glycogen content (A) and GPho-asectivity (B) in heat-preconditioned intact and diabetic rats treated with aspirin. Legend as a Fig. 1.

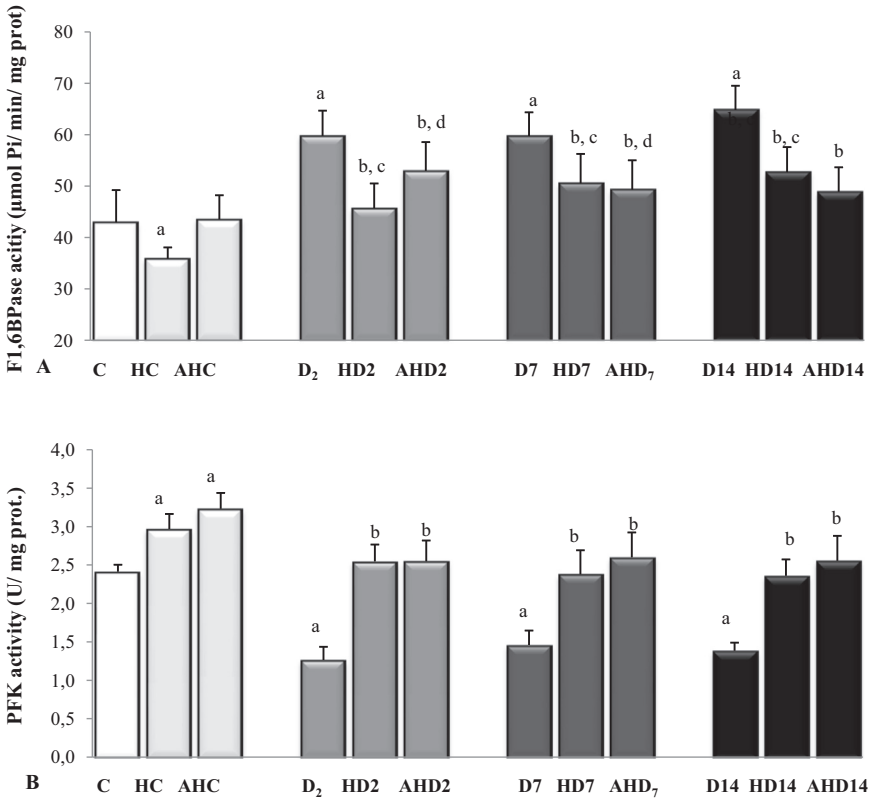

Fig. 5. Changes in hepatic fructose – 1,6- bisphospatase activity (A) and PFK activity (B) in heat-preconditioned intact and diabetic rats treated with aspirin. Legend as a Fig. 1.

Table 4
Hepatic and pancreatic HSP70 protein level (average ± SD) in a function of ASA treatment, heat stress and duration of diabetes.

	Hepatic HSP70 level (ng/mg prot)	Pancreatic HSP70 level (ng/mg prot)
C	38.9 ± 4.3	35.8 ± 4.2
HC	212.2 ± 22.1[a]	132.1 ± 5.9[a]
AHC	309.6 ± 25.9[a,c]	248.5 ± 16.9[a,c]
D2	16.7 ± 0.4[a]	98.62 ± 9.7[a]
HD2	88.9 ± 11.1[b,c]	156.7 ± 27.8[b]
AHD2	127,0 ± 28.0[b,c,d]	213.9 ± 17.8[b,c,d]
D14	17.3 ± 0.9[a]	82.8 ± 8.1[a]
HD14	26.1 ± 1.7[b,c]	52.7 ± 4.1[b,c]
[A]HD14	43.4 ± 6.5[b,c,d]	59.2 ± 14.7[b,c,]

Legend:
C- Control (intact) animals.
HC - Control animals exposed to HS, allowed recovering 24 h at room temperature.
AHC - Control animals treated with aspirin, 1 h before HS, allowed recovering 24 h at room temperature.
D2, D7, D14 - Diabetic animals.
HD2, HD7, HD14 - Diabetic animals exposed to HS, allowed to recover 24 h at room temperature before induction of diabetes.
AHD2, AHD7, AHD14 - diabetic animals treated with aspirin, 1 h before HS, allowed to recover 24 h at room temperature before induction of diabetes.
Significant difference ($p < 0.050$): .
[a] Relative to C animals (C: HC, C: D2, C: D7, C: D14).
[b] Relative to D animals (D2: HD2, D7: HD7, D14: HD14, respectively).
[c] Relative to HC animals (HC: AHC, HC: HD2, HC: HD7, HC: HD14).
[d] Relative to HD animals (HD2: AHD2, HD7: AHD7, HD14: AHD14, respectively).

content and decrease of GPho-ase activity. Finally, the glyconeogenic F16BP-ase was also decreased, but glycolytic PFK activity was significantly increased.

In other similar estimations (Miova et al., 2014) we estimated the effect of acute HS with duration of 1 h at 41 ± 0.5 °C, with a different period of recovery (1 h and 24 h) at room temperature, with more evident changes in carbohydrate metabolism, especially in animals that were recovered only 1 h after the HS. Previous investigations demonstrated that insulin concentrations gradually increase in lactating heat-stressed cows (Wheelock et al., 2010) and have confirmed this in growing heat-stressed calves (Baumgard and Rhoads, 2013; O'Brien et al., 2010) and pigs (Pearce et al., 2011). Also, we found a decreased glucose level and increase of the insulin concentration in subjects adapted to for 2 h at 40 °C (Hargreaves et al., 1996). We assume that depletion of glycogen stores occurs as a result of the increased need for energy substrates during HS exposure (Kameyama et al., 1981; Torlinska et al., 1984).

Furthermore, it is important to note that short term exposure to HS (45 min, 41 ± 0.5 °C) resulted in a significant increase of rectal temperature of approximately 3.7 °C, which returned to control values after 24 h recovery at room temperature. This elevated rectal temperature in the same intact animals (our previous results) led to a significant increase of HSP70 protein levels up to 3- and 10-fold in the pancreas and the liver, respectively (Table 4). Since the present results are a part of the same experiment, we assume that most of the obtained changes are based on the increament of the HSP70 level. Namely, a number of studies suggest that HSP70 has a protective functioned in the prevention of cellular damage in thermal injury (Horowitz, 2002) and that its synthesis is a crucial part of the whole physiological response to the stressor (Feder and Hofmann, 1999).

4.2. Experimental diabetes (2, 7 or 14 days after STZ administration)

As for experimental diabetes, our results showed that besides the well known hyperglycemia, there was also a reduction in hepatic glycogen and G6P concentration, decrease in glycogenolytic/ glycolytic enzymes (GPho-ase, HK and PFK) acitivity and an elevation in gluconeogenic enzyme activity (G6P-ase and F1,6BPase) in rats. Still, there were no significant changes regardless of duration of diabetes. Diabetes mellitus, as a complex and heterogenic metabolic syndrome, is characterized by extensive metabolic disturbances in different metabolic pathways, with special respect to carbohydrate metabolism (Delaney et al., 1993) and cell vulnerability (Tytell and Hooper, 2001), most obviously because of the down-regulated heat shock proteins (HSP) synthesis (Atalay et al., 1993; Hooper, 1993; Kurucz et al., 2002). Concerning carbohydrate metabolism, it is well known that diabetes mellitus impair the normal capacity of liver to synthesize glycogen (Vats et al., 2003; Whitton and Hems, 1975), caused suppression of hepatic glycolysis (decreased HK, glucokinase and PFK activity) (Pari and Amarnath-Satheesh, 2006; Vats et al., 2003), increase of the activity of gluconeogenic enzymes – G6P-ase and PEPCK (Li et al., 1999; Marzban et al., 2002; Mithieux et al., 1996,) and F1,6BP-ase (Mithieux, 1996; Whitton and Hems, 1975), which finally results in elevation of blood glucose level.

In addition to these results are the changes in HSP70 protein level in liver as well as in pancreas. Namely, our previous results showed that in the same animals there was a significant increase of HSP70 protein levels in the pancreas and a decrease in the liver (Table 4; Dimitrovska et al., 2017). Namely, in organs with high secretory capacity, such as the liver and pancreas, HSPs are vital for a normal physiology of the organism, and the vulnerability of diabetic animals is due heavily to disturbances of HSPs (Hooper, 2007).

4.3. Heat preconditioning of diabetic animals

Our results showed that compared to diabetic animals from room temperature, HP diabetic animals have lower liver glucose concentration, but higher HK activity (Fig. 2A, B) and lower G6P-ase (Fig. 3B); very evident and significantly higher liver glycogen content and GPho-ase activity (Fig. 4A, B), as well as very evident and significantly lower F16PB-ase and higher PFK activity (Fig. 5A, B).

We consider that this moderation of the diabetic disturbances in carbohydrate metabolism is based on the protective effect of heat preconditioning and increased synthesis of HSP70 both in liver and pancreas (Dimitrovska et al., 2017). The main mechanism of heat preconditioning is based on production of cellular HSPs, which have strong cytoprotective effects and speed up recovery from additional stress. The changes were especially evident in HP diabetic animals which were sacrificed 2 days after induction of STZ and less evident if the animals were sacrificed 14 days after STZ administration. Namely, we thought that these changes are in accordance to some previous findings that abundant HSP70 protein level in the first 24–48 h after the single HS, and further decrement after the 72 h after the HS (both in rats (Horowitz, 2003) and in cells (Miova et al., 2015). Namely, in HepG2 cells, abundant level of HSP70 and BCl-2 was observed 24–48 h after the single HS, which indicates that HS might be a "physiological conditioner" and might obtain cytoprotection against an additional stress (Miova et al., 2015). Taking in consideration the above changes in HSP70 protein level in the same animals, the moderation of the changes in carbohydrate metabolism are a logical consequence.

Besides short-term diabetes that we have in our experiment, there were also data with more severe diabetes. Namely, it was found that HSP70/HSC70 expression in the liver of warm-exposed diabetic rats (by achieving rectal temperature of 42 °C during 15 min) was higher than in non-exposed diabetic rats, where the experiments were made 1, 2 and 3 months after injection of STZ (Yamagishi et al., 2001). Also, hot tub therapy-treated diabetic rats (immersed up to midsternum in a bath of circulating water, 42 °C, 30 min, to obtain a core body temperature of 41 °C, three times a week for 5 months) showed a significant improvement in the lipid profile, antioxidant capacity, insulin secretion and serum HSP70 level and a significant decrease in advanced glycation

end-product (AGE) formation when compared to untreated diabetic rats, while only a borderline significant effect on weight and fasting blood glucose was observed (Bathaie et al., 2010).

Moreover, HP of diabetic rats resulted in a significant increase in total GSH concentration, as well as an increase in glutathione reductase and catalase activity compared to diabetic rats (Dimitrovska et al., 2017). According to Panchnadikar and Bhonde (2003), thermoresistent cells are generally resistant to environmental stress and death.

4.4. Aspirin (ASA) pretreatment of HP–diabetic animals

Previous aspirin treatment of HP-diabetic animals in our results is manifested with more intensive reductions of the diabetic disturbances in carbohydrate metabolism. Namely, ASA- treated HP diabetic animals have resulted in significantly lower blood glucose and especially liver glucose, compared both to diabetic and HP-diabetic animals, especially AHD7 and AHD14 groups. These changes were accompanied with significantly higher G6P concentration, significantly lower G6P-ase and HK activity. ASA-treated HP-diabetic animals have also significantly higher Glk content and GPho-ase activity, compared both to diabetic and HP-diabetic animals, especially AHD7 and AHD14 groups. Finally, gluconeogenic F1,6BP-ase and glycolitic PFK activity did not manifested significant changes after ASA-treatment, but they are still significantly lower and significantly higher compared to diabetic animals, with almost a tendency to normalize the control values. Some studies indicated that sodium salicylate has been used as a hypoglicaemic agent in treatment of diabetes (Zhanguo et al., 2003) and that the hypoglicemic effects of salicylates is mediated at least in part by enhanced insulin secretion, or changes in insulin sensitivity (Baron, 1982). According to Hundal (2002), high dose ASA treatment improved both fasting and postprandial hyperglycemia in patients with noninsulin-dependent diabetes mellitus (NIDDM), an effect that could be attributed to decreased basal rates of hepatic glucose production, enhanced peripheral insulin sensitivity and decreased insulin clearance.

It was found that diabetic hyperglycemia was reported to increase human b-cell interleukin-1b and that this cytokine can induce COX-2 expression (Persaud et al., 2004). It is known that interleukin-1b and prostaglandin E2 (PGE2) both inhibit glucose-induced insulin secretion from the pancreatic islet (Eizirik, . et al., 1991; Sjoholm, 1996). In this sense, according to Tran et al. (2002), the sites of action through which sodium salicylate inhibits these negative effects of IL-1b on b-cell function include activation of NF-kB as well as generation of prostaglandin E2 (PGE2) by COX-2. ASA inhibition of COX-2 in human islets could be one route through which NSAID can moderate diabetic hyperglycemia. Moreover, in the previous data of Jafarnejad et al. (2008), high-dose (100 mg/kg in drinking water) and long-term ASA (5 mounts) therapy of type-1 diabetic rats cause a decrease in the glucose level and AGE and HbA1c formation as well as a slight and insignificant increase in the serum insulin level. There was also improvement of the lipid profile, HDL functionality, and the antioxidant capacity, induced serum HSP70, and overall decreased mortality of diabetic rats in comparison with the group without treatment.

We also observed that aspirin administration given as a pre-treatment to acute HS enhanced the rise of rectal temperature by about 4.5 °C in ASA-treated HP–control rats. Our assumption is that this is a main trigger for additional production of HSP70 in both the liver and pancreas with respect to non-ASA-treated HP–control animals (Table 4; Dimitrovska et al., 2017). It is known that aspirin treatment causes a slight rise in body core temperature in rats, even in an absence of heat, but treatment with aspirin prior to HS markedly enhances heat-induced HSP70 levels (Fawcett et al., 1997). According to our results (Dimitrovska et al., 2017), increased HSP70 synthesis due to ASA pre-treatment was accompanied by a significantly higher PARP activity (about 32.6%) with respect to non-treated HP animals, but enzyme activity was still significantly lower than that of control animals. Furthermore, our previous results demonstrate that ASA pre-treatment reversed both GSH concentration and enzyme activity in the liver and pancreas of HP–animals to almost control levels (Dimitrovska et al., 2017). In this sence, we strongly recall suggestions by Jafarnejad et al. (2008) for usefullness of ASA in a prevention of in vivo and in vitro protein glycation, protein stability and folding patterns, as well as chemical chaperone-like activity in protecting the structure and funcition of the proteins involeved in glucose metabolism.

5. Conclusion

In conclusion, both HP and aspirin, as physiological and pharmacological inductors of HSP70, respectively, improve the carbohydrate disturbances in diabetic rats, with almost tendency to normalize the control values for most of the estimated parameters. Moreover, one can see the cumulative effects of ASA in a combination to HS and its beneficial effects on amelioration of diabetic metabolic changes through HSP70 induction patterns.

We confirm that all authors have approved the final version of the paper.

We confirm that there is no conflict of interest between authors.

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References

Arvanitakis, C., Chen, G.H., Folscroft, J., Greenberger, N.J., 1977. Effect of aspirin on intestinal absorption of glucose, sodium, and water in man. Gut 18, 187–190.
Atalay, M., Oksala, N.K., Laaksonen, D.E., Khanna, S., Nakao, C., Lappalainen, J., et al., 1993. Exercise training modulates heat shock protein response in diabetic rats. J. Appl. Physiol. 97, 605–611.
Balasubramanian, A., Ramakrishnan, S., 1980. Effect of aspirin and prostaglandins on the carbohydrate metabolism in albino rats: glucose oxidation through different pathways and glycolytic enzymes. Indian J. Med. Res. 71, 416–420.
Baron, S.H., 1982. Salicylates as hypogllycamic agents. Diabetes Care 5 (1), 64–71.
Bathaie, S.Z., Jafarnejad, A., Hosseinkhani, S., Nakhjavani, M., 2010. The effect of hot-tub therapy on serum Hsp70 level and its benefit on diabetic rats: a preliminary report. Int. J. Hyperth. 26, 577–585.
Batulan, Z., Nalbantoglu, J., Durham, H.D., 2005. Nonsteroidalanti-inflammatory drugs differentially affect the heat shock response in cultured spinal cord cells. Cell Stress Chaperon-. 10, 185–196.
Baumgard, L.H., Rhoads Jr., R.P., 2013. Effects of heat stress on postabsorptive metabolism and energetics. Annu. Rev. Anim. Biosci. 1, 311–337.
Bergmeyer, U., Michal, G., 1974. In: Bergmeyer, H.U. (Ed.), Methods of Enzymatic Analysis Vol. 1 Academic, New York.
Bontemps, F., Hue, L., Hers, H.G., 1978. Phosphorylation of glucose in isolated hepatocytes. Biochem. J. 774, 603–611.
Chung, J., Nguyen, A.K.K., Henstridge, D.C., Holmes, A.G., Chan, M.H.S., Mesa, J.L., et al., 2008. HSP72 protects agains to besity-induced insulin resistance. Proc. Natl. Acad. Sci. USA 105, 1739–1744.
Delaney, C.A., Dunger, A., Di Matteo, M., Cunningham, J.M., Green, M.H., Green, I.C., 1993. Comparison of inhibition of glucose-stimulated insulin secretion in rat islets of Langerhans by streptozotocin and methyl and ethyl nitrosoureas and methanesulphonates. Lack of correlation with nitric oxide-releasing or 6-alkylating ability. Biochem. Pharmacol. 50, 2015–2020.
Dimitrovska, M., Dervisevik, M., Cipanovska, N., Gerazova, K., Dinevska- Kjovkarovska, S., Miova, B., 2017. Physiological and pharmacological inductors of HSP70 enhance the antioxidative defense mechanisms of the liver and pancreas in diabetic rats. Can. J. Physiol. Pharmacol. 96, 158–164.
Dinevska- Kjovkarovska, S., Miova, B., Mitev, S., Popeska, Z., 2007. Some aspects of hepatic carbohydrate metabolism in heat-acclimated diabetic rats. Biol. Maced. 60, 37–51.
Eizirik, D.L., Bendtzen, K., Sandler, S., 1991. Short exposure of rat pancreatic islets to interleukin-1 beta induces a sustained but reversible impairment in beta-cell function: influence of protease activation, gene transcription, and protein synthesis. Endocrinology 128, 1611–1616.
Fawcett, T.W., Xu, Q., Holbrook, N.J., 1997. Potentiation of heat stress induced hsp70 expression in vivo by aspirin. Cell Stress Chaperon-. 2, 104–109.
Feder, M.E., Hofmann, G.E., 1999. Heat-shock proteins, molecular chaperones and the stress response: evolutionary and ecological physiology. Annu. Rev. Physiol. 61, 243–282.
Fiske, C.A., Subbarow, Y., 1978. The colorimetric determination of phosphorus. J. Biol. Chem. 66, 357–400.
Freedland, R.A., Harper, A.E., 1959. Metabolic adaptations in higher animals. V. The

study of metabolic pathways by means of metabolic adaptations. J. Biol. Chem. 243, 1350–1354.
Gupte, A.A., Bomhoff, G.L., Swerdlow, R.H., Geiger, P.C., 2009. Heat treatment improves glucose tolerance and prevents skeletal muscle insulin resistance in rats feda high-fat diet. Diabetes 58, 567–578.
Hargreaves, M., Angus, D., Howlett, K., Marmy Conus, N., Febbraio, M., 1996. Effect of heat stress on glucose kinetics during exercise. Appl. Physiol. 81, 1594–1597.
Hers, H.G., 1959. Glycogen storage disease. In: Levine, R., Luft, R. (Eds.), Advances in Metabolic Disorders. Academic press, New York, London, pp. 36–85.
Hooper, P.L., 2007. Insulin signaling, GSK-3, heat shock proteins and the natural history of type 2 diabetes mellitus: a hypothesis. Metab. Syndr. Relat. Disord. 5, 220–230.
Hooper, P.L., 1999. Hot-tub therapy for type 2 diabetes mellitus. N. Engl. J. Med. 341, 924–925.
Hooper, P.L., 1993. Diabetes, Nitric Oxide and Heat Shock Proteins. Diabetes Care 26, 951–952.
Horowitz, M., 2003. Matching the heart to heat-induced circulatory load: heat acclimatory responses. News Physiol. Sci. 8, 215–221.
Horowitz, M., 2002. From molecular and cellular to integrative heat defense during exposure to chronic heat. Comp. Biochem. Physiol. Part A: Mol. Integr. Physiol. 131 (3), 475–483.
Hundal, R.S., Petersen, K.F., Mayerson, A.B., Randhawa, P.S., Inzucchi, S., Shoelson, S.E., Shulman, G.I., 2002. Mechanism by which highdose aspirin improves glucose metabolism in type 2 diabetes. J. Clin. Investig. 109, 1321–1326.
Jafarnejad, A., Bathaie, S.Z., Nakhjavani, M., Hassan, M.Z., 2008. Investigation of the mechanisms involved in the high-dose and long-term acetyl salicylic acid therapy of type I diabetic rats. J. Pharmacol. Exp. Ther. 324 (2), 850–857.
Joyeux, M., Faure, P., Godin-Ribuot, D., Halimi, S., Patel, A., Yellon, D.M., et al., 1999. Heat stress fails to protect myocardium of streptozotocin-induced diabetic rats against in farction. Cardiovasc. Res. 43, 939–946.
Kameyama, T., Nabeshima, T., Nagata, H., 1981. Hypoglycemia in mice exposed to an environment of high temperature and humidity. Res. Comm. Chem. Pathol. Pharmacol. 32 (2), 261–279.
Kelton, J.G., Hirsh, J., Carter, C.J., Buchanan, M.R., 1978. Thrombogenic effect of high-dose aspirin in rabbits. Relationship to inhibition of vessel wall synthesis of prostaglandin I2-like activity. J. Clin. Investig. 62, 892–895.
Keppler, D., Decker, K.,1974. Glycogen determination with amyloglucosidase. In: Methods of Enzymatic Analysis. Academic Press, New York, vol. 3, pp. 1127–1131.
Kokura, S., Adachi, S., Manabe, E., Mizushima, K., Hattori, T., Okuda, T., et al., 2007. Whole body hyperthermia improves obesity-induced insulin resistance in diabetic mice. Int. J. Hyperth. 23, 259–265.
Kurucz, I., Morva, A., Vaag, A., Eriksson, K.F., Huang, X., Groop, L., et al., 2002. Decreased expression of heat shock protein 72 in skeletal muscle of patients with type 2 diabetes correlates with insulin resistance. Diabetes 51, 1102–1109.
Li, Y., Méchin, M.C., van de Werve, G., 1999. Diabetes affects similarly the catalytic subunit and putative G6P translocase of glucose 6-phosphatase. J. Biol. Chem. 274, 33866–33878.
Locke, M., Atance, J., 2000. The myocardial heat shock response following sodium salicylate treatment. Cell Stress Chaperon-. 5 (4), 359–368.
Lowry, O.H., Rosenbrough, J.N., Ffffarr, L.A., Rrandall, J.R., 1951. Protein measurement with the folin phenol reagent. J. Boil. Chem. 193, 265–275.
Marzban, L., Roshanak, R., Brownsey, R.W., M'cneill, J.H., 2002. Mechanisms by which Bis(Maltolato) Oxovanadium (IV) normalizes phosphoenolpyruvate carboxykinase and G6P-ase expression in streptozotocin- diabetic rats in vivo. Endocrinology 143, 4636–4645.
Miova, B., Dinevska, Kj, S., Esplugues, J.V., Apostolova, N., 2015. Heat stress induces extended plateau of Hsp70 accumulation — a possible cytoprotection mechanism in hepatic cells. J. Cell. Biochem. 116, 2365–2374.
Miova, B., Dinevska, Kj.S., Djimrevska, A., Mitev, S., 2014. Prior heat stress induces moderation of diabetic alterations in glycogen metabolism of rats. Cent. Eur. J. Biol. 9 (3), 249–259.
Miova, B., 2010. Acclimation to moderate heat and gene expression of the key carbohydraterelated enzymes in rats with nicotinamide/streptozotocine induced diabetes. Doctoral thesis, Faculty of natural sciences and mathematics. Skopje, R. Macedonia.
Mithieux, G., Vidal, H., Zitoun, C., Bruni, N., Daniele, N., Minassian, C., 1996. G6P-ase mRNA and activity are increased to the same extent in kidney and liver of diabetic rats. Diabetes 45, 891–896.
Najemnikova, E., Rodgers, C.D., Locke, M., 2007. Altered heat stress response following streptozotocin-induced diabetes. Cell Stress Chaperon-. 12, 342–352.
O'Brien, M.D., Rhoads, R.P., Sanders, S.R., Duff, G.C., Baumgard, L.H., 2010. Metabolic adaptations to heat stress in growing cattle. Domest. Anim. Endocrinol. 38, 86–94.
Panchnadikar, A., Bhonde, R., 2003. Can stress provide protection to pancreatic - cells and prevent diabetes? Med. Hypotheses 60 (3), 356–359.
Pari, L., Amarnath-Satheesh, M., 2006. Effect of pterostilbene on hepatic key enzymes of glucose metabolism in streptozotocin- and nicotinamideinduced diabetic rats. Life Sci. 79, 641–646.
Pearce, S.C., Upah, N.C., Harris, A.J., Gabler, N.K., Ross, J.W., Rhoads, R.P., et al., 2011. Effects of heat stress on energetic metabolism in growing pigs. FASEB J. 25, pp. 1052–1055.
Persaud, S.J., Burns, C.J., Belin, V.D., Jones, P.M., 2004. Glucose induced regulation of COX-2 expression in human islets of langerhans. Diabetes 53, 190–192.
Sjoholm, A., 1996. Prostaglandins inhibit pancreatic beta-cell replication and long-term insulin secretion by pertussis toxininsensitive mechanisms but do not mediate the actions of interleukin-1 beta. Biochim. Biophys. Acta 1313, 106–110.
Smith, M.J., Meade, B.W., Bornstein, J., 1952. The effect of salicylate on glycosuria, blood Glucose and liver glycogen of the alloxandiabetic rat. Biochem. J. 51 (1), 18–20.
Stalmans, W., Wuif, H., Hue, L., Hers, H.G., 1974. The sequential inactivation of GPhoasend activation of glycogen syntethase after the administration of glucose to mice and rats. The mechanism of the hepatic threshold to glucose. Eur. J. Biochem. 41, 117–134.
Streffer, C., 1988. Aspects of metabolic change after hyperthermia. Cancer Res. 107, 7–16.
Torlinska, T., Paluszak, J., Waliszevska, A., Banach, R., 1984. Effect of hyperthermia on blood levels of corticosterone and certain metabolites in rats during thiobutabarbital anaesthesia. Acta Physiol. Pol. 35 (3), 243–247.
Tran, P.O.T., Gleason, C.E., Robertson, R.P., 2002. Inhibition of Interleukin-1-induced COX-2 and EP3 gene expression by sodium salicylate enhances pancreatic islet-cell function. Diabetes 51, 1772–1778.
Tytell, M., Hooper, P.L., 2001. Heat shock proteins: new keys to the development of cytoprotective therapies. Expert Opin. Ther. Targets 5, 267–287.
Yamagishi, H, Nakayama, K, Wakatsuki, T, Hatayama, T., 2001. Characteristic changes of stress protein expression in streptozotocin-induced diabetic rats. Life Sci. 69, 2603–2609.
Vats, V., Yadav, S.P., Grover, J.K., 2003. Effect of T. foenumgraecum on glycogen content of tissues and the key enzymes of carbohydrate metabolism. J. Ethnopharmacol. 85, 237–242.
Wheelock, J.B., Rhoads, R.P., Van Baale, M.J., Sanders, S.R., Baumgard, L.H., 2010. Effects of heat stress on energetic metabolism in lactating Holstein cows. J. Dairy Sci. 93, 644–655.
Whitton, P.D., Hems, D.A., 1975. Glycogen synthesis in the perfused liver of streptozotocin-diabetic rats. Biochem. J. 150, 153–165.
Winegarden, N.A., Wong, K.S., Sopta, M., Westwood, J.T., 1996. Sodium salicylate decreases intracellular ATP, induces both heat shock factor binding and chromosomal puffing, but does not induce hsp 70 gene transcription in Drosophila. J. Biol. Chem. 271, 26971–26980.
Winters, R.W., Morrill, M.F., 1955. Carbohydrate metabolism in experimental salicylism. Proc. Soc. Exp. Biol. Med. 88, 409–411.
Zhanguo, G., Aamir, Z., Michael, J.Q., Zigang, D., Jailping, Y., 2003. Aspirin inhibits serine phosphorylation of insulin receptor substrate 1 in tumor necrosis factor-treated cells through targeting multiple serine kinases. J. Boil. Chem. 278, 24944–24950.