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The assessment of glucometabolic hormones in blood serum of aging rats fed with high-fat diet

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Introduction

The field of ageing research has been rapidly advancing in recent decades and it had provided insight into the complexity of ageing phenomenon [1,2]. The biological age of an individual is relevantly influenced by metabolic state of the body, which is, in turn, linked to a life style and nutritional habits [3]. Nutritional interventions been shown to provide health benefits, including the life prolonging effect, in a range of experimental animals [4]. The results of recent human studies show that e.g. mild caloric restrictions can have beneficial effects in humans [5,6]. Since the nutrition is an important and readily modifiable risk factor for disease prevention, many studies have consistently proven steady relationship between diet and health including in older adults [7-9]. The regulatory hormones of glucagon and glucagon-like peptide 1 (GLP-1), both play an important role in glucose homeostasis [10]. The plasminogen activator inhibitor type 1 (PAI-1) is the major determinant of fibrinolytic activity and its concentrations are elevated in obesity, type 2 diabetes and metabolic syndrome [11].

In order to investigate the potential alterations in glucose metabolism regulation within aging, the blood serum levels of glucometabolic hormones were assessed on offspring of Sprague Dawley (SD) rats. SD is an albino outbred rats strain widely used in biomedical research including toxicology and pharmacology for its common attributes providing satisfactory scale-up outcomes for pre-clinical predictions [12]. istarTo mimic the nutritional preferences of the western-style diet rich in high-fat, first we fed parental generation either with control (C) standard diet or high-fat diet (H, 1% cholesterol and 7.5% lard). Then, the type of diet continued within offspring generation: male SD rats were continously fed with either control standard (C-C) or high fat diet (H-H, 1% cholesterol and 7.5% lard) until adulthood, almost two years.

The main goal of our study was to assess and evaluate the levels of glucometabolic hormones glucagon, GLP-1 and PAI-1 in aging offspring SD rats in respect of the administered nutritional differences with either standard or high-fat diets.

Material and Methods

All experimental procedures involving animals were approved by the Ethical Committee of the Institute of Experimental Pharmacology and Toxicology, Animal Health and Animal Welfare Division of the State Veterinary and Food Diet Administration of the Slovak Republic (the number of the permit 3693/19-221/3) and they conformed to Directive 2010/63/EU on protection of

animals used for scientific purposes. SD rats were from the certified Breeding Animal Facility (Velaz, Czech Republic). The rats had free access to water and food and were kept on 12h/12h light/dark cycle and housed 5 animals per cage. Animals were divided into experimental groups (n = 10 rats/group). Parents animals (male and female) were fed with either control standard (C) or high-fat diet (H, 1% cholesterol and 7.5% lard) for 8 weeks prior mating, and then during pregnancy and lactation. Subsequently, the posterity continued in the type of parent´s diet so it was fed with either control standard (C-C) or high fat diet (H-H, 1% cholesterol and 7.5% lard) until adulthood, up to 21st month.

The blood was collected from *plexus chorioideus* at the indicated time points of offspring life span (1st, 15th and 21st month). The serum levels of glucagon, GLP-1 and PAI-1 were thoroughly assessed by Multiplex magnetic bead–based immunoassays on Bio-Plex 200 systems (Bio-Rad, U.S.).

The data were statistically evaluated using GraphPad Prism 6 Software (La Jolla, USA). Data were expressed as means ± SEM. One-way analysis of variance (ANOVA) was used to evaluate the difference among all experimental groups (using the Bonferroni multiple comparison test). The level of $p < 0.05$ was considered as statistically significant difference. When comparing C-C *vs* H-H rats at all of the selected time points, the asterisks were used to mark significancy as follows: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ and $^{****}p < 0.001$. For further comparisons the other types of symbols were used as listed here: $^{\#}$ H-H: 15th *vs* 1st resp. C-C: 15th *vs* 1st; √ H-H: 21st *vs* 1st resp. C-C: 21st *vs* 1st; ^ H-H: 21st *vs* 15th resp. C-C: 21st *vs* 15th.

Results and Discussion

The assessment of glucometabolic hormones levels in blood serum of aging offspring SD rats were evaluated at specified time points such resembled the life span of rodents as follows: 1st month represented for pups, then 15th month constituted for adults animals and finally 21st month established for old ones. The appraisal of the acquired data was done in respect of the administered nutritional differences while fed either control standard or high-fat diet. The corresponding values are listed in **Tab. 1**.

Tab. 1.: The levels of glucometabolic hormones within the offspring of aging SD rats.

	GLP-1 *(pg/ml)*	**Glucagon** *(pg/ml)*	**PAI-1** *(pg/ml)*
C-C-1st	235.5 ± 52.4	3780.9 ± 176.8	61.3 ± 4.9
H-H-1st	359.4 ± 21.5 *	7137.7 ± 149.9 ****	534.7 ± 57.9 ****
C-C-15th	247.3 ± 10.9	8975.1 ± 3493.6 $^{\#\#\#\#}$	258.4 ± 18.4 $^{\#\#\#\#}$
H-H-15th	952.3 ± 96.7**** $^{\#\#\#}$	25375.9 ±5291.7**** $^{\#\#\#\#}$	1449.9 ± 44.8**** $^{\#\#\#\#}$
C-C-21st	193.7 ± 5.1 $^{\wedge\wedge\wedge}$	14402.5 ± 701.9 $^{\surd\surd\surd\surd\ \wedge\wedge\wedge\wedge}$	921.1 ± 76.5 $^{\surd\surd\surd\surd\ \wedge\wedge\wedge\wedge}$
H-H-21st	1953.4 ± 231.8**** $^{\surd\surd\surd\surd\ \wedge\wedge\wedge}$	23782.7 ± 859.0**** $^{\surd\surd\surd\surd}$	3796.5 ± 247.2**** $^{\surd\surd\surd\surd\ \wedge\wedge\wedge\wedge}$

When comparing H-H *vs* C-C experimental goups, at all selected time points (1st, 15th and 21st months of age) there were remarkable shifts upwards within all of the measured parameters, namely glucagon, GLP-1 and PAI-1. Moreover, there was also obvious certain dynamics of glucose metabolims correlated with the age progression of W offspring rats: e.g. *i)* the level of GLP-1 in H-H group was progressively increasing, correlating with the duration of the experiment, while in C-C group stays stable; *ii)* similarly the level of PAI-1 in both C-C and H-H group were gradually increasing, correlating with the duration of the experiment; *iii)* the level of glucagon dramatically increased when comparing H-H:15th *vs* 1st month of the experiment, then stays rigid, however while considering C-C group correlated along with the proceeding age of the animals.

By monitoring nutrient-induced GLP-1 secretion over time during diet-induced obesity development in rats, Hira *et al.,* showed that GLP-1 secretion was enhanced in diet-induced obese rats compared with control rats [13]. Thus, the postprandial GLP-1 response is likely to play a protective role against glucose intolerance [14].

PAI-1 is closely related to the development of metabolic syndrome, glucose homeostasis impairment and lipid metabolism disbalances [15]. Recent studies showed that PAI-1 might contribute to the development of glucose intolerance, and have pro-atherogenic properties [16].

Our findings are in good agreement with studies supported the importance of the evaluation of glucometabolic hormones levels within pathophysiology of aging [17], especially when combined and potentiated with western-type diet rich in fat [18]. These data from long-term *in vivo* experiment on aging offspring of SD rats might possibly elucidate the glucose metabolism variations related to the nutritional preferences. Moreover, the further insights into mechanisms of senescense might be clarified with perspective towards the research of various civilization and metabolic diseases. Additional impact on clinical studies tendencies might be also considered.

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