SHR-*Zbtb16* minimal congenic strain reveals nutrigenetic interaction between *Zbtb16* and high-sucrose diet

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SUMMARY

Both prenatal and postnatal excessive consumption of dietary sucrose or fructose was shown to be detrimental to health and contributing to pathogenesis of metabolic syndrome. Our knowledge of genetic determinants of individual sensitivity to sucrose-driven metabolic effects is limited. In this study, we have tested the hypothesis that a variation of metabolic syndrome-related gene, *Zbtb16* (Zinc Finger and BTB Domain Containing 16 will affect the reaction to high-sucrose diet (HSD) content in "matched" nutritional exposition settings, i.e. maternal HSD with re-exposition to HSD in adulthood vs. standard diet. We compared metabolic profiles of adult males of spontaneously hypertensive rats (SHR) and a single-gene, minimal congenic strain SHR-*Zbtb16* fed either standard diet or exposed to HSD prenatally throughout gestation and nursing and again at the age of 6 months for the period of 14 days. HSD exposition led to increased adiposity in both strains and decrease of glucose tolerance and cholesterol (Ch) concentrations in majority of low-density lipoprotein (LDL) particle classes and in very large and large high-density lipoprotein (HDL) in SHR-*Zbtb16* male offspring. There was a similar pattern of HSD-induced increase of triacylglycerols in chylomicrons and very low-density lipoprotein (VLDL) of both strains, though the increase of (triacylglycerol) TAG content was clearly more pronounced in SHR. We observed significant STRAIN*DIET interactions for the smallest LDL particles as their TAG content decreased in SHR-*Zbtb16* and did not change in SHR in response to HSD. In summary, we provide evidence of nutrigenetic interaction between *Zbtb16* and HSD in context of pathogenesis of metabolic syndrome.

MAIN BODY OF THE TEXT

The quantitative and qualitative changes of nutrition in early development (Kunes *et al.* 2015, Nicholas and Ozanne 2019) as well as postnatally (Fabiani *et al.* 2019) were shown to be tightly connected with pathophysiology of metabolic syndrome (MetS), a clustering of dyslipidemia, hypertension, obesity and insulin resistance. Apart from the traditional environmental factors (diet, physical activity, smoking etc.), a growing body of evidence supports the role of fetal and early life environment in the determination of disease in adulthood, including MetS. Studies in human subjects and animal models have shown that the early environment modulates, or "programs" key hormonal systems and pathways involved in growth, metabolism, coping with stress or reproduction (Fleming *et al.* 2018). Although the prevalence of MetS and hypertension is highest in the "westernized" societies where maternal excess caloric intake rather than malnutrition is likely to be the prenatal environment, this modality received so far only limited attention. Also, despite the particularly detrimental effect of sucrose and fructose overconsumption in terms of MetS pathogenesis (Khan and Sievenpiper 2016, Dehghan *et al.* 2017), most experimental studies utilized high-fat, or mixed high-fat high-sucrose diet. It is generally perceived that adverse environment during development changes gene expression via epigenomic modulation (DNA methylation, histone acetylation, chromatin remodeling) and leads to physiological or morphological phenotypes associated with disease (Hoffman *et al.* 2017). On the other hand, we and others observed that there is a tighter interaction between epigenomic and genomic components in the pathogenetic process as some of the outcomes of epigenomic modulations depend on genomic constitution (Pausova *et al.* 2003, Buresova *et al.* 2006). In this study, we have tested the hypothesis that a variation of MetS-related gene, *Zbtb16* (Zinc Finger and BTB Domain Containing 16) (Seda *et al.* 2017b, Bendlova *et al.* 2017) will affect the reaction to HSD in "matched" nutritional exposition settings (Hanson and Gluckman 2014), i.e. maternal HSD with re-exposition to HSD in adulthood vs. standard diet.

All henceforth-described experiments were approved by the Ethical Committee of the First Faculty of Medicine of the Charles University and by the Ministry of Education, Youth and Sports (protocol no. MSMT-14076/2015-14). We used the spontaneously hypertensive rat (SHR/OlaIpcv, RGD ID 631848) and the SHR-*Lx*.PD5$^{PD-Zbtb16}$ single congenic strain (SHR-*Zbtb16* hereafter), which was derived from polydactylous rat (PD/Cub, RGD ID 728161) (Sedova *et al.* 2000, Seda *et al.* 2002) and carries the *Zbtb16* gene of PD origin on the SHR genomic background. As we showed previously, the two strains thus differ only in the variant *Zbtb16* gene (Zinc Finger and BTB Domain Containing 16) (Krupkova *et al.* 2018, Liska *et al.* 2014). Both strains are highly inbred and maintained by brother x sister mating at the Institute of Biology and Medical Genetics.

All animals were held under controlled conditions (temperature, humidity) with free access to food and water. Rat dams of SHR and SHR-*Zbtb16* strains (n=6/strain) were fed standard diet till breeding with corresponding (SHR x SHR, SHR-*Zbtb16* x SHR-*Zbtb16*) males fed standard diet. After mating, rat dams were placed in the cages individually and fed a corresponding diet – standard diet (STD, ssniff RZ, ssniff Spezialdiäten GmbH, Soest, Germany) for control group and HSD (proteins (19.6 cal%), fat (10.4 cal%), carbohydrates (sucrose, 70 cal%) for experimental group throughout pregnancy and lactation. The diets differed in the carbohydrate fraction only, with starch in STD vs. sucrose in HSD as a source of carbohydrates; otherwise they contained equal amounts of macro- and micronutrients. The litter size was restricted to 8 pups both in SHR and SHR-*Zbtb16* offspring which were weaned after 28 days and fed standard diet till the age of six months. At that time, offspring of the experimental groups of dams (n=6/strain) were re-exposed to HSD feeding for 14 days, the offspring of control dams were fed STD. At the end of experiment, all male offspring were subjected to blood draw for metabolic and lipid profile assessment after an 14-hour fast followed by oral glucose tolerance test (OGTT) - intragastric glucose administration to

conscious rats by oral gavage (3 g/kg body weight, 30% aqueous solution); Ascensia Elite Blood Glucose Meter; Bayer HealthCare, Mishawaka, IN, USA; validated by the Institute of Clinical Biochemistry and Laboratory Diagnostics of the First Faculty of Medicine). and sacrifice.. The lipid profile was assessed using high performance liquid chromatography (HPLC) for determining TAG and Ch concentrations in 20 lipoprotein fractions and the size of major classes of lipoprotein particles as described previously (Usui *et al.* 2002, Seda *et al.* 2017a). All statistical analyses were performed using STATISTICA 13.5 (TIBCO Software Inc.). When comparing morphometric and biochemical variables between groups, two-way ANOVA with STRAIN and DIET as major factors were used, followed by post-hoc Fisher's test for comparison of the specific pairs of variables. Null hypothesis was rejected whenever $p > 0.05$.

There was no difference in body weight among the male offspring groups. HSD resulted in an increase in visceral and retroperitoneal white adipose tissue mass as well as interscapular brown fat in both strains and in decrease of kidney weight exclusively in SHR (**Tab. 1**). The comparison of glucose tolerance revealed the effect of HSD, which was particularly prominent in SHR-*Zbtb16*, showing significant increase of area under the glycaemic curve (**Fig. 1A**) despite rise in fasting insulin (**Tab. 1**). Maternal HSD with re-exposition to the same diet in adulthood led to elevated glycemia between 30th and 90th minute after glucose bolus administration in SHR-*Zbtb16*, while in SHR we observed a distinct reaction pattern with no significant differences between the HSD-exposed and control groups. In this context, we identified a STRAIN*DIET interaction at 60th ($p = 0.00009$) and 90th ($p = 0.007$) minutes of OGTT: in control groups, SHR showed higher glucose concentration at 60th minute compared to SHR-*Zbtb16*, whereas opposite was true for the HSD-exposed groups (**Fig. 1A**). This strain-specific effect could be in part attributable to greater rise in fasting insulin in HSD-exposed SHR animals (78% compared to 44% increase in SHR-*Zbtb16*). We have previously described

particular sensitivity of SHR-*Zbtb16* to diabetogenic effect of dexamethasone (Krupkova *et al.* 2018). Since we have previously shown a lack of effect of maternal HSD on standard chow-fed adult male offspring of PD (Sedova *et al.* 2007) and SHR-*Zbtb16* rats and a modest one in SHR (Skolnikova *et al.* 2017), the observed differences in glycemia course in both SHR and SHR-*Zbtb16* experimental groups are most likely attributable to postnatal HSD feeding. While the mechanism connecting the variant *Zbtb16* gene comprising an intronic deletion and one conserved nonsynonymous substitution (Liska *et al.* 2014) to the specific nutri- and pharmacogenetic response is not clear, there is prior evidence indicating crucial role of *Zbtb16* in glucose homeostasis, particularly in regulating hepatic gluconeogenesis (Chen *et al.* 2014). We could speculate that a dysregulation of peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) /glucocorticoid receptor - Zbtb16 crosstalk may lead to increased or more sustained Zbtb16 effect triggering greater induction of hepatic gluconeogenesis and more effective impairment of insulin signaling (Chen *et al.* 2014). The activation hypothesis is further supported by our observation in heterozygous SHR-*Zbtb16* knockout rats showing improved glucose tolerance and increased insulin sensitivity of peripheral tissues (Liska *et al.* 2017).

Total Ch was higher in control SHR-*Zbtb16* compared to control SHR. Exposure to HSD led to total Ch decrease exclusively in SHR-*Zbtb16* and no change in SHR, resulting in comparable Ch concentrations in HSD-exposed groups (**Tab. 1**). Upon closer examination in 20 lipoprotein subclasses, the decrease of cholesterol concentrations in SHR-*Zbtb16* was most evident in majority of LDL particle classes and in very large and large HDL (**Fig. 1B**). The cholesterol content of medium to small LDL was decreased also in HSD-exposed SHR, however, the strain-specific (nutrigenetic) interaction was particularly apparent in very large and large HDL classes, containing most of the cholesterol (**Fig. 1B**). Total TAG were significantly higher in HSD-exposed SHR compared to the control group, the change of total TAG in SHR-*Zbtb16* did not reach the significance threshold. As presented in Figure 1C, the

pattern of reaction to HSD exposure was similar between the two strains for chylomicrons and VLDL, though the increase of TAG content was clearly more pronounced in SHR. We observed significant STRAIN*DIET interactions for the two classes of smallest LDL particles as their TAG content decreased in SHR-*Zbtb16* and did not change in SHR in response to HSD. Rise in TAG and no change or even decrease of cholesterol in response to sucrose-rich diet has been reported earlier (Cahova *et al.* 2007, Jameel *et al.* 2014). Heterozygous targeted SHR-*Zbtb16*^{+/-} male rats showed decreased concentrations of total serum and hepatic cholesterol and TAG (Liska *et al.* 2017).*ZBTB16* gene variation was also associated with total and LDL cholesterol levels in a cohort of Caucasian adults (Bendlova *et al.* 2017). The obtained results do not provide a direct mechanistic explanation connecting the variation in *Zbtb16* gene to the observed strain-specific metabolic effects of HSD. However, given the important role of Zbtb16 in adipose tissue metabolism (Plaisier *et al.* 2012, Wei *et al.* 2018), it is possible that distinct changes in substrate utilization in the two compared strains resulted in the observed shifts of lipid profiles.

The presented results reflect particular developmental stages and durations of exposition to HSD based on our previous results (Sedova *et al.* 2007, Skolnikova *et al.* 2017); different designs would be necessary to address the importance of specific "developmental windows" (Kunes and Zicha 2006). In summary, we provide evidence of nutrigenetic interaction between *Zbtb16* and high-sucrose diet in context of pathogenesis of metabolic syndrome in an experimental model.

Conflict of Interest

There is no conflict of interest.

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Table 1.

	SHR control	SHR-*Zbtb16* control	SHR prog. + HSD	SHR-*Zbtb16* prog. + HSD	P ANOVA (STRAIN)	P ANOVA (DIET)	P ANOVA (STRAIN * DIET)
Weight (g)	293 ± 7	303 ± 4	309 ± 6	298 ± 7	ns	ns	ns
Heart g/100g b.wt.	0.38 ± 0.005	0.39 ± 0.003	0.41 ± 0.019	0.40 ± 0.001	ns	ns	ns
Liver g/100g b.wt.	2.72 ± 0.03	2.77 ± 0.03	2.88 ± 0.04*	2.91 ± 0.04*	ns	0.000124	ns
Kidneys g/100g b.wt.	0.71 ± 0.009	0.66 ± 0.006^C	0.65 ± 0.009***	0.65 ± 0.003	0.006615	0.000138	0.009447
Adrenals g/100g b.wt.	0.014 ± 0.001	0.014 ± 0.001	0.013 ± 0.001	0.015± 0.001	ns	ns	ns
RPFP g/100g b.wt.	0.97 ± 0.07	0.74 ± 0.04	1.17 ± 0.04*	1.12 ± 0.11**	0.028680	0.000038	ns
EFP g/100g b.wt.	0.88 ± 0.03	0.76 ± 0.01^A	1.02 ± 0.03*	0.99 ± 0.04***	0.012476	6.60E-08	ns
BFP g/100g b.wt.	0.07 ± 0.004	0.05 ± 0.004^B	0.10 ± 0.004***	0.10 ± 0.002***	0.029883	5.30E-12	ns
fasting glucose (mmol/l)	2.9 ± 0.1	2.6 ± 0.1	2.7 ± 0.1	2.6 ± 0.1	ns	ns	ns
fasting insulin (nmol/l)	48.5 ± 3	52.9 ± 4	85.4 ± 7***	76 ± 6*	ns	0.000009	ns
fasting total cholesterol (mmol/l)	32.5 ± 2	39.1 ± 2**	31.1 ± 1	33.7 ± 2*	0.005575	0.031042	ns
fasting total triacylglycerols (mmol/l)	17.8 ± 2	24.6 ± 2	28.6 ± 2*	29.5 ± 3	ns	0.002092	ns

Table footnotes. HSD: high-sucrose diet, b.wt.: body weight, *prog*: programmed (exposed to maternal HSD feeding), RPFP: retroperitoneal fat pad, EFP: epididymal fat pad, BFP: interscapular brown fat pad, ns: not significant. The levels of significance of pair-wise comparisons (post-hoc Tukey's test) are indicated as follows: * p<0.05, ** p<0.01, *** p<0.001 for programming effect within a single strain; A p<0.05, B p<0.01, C p<0.001 for SHR vs. SHR-*Zbtb16* comparison within the same dietary regimen.
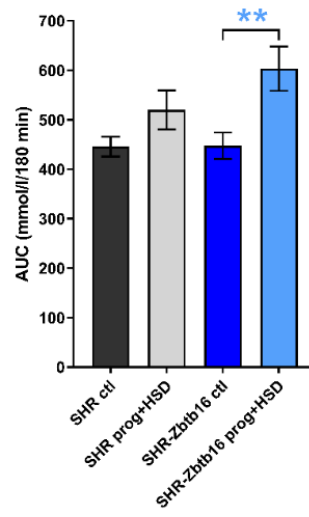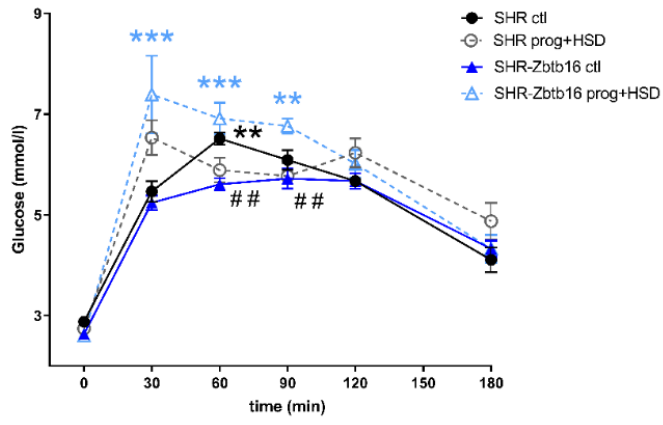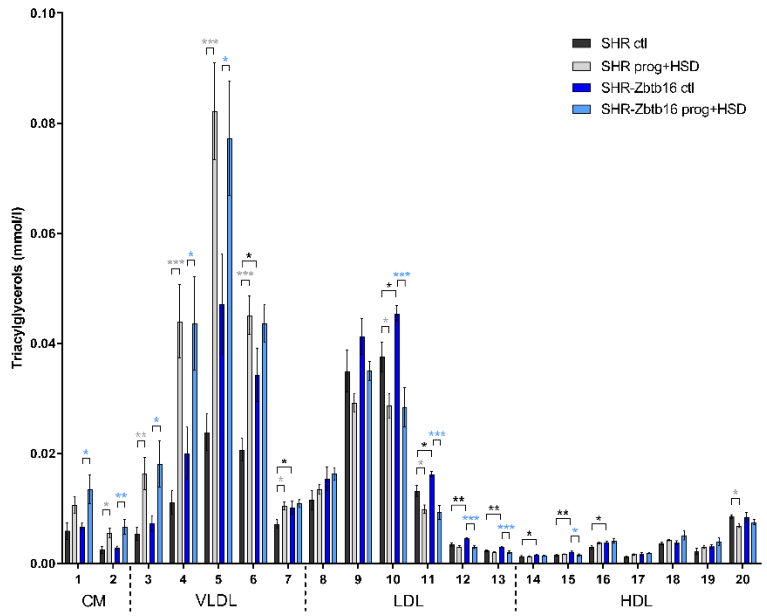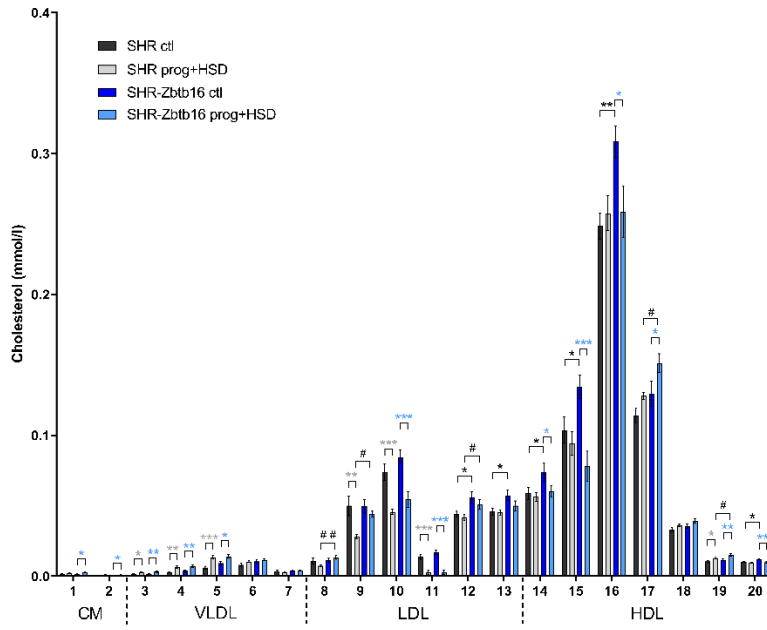
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Figure 1. Panel A. The oral glucose tolerance test . The course of glycaemic curves in SHR male offspring (control – black circles, programmed (prog) + HSD – gray empty circles), SHR-*Zbtb16* male offspring (control – dark blue triangles, prog + HSD – light blue empty triangles) during OGTT. Data are expressed as mean ± SEM. The significance levels for strain comparison using the post-hoc Fisher's least significant difference test of two-way ANOVA for STRAIN and DIET as major factors are indicated as follows: ** p<0.01, *** p<0.001. Effect of HSD exposure in SHR-*Zbtb16* strain shown by blue asterisks (*). **(A)** Black asterisk (*) represents STRAIN differences between SHR and SHR-*Zbtb16* control males, t=60 min **, which was higher in SHR control males. Black hashtag (#) represents STRAIN differences between SHR and SHR-*Zbtb16* males exposed to HSD, t=60 min **, t=90 min **, which were both higher in SHR-*Zbtb16*.

Panel B, C. Lipoprotein profile. The cholesterol and triacylglycerol content in 20 lipoprotein subfractions in adult SHR control male offspring (dark grey bars), SHR-*Zbtb16* control male offspring (dark blue bars), programmed SHR male offspring exposed to HSD (gray bars), programmed SHR-*Zbtb16* male offspring exposed to HSD (light blue bars). Data are expressed as mean ± SEM. The significance levels for strain comparison using the post-hoc Fisher's least significant difference test of the two-way ANOVA for STRAIN and DIET as major factors are indicated as follows: * p<0.05, ** p<0.01, *** p<0.001. Effect of HSD exposure in SHR strain is represented by gray asterisks (*), in SHR-*Zbtb16* strain by blue asterisks (*). **(B)** Black asterisk (*) represents STRAIN differences between SHR and SHR-*Zbtb16* control males, C12 *, C13 *, C14 *, C15 *, C16 **, C20 *, which were all higher in SHR-*Zbtb16* control males. Black hashtag (#) represents STRAIN differences between SHR and SHR-*Zbtb16* males exposed to HSD, C8 **, C9 *, C12 *, C17 *, C19 *, which were all higher in SHR-*Zbtb16*. **(C)** Black asterisk (*) represents STRAIN differences between SHR and SHR-*Zbtb16* control males, TG6 *, TG7 *, TG10 *, TG11 *, TG12 **, TG13 **, TG14 *, TG15 **, TG16 *, which were all higher in SHR-*Zbtb16*.

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