# Region of Rat Chromosome 8 Determines Complex Nutrigenetic Interactions under Conditions of Sucrose and Cholesterol Diets

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**Abstract:** We have previously established a congenic strain SHR-*Lx* that carries a differential segment of rat chromosome 8 introgressed from a model of metabolic syndrome – the polydactylous rat strain – on the genomic background of spontaneously hypertensive rat (SHR). We compared the glucose tolerance and lipid profile of adult SHR and SHR-*Lx* males under conditions of standard diet and diets enriched in sucrose and cholesterol, respectively. While there was no evident difference between the SHR and SHR-*Lx* on standard diet, the one-week sucrose administration revealed the congenic strain sensitivity to carbohydrate-induced dyslipidemia conferred by the differential segment with only mild derangement of glucose tolerance. On the other hand, the high-cholesterol diet administration for three-weeks resulted in a contrasting pattern as the congenic strain displayed significantly lower concentrations of free fatty acids and improved glucose tolerance compared to SHR. After one-month washout period, the SHR-*Lx* showed higher insulin, triglyceride and cholesterol concentrations together with diminished insulin sensitivity of visceral adipose tissue. In summary, we have identified a genomic region syntenic to human chromosome 11q23, which determines complex nutrigenetic interactions under conditions of sucrose- and cholesterol-enriched diets.

## Introduction

Recently we are witnessing a steep rise in incidence and prevalence of complex metabolic diseases like atherosclerosis, type 2 diabetes, hypertension or obesity. Their most common forms share the existence of almost equally strong genetic and environmental components participating and interacting in their pathogenesis. Dietary habits belong among the most influential environmental factors, yet still acting partly interdependently through the genomic setup of an individual. The genetic architecture of human multifactorial traits is currently often envisaged as complex set of modular networks. Those in turn are thought to be composed of wide variety of interacting genomic features, including protein-coding genes, regulatory RNAs, short- and long-range acting modulators of expression etc. This complicated structure is far from being static. So, changes in the "environment" elicit adaptive and predictive responses of the organism in the form of rearrangement and switching between available modules (genes, transcription factors, metabolic pathways) resulting in a state of the *dynamic* genetic architecture [1] of a given trait. It follows that the ongoing search for the genomic components of prevalent multifactorial diseases will face another hurdle. Some of the associations and linkage signals for alleles influencing the above mentioned traits may rather represent a reflection of the allele's involvement in the particular genotype-environment setting. These issues are currently starting to receive more attention also in the study design [2]. Here, the genetically defined models will probably become crucial tools in the process of deciphering of the importance of particular interactions for pathophysiology and, eventually, the therapeutical modulation of complex diseases within the paradigm of personalized medicine.

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The genes present in the region of human chromosome 11q23, syntenic to rat chromosome 8q22, were shown in several studies involving human subjects [2–4] and rat and mouse models [5, 6] to carry alleles influencing all of the metabolic syndrome components including their outcomes [7]. We have previously established a congenic strain SHR-*Lx* [8], in which the mentioned region was introgressed onto the spontaneously hypertensive rat (SHR) genetic background from the polydactylous rat (PD/Cub) [9], an inbred model of metabolic syndrome [1, 10]. Compared to the SHR progenitor, the SHR-*Lx* congenic strain displays significantly lower blood pressure and heart weight [8]. When the segment of PD/Cub origin is introgressed into the Brown Norway (BN/Cub) background, it affects parameters of metabolic profile on standard and high sucrose diets, evidenced by the deranged metabolic profile of the BN-*Lx* congenic strain [11]. As the ultimate effect of genomic component within the nutrigenetic interaction results only from the complex network of interactions among genes present in the differential segment and the allelic setup of the genetic background, we subjected the SHR-*Lx* strain to series of nutritional challenges in order to investigate potential role of the introgressed segment within the nutrigenetic interactions.

**Materials and Methods**
*Rat strains*
The spontaneously hypertensive rat (SHR/OlaIpcv) was derived by recurrent selective breeding of Wistar rats by Japanese authors Okamoto and Aoki in Kyoto, Japan [12]. The SHR colony in Prague was originally obtained from the National Institutes of Health of USA >25 years ago and since then it has been maintained by brother × sister mating at the Academy of Sciences of the Czech Republic as well as at Institute of Biology and Medical Genetics of the First Faculty of Medicine, Charles University in Prague.

SHR.PD(*D8Mgh9-D8Rat149*)/Cub (SHR-*Lx* hereafter) congenic strain was derived by introgressing RNO8 differential segment of the PD/Cub origin onto SHR genetic background [8, 13]. The original SHR-*Lx* derivation was previously described [8]. In short, one of the recombinant inbred strains (BXH11) that inherited a large segment of chromosome 8 including the *Lx* mutation from the BN-*Lx* strain was used to introgress this region of chromosome 8 onto the SHR background by backcross breeding. After the equivalent of 12 generations of selective backcrossing to the SHR progenitor strain, the differential chromosome segment in the vicinity of *Lx* was fixed and maintained in the homozygous state by brother × sister mating and selective inbreeding of the polydactylous offspring. Eventually, the total genome scan confirmed the congenicity of the SHR-*Lx* [8].

*Experimental protocol*
All experiments were performed in agreement with the Animal Protection Law of the Czech Republic (311/1997) which is in compliance with the European

Community Council recommendations for the use of laboratory animals 86/609/ECC and were approved by the ethical committee of the First Faculty of Medicine. Male SHR (n = 6) and SHR-*Lx* (n = 5) rats were fed standard laboratory chow *ad libitum*. At the age of 4 months, blood samples were drawn and the oral glucose tolerance test (OGTT) was performed at baseline (standard diet), after one week of high-sucrose diet (70% calories as sucrose) and after another three weeks of high-cholesterol diet (2% cholesterol) administration. Then the rats were returned to the original, standard diet for four weeks. In the end of experiment, the rats were sacrificed and the weights of liver, kidneys and epididymal fat pads were determined. The diaphragm and adipose tissues were used for *in vitro* assessment of insulin sensitivity.

*Metabolic measurements*
The oral glucose tolerance test (OGTT) was performed after overnight fasting. Blood for glycemia determination was drawn from the tail at intervals of 0, 30, 60 and 120 minutes after the intragastric glucose administration to conscious rats (3g/kg total body weight, 30% aqueous solution). The serum concentrations of triglycerides (TG), cholesterol (CH), free fatty acids (FFA), insulin and glucose were determined as described previously [10]. In short, commercially available analytical kits were employed to determine plasma glucose and serum triglyceride concentrations (Lachema, Brno, Czech Republic). Serum FFAs were determined using an acyl-CoA oxidase-based colorimetric kit (Roche Diagnostics GmbH, Mannheim, Germany). Serum insulin concentration was determined using an RIA kit for rat insulin assay (Amersham Pharmacia Biotech, Little Chalfont, UK).

 *Insulin-Stimulated Glycogen Synthesis.* Basal and insulin- stimulated glucose incorporation into glycogen (conversion of [$^{14}$C]glucose to [$^{14}$C]glycogen) was determined in diaphragm and epididymal adipose tissue as described previously [14, 15].

**Table 1 – Nutrigenetic interactions of standard, high-sucrose and high-cholesterol diets in SHR vs. SHR-*Lx* rats**

| Phenotype | STRAIN | DIET | S*D |
|---|---|---|---|
| Triglycerides (f) | 0.018 | <0.0001 | 0.0040 |
| Triglycerides (nf) | 0.006 | <0.0001 | *0.1200* |
| Free fatty acids (f) | *0.870* | *0.1900* | 0.0030 |
| Free fatty acids (nf) | *0.360* | 0.0010 | *0.2700* |
| Glucose (f) | 0.001 | *0.0500* | <0.0001 |
| Glucose – 30 min OGTT | *0.070* | 0.0110 | *0.4600* |
| Glucose – 60 min OGTT | *0.200* | 0.0040 | *0.8800* |
| Glucose – 120 min OGTT | *0.650* | <0.0010 | *0.4300* |
| AUC$_{120min}$ – OGTT | *0.460* | 0.0030 | 0.0130 |

The significance levels of two-way ANOVA's STRAIN, DIET and STRAIN*DIET (S*D) factor interactions are shown (significant p values in bold, non-significant in italics). OGTT – oral glucose tolerance test, AUC – area under the glycaemic curve.

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*Insulin-stimulated Lipogenesis.* Basal and insulin-stimulated incorporation of $^{14}$C-U glucose into total lipids of rat adipose tissue and diaphragm *in vitro* (lipogenesis) was determined. In short, after decapitation, distal parts of the epididymal adipose tissue (200 mg) were incubated in Krebs-Ringer bicarbonate buffer under conditions described above. Total adipose tissue lipids were extracted according to [16] and the radioactivity was determined as described previously [15].

*Statistical analysis*
Two-way ANOVA with STRAIN and DIET as major factors was used with the post-hoc LSD test for comparison of the specific pairs of variables. Null hypothesis was rejected whenever $p < 0.05$ (Table 1).

**Results**
When fed standard diet, the adult male rats of both SHR and SHR-*Lx* strains displayed comparable levels of both fasting and postprandial triglyceridemia, free fatty acids (FFA) and glucose tolerance (Figure 1), even though the congenic strain tended towards higher fasting plasma glucose concentrations (5.0±0.1 mmol/l vs. 4.4±0.1 mmol/l in SHR-*Lx* and SHR, respectively; $p = 0.05$). The acute, one-week administration of high-sucrose diet (HSD) resulted in disproportionate increase in the index of glucose tolerance, the area under the glycaemic curve (AUC). In SHR-*Lx* the AUC rose by 66% in comparison to only 33% rise in the SHR (Figure 1). Nevertheless, the difference in the global glucose tolerance was not significant ($p = 0.11$). Along similar pattern, both fasted (1.4±0.1 mmol/l vs. 1.1±0.1 mmol/l in SHR-*Lx* and SHR, respectively; $p < 0.001$) and postprandial (Figure 2) triglyceridemia rose to much larger extent in the congenic strain, reaching significantly higher values compared to those found in SHR. While we observed no difference in postprandial levels of free fatty acids of sucrose-fed rats (0.48±0.06 mmol/l vs. 0.48±0.03 mmol/l in SHR-*Lx* and SHR, respectively; $p = 0.93$), the mobilization of FFA by the overnight fasting was more pronounced in SHR-*Lx*, reaching thus significantly higher concentrations.

The subsequent three-week administration of high-cholesterol diet revealed a distinct pattern: first, the glucose tolerance was significantly more impaired in SHR
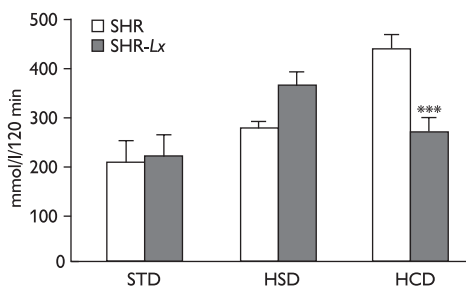


Figure 1 – Glucose tolerance in response to dietary challenges in SHR vs. SHR-Lx rats.

compared to the SHR-*Lx* congenic strain (Figure 1) with e.g. the fasting glycaemia being significantly higher (4.0±0.1 mmol/l vs. 5.9±0.7 mmol/l in SHR-*Lx* and SHR, respectively; p < 0.001). Second, the fasting free fatty acid concentrations were significantly higher in SHR (Figure 2). On the other hand, both fasting and postprandial triglyceride levels were higher in the SHR-*Lx* already after two weeks of high-cholesterol diet administration (1.37±0.02 mmol/l vs. 0.92±0.03 mmol/l in SHR-*Lx* PD5 and SHR, respectively; p < 0.0001) as well as after an additional week (Figure 2). The postprandial cholesterol concentrations were not significantly different between the two strains both after two and three weeks of HCD administration (2.91±0.25 mmol/l vs. 2.72±0.15 mmol/l in SHR-*Lx* and SHR, respectively; p = 0.50). The fasting cholesterol concentration was significantly higher in SHR-*Lx* compared to SHR (1.68±0.03 mmol/l vs. 1.48±0.06 mmol/l in SHR-*Lx* and SHR, respectively; p = 0.03).

After one-month period of "washout", the postprandial concentrations of triglycerides, cholesterol and insulin were found to be significantly higher in SHR-*Lx* compared to SHR, while free fatty acid concentrations were comparable between the strains (Figure 3). The *in vitro* analyses of insulin sensitivity of peripheral tissues showed that the visceral adipose tissue has a decreased sensitivity to the insulin stimulation of lipogenesis (Figure 4). The insulin sensitivity of skeletal muscle did not significantly differ between the strains (Figure 5).

Compared to the SHR, the SHR-*Lx* rats displayed lower total body weight but comparable absolute and relative weights of internal organs and adipose tissue depots.
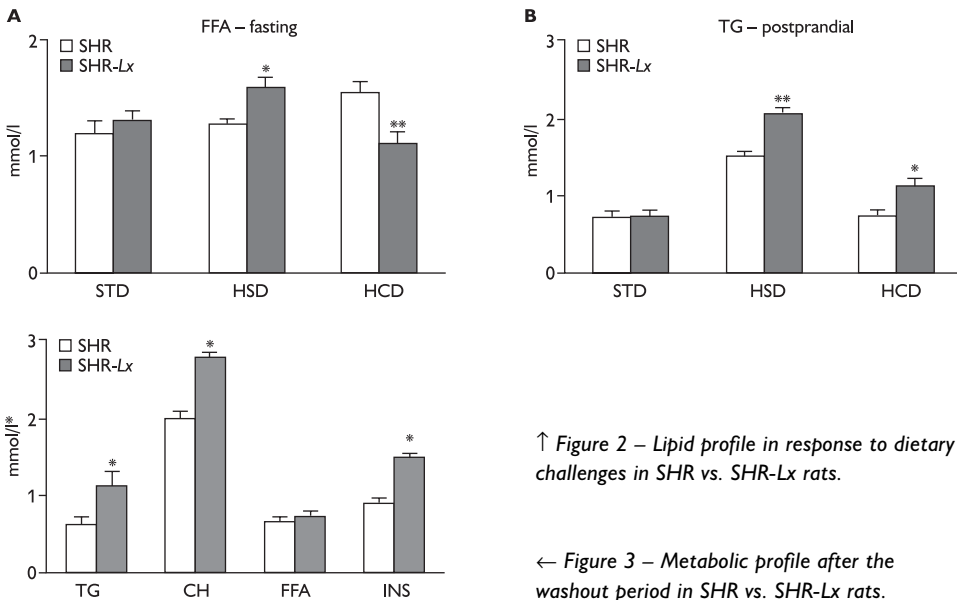


↑ Figure 2 – Lipid profile in response to dietary challenges in SHR vs. SHR-Lx rats.

← Figure 3 – Metabolic profile after the washout period in SHR vs. SHR-Lx rats.

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## Discussion

The spontaneously hypertensive rat and the polydactylous rat are two established rodent models of specific variants of human metabolic syndrome [1, 10, 17]. Introgression of ca. 30 cM region of rat chromosome 8 from PD/Cub onto the genetic background of SHR was previously shown to cause the polydactyly-luxate syndrome (PLS) and decrease the blood pressure in the SHR-*Lx* congenic strain. Our current results provide evidence that the introgressed region carries genetic determinants of complex nutrigenetic interactions involving high-sucrose and high-cholesterol diets. While there was no evident difference in metabolic profile between the SHR and SHR-*Lx* kept on standard diet, the one-week sucrose administration revealed the sensitivity to carbohydrate-induced dyslipidemia conferred by the differential segment with only mild derangement of glucose tolerance (most probably due to the short time of sucrose challenge). Interestingly, when the same differential segment of PD/Cub origin was previously introgressed in the Brown Norway genetic background in the BN-*Lx* congenic strain, the differences were observable under both standard and sucrose diet feeding conditions with no difference in reaction to the sucrose diet administration.
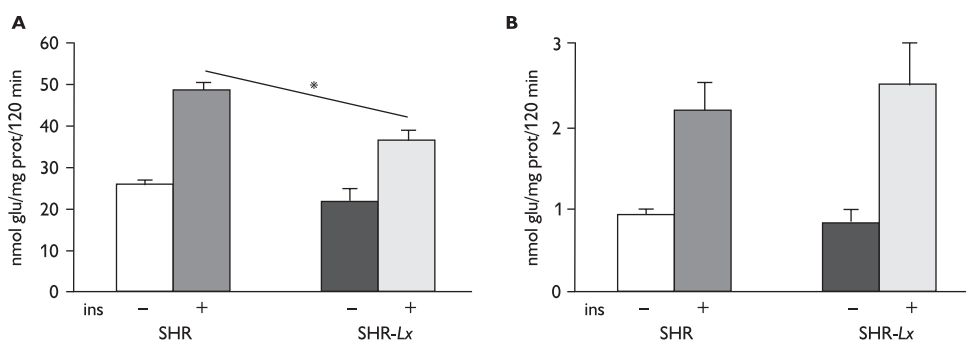


Figure 4 – Insulin sensitivity of visceral adipose tissue in SHR vs. SHR-Lx rats.
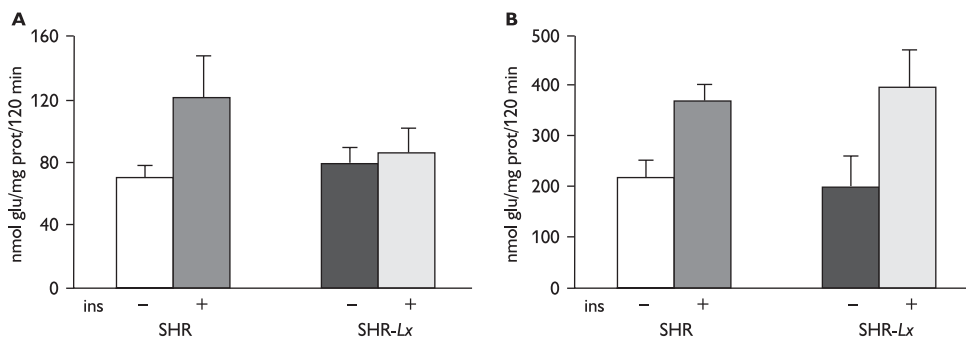


Figure 5 – Insulin sensitivity of skeletal muscle in SHR vs. SHR-Lx rats.

The metabolic consequences of the subsequent high-cholesterol diet feeding illustrate the complexity of the dynamic genetic architecture of metabolic traits. The same allelic combination present in the SHR-*Lx* genome that sensitised the congenic strain towards the sucrose became relatively protective when the environment changed to the one enriched in cholesterol, yet became again partially detrimental after the diet withdrawal. It is impossible from the presented results to conclude whether the observed interactions stem from the identical alleles, different alleles of the same genes or different genes within the differential segment. Nevertheless, within the region of rat chromosome 8 of PD/Cub origin present in the differential segment of the SHR-*Lx* strain there are several genes that can be considered interesting candidates. First, there is the gene most likely responsible for the polydactyly-luxate syndrome of PD/Cub, the promyelocytic leukemia zinc-finger *Plzf* (*Zbtb16*) transcription repressor. Although this gene has been studied so far mostly because its substantial role in tumorigenesis, stem cell renewal and limb development [18–21], several recent lines of evidence clearly indicate that major pathways relevant for metabolic syndrome attributes converge to this gene. Apart from being an inhibitor of nuclear receptor RXR heterodimerization, its role have been described in the angiotensin II receptor – mediated cardiac hypertrophy, a link was established with corticoid and androgen actions as well as sirtuin, PI-3 kinase or Ras and Wnt pathways [22,23]. Although the hypothesis relating the functional genomic variation of *Plzf* gene with the aberration of metabolic traits is still to be confirmed, the gene may be envisaged as a novel promising candidate for one of the network hubs in the systems biology view of metabolic syndrome.

   Second, there is the *ApoA-I/ApoC-III/ApoA-IV/ApoA-*V gene cluster. The impact of this cluster and its individual members on lipid metabolism and atherogenesis has been documented in human studies as well as in rat and mouse models. Especially, apolipoproteins A-III and A-V are considered to represent major determinants of lipid metabolism [24–28]. It is of interest that in another SHR-PD.RNO8 congenic strain – SHR-*Lx* PD5 [SHR.PD(*D8Rat42-D8Arb23*)/Cub] with differential segment spanning only 1.4 Mb (including the *Plzf* gene but not the apolipoprotein cluster), several distinct metabolic features observed in the SHR-*Lx* congenic were still manifested [29]. Several other genes within the differential segment were also associated with metabolic syndrome features. The serotonin receptors Htr3a and Htr3b were shown to influence the sympathoadrenal axis [30], an important player in the development of metabolic syndrome. Also, several polymorphisms of the dopamine D2 receptor (*Drd2*) have been proposed to play role in obesity and hypertension [31, 32]. In summary, we have identified a genomic region synthetic to human chromosome 11q23, which, apart from causing the polydactyly-luxate syndrome, determines complex nutrigenetic interactions when conditioned by sucrose- and cholesterol-enriched diets. The SHR-*Lx* thus displays a specific subset of metabolic syndrome, combining dyslipidemia, insulin resistance with normal to mildly elevated blood pressure and adiposity similar to the SHR model. Moreover,

the present study strongly supports the importance of this region for gene-environment interactions and the SHR-*Lx* is indeed comparative genomic model of choice for its future nutrigenomic and pharmacogenomic interrogations.

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