

MINIREVIEW

Hereditary Hypertriglyceridemic Rat: a Suitable Model of Cardiovascular Disease and Metabolic Syndrome?

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Summary

Hypertriglyceridemia and hypertension seem to be very important cardiovascular risk factors. The Prague hereditary hypertriglyceridemic (hHTG) rat was developed as a model of human hypertriglyceridemia. It was demonstrated that these rats are not obese, they are hypertensive and insulin resistant and they have some disturbances in glucose metabolism. Several QTLs were identified for blood pressure, its particular components (dependent on major vasoactive systems) and plasma triglycerides throughout the genome of hHTG rats by using of F₂ hybrids strategy. It is evident that hHTG rats are a suitable model for the study of metabolic disturbances in relation to blood pressure as well as for the search of genetic determinants of these abnormalities. Numerous abnormalities of blood pressure regulation as well as alterations in the structure and function of cardiovascular apparatus (heart, conduit and resistance arteries) were found in hHTG rats. A special attention was paid to possible changes in the efficiency of various vasoactive systems such as nitric oxide, renin-angiotensin-aldosterone system and sympathetic nervous system, which seem to contribute substantially to cardiovascular and/or metabolic abnormalities observed in Prague hereditary hypertriglyceridemic rats.

Key words

Hereditary hypertriglyceridemic rat • Insulin resistance • Hypertension • Cardiovascular system • Metabolic alterations

Introduction

Hypertriglyceridemia is an independent risk factor for coronary artery disease and it participates in the development of atherosclerosis and hypertension. Moreover, hypertriglyceridemia is associated with several other metabolic disorders, such as insulin resistance,

decreased HDL-cholesterol and hyperinsulinemia. Reaven (1988) designated this cluster of related abnormalities as a syndrome X, which was later named as metabolic syndrome or insulin resistance syndrome (for more details see Sarafidis and Nilsson 2006). The history of the existence of metabolic syndrome has its beginning in the twenties of the last century. In spite of the

extensive research in this field, there is still no precise and generally accepted definition describing what is included in this syndrome (Federspil *et al.* 2006). Recently, several recommendations for definition of the metabolic syndrome have been proposed. The Adult Treatment Panel III (2001) of the National Cholesterol Education Program proposed criteria, which are as followed: abdominal obesity (men: WC >102 cm, women: WC >89 cm), fasting glucose (≥ 6.1 to < 7.0 mmol/l), blood pressure ($\geq 130/80$ mm Hg), TGs (1.7 mmol/l) and HDL-C (men: < 1.04 mmol/l, women: < 1.3 mmol/l). The International Diabetes Federation recommended the similar definition (www.idf.org). When three or more of these five criteria are present, one could speak about the metabolic syndrome. Nevertheless, Reaven (2005) raises the question „Does the ATP III concept of the metabolic syndrome have any redeeming virtues?“.

Some interrelated symptoms of the metabolic syndrome such as insulin resistance, hypertriglyceridemia and hypertension seem to be very important as cardiovascular risk factors and need to be intensively studied. It has been postulated that insulin resistance and the concomitant compensatory hyperinsulinemia contribute to the pathogenesis of hypertension, possibly by stimulating the sympathetic nervous system, promoting renal sodium reabsorption, modulating cation transport, and/or stimulating vascular smooth muscle hypertrophy. Several experimental models have been intensively studied for the presence of insulin resistance and related abnormalities including hypertension. One of these models is Prague hereditary hypertriglyceridemic rat, which was developed as a model of human hypertriglyceridemia in eighties (Vrána and Kazdová 1990).

The purpose of this article is to present a comprehensive up-to-date review of the literature and critically examine the relationship between insulin resistance/hypertriglyceridemia and hypertension in Prague hHTG rats.

Animal models of metabolic syndrome

Almost all animal models used for the study of hypertension have also other symptoms of metabolic syndrome. The most frequently used model with genetic hypertension, the spontaneously hypertensive rats (SHR), have fasting hyperglycemia, impaired oral glucose tolerance, hyperinsulinemia and hypertriglyceridemia in

comparison with the normotensive Wistar-Kyoto (WKY) rats (Yamori *et al.* 1978, Reaven and Chang 1991). Moreover, the resistance to insulin-stimulated glucose uptake in adipocytes isolated from SHR was demonstrated *in vitro* (Reaven *et al.* 1989, Reaven and Chang 1991). On the contrary, Bader *et al.* (1992) who measured the insulin receptor tyrosine kinase activity and glucose transporter (GLUT4) level in skeletal muscle of SHR did not find any difference in comparison to Lewis or Wistar rats. The discrepant results of different studies in SHR could be explained by the fact that some experiments were performed in stressed anesthetized or restrained animals, whereas the other experiments used unstressed and/or unrestrained rats. It was demonstrated that SHR show a more enhanced response of sympathetic nervous system to stress than WKY (McMurty and Wexler 1981), which could lead to a great impairment of glucose metabolism in SHR.

The genetic determinants of hypertension and metabolic defects in SHR were studied mainly by the panel of recombinant inbred strains derived by reciprocal crossing of SHR/Ola and Brown Norway-Lx/Cub progenitors (Pravenec *et al.* 1989). It was demonstrated that QTL underlying complex metabolic disorders in the SHR can be identified on molecular level (for review see Pravenec *et al.* 2004). Very promising was the sequence analysis of multiple positional candidate genes, which revealed two unique mutations in *CD36* gene on chromosome 4. Finally, this gene was identified as a prominent candidate gene because of its reduced expression in the SHR as revealed by microarray cDNA chips (Aitman *et al.* 1999). Its role in SHR was proven by using of congenic and transgenic rats (Pravenec *et al.* 2001, 2003, Qi *et al.* 2002, Šeda *et al.* 2003). However, one should keep in mind that the genetic determinants could be strain-specific and very dependent on the techniques used for phenotyping. As was demonstrated in the case of *CD36*, this gene is candidate gene for disordered fatty-acid metabolism, glucose intolerance and insulin resistance only in NIH-derived SHR, because this gene is normal in original stock of the SHR kept in Japan (Gotoda *et al.* 1999) and there is no polymorphism in this gene among other normotriglyceridemic and hypertriglyceridemic strains of rats (Kadlecová *et al.* 2004).

Other genetic model of experimental hypertension is Dahl salt-sensitive (DS) rat (Dahl *et al.* 1962), which is also used for the study of insulin resistance (Reaven *et al.* 1991). It was described that

maximal insulin-stimulated glucose transport was significantly lower in adipocytes isolated from DS rats than in those from Sprague-Dawley (Reaven *et al.* 1991). A number of studies have established an association between salt sensitivity and insulin resistance in both normotensive and hypertensive subjects (Sharma *et al.* 1991, Ferri *et al.* 1994, Fuenmayor *et al.* 1998). Therefore, Dahl salt-sensitive rats were studied to prove whether the same genetic factors are responsible for salt-induced increase of blood pressure and for salt-induced insulin resistance. It was demonstrated that salt loading did not affect the fasting blood glucose or plasma insulin levels in either DS or DR rats (Ogihara *et al.* 2002). However, when whole body insulin sensitivity was evaluated using the hyperinsulinemic-euglycemic clamp, it was found that glucose infusion and utilization rates were lower in DS rats fed a high-salt diet than in those fed a normal diet (Ogihara *et al.* 2003).

Several other models with combination of hypertension, insulin resistance, obesity or diabetes are used (Klimeš *et al.* 1997, Chen and Wang 2005) including Prague hereditary hypertriglyceridemic rats.

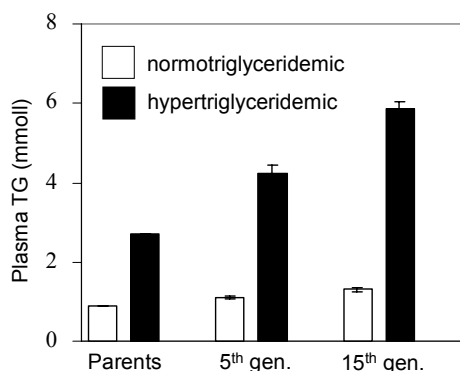


Fig. 1. Plasma triglyceride levels in normotriglyceridemic and hereditary hypertriglyceridemic rats on high sucrose diet in different generations (adapted from Vrána and Kazdová 1990).

Prague hereditary hypertriglyceridemic rats

Model development and characterization

Prague hereditary hypertriglyceridemic rats were developed as a genetic model of human hypertriglyceridemia from the colony of Wistar rats (Vrána and Kazdová 1990). During his studies, Dr. Antonín Vrána observed that high-carbohydrate intake produced a number of metabolic and other changes that can have pathological consequences (Vrána and

Kazdová 1970, Vrána *et al.* 1982). The major effect was on plasma triglycerides level and tissue glucose utilization. Because the variation of plasma triglycerides in outbred Wistar rats was too high, three breeding pairs with the lowest plasma triglycerides and three pairs with the highest plasma triglycerides were selected and bred by brother x sister mating to established “normotriglyceridemic” and “hypertriglyceridemic” lines (Vrána and Kazdová 1990). As can be seen from the Figure 1, plasma level of triglycerides was more than four times higher in the 15th generation of hHTG rats in comparison to normotriglyceridemic controls. At present hHTG rats are bred for more than 35 generations. However, the normotriglyceridemic line was poorly fertile and became extinct (Vrána *et al.* 1993). Therefore, outbred Wistar rats or several inbred strains have been used as controls (see below).

We have demonstrated that hHTG rats are hypertensive (Štolba *et al.* 1992). This study confirmed that hypertriglyceridemia and hypertension could have some similar regulatory mechanisms. It is important to know that other hypertensive strain, Lyon hypertensive rat, which was selected on the base of blood pressure level, is also hypertriglyceridemic (Vincent *et al.* 1984). We have also found the positive correlation between blood pressure and plasma triglyceride concentration in hHTG and Wistar rats (Štolba *et al.* 1992) ($r = 0.585$, $n = 40$, $p < 0.001$) and this was confirmed even in F₂ hybrids derived from hHTG and Lewis progenitors (Kuneš *et al.* 1995) ($r = 0.420$, $n = 131$, $p < 0.0001$).

Elevated triglyceride levels seem to be an important determinant of numerous alterations in the structure and function of cell membrane in hHTG rats (for review see Devynck *et al.* 1998b, Zicha *et al.* 1999). Although membrane microviscosity was similar in hHTG rats and their normotensive Wistar controls, the relationship of plasma triglycerides to the microviscosity determined either in the lipid core or in the outer leaflet of platelet membrane was strikingly different in the two rat strains (Devynck *et al.* 1998a). The response of cell Ca²⁺_i and pH_i to thrombin stimulation (Zicha *et al.* 1996) as well as thrombin-induced platelet aggregability (Kuneš *et al.* 1997) were reduced in hHTG rats (proportionally to the elevation of plasma triglycerides). Chronic interventions leading to the increase (fructose intake) or reduction of plasma triglyceride levels (gemfibrozil) modify membrane microviscosity (Kuneš *et al.* 2000) and cyclic nucleotide metabolism in platelets of hHTG rats (Pernollet *et al.* 2001). Thus hHTG rats represent a useful

Table 1. QTLs detected by the study of F₂ hybrids derived either from hHTG and Lewis progenitors (a) or from hHTG and Brown-Norway progenitors (b).

Phenotype	Chromosome	Marker	LOD score	P
Baseline MAP (mm Hg)	1	D1Got228 ^a	2.7	0.0015
	3	D3Rat186 ^a	3.8	0.0001
	5	D5Wox25 ^a	3.6	0.0003
Systolic BP (mm Hg)	3	D3Rat126 ^b		0.0086
	5	D5Mgh9 ^b		0.0010
Diastolic BP (mm Hg)	15	D15Wox3 ^b		0.0009
Plasma TG (mmol/l)	2	D2Rat61 ^a	3.1	0.0035
	2	D2Rat210 ^a	4.4	0.0001
	13	D13Rat34 ^a	3.3	0.0011
	4	D4Wox8 ^b	6.5	0.0001
	8	D8Mit13 ^b	≅4	0.0003
	8	D8Mgh6 ^b	≅4	0.0019
Plasma TC (mmol/l)	5	D5Rat108 ^a	5.6	<0.0001
	12	D12Rat20 ^a	3.8	0.0011

TG – triglycerides, TC – total cholesterol, for more details see: ^a – Ueno *et al.* (2003a, 2004), ^b – Klimeš *et al.* (2003).

model for the analysis of disturbances of lipid metabolism both at whole-body level and at the level of cell membrane.

Surprisingly, it has been demonstrated that hHTG rats are not obese (Štolba *et al.* 1992, Šeböková *et al.* 1995) suggesting that the relation of particular symptoms of metabolic syndrome is not too tight. Numerous metabolic disturbances have been demonstrated in hHTG rats in comparison with controls (for review see Klimeš *et al.* 1995).

Genetic analysis

Genetic determinants of hypertriglyceridemia and hypertension were intensively studied in two sets of F₂ hybrids derived either from hHTG and Lewis progenitors (Kuneš *et al.* 1995, Ueno *et al.* 2003a,b, 2004) or from hHTG and Brown-Norway parents (Klimeš *et al.* 2003). The positive relation of blood pressure with ion transport abnormalities and membrane alterations lead us to search for alterations of ion transport in erythrocytes of the hHTG rats (Kuneš *et al.* 1994). We have demonstrated a mild elevation of Na⁺ content in erythrocytes from hHTG rats, which was caused by an increase in ouabain-resistant Na⁺ net uptake due to the augmentation of both bumetanide-sensitive and bumetanide-resistant Na⁺ net fluxes. There was not only the correlation of plasma TG with blood pressure but also with Na⁺ content in erythrocytes and some particular

components of sodium transport (Zicha *et al.* 1995, 1997). This is with a good agreement with our previous results found in recombinant inbred strains derived from SHR and Brown-Norway progenitors (Bin Talib *et al.* 1992).

Recently we have carried out the total genome scan in 266 F₂ hybrids derived from hHTG and Lewis progenitors (Ueno *et al.* 2003a,b, 2004). Several loci were disclosed on different chromosomes for blood pressure, plasma triglycerides (TG) and total cholesterol (TC) (Table 1). The using of other set of F₂ hybrids derived from hHTG and Brown-Norway rats, Klimeš *et al.* (2003) also disclosed several QTLs for blood pressure and metabolic parameters. However, QTLs that were found for plasma TG in our studies are localized on different chromosomes than those found in the study of Klimeš *et al.* (2003). It is evident that the genetic regulation of plasma TG concentration in hHTG rats is complex and thus the finding of multiple QTLs is reasonable. Very interesting results should be drawn from the comparative map of rat, human or mouse chromosome regions. It was demonstrated that our loci for plasma TG on rat chromosome 2 are syntenic to previously reported human loci for the increased cholesterol concentration of small, dense LDL on chromosomes 3 and 4, one of clinical characteristics of human familial combined hyperlipidemia (Rainwater *et al.* 1999). Several QTLs were also disclosed for relative

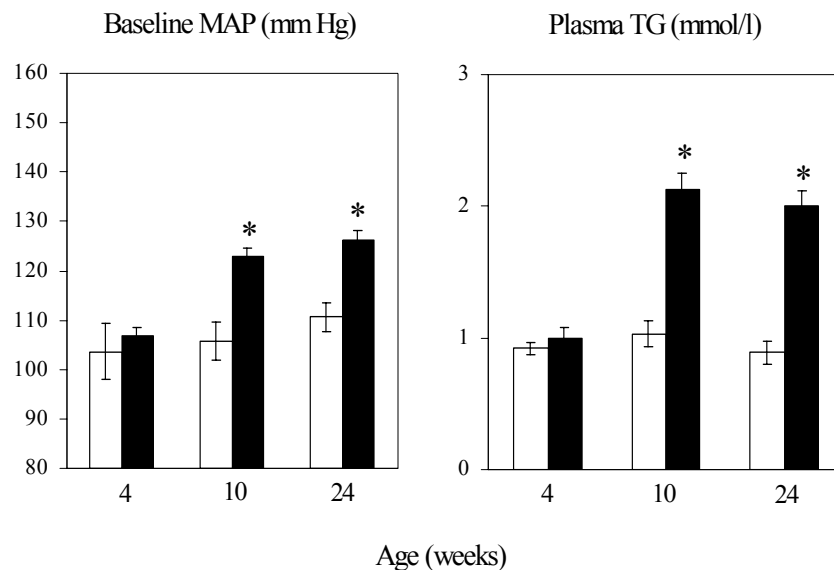


Fig. 2. Age-dependent changes of basal blood pressure (left panel) and plasma triglycerides (right panel) in control (open bars) and hereditary hypertriglyceridemic rats (full bars). Data are means \pm S.E.M., * $p < 0.01$ with respect to the controls.

heart and kidney weight by our panel of F₂ hybrids (Ueno *et al.* 2003b). Moreover, because two reciprocal crosses, hHTG \times LEW and LEW \times hHTG were produced, this enabled to study gender-specific determinants of studied phenotypes. Thus several gender-specific QTLs for blood pressure and relative organ weight were already found (Ueno *et al.* 2003b).

Changes of SNS/NO balance in blood pressure control of hHTG rats

Hypertension in hHTG rats appears in parallel with the increase of plasma triglycerides (TG) (Fig. 2). Weanling (4-week-old) hHTG rats are still normotensive and normotriglyceridemic, but their BP and TG begin to rise during the second postnatal months (Kuneš *et al.*, to be published). Nevertheless, some abnormalities characteristic for adult hHTG animals can be observed even in weanling rats. Residual BP (recorded after the consecutive blockade of renin-angiotensin system and sympathetic nervous system) is elevated by 15 mm Hg, BP rise after the acute L-NAME administration is augmented by about 30 mm Hg and the rate of BP rise during acute NOS blockade is almost doubled compared to weanling Lewis rats (Zicha *et al.*, to be published). Blood pressure tends to be higher in male than female hHTG rats so that hypertensive values are more frequently found in males (Kuneš *et al.* 2002). Maximal BP values are reached at the age of about 12 months (SBP/DBP: 174 \pm 10/114 \pm 8 mm Hg vs. Lewis 135 \pm 2/80 \pm 2 mm Hg) (Dobešová *et al.*, to be published).

Adult hHTG rats tend to have greater BP changes after the acute blockade of renin-angiotensin system and sympathetic nervous system, but the most pronounced abnormality of hHTG rats is the elevation of residual BP. Male (but not female) hHTG rats display a greater degree of relative NO deficiency compared to normotensive Lewis controls (Kuneš *et al.* 2002, 2004). The analysis of a large cohort of F₂ hybrids (derived from hHTG and Lewis progenitors) revealed that baseline BP elevation was proportional to the elevation of residual BP and to the augmentation of sympathetic BP component (pentolinium-induced BP change). It should be noted that the magnitude of sympathetic BP component in hypertensive F₂ rats was increased not only absolutely but also relatively, i.e. in percentage of their baseline BP. This study also confirmed the importance of relative NO deficiency for BP elevation in F₂ rats because baseline BP correlated positively with SNS/NO ratio (ratio of BP changes elicited by pentolinium and L-NAME) and negatively with BP changes induced by L-NAME when expressed in percentage of initial blood pressure (Kuneš *et al.* 2002). It is evident from the above data that increased SNS/NO ratio and elevated residual BP play a similar role in the maintenance of high BP in genetic hypertension as was described in salt-loaded F₂ hybrids of Dahl rats (Dobešová *et al.* 2002).

The relative NO deficiency in hHTG rats is a highly interesting phenomenon which must always be considered in terms of existing sympathetic hyperactivity because there are no signs of absolute NO deficit in

hHTG rats. The dose-dependent BP response to intravenous acetylcholine injection was substantially enhanced in hHTG rats. These NO changes correlated positively with augmented BP response to intravenous arginine administration in the same rats subjected to previous L-NAME-induced NOS inhibition. Moreover, BP rise elicited by acute NOS inhibition was enhanced in hHTG rats even at lower L-NAME doses (1-10 mg/kg b.w.) (Zicha *et al.*, to be published). It should also be mentioned that in both hHTG and Lewis rats the whole extent of fully developed BP rise after acute NOS inhibition was transiently abolished by intravenous arginine injection (Kuneš *et al.* 2002). However, Kuneš *et al.* (2002) also demonstrated that the acute administration of tempol (a membrane-permeable mimetic of superoxide dismutase) caused greater BP reduction in hHTG than in Lewis rats and this BP reduction was inversely proportional to initial BP level.

An attempt was also made to estimate the participation of various NOS isoforms and endothelium-derived hyperpolarization factor (EDHF) in vasodilator mechanisms of hHTG rats (Zicha *et al.*, to be published). Using the animals subjected to previous ganglionic blockade we have observed greater pressor effects of intravenous administration of neuronal NOS inhibitor S-methyl-thiocitrulline (1-2 mg/kg b.w.) in hHTG than in Lewis rats, although BP changes elicited by L-NAME were comparable. Similarly, aminoguanidine, which is generally considered to be inducible NOS inhibitor, caused greater BP rise in hHTG rats than in normotensive animals. Finally, hHTG rats responded to acute injection of tetraethylammonium (inhibitor of calcium-dependent K^+ channels opened by EDHF) by 2-3 fold greater BP elevation compared to Lewis rats, although BP response to L-NAME was similar in both rat strains. The latter observation suggests a considerable participation of EDHF in vasodilator mechanisms of hHTG rats.

As far as chronic dietary interventions are concerned, blood pressure of adult hHTG rats does not seem to be sensitive to high salt intake (Dobešová *et al.*, unpublished data). On the other hand, in some studies we have observed moderate BP lowering effects of chronic gemfibrozil treatment, which considerably decreased the levels of circulating triglycerides (Kuneš *et al.*, unpublished data). Surprisingly, chronic fructose intake, which doubled plasma TG level did not affect BP of hHTG rats (Kuneš *et al.* 2000, Kadlecová *et al.* 2004).

Myocardial hypertrophy and the effect of ACE inhibition in hHTG rats

Despite the fact that pressure overload should be expected to induce the adaptive growth of the heart and alteration of myocardial and vascular structure and function, the data of this kind are sparse in the hHTG model of hypertension.

The increase of systolic blood pressure by 20 mm Hg (measured by the tail cuff method – Šimko *et al.* 2002) together with the diastolic BP increase by about 20-40 mm Hg (measured invasively – Klimeš *et al.* 1997) represent a hemodynamic overload in hHTG rats that induced left ventricular hypertrophy (LVH) (the weight increase by 25 %). However, there was also a hypertrophy of the right ventricle (Šimko *et al.* 2005). The concentration of metabolic proteins (mitochondrial and cytoplasmatic enzyme proteins) was increased in both ventricles, suggesting that the hypertrophic response of the heart ventricles reflects greater demands on energy production and calcium transport (Šimko *et al.* 2005). Despite long lasting pressure overload, neither the concentration of total collagenous proteins, nor the hydroxyproline in total collagen changed in either ventricle of hHTG animals, indicating that the hHTG model of hypertension is not associated with the fibrosis of either ventricle. However, alterations in individual fractions of cardiac collagen were detected. They were characterized by a higher amount of highly cross-linked collagen in a pepsin-insoluble fraction in the LV and lower amount of newly synthesized collagen (in the pepsin-soluble fraction) in both ventricles. Although diastolic properties were not analyzed, these collagen fractions alterations may have modified diastolic heart function, despite the absence of significant changes of the total extracellular matrix protein (Šimko *et al.* 2005).

Prominent left ventricular fibrosis is demonstrated in models of arterial hypertension associated with the activation of circulating or local renin-angiotensin-aldosterone system (RAAS), since angiotensin II and aldosterone stimulate fibrotic tissue growth (Šimko 2002, Weber 1997). However, in the hHTG model of hypertension, the neurohormonal alteration has a specific nature. Increased norepinephrine (Lichardus *et al.* 1993) and epinephrine (Štolba *et al.* 1993) plasma concentrations, and increased excretion of vanilmandelic acid (Štolba *et al.* 1993) reflecting sympathoadrenal system activation, are associated with normal levels of renin and aldosterone (Lichardus *et al.* 1993). Thus, angiotensin II is not considered to

participate in left ventricular remodeling in hHTG rats.

There are two facts supporting the idea that in hHTG model of hypertension, beside increased pressure overload, also volume hemodynamic overload may be a substantial mechanism participating in hypertrophy development. First, the absence of fibrosis in a hypertrophied left ventricle is typical for models of volume overload such as aortic insufficiency (Fizelová and Fizel 1969), anemia, septal defect (Beznak *et al.* 1969) or aortocaval fistula (Šimko 2002). Besides the lack of fibrosis, these volume overload-induced left ventricular hypertrophy models are also characterized by a concomitant hypertrophy of the right ventricle (Fizelová and Fizel 1969) what is also the case of hHTG model. Insulin resistance with Na⁺ retention and subsequent circulating volume enlargement may represent the underlying mechanism (Klimeš *et al.* 1997).

ACE inhibitor captopril eliminated both hypertension and LVH. Although the RAAS apparently is not activated in hHTG rats, the reduction of hypertension, regression of LVH and normalization of protein fractions by ACE inhibition is not surprising. It has been previously shown that ACE inhibition was able to reduce blood pressure and left ventricular weight not only in states with hemodynamic overload but also in healthy control individuals, in which no activation of RAAS was anticipated (Šimko *et al.* 2002). It seems also comprehensible that normalized blood pressure resulted in normalization of LV weight. The fact that RV was not influenced by captopril treatment suggests a different mechanism in the hypertrophic growth in either ventricle – potentially the combination of pressure and volume overload in the LV and volume overload in the RV (Šimko *et al.* 2005).

Functional alterations of heart and vessels of hHTG rats

Hereditary hypertriglyceridemia in rats was found to be associated with several metabolic abnormalities (hyperinsulinemia, insulin resistance, hypertriglyceridemia) and elevation of blood pressure (Klimeš *et al.* 1997). Each of these abnormalities has independently been shown to be associated with impaired endothelial function, as demonstrated by decreased endothelium-dependent relaxation (Pieper *et al.* 1995, Katakam *et al.* 1998, Bartus *et al.* 2005).

High plasma triglyceride level is a major independent risk factor of coronary heart disease. Acute hypertriglyceridemia in Wistar rats was accompanied with the impairment of NO-dependent relaxation in aorta

as well as in the mesenteric resistance artery (Bartus *et al.* 2005). Impaired endothelial function has been found within several hours after triglyceride load in both animals and humans (Bae *et al.* 2001, Gudmundsson *et al.* 2000).

In adult hHTG rats blood pressure is moderately increased (140-150 mm Hg) compared to age-matched normotensive Wistar rats. Impaired endothelium function has been documented by finding of reduced endothelium-dependent relaxations of aorta to acetylcholine. This is accompanied by marked changes in vascular structure, mainly by significant thickening of the aortic wall. Administration of L-NAME to hHTG rats resulted in additional enhancement of inhibition of acetylcholine-induced relaxation and further thickening of aortic wall (Török *et al.* 2002). This suggests that in hHTG rat there was still a capacity of endothelial cells to produce and release NO. Reduction of endothelium-dependent relaxation in hypertriglyceridemic rats was not limited only to aorta but it was found in other conduit (mesenteric, iliac, carotid arteries) and mesenteric resistance arteries as well (Kusterer *et al.* 1999, Čačányiová *et al.* 2005, Bartus *et al.* 2005). The impairment of endothelial function is not species-dependent, since it was demonstrated also in humans (Lundman *et al.* 2001) and Japanese white rabbits (Shishido *et al.* 2004).

Besides Prague hereditary hypertriglyceridemic rat model it has been shown that normotensive Wistar rats fed a high-sucrose or high-fructose diets will also become hypertriglyceridemic and hypertensive (Reil *et al.* 1999, Erlich and Rosenthal, 1996, Bartus *et al.* 2005). In arteries of these rats the endothelium-dependent relaxation was impaired, but the endothelial function was restored with the change to a normal diet. It suggests that the overall dyslipidemia could contribute to an elevation of vascular tone and to hypertension. Shishido *et al.* (2004) have demonstrated that hypertriglyceridemia aggravates functional impairment induced by hypercholesterolemia in endothelial and smooth muscle cells.

It has been shown that acetylcholine-induced relaxation in the dog thoracic aorta was fully operative during whole perinatal period and its extent was not decreased up to 6th week of life (Török and Gerová 1997). The existence of profound endothelium-dependent relaxation was also found in thoracic aorta of hHTG rats aged 4 weeks (Török *et al.* 2003a, 2006). It indicates that the enzymatic equipment of endothelial cells required for

NO production is fully developed in first few weeks after birth. The endothelial dysfunction leading to impaired relaxation in hHTG rats develops in parallel with the progress of hypertension.

The contractile response of thoracic aorta to noradrenaline was not different in adult hHTG rats as compared to age-matched Wistar controls (Török *et al.* 2002). On the other hand, in 4-week-old hHTG rats the maximal isometric contraction of the thoracic aorta to noradrenaline was reduced and the concentration-response curve to noradrenaline was shifted to the right indicating decreased sensitivity of smooth muscle to noradrenaline as compared to age-matched normotensive young Wistar rats. The reduced contractility of thoracic aorta could be explained by decreased thickness, cross-sectional area and inner diameter of this vessel (Török *et al.* 2006).

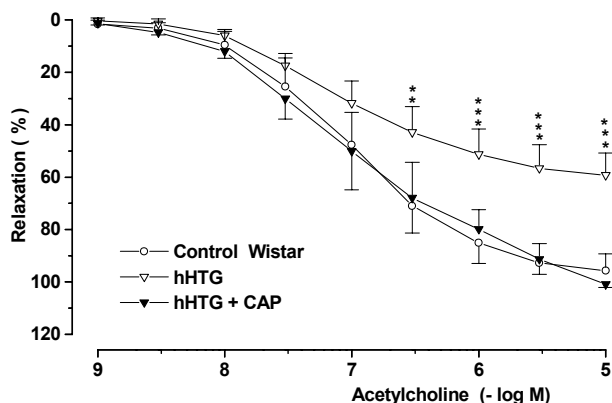


Fig. 3. Endothelium-dependent relaxation of rat thoracic aorta induced by acetylcholine in control Wistar and hereditary hypertriglyceridemic (hHTG) rats with and without captopril treatment (hHTG + CAP). ** $p < 0.01$, *** $p < 0.001$ with respect to the control and hHTG + CAP rats. (adapted from Török *et al.* 2002).

Chronic treatment of hHTG rats with captopril, the ACE inhibitor, prevented the impairment of aortic vascular relaxation (Fig. 3), together with preserving normal blood pressure (Török *et al.* 2002). Beneficial effect of captopril on functional changes in the thoracic aorta was probably nonspecific, because in the state of selective NO deficiency without hypertriglyceridemia, captopril effectively improved endothelial function as well (Küng *et al.* 1995).

Long-term treatment of hHTG rats with L-arginine, precursor of NO production, influenced neither systolic blood pressure nor endothelium-dependent relaxation of thoracic aorta. On the other hand,

simvastatin, inhibitor of 3-hydroxy-3-methylglutaryl-CoA reductase, normalized both the systolic blood pressure and endothelium-dependent relaxation of this vessel (Török *et al.* 2003b).

Endothelial dysfunction results mainly from hemodynamic alterations and its correction by antihypertensive treatment is not only a consequence of blood pressure reduction but it might be caused by other mechanisms influencing RAS and triglyceride metabolism.

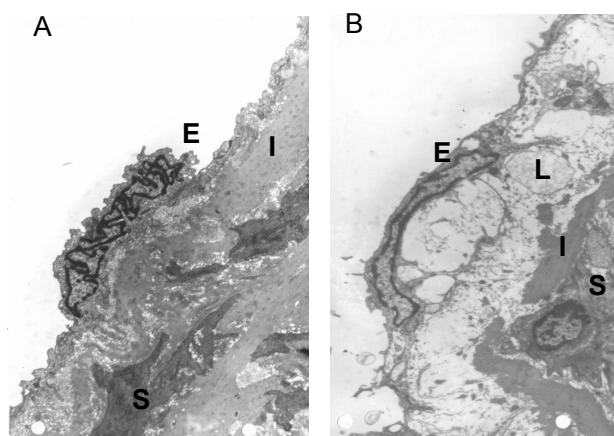


Fig. 4. Luminal part of the arterial wall of normotensive Wistar rats (A) and hereditary hypertriglyceridemic rats (B). E – endothelial cell, I – internal elastic lamina, S – smooth muscle cell, L – deposits of remnants of lipid metabolism. Magnification 5 000 x

Structural alterations of the cardiovascular system in hHTG rats

Conduit arterial wall of hHTG rats is already altered from the beginning of their ontogenic development (4-week-old rats). Pathological alterations were also observed in adult animals (aged 17 weeks) and one-year-old hHTG rats. In comparison with age-matched control rats we found increased wall thickness and a pronounced increase of the arterial wall mass (tunica intima + tunica media) in thoracic aorta, carotid and iliac arteries. Hypertrophy of the arterial wall was also documented by an increase of wall thickness/inner diameter ratio (Čačányiová *et al.* 2005, Cebová *et al.* 2006). Changes in geometry of conduit arteries were accompanied by ultrastructural abnormalities of arterial wall. As we have shown earlier (Kristek *et al.* 1997) ultrastructural changes took place predominantly in innermost layers of the arterial wall. We found pronounced accumulation of products of lipid metabolism, which was localized mainly in luminal part

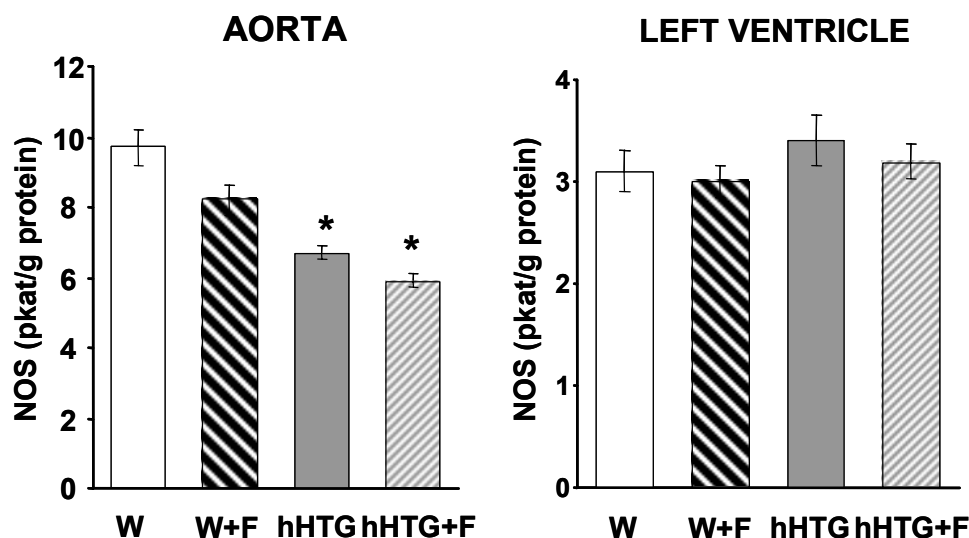


Fig. 5. The effect of fructose (F) treatment on nitric oxide synthase (NOS) activity in aorta (left panel) and left ventricle (right panel) of Wistar-Kyoto (W) and hHTG rats. * $P < 0.05$ compared to control and fructose-treated W rats.

of the arterial wall (endothelial cells and subendothelial space). Less lipids were found in smooth muscle cells, and there was only a sporadic occurrence in intercellular space among smooth muscle cells (Fig. 4). Thus there was a gradient in lipid deposition in the arterial wall. It seems that relatively compact layer of internal elastic lamina acted as protective coat, preventing penetration of remnants of lipid metabolism deep into the tunica media.

Similar accumulation of lipid droplets in the cellular and extracellular space has also been observed in hereditary hyperlipidemic rabbits (Rosenfeld *et al.* 1987). The organization and orientation of smooth muscle cells were essentially intact. We suggest that ultrastructural changes in endothelial cells and smooth muscle cells could be at least partly responsible for changes in the vascular responsiveness of hHTG rats. Localization of pathological alterations in the luminal part of arterial wall particularly in endothelial cells could be in a close relation to decreased endothelial dependent relaxation which we observed in iliac artery from hHTG rats (Čačányiová *et al.* 2005). The impairment of endothelium-dependent relaxation was also observed in aorta and mesenteric and carotid arteries, as well as in the mesenteric resistance arteries of Wistar rats fed diets leading to the elevation of plasma triglycerides (Banos *et al.* 1997, Kusterer *et al.* 1999, Bartus *et al.* 2005). The decreased relaxation to acetylcholine was documented in forearm circulation of hypertriglyceridemic humans (Lewis *et al.* 1999). Beside the decreased endothelium dependent relaxation to acetylcholine we have found enhanced noradrenaline-induced contraction of iliac

artery of hHTG rats (Čačányiová *et al.* 2005). This is in agreement with observations made in the thoracic aorta and mesenteric artery isolated from hypertriglyceridemic rats, where adrenergic contractions were found to be increased (Banos *et al.* 1997). We supposed that the increase in maximal active tension could correspond to the hypertrophy of arterial wall observed in different hHTG conduit arteries (Čačányiová *et al.* 2005, Šimko *et al.* 2005, Cebová *et al.* 2006). These data suggest that the ultrastructural changes in tunica media were not so pronounced to decrease active tension of smooth muscle cells to noradrenaline. Taking into account Laplace's law it could be suggested that the changes in the arterial geometry and ultrastructure may exert a negative effect on physiological parameters and their functional consequences lead to negative effects on nutritional supply of the respective areas.

NO/ROS balance in the cardiovascular system of hHTG rats

In humans, hypertriglyceridemia induces endothelial dysfunction leading to the atherosclerosis. In contrast, rats are resistant to atherosclerosis. The study of Bartus *et al.* (2005) documented, however, that hypertriglyceridemia causes impairment of NO-dependent vasodilation also in Wistar-Kyoto and spontaneously hypertensive rats. The increased production of reactive oxygen species and decreased availability of nitric oxide have been suggested to be responsible for endothelial dysfunction in hypertriglyceridemic rats (el Hafidi and Banos 1997,

Erllich and Rosenthal 1996).

However, hereditary hypertriglyceridemic rats were studied much less from this point of view. Recently, we have documented a decreased acetylcholine-induced relaxation of thoracic aorta isolated from hHTG rats (Šimko *et al.* 2005). Further analysis of decreased NO-dependent relaxation showed marked changes in nitric oxide synthase activity of aorta in hHTG rats. We have demonstrated a significant decrease of total NO synthase activity in the aorta of 7-month-old hHTG rats compared to Wistar Kyoto rats. Fructose treatment of both Wistar-Kyoto and hHTG rats did not further affected the activity of NO synthase. However, no changes in basal NO synthase activity were found in the heart (Fig. 5). Similarly, the decreased total NO synthase activity was demonstrated in the kidney and brain of 7-month-old hHTG rats (Pecháňová *et al.*, to be published). Moreover, Kazdová *et al.* (2001) documented a decreased cGMP concentration in the aorta of hHTG rats, which was further depressed by L-NAME treatment.

Chronic administration of captopril normalized the decreased NO synthase activity in the aorta of hHTG rats. It had, however, no significant effect on NO synthase activity decreased by L-NAME treatment. Western blot analysis demonstrated a reduced expression of endothelial NO synthase protein in the hHTG rats. Captopril administration was not able to modify this expression (Šimko *et al.*, to be published). Thus, it is hypothesized that captopril increased NO synthase activity probably by decreasing level of reactive oxygen species. Both decreased production of angiotensin II and increased concentration of thiol groups due to the captopril treatment may be responsible for this effect (Pecháňová *et al.* 2006). On the other hand, treatment with simvastatin increased the expression of endothelial NO synthase protein leading to the improvement of endothelial function of aorta in hereditary hypertriglyceridemic rats (Török *et al.* 2003b).

Previously it was reported that hHTG rats exhibit some signs of oxidative stress including increased lipoprotein oxidability and lipid peroxidation (Kazdová *et al.* 1997). When these rats were fed a high-sucrose diet, these metabolic defects were strongly aggravated (Vrána *et al.* 1993). Indeed, the concentration of conjugated dienes, a marker of oxidative membrane damage, was significantly increased in the kidney of both 3- and 7-month-old hHTG rats (Pecháňová *et al.*, to be published). Oxidative stress manifestation was further enhanced by high-sucrose diet as demonstrated by

increased TBARS and conjugated diene content, decreased GSH levels and glutathione peroxidase activity in blood and liver of hHTG rats. Phenolics-rich extracts from the plants were able to improve antioxidant status in blood and liver and positively affect plasma lipoprotein profile in this experimental model (Škottová *et al.* 2004).

The decreased NO synthase activity and cGMP concentration as well as increased oxidative stress are in good agreement with decreased acetylcholine-induced relaxation of thoracic aorta in hHTG rats. Thus, it is hypothesized that antioxidants with simultaneous ability to increase NO synthase activity may interfere successfully with this form of metabolic syndrome.

Conclusions and perspectives

The development of hHTG rats was very helpful for the study of the particular symptoms of metabolic syndrome. Although obesity belongs to the one of major symptoms of human metabolic syndrome, hHTG rats are not obese. It suggests a possibility of dissociation of these phenomena. The total genome scan of two different sets of F₂ hybrids derived from hHTG and respective control strain revealed the complexity of metabolic syndrome as well as hypertriglyceridemia. Several candidate genes of hypertriglyceridemia could be studied on the bases of syntenicity among rat, mice and human genome. The findings that some QTLs found in hHTG rats are similar to human locus for human familial combined hyperlipidemia are rather optimistic. Recently we have found a strong relation between polymorphism in *Igf2* gene and plasma levels of triglycerides and cholesterol in our set of F₂ hybrids (Kadlecová *et al.* 2003).

Hypertension in hHTG rats appears in parallel with the increase of plasma triglycerides (TG), which is in accordance with a strong correlation of plasma TG with BP in adult F₂ hybrids. There is a considerable dysbalance between pressor and depressor systems indicating a relative NO deficiency in hHTG rats. Increase in blood pressure was accompanied by the impairment of endothelium-dependent relaxation of thoracic aorta. ACE inhibition by captopril normalized both blood pressure and endothelium-dependent relaxation. A possible relationship between functional and morphological changes was suggested. The accumulation of lipid metabolism products in intimal part of arterial wall (especially in endothelial cells) resulted in a decrease of endothelium-dependent relaxation to acetylcholine and an increase in maximal active tension

of the arterial wall, which corresponded with vascular smooth muscle hypertrophy in hHTG vessels. The decreased NO synthase activity and cGMP concentration and increased oxidative stress are in a good agreement with decreased acetylcholine-induced relaxation of thoracic aorta in hHTG rats. Moreover, it is hypothesized that antioxidants with simultaneous ability to increase NO synthase activity may interfere successfully with this form of metabolic syndrome.

Taking together all results of this paper it is evident that hereditary hypertriglyceridemic rats are a suitable model for phenotyping and genotyping such complex diseases as hypertension, hypertriglyceridemia,

insulin resistance, etc. which represent components of metabolic syndrome. A more detailed analysis of particular physiological and pathophysiological mechanisms is necessary for the search for genetic determinants of this complex disease.

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