Physiol. Res. 53: 265-271, 2004

Does *Cd36* Gene Play a Key Role in Disturbed Glucose and Fatty Acid Metabolism in Prague Hypertensive Hypertriglyceridemic Rats?

M. KADLECOVÁ, J. ČEJKA, J. ZICHA, J. KUNEŠ

Institute of Physiology, Academy of Sciences of the Czech Republic and Center for Experimental Research of Cardiovascular Diseases, Prague, Czech Republic

Received September 30, 2003
Accepted December 20, 2003

Summary

Close links between hypertension, hypertriglyceridemia, insulin resistance and other symptoms of metabolic syndrome was demonstrated in humans and experimental animals. Quantitative trait loci for defects in glucose and fatty acid metabolism, hypertriglyceridemia and hypertension were mapped in spontaneously hypertensive rats (SHR) on chromosome 4 and defective *Cd36* gene was identified in this region. Here we investigated the polymorphism of *Cd36* gene in Prague hereditary hypertriglyceridemic (HTG) rats, which represent another model of genetic hypertension and metabolic syndrome. These animals were compared with NIH-derived SHR and two different normotensive control strains (WKY, LEW). In spite of the fact that HTG and SHR rats had similar metabolic disturbances, genotype analysis of PCR products has shown that *Cd36* mutation was not present in HTG rats. In conclusion, we have revealed that defective *Cd36* is probably a candidate gene for disorded fatty-acid metabolism, glucose intolerance and insulin resistance in NIH-derived SHR, but other genes might play a role in pathogenesis of metabolic syndrome in Prague hereditary hypertriglyceridemic rats. This is in accordance with the absence of defective *Cd36* gene in original SHR from Japan.

Key words

Hypertension • Insulin resistance • Fatty acid • Oral glucose test • Metabolic syndrome

Introduction

Several studies have demonstrated the existence of insulin resistance and hyperinsulinemia in patients with untreated hypertension (Ferrannini *et al.* 1987, Shen *et al.* 1988) as well as in rats with spontaneous hypertension (Reaven and Chang 1991) or in rats with hypertension induced by fructose-enriched diet (Hwang *et al.* 1987, Reaven *et al.* 1988). The spontaneously hypertensive rat (SHR) is considered as a model of human insulin-resistance syndrome and/or metabolic syndrome because of the presence of hypertension, hyperinsulinemia, hypertriglyceridemia, glucose intolerance and visceral type of obesity. Numerous different colonies of SHR, mostly derived from a colony at the Institute of Health (USA), are widely used for the study of basic mechanisms responsible for essential hypertension and metabolic syndrome around the world. A deletional mutation in fatty acid translocase gene *Cd36* was proposed as the underlying mechanism of the

Fax +420 241 062 164
E-mail: physres@biomed.cas.cz http://www.biomed.cas.cz/physiolres

insulin-resistant phenotype in SHR (Aitman *et al.* 1999). Recently, overexpression of wild type *Cd36* gene in SHR was shown to ameliorate their insulin resistance (Pravenec *et al.* 2001). Nevertheless, the *Cd36* mutation is absent in the original SHR strain (maintained since their development in Japan) in which the insulin resistance is also present (Gotoda *et al.* 1999). These discrepant results documented that other mechanisms based upon other genes could also play a role in metabolic syndrome of different rat strains.

At the beginning of the nineties a new model of human hypertriglyceridemia was developed by Vrána and Kazdová (1990) from the colony of Wistar rats. We have demonstrated that these hereditary hypertriglyceridemic (HTG) Prague rats with disturbed glucose and lipid metabolism are also hypertensive (Štolba *et al.* 1992). Several membrane abnormalities were demonstrated in HTG rats (Devynck *et al.* 1998) and the close links between hypertension, hypertriglyceridemia and altered membrane electrolyte transport were supported by the demonstration of their association in F_2 hybrids of HTG and Lewis rats (Zicha *et al.* 1997). It has been shown that cross-sectional area and inner diameter of conduit arteries were decreased in HTG rats during the whole ontogeny (Kristek *et al.* 2003), but this was not accompanied by endothelial dysfunction (Török *et al.* 2003). HTG rats are also very useful for the search of candidate genes of hypertension and metabolic syndrome (Kadlecová *et al.* 2003) as well as for the search of genetic determinants of hypertension and/or dyslipidemia (Ueno *et al.* 2003a,b, 2004).

In the present study, we have searched for the possible polymorphism of *Cd36* gene in HTG rats – another rat model of metabolic syndrome and hypertension. We have also compared HTG rats with NIH-derived SHR in order to investigate the relation of *Cd36* gene polymorphism to alterations in glucose and fatty acid metabolism.

Methods

Animals

Age-matched inbred hereditary hypertriglyceridemic (HTG), spontaneously hypertensive (SHR/Ola), Wistar-Kyoto (WKY) and Lewis (LEW) male rats were received from the colonies of the Institute of Physiology AS CR (Prague). Lewis rats are used as a control strain for HTG rats because they are inbred and Wistar-derived as HTG rats are. All animals were given laboratory chow (ST1) and water *ad libitum* and maintained on a 12 h (7:00 - 19:00 h) light-dark cycle. A part of animals was drinking 10 % fructose solution from the 9th week of age for three weeks to enhance metabolic abnormalities. Ethical Committee of the Institute of Physiology AS CR approved all procedures.

Blood pressure determination

Systolic, diastolic and mean arterial blood pressures were measured at the age of 12 weeks under light ether anesthesia by direct puncture of the left carotid artery using Statham P23Db transducer and Hewlett-Packard 7754A recorder.

Biochemical measurements

Tail blood samples were taken, blood was centrifuged and serum was frozen for later diagnostics. The total serum triglycerides and cholesterol were assayed by commercial kits from Lachema (Brno, Czech Republic) and non-esterified fatty acids were measured by commercial test from Roche Diagnostics (Mannheim, Germany). Oral glucose tolerance test was performed in animals under basal conditions or after receiving of 10 % fructose solution (instead of water) as a drinking fluid for three weeks. Oral glucose tolerance test was started by a glucose load of 100 mg/100 g b.w. (water drinking rats) or 300 mg/100 g b.w. (fructose drinking rats) after overnight (14 h) fasting. Blood for glycemia determination was withdrawn from the tail at the intervals of 0, 30, 60 and 120 min after the intragastric glucose administration. Glucose concentration was measured by Glucotrend diagnostic kit (Roche Diagnostics).

DNA analysis

Genomic DNA was extracted from the liver by phenol/chloroform method. The PCR was performed with 50 ng genomic DNA and 25 pmol of each primer (RCD36FAT355F 5'-AGT TCG CTA TTT AGC CAA GG-3' and RCD36FAT675F 5'-AGG ATA AAA CAC ACC AAC TGT-3'). The DNA was amplified for 45 cycles with denaturation at 94 °C for 40 s, annealing at 60 °C for 30 s, and extension at 42 °C for 50 s. PCR products were analyzed by electrophoresis on 4 % agarose gel containing ethidium bromide and visualized by ultraviolet illumination.

Statistical analysis

Data are expressed as means ± S.E.M. Significance of the differences between groups was assessed by ANOVA. $P<0.05$ value was considered as significant.

Results

Table 1 summarizes the basal data about the four strains used. It is evident that blood pressure was significantly higher in both hypertensive groups in comparison with corresponding controls. Relative heart and kidney weights were significantly increased in SHR when compared to WKY rats. On the other hand, the relative kidney weight was significantly lower in HTG rats as compared to LEW control rats. Drinking of 10 % fructose for three weeks did not influence significantly body weight, relative organ weight and blood pressure in all strains studied (Table 1) except of HTG rats. There was significant decrease of body weight and increase of relative heart and kidney weight in HTG rats drinking 10 % fructose solution in comparison with HTG rats drinking water.

Table 1. Body weight, relative organ weight and blood pressure in spontaneously hypertensive (SHR), Wistar-Kyoto (WKY), Prague hereditary hypertriglyceridemic (HTG) and Lewis (LEW) rats.

	BW	HW/BW	KW/BW	SBP	MAP	DBP	PP
	g	mg/100 g b.w.		mm Hg			
Water drinking							
SHR	305±9	333±9**	655±15*	198±9**	160±7**	127±8**	70±5**
WKY	284±8	260±2	616±9	145±3	114±5	95±5	51±3
HTG	294±14	233±2	537±14**	162±8**	135±6**	115±5**	46±4
LEW	321±22	248±9	704±26	129±5	100±3	84±3	46±4
Fructose drinking							
SHR	301±10	331±2**	645±3	206±8**	167±6**	134±5**	72±5**
WKY	272±11	265±7	633±7	141±4	114±2	94±3	46±6
HTG	242±2**$^{+}$	292±4**$^{+}$	742±21^{+}	168±5**	137±5**	110±5**	58±1
LEW	366±12	242±4	700±18	144±2	107±3	84±2	60±4

Data are means ± S.E.M., BW – body weight, HW – heart weight, KW – kidney weight, SBP – systolic blood pressure, MAP – mean arterial pressure, DBP – diastolic blood pressure, PP – pulse pressure, *p<0.05, **p<0.01 from respective controls, $^{+}$p<0.01 from water drinking rats.

Table 2. Metabolic parameters measured in animals of four different strains drinking either water or 10 % fructose solution.

		SHR (n=6)	WKY (n=5)	HTG (n=6)	LEW (n=5)
TG (mmol/l)	H_2O	0.49±0.03**	0.36±0.03	2.37±0.21**	0.69±0.08
	F	1.27±0.21^{+}	1.08±0.10^{+}	3.57±0.22**$^{+}$	1.31±0.12^{+}
CHOL (mmol/l)	H_2O	2.05±0.18	2.34±0.29	1.84±0.06**	2.72±0.18
	F	4.05±0.44**$^{+}$	2.84±0.48	4.26±0.24^{+}	4.61±0.37^{+}
NEFA (mmol/l)	H_2O	1.39±0.17*	0.85±0.08	1.26±0.27	0.93±0.19
	F	0.74±0.03^{+}	0.68±0.13	0.60±0.08	0.78±0.09
Glucose (mmol/l)	H_2O	3.83±0.10	3.65±0.08	3.26±0.08	3.24±0.07
	F	6.26±0.14^{+}	5.60±0.39^{+}	5.90±0.52^{+}	5.13±0.35^{+}

Data are means ± S.E.M., TG – triglycerides, CHOL – total cholesterol, NEFA – non-esterified fatty acids, F – 10 % fructose as a drinking fluid, *p<0.05, **p<0.01 from respective controls, $^{+}$p<0.01 from water drinking rats.

There was significantly higher level of plasma triglycerides in both hypertensive strains in comparison with corresponding controls (Table 2). On the other hand, total plasma cholesterol level was lower in HTG

compared to LEW rats, but it was not different between SHR and WKY rats. The level of non-esterified fatty acids tended to be higher in both hypertensive strains, but the only significant difference was found between SHR and WKY rats. Drinking of 10 % fructose for three weeks influenced metabolic parameters to a higher extent (Table 2) than blood pressure or weight parameters (Table 1). Plasma levels of triglycerides, total cholesterol and glucose were significantly increased by fructose consumption in all four strains studied. In contrast, the level of non-esterified fatty acids tended to be decreased in all strains, but the significant difference was reached in SHR rats only.

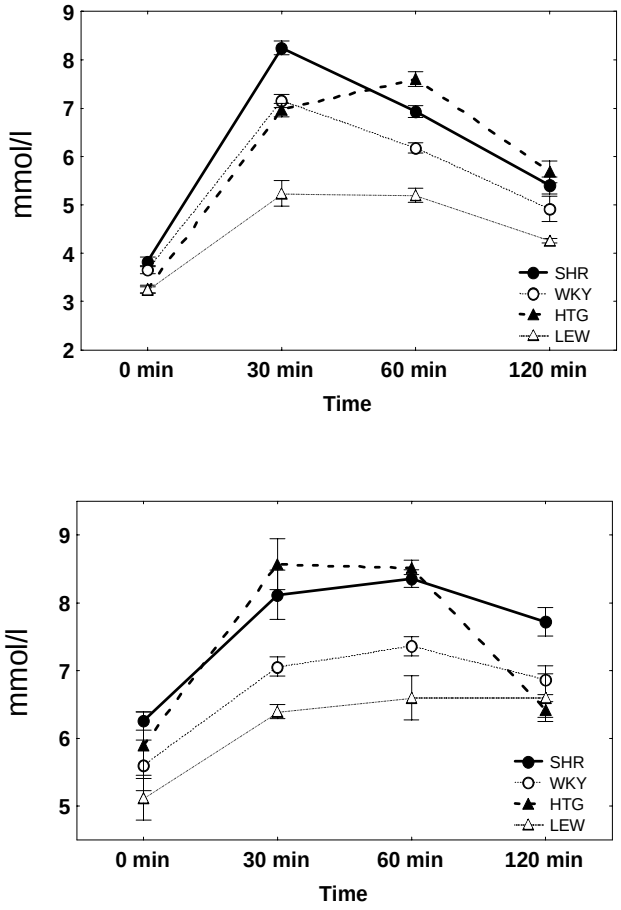

Fig. 1 Oral glucose tolerance test curves obtained in four different strains drinking either water (upper panels) or 10 % fructose solution (lower panels). Rats, fasted overnight, were given glucose gavage as mentioned in Material and Methods. Data for hypertensive strains (HTG and SHR, full symbols) and normotensive controls (LEW and WKY, open symbols) represent means ± S.E.M. with n = 5-6 rats per groups.

To investigate whether differences in *Cd36* gene affect glucose metabolism, the glucose tolerance test was performed in water or 10 % fructose drinking animals of all strains studied. Serum glucose levels were significantly higher in both hypertensive strains than in respective controls at 30 and 60 min after glucose loading (Fig. 1, upper panel). Drinking of 10 % fructose shifted glucose tolerance test curves to higher glucose levels in all strains, but the difference between hypertensive and normotensive strains remained preserved (Fig. 2, lower panel). Area under the curve (AUC) was not different between the two hypertensive strains, but it was significantly higher in hypertensive rats in comparison with both control strains (Table 3). Drinking of 10 % fructose instead of water increased significantly AUC in all four strains studied.

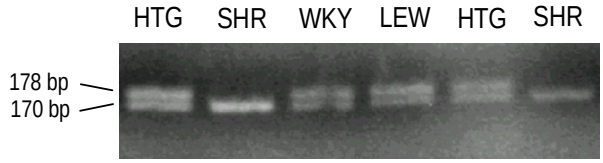

Fig. 2 Genotype analysis of PCR products derived from *Cd36* shown an 8-bp polymorphism between HTG and SHR/Ola strains. PCR products were run on 4 % agarose gel and visualized by ethidium bromide. A single band was seen in NIH-derived SHR/Ola strain, whereas two identical bands were identified in HTG and both normotensive control strains.

Genotype analysis of PCR products has shown that *Cd36* mutation was not present in HTG rats (Fig. 2) because two identical bands of PCR products were found in DNA of HTG, LEW and WKY rats. As expected, only one band was present in samples from NIH-derived SHR rats (SHR/Olac).

Discussion

Our study confirmed the presence of similar metabolic abnormalities in both hypertensive strains – HTG and SHR, but *Cd36* gene mutation was found in SHR only.

HTG rats were developed as a model of human hypertriglyceridemia (Vrána and Kazdová 1990). This model is, however, very useful for the study of the relationship among particular phenotypes of metabolic syndrome (e.g. hypertension vs hypertriglyceridemia, hypertension vs insulin resistance, etc.) as well as for the search of genetic background of these abnormalities. Recently we have demonstrated by using of HTG x LEW F_2 hybrids that sympathetic hyperactivity is one of the major pathogenetic factors in this form of genetic hypertension (Kuneš *et al.* 2002). Wide-range

phenotyping of these F_2 hybrids together with DNA analysis disclosed several significant quantitative trait loci for hypertension and hypertriglyceridemia (Ueno *et al.* 2003a,b).

Table 3. The area under the curve values (mmol/l/min) calculated after oral glucose tolerance test for four different rat strains drinking either water or 10 % fructose solution.

	SHR	WKY	HTG	LEW
Water drinking	779 ±9*$^{\#}$	695 ±13	770 ±13*$^{\#}$	568 ± 17
Fructose drinking	945 ±17*$^{\#+}$	833 ±14^{+}	922 ±17*$^{\#+}$	764 ± 18^{+}

Data are means ± S.E.M., Fructose – 10 % fructose as a drinking fluid, *p<0.01 from WKY, $^{\#}$p<0.01 from LEW, $^{+}$p<0.01 from water drinking rats.

As can be seen from our results, both HTG and SHR rats are hypertriglyceridemic in comparison with appropriate controls. This difference is even more pronounced in animals drinking 10 % fructose instead of water. On the other hand, plasma glucose level of fasting animals was the same in all four strains studied. If *Cd36* should play a unique role in carbohydrate metabolism and insulin resistance, one would expect the differences in oral glucose tolerance curves between HTG and SHR. This was not true because glucose tolerance test curves have shown similar characteristics in both HTG and SHR rats. Moreover, the comparison of area under the curve has indicated that differences between hypertensive and normotensive rats exist independently of control strain used (Table 3). In spite of the fact that the genetic background of both HTG and SHR originates from Wistar rats, it is evident that the genetic basis of insulin resistance in HTG and SHR is not the same.

It has been demonstrated in spontaneously hypertensive rats (SHR) that spontaneous deletion in *Cd36* gene (fatty acid translocator) is linked to the transmission of insulin resistance and defective fatty acid metabolism (Aitman *et al.* 1999, Pravenec *et al.* 2001). This seems to be true for NIH-derived SHR strains only because Gotoda *et al.* (1999) did not find the *Cd36* gene mutation in the original SHR maintained since their development in Izumo University (Japan). Our results have shown that *Cd36* mutation was not present in HTG rats despite the fact that their metabolic parameters are similar to those in SHR. Moreover, two identical bands were disclosed in HTG and both Wistar-Kyoto (WKY) and Lewis (LEW) normotensive control strains suggesting that *Cd36* can play a significant role in metabolic disturbances of NIH-derived SHR only.

The findings on the role of CD36 protein in the development of hypertension are discrepant. Pravenec *et al.* (2001, 2003) failed to reveal any consistent changes in blood pressure measured by radiotelemetry after the transfer of wild-type *Cd36* gene to NIH-derived SHR. On the contrary, Greenwalt *et al.* (1995) demonstrated increased level of CD36 protein in animal hypertensive models, suggesting that CD36 may be involved in blood pressure regulation. Recently, it was demonstrated in CD36 null mice fed a normal chow or high fat diet that the absence of CD36 did not affect resting blood pressure (Kincer *et al.* 2002). This is in a good agreement with our study in which HTG rats are hypertensive independently of the absence of *Cd36* gene mutation.

In conclusion, we have confirmed that defective *Cd36* gene is probably a candidate gene for disorded fatty-acid metabolism, glucose intolerance and insulin resistance in NIH-derived SHR, but other genes might play a role in pathogenesis of metabolic syndrome in Prague hereditary hypertriglyceridemic rats.

Acknowledgements

This work was supported by a research grant of Grant Agency of the Czech Republic (305/03/0769), by research project LN00A069 (Ministry of Education) and by the research project AVOZ 5011922.

References

AITMAN TJ, GLAZIER AM, WALLACE CA, COOPER LD, NORSWORTHY PJ, WAHID FN, AL-MAJALI KM, TREMBLING PM, MANN CJ, SHOULDERS CC, GRAF D, ST LEZIN E, KURTZ TW, KŘEN V, PRAVENEC M, IBRAHIMI A, ABUMRAD NA, STANTON LW, SCOTT J: Identification of Cd36 (FAT) as

an insulin-resistance gene causing defective fatty acid and glucose metabolism in hypertensive rats. *Nat Genet* **21**: 76-83, 1999.

DEVYNCK M-A, KUNEŠ J, LE QUAN SANG KH, ZICHA J: Membrane microviscosity and plasma triacylglycerols in the rat. *Clin Sci* **94**: 79-85, 1998.

FERRANNINI E, BUZZIGOLI G, BANADONNA R, GIORICO MA, OLEGGINI M, GRAZIADEI L, PEDRINELLI R, BRANDI L, BEVILACQUA S: Insulin resistance in essential hypertension. *N Engl J Med* **317**: 350-357, 1987.

GOTODA T, IIZUKA Y, KATO N, OSUGA J, BIHOREAU M-T, MURAKAMI T, YAMORI Y, SHIMANO H, ISHIBASHI S, YAMADA N: Absence of Cd36 mutation in the original spontaneously hypertensive rats with insulin resistance. *Nat Genet* **22**: 226-228, 1999.

GREENWALT DE, SCHECK SH, RHINEHART-JONES T: Heart CD36 expression is increased in murine models of diabetes and in mice fed a high fat diet. *J Clin Invest* **96**: 1382-1388, 1995.

HWANG I-S, HO H, HOFFMAN BB, REAVEN GM: Fructose-induced insulin resistance and hypertension in rats. *Hypertension* **10**: 512-516, 1987.

KADLECOVÁ M, DOBEŠOVÁ Z, ZICHA J, KUNEŠ J: Nitric oxide synthase isoforms in Prague hypertriglyceridemic rats (HTG), gene polymorphisms, protein expression and participation in blood pressure regulation. *Physiol Res* **52**: 51P, 2003.

KINCER JF, UITTENBOGAARD A, DRESSMAN J, GUERIN T, FEBBRAIO M, GUO L, SMART EJ: Hypercholesterolemia promotes a CD36-dependent and endothelial nitric-oxide synthase-mediated vascular dysfunction. *J Biol Chem* **277**: 23525-23533, 2002.

KRISTEK F, URMANSKÁ M, KUNEŠ J, DOBEŠOVÁ Z: Geometry of conduit arteries of hypertriglyceridemic rats during ontogenic development. *Physiol Res* **52**: 51P, 2003.

KUNEŠ J, DOBEŠOVÁ Z, ZICHA J: Altered balance of main vasopressor and vasodepressor systems in rats with genetic hypertension and hypertriglyceridaemia. *Clin Sci* **102**: 269-277, 2002.

PRAVENEC M, LANDA V, ZÍDEK V, MUSILOVÁ A, KŘEN V, KAZDOVÁ L, AITMAN TJ, GLAZIER AM, IBRAHIMI A, ABUMRAD NA, QI N, WANG J-M, ST LEZIN EM, KURTZ TW: Transgenic rescue of defective Cd36 ameliorates insulin resistance in spontaneously hypertensive rats. *Nat Genet* **27**: 156-158, 2001.

PRAVENEC M, LANDA V, ZÍDEK V, MUSILOVÁ A, KAZDOVÁ L, QI N, WANG J, ST LEZIN E, KURTZ TW: Transgenic expression of CD36 in the spontaneously hypertensive rat is associated with amelioration of metabolic disturbances but has no effect on hypertension. *Physiol Res* **52**: 681-688, 2003.

REAVEN GM, CHANG H: Relationship between blood pressure, plasma insulin and triglyceride concentration, and insulin action in spontaneous hypertensive and Wistar-Kyoto rats. *Am J Hypertens* **4**: 34-38, 1991.

REAVEN GM, HO H, HOFFMAN BB: Attenuation of fructose-induced hypertension in rats by exercise training. *Hypertension* **12**: 129-132, 1988.

SHEN DC, SHIEH SM, FUH MM, WU DA, CHEN YD, REAVEN GM: Resistance to insulin-stimulated-glucose uptake in patients with hypertension. *J Clin Endocrinol Metab* **66**: 580-583, 1988.

ŠTOLBA P, DOBEŠOVÁ Z, HUŠEK P, OPLTOVÁ H, ZICHA J, VRÁNA A, KUNEŠ J: The hypertriglyceridemic rat as a genetic model of hypertension and diabetes. *Life Sci* **51**: 733-740, 1992.

TÖRÖK J, KRISTEK F, GEROVÁ M, KUNEŠ J, DOBEŠOVÁ Z: Endothelial function of conduit artery in newborn rats with hereditary hypertriglyceridemia. *Physiol Res* **52**: 52P, 2003.

UENO T, TREMBLAY J, KUNEŠ J, ZICHA J, DOBEŠOVÁ Z, PAUSOVÁ Z, DENG AY, SUN Y-L, JACOB HJ, HAMET P: Resolving the composite trait of hypertension into its pharmacogenetic determinants by acute pharmacological modulation of blood pressure regulatory systems. *J Mol Med* **81**: 51-60, 2003a.

UENO T, TREMBLAY J, KUNEŠ J, ZICHA J, DOBEŠOVÁ Z, PAUSOVÁ Z, DENG AY, SUN Y, JACOB HJ, HAMET P: Gender-specific genetic determinants of blood pressure and organ weight: pharmacogenetic approach. *Physiol Res* **52**: 689-700, 2003b.

UENO T, TREMBLAY J, KUNEŠ J, ZICHA J, DOBEŠOVÁ Z, PAUSOVÁ Z, DENG AY, SUN YL, JACOB HJ, HAMET P: Rat model of familial combined hyperlipidemia as a result of comparative mapping. *Physiol Genomics* **17**: 38-47. 2004.

VRÁNA A, KAZDOVÁ L: The hereditary hypertriglyceridemic nonobese rat: an experimental model of human hypertriglyceridemia. *Transplant Proc* **22**: 2579, 1990.

ZICHA J, DOBEŠOVÁ Z, KUNEŠ J: Plasma triglycerides and red cell ion transport alterations in genetically hypertensive rats. *Hypertension* **30**: 636-640, 1997.

Reprint requests

Jaroslav Kuneš, DSc, Institute of Physiology AS CR, Vídeňská 1083, 142 20 Prague 4, Czech Republic. Fax: +420 2 4106 2488. E-mail: kunes@biomed.cas.cz