*Table 1. Characteristics of experimental rat strains*

| | Strains | | |
|---|---|---|---|
| | **PD/Cub** | **SHR/OlalpcvCub** | **BN/Cub** |
| Number of animals | 9 | 8 | 9 |
| Body weight (g)* | 361 ± 10[a] | 358 ± 12 | 332 ± 12 |
| Blood pressure (mm Hg)* | 138 ± 3 | 186 ± 4 | 122 ± 4 |
| Liver weight (% of body weight)* | 2.98 ± 0.08 | 3.12 ± 0.07 | 2.54 ± 0.03 |
| Kidney weight (% of body weight)* | 0.456 ± 0.010 | 0.546 ± 0.005 | 0.528 ± 0.016 |
| Epididymal fat weight (% of body weight)* | 1.559 ± 0.046 | 0.838 ± 0.044 | 0.838 ± 0.024 |
| Serum triglycerides (mmol/l)* | 3.55 ± 0.29 | 1.36 ± 0.09 | 1.02 ± 0.06 |
| Serum cholesterol (mmol/l) | 2.79 ± 0.14 | 2.50 ± 0.08 | 2.59 ± 0.16 |
| Serum FFA (mmol/l)* | 0.812 ± 0.085 | 0.734 ± 0.047 | 0.56 ± 0.029 |
| Serum glucose (mmol/l)* | 6.61 ± 0.19 | 6.22 ± 0.17 | 5.61 ± 0.20 |
| Serum insulin (pmol/l)* | 314 ± 26 | 282 ± 24 | 172 ± 18 |

[a]mean ± SEM
*these values significantly differ among strains (discussed in Results)

*Table 2. Effect of insulin on $^{14}$C-U glucose incorporation into adipose tissue total lipids in vitro (nmol/mg proteins/2 hours)*

| | Strains | | |
|---|---|---|---|
| | **PD/Cub** | **SHR/OlalpcvCub** | **BN/Cub** |
| Number of animals | 9 | 8 | 9 |
| Concentration of insulin (μU/ml buffer) | | | |
| 0* | 298.1 ± 0.24[a] | 326.9 ± 67.1 | 505.6 ± 14.2 |
| 250* | 556.4 ± 46.7 | 1116.2 ± 162 | 924 ± 34.0 |

[a]mean ± SEM
*these values significantly differ among strains (discussed in Results)

polymorphism between PD and SHR was found in 95 markers and in 115 markers between PD and BN strains.

Altogether 16 microsatellite markers were tested on chromosome 4, from which three are demonstrated in Fig. 1A (*D4Brol*), Fig. 1B (*Cd36/Fat*) and Fig. 1C introducing the polymorphism of the *Pparγ* gene. The *D4Brol* marker exhibits polymorphism in PD/Cub, SHR and BN strains, having clearly different lengths of PCR products (Fig. 1A). *Cd36/Fat* was also tested, because it was shown to be involved in the control of free fatty acids and insulin resistance (Aitman et al., 1999). The PCR product of *Cd36/Fat* was cleaved by *Hinf*I, resulting in different number of fragments in SHR (2 fragments) and BN (3 fragments), respectively. PD/Cub had the same number of fragments as the BN strain, which had no alterations in lipid metabolism (Fig. 1B). The polymorphism was revealed in *Pparγ* after *Rsa*I restriction (Fig. 1C); PD/Cub differs from both SHR and BN strains.

Eighty-four markers were evaluated on chromosome 8. The most important candidate for altered lipid metabolism on chromosome 8 was the *ApoaI/ApocIII/ApoaIV* gene cluster. In Fig. 1D we show the polymorphism of the microsatellite marker *D8Mit7* defining the *ApocIII* gene among PD/Cub, SHR and BN strains.

### *Metabolic Profile of PD/Cub, SHR and BN Strains*

Characteristics of several metabolic and physiologic phenotypes were determined in PD/Cub, SHR and BN rat strains (Tab. 1, Tab. 2). SHR had significantly (P < 0.0001) higher blood pressure as compared to both PD/Cub and BN strains. The PD/Cub strain had mildly elevated (P = 0.0004) blood pressure as compared to the normotensive BN strain. Liver weight (measured as % of body weight) was significantly (P < 0.0001) higher in both PD/Cub and SHR strains as compared to the BN strain. Kidney weight (measured as % of body weight) was significantly lower in the PD/Cub strain as compared to both SHR (P < 0.0001) and BN (P = 0.0015) strains. SHR had significantly higher kidney weight (P = 0.0058) as compared to the BN strain. Epididymal fat weight (measured as % of body weight) was significantly higher in PD/Cub (P < 0.0001) as compared to both SHR and BN strains (Fig. 2). Serum triglycerides were significantly (P < 0.0001) higher in PD/Cub as compared to both SHR and BN strains (Fig. 3). SHR had significantly (P = 0.0058) higher serum triglycerides as compared to the BN strain. Serum cholesterol did not significantly differ among strains. Serum free fatty acids (FFA) were significantly elevated in the

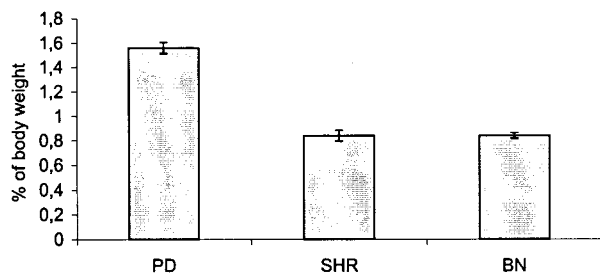


*Fig. 2.* Epididymal fat weight (mean ± SEM) in PD/Cub, SHR and BN strains. PD/Cub has significantly ($P < 0.0001$) higher epididymal fat weight as compared to both BN and SHR strains.

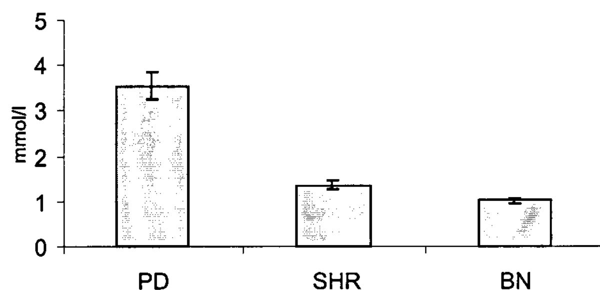


*Fig. 3.* Serum triglyceride concentrations (mean ± SEM) in PD/Cub, SHR and BN strains. Serum triglycerides are significantly ($P < 0.0001$) higher in PD/Cub as compared to both SHR and BN strains.

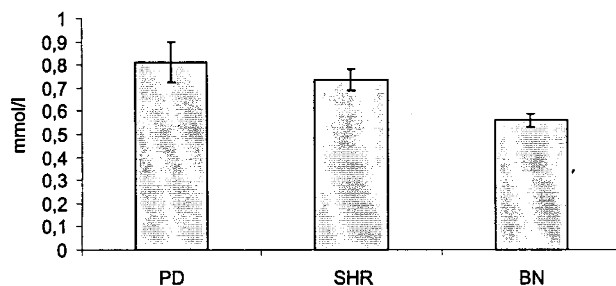


*Fig. 4.* Serum free fatty acids (mean ± SEM) in PD/Cub, SHR and BN strains. Serum FFA are significantly elevated in the PD/Cub strain ($P = 0.0094$) and SHR ($P = 0.0058$) as compared to BN.

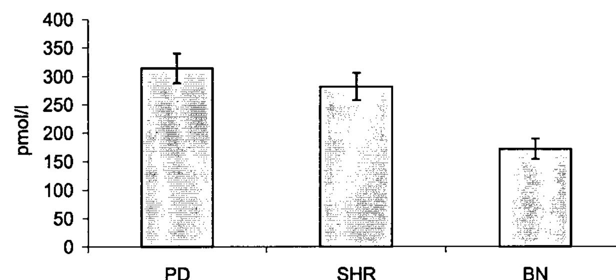


*Fig. 5.* Serum insulin concentrations (mean ± SEM) in PD/Cub, SHR and BN strains. Serum insulin concentrations were significantly higher in both PD/Cub ($P = 0.0004$) and SHR ($P = 0.0021$) strains.

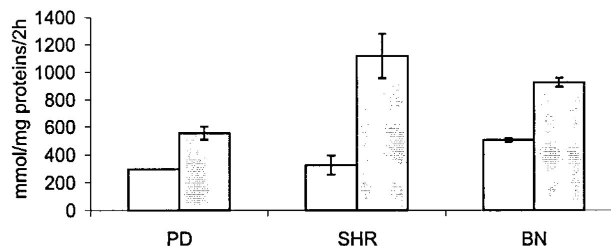


*Fig. 6.* Incorporation of $^{14}$C-U glucose into total lipids of the epididymal adipose tissue without (white bars) and with (grey bars) insulin stimulation (mean ± SEM) in PD/Cub, SHR and BN strains. The lowest basal and insulin stimulated incorporation was found in the PD/Cub strain epididymal adipose tissue ($P < 0.0001$) as compared to both SHR and BN strains.

PD/Cub strain ($P = 0.0094$) and SHR ($P = 0.0058$) as compared to BN (Fig. 4). Serum glucose was slightly, but significantly elevated in both PD/Cub and SHR strains. Serum insulin concentrations were significantly higher in both PD/Cub ($P = 0.0004$) and SHR ($P = 0.0021$) strains as compared to the BN control strain (Fig. 5). Significant differences were observed in $^{14}$C-U glucose incorporation into total lipids of the epididymal adipose tissue without or with insulin stimulation (Fig. 6). The lowest basal and insulin stimulated incorporation was found in PD/Cub strain epididymal adipose tissue ($P < 0.0001$) as compared to both SHR and BN strains.

## Discussion

Precisely defined animal models are essential for functional genomics and especially the rat offers many advantages for identification of gene functions in common human diseases, for which many rat models were developed (Jacob, 1999). The typical example of such a model strain for cardiovascular research is the spontaneously hypertensive SHR rat, which is also suggested to be a model strain of insulin resistance and hypertriglyceridemia (Aitman et al. 1999). However, the insulin resistance was not found in all the SHR substrains (Furukawa et al. 1998), although it always depends on the control strains used for comparison, as discussed by Pravenec et al. (1999a).

Insulin resistance, defined as decreased ability of insulin to stimulate glucose uptake, is a major feature of several common human disorders including type 2 diabetes, obesity, essential hypertension and hypertriglyceridemia. This group of disorders is sometimes referred to as the metabolic syndrome X (Reaven's syndrome). Insulin resistance is often considered to be the link between the metabolic syndrome X attributes. However, the molecular basis of defective insulin action is not yet fully understood (Reaven and Chen, 1988, 1995). All the traits of the X syndrome are not always expressed together in one strain. Our SHR substrain originally

transferred from Ola to Ipcv and then to Cub does not exhibit strong insulin resistance in the glucose uptake test; on the other hand, it has significantly elevated serum glucose concentrations and serum insulin concentrations as compared to the control BN strain, but does not reach the values of the PD/Cub strain. The variability in the expression of insulin resistance among different SHR substrains seems to indicate continuous distribution of this trait and thus strongly suggests multifactorial determination of this metabolic disorder. Syndrome X traits in the PD/Cub strain include mildly elevated blood pressure as compared to the BN strain, significantly increased amount of epididymal adipose tissue and elevation of plasma triglycerides, significantly elevated serum FFA, elevation of serum insulin levels and the lowest basal as well as insulin-stimulated incorporation of $^{14}$C-U glucose into total lipids of the epididymal adipose tissue (defining insulin resistance). The polygenic and/or multifactorial nature of syndrome X or familial combined hyperlipidemia (FCHL) requires to exploit any polymorphism revealed among putative candidate genes which any particular model offers in searching for the associations with distinct metabolic phenotypes. The *Cd36/Fat* gene was shown to control free fatty acid metabolism and is also involved in the determination of blood pressure and insulin resistance (Aitman et al., 1999). On the other hand, it was shown that the deletion variant of *Cd36/Fat* need not be directly connected with elevated blood pressure, because it was not found in stroke-prone SHR (Pravenec et al., 1999a). The *Cd36/Fat* mutation is not the necessary condition for insulin resistance, either (Gotoda et al., 1999), because the wild allele of *Cd36/Fat* was found in one SHR substrain with the clear-cut insulin resistance syndrome. *Cd36/Fat* is obviously not the only transporter protein for long chain fatty acids (Abumrad et al., 1999; Febbraio et al., 1999) and thus not the only determinant in fatty acid metabolism. Even higher levels of serum FFA following sucrose diet were proven in the PD/Cub strain compared to SHR, despite that the PD/Cub strain carries the wild allele of *Cd36/Fat.* Another gene involved in the regulation of lipid and carbohydrate metabolism is *Pparγ*, which is a member of the nuclear hormone receptor superfamily. *Pparγ* modulates expression of target genes by binding to peroxisome proliferator-activated receptor (PPAR) response elements as heterodimer with the retinoid X receptors (Kliewer et al., 1992). PPAR response elements have been identified in the regulatory regions of several genes encoding proteins involved in energy balance (Keller et al. 1993). *Pparγ* was found to mediate adipocyte differentiation (Tontonoz et al., 1994) and modulates insulin sensitivity and cell proliferation (Willson et al., 1996; Miles et al., 2000). It is therefore of interest to find out polymorphism of the *Pparγ* gene among our examined strains. It would be worth analyzing whether the PD allele of the *Pparγ* gene is somehow engaged in the development of dyslipidemia and insulin resistance of the PD/Cub strain. Such analysis might be accomplished by the derivation of BN × PD segregating population for association studies.

Several experimental data are supporting the role of the *APOAI/APOCIII/APOAIV* gene cluster in familial combined hyperlipidemia (FCHL) (Rees et al., 1983; Rees et al., 1986; Ordovas et al., 1991; Wojciechowski et al., 1991; Xu et al., 1994; Hegele et al., 1995; Shoulders et al., 1996). FCHL was described by Goldstein et al. (1973) as a distinctive genetic lipid disorder resulting in dramatically elevated plasma triglyceride levels and a secondary effect on plasma cholesterol levels. Subsequent genetic analyses indicated that the inheritance pattern was more complex and non-Mendelian, with the major gene acting at triglyceride levels (Iselius, 1981; Cullen et al., 1994). Genetic defects underlying FCHL are yet far from being fully understood. The strongest evidence for involvement in FCHL is associated with three important apolipoproteins, namely APOAI, APOCIII, and APOAIV, which are major constituents of high-density and triglyceride-rich lipoproteins, and are located within a 15 kilobase gene cluster on the long arm of human chromosome 11 (Karathanasis et al., 1985; Bruns et al., 1984). In the rat two significant quantitative trait loci (QTLs) on chromosomes 1 and 8 were identified in a backcross population of (OLETF × BN) × OLETF that are related to fasting triglyceride levels (Okuno et al., 1999). The chromosome 8 QTL was localized to the *D8Mit2* marker in a region carrying the apolipoprotein gene cluster including the *ApocIII* locus. It should be mentioned that *ApocIII*-defining marker was found closely linked to the *Lx* mutation, which is determining polydactyly in PD/Cub strain on chromosome 8. The segment of rat chromosome 8 with *ApocIII* exhibits homology with human chromosome 11q23. Moreover, transgenic mouse lines expressing the human *APOCIII* gene were created and found to have elevated triglyceride concentrations (Ito et al., 1990). This suggests an important role for *APOCIII in vivo.* On the other hand, in a recent paper on human FCHL (Aouizerat et al., 1999), a linkage with a locus on the short arm of human chromosome 11, which does not contain any strong candidate genes, is indicated and proclaims support for the concept that FCHL is complex and heterogenous.

The extensive genotyping of chromosome 8 with 84 microsatellite markers has its reason in the characterization of differential segments in several SHR.BN/PD congenic sublines (Křen et al., 2000). Differential segments of congenic sublines contain several candidate genes for blood pressure control, cardiac mass (Koike et al., 1998; Křen et al., 1997) and hypertriglyceridemia (Vrána et al., 1993) as well as leg malformation (Křen et al., 1996). For the same reason another two chromosomes (4 and 10) were also genotyped with more markers (16 and 10) in order to reveal polymorphisms between PD, and SHR and BN strains, respectively.