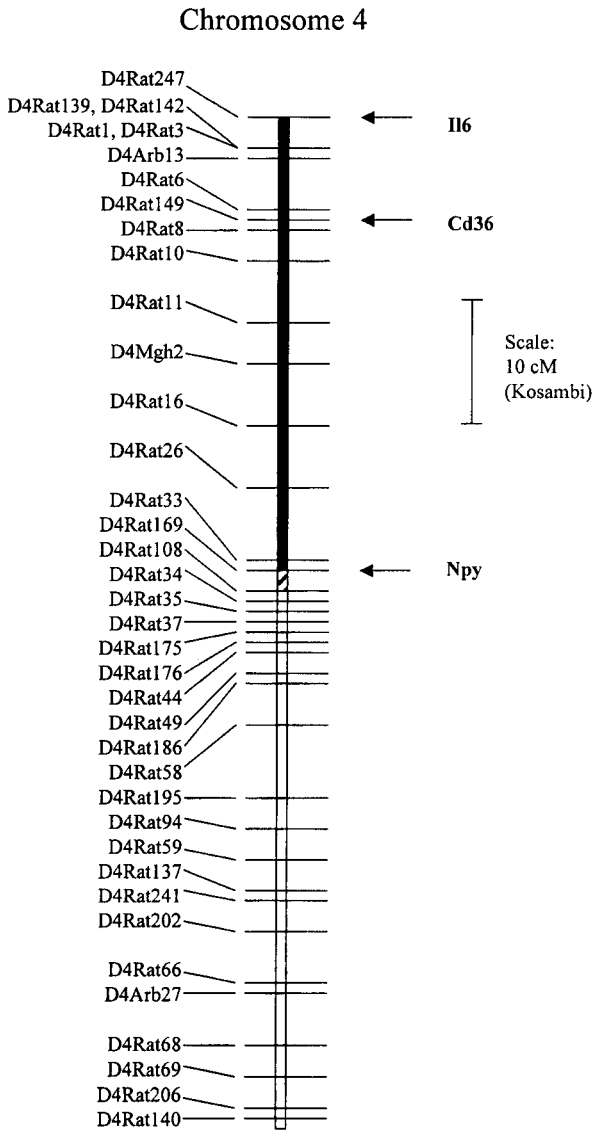

Fig. 2. Linkage map showing the transferred segment of chromosome 4 in the SHR.BN-Il6/Npy (SHR-4) congenic strain. The solid bar denotes the chromosome region from the BN strain that has been fixed in the homozygous state on the SHR background. The cross-hatched bar denotes a region in which some residual heterozygosity may exist within the congenic strain. The open region denotes the flanking segment of SHR chromosome. Map distances are adapted from chromosome 4 maps of Jacob et al., (1995), Pravenec et al. (1996), and Steen et al. (1999).
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fasting glucose levels between fructose-fed SHR and fructose-fed congenic rats. However, after intraperitoneal injection of a glucose load, the peak glucose levels in the SHR progenitor rats were significantly higher than in SHR-4 congenic rats (Pravenec et al., 1999) (Fig. 4).

In addition to glucose tolerance testing, we measured plasma insulin/glucose ratios before and after feeding the high-fructose diet to 8-week-old rats for 15 days. Before fructose loading, the fasting insulin/glucose ratios were similar in the SHR progenitor and SHR-4 congenic rats (Fig. 5). However, after fructose loading, the SHR exhibited a marked increase in insulin/glucose ratios whereas the SHR-4 congenic rats did not. The insulin/glucose ratios of the fructose-fed SHR progenitor rats were significantly greater than in the SHR-4 congenic rats (Fig. 5) (Pravenec et al., 1999). These results proved that a transfer of a segment of chromosome 4 from the BN strain onto the SHR genetic background induced a significant amelioration of glucose intolerance and a decrease in the insulin/glucose ratio after feeding a high-fructose diet. Glucose tolerance testing and determination of the insulin/glucose ratios is, however, not sufficient to prove insulin resistance at the cellular level. Therefore, we tested the effects of insulin directly at the cellular level, in isolated adipocytes.

Adipocytes isolated from SHR-4 congenic rats fed a normal diet demonstrated significantly greater insulin-mediated glucose uptake than those isolated from SHR progenitor rats also fed the normal diet (Fig. 6). Fat-cell volume and body weight were similar in the SHR progenitor and SHR-4 congenic strains, excluding fat-cell volume as a possible cause of this metabolic defect (Aitman et al., 1999). These results confirmed that transfer of a segment of chromosome 4 from the BN strain into the SHR increases insulin-stimulated glucose uptake and ameliorates insulin resistance at the cellular level.

In subsequent *in vivo* studies in SHR progenitor and SHR-4 congenic rats fed a high-fructose diet for 15 days, we have also found that plasma nonesterified fatty acid (NEFA) levels were significantly lower in the congenic strain than in the SHR progenitor strain. Serum triglycerides were also lower in SHR-4 congenic rats versus SHR progenitor rats (Fig. 7) (Pravenec et al., 1999).

Mutations in Cd36 are linked to defects in insulin action and fatty acid metabolism in the SHR

In the SHR, a significant advance has recently been made in understanding the molecular basis for disordered carbohydrate and lipid metabolism that could shed light on the clustering of systemic cardiovascular risk factors in human essential hypertension. Given that the congenic segment of chromosome 4 was known to regulate carbohydrate and lipid metabolism in isolated adipocytes, it was elected to use cDNA microarray analysis of adipose tissue to search for genes differentially expressed in fat between the SHR progenitor strain and the SHR-4 congenic strain. In the gene-profiling studies of adipose tissue, one particular gene was observed to show a dramatic difference in expression between the SHR progenitor strain and SHR-4 congenic strain (see Fig. 8). No other genes showed this marked degree of differential expression (10 fold) and therefore, attention was immediately focused on this gene. The gene was found to be Cd36, which mapped directly back within the differential chromosome segment. Subsequent molecular studies

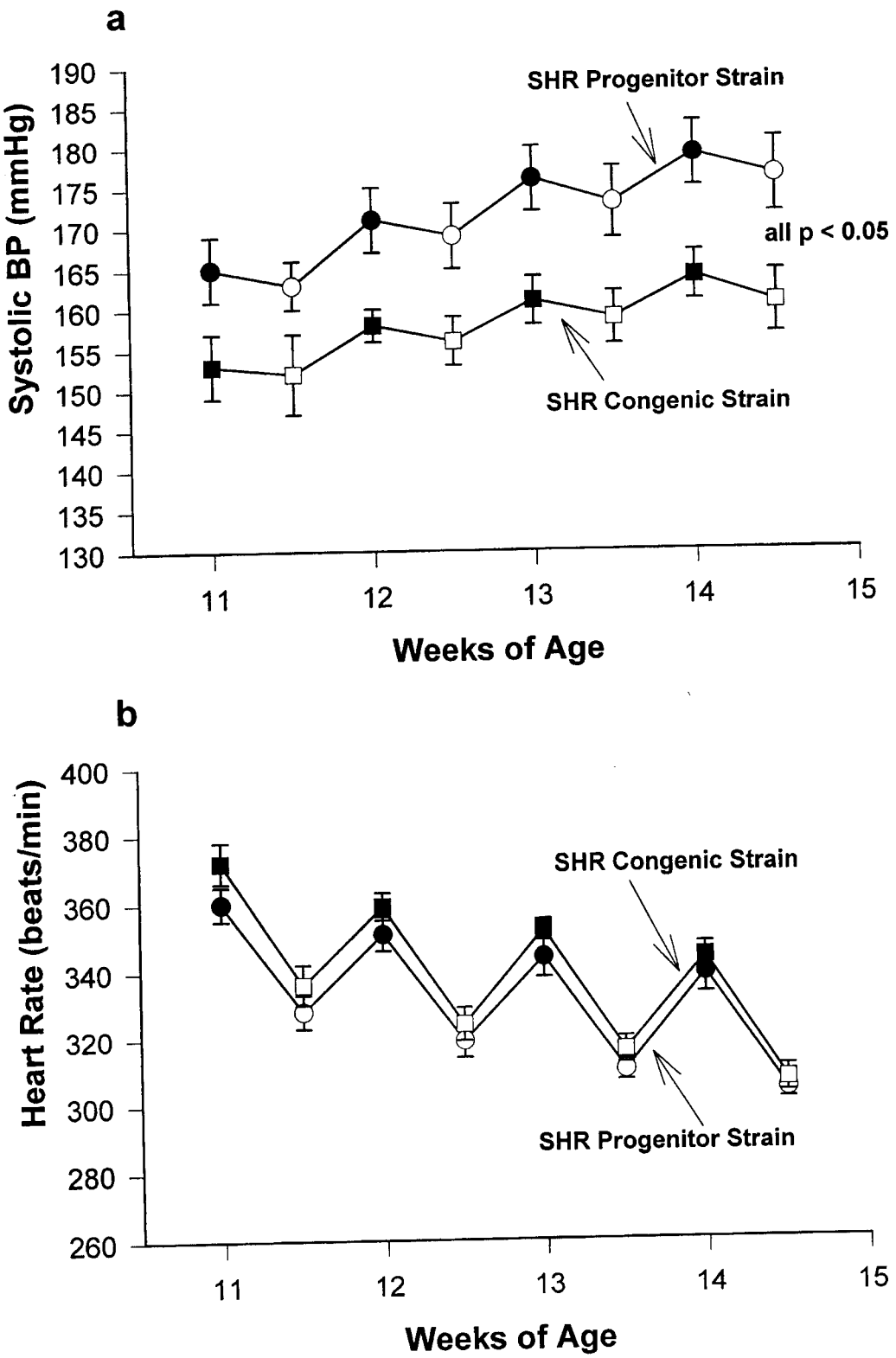

Fig. 3. Daytime and nighttime average systolic blood pressures (panel a) and heart rates (panel b) determined by radiotelemetry over a period of 4 weeks in the SHR progenitor strain and the SHR-4 congenic strain. Each data point represents the weekly daytime (open symbols) or nighttime (solid symbols) average blood pressure or heart rate (mean ± SEM) in the SHR progenitor strain (circles, n = 7) and the SHR-4 congenic strain (squares, n = 7). The saw-tooth pattern reflects the circadian variation in blood pressure and heart rate. Systolic blood pressure was significantly lower in the SHR-4 congenic strain than in the SHR progenitor strain during all daytime and nighttime periods. The heart rates were not significantly different between the two strains.
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