Segment of Rat Chromosome 20 Regulates Diet-Induced Augmentations in Adiposity, Glucose Intolerance, and Blood Pressure

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Abstract—Previous linkage and association studies have suggested that a region of human chromosome 6 containing the tumor necrosis factor (TNF)-α gene is involved in the pathogenesis of obesity and obesity-associated hypertension. The aim of the present investigation was to establish whether a segment of rat chromosome 20 (RNO20), which also contains the TNF-α gene, determines diet-induced changes in adiposity and blood pressure (BP). The results showed that a transfer of the RNO20 segment from the normotensive Brown Norway (BN) rat onto the background of the spontaneously hypertensive rat (SHR) is associated with a significantly greater increase in adiposity, glucose intolerance, circulating leptin levels, and BP during 12-week, high-fat-diet feeding. In contrast, the transfer is not associated with significant changes in these variables during 12-week, normal-diet feeding. In addition, sequencing of the TNF-α gene revealed differences between SHR and BN in the 5′- and 3′-regulatory regions of the gene. Subsequent analyses of TNF-α gene expression in fat, muscle, and liver, however, did not provide support for the functional involvement of these differences. In summary, the investigated RNO20 segment contains 1 or more gene variants that affect adiposity, glucose tolerance, serum leptin levels, and BP, but only when the animals are exposed to a particular environment, ie, high-fat-diet feeding. Further studies are needed to identify genes mediating these effects. Considering current changes in our lifestyle involving an increased calorie and fat intake, we believe that gene-environment interactions, such as those described here, play an important role in the current epidemic of obesity and obesity-associated hypertension. (Hypertension. 2003;41:1047-1055.)

Key Words: hypertension, obesity ■ diet ■ genetics ■ glucose ■ tumor necrosis factor ■ gene-environment interaction

Obesity is a leading risk factor for a number of metabolic and cardiovascular disorders, including diabetes, dyslipidemia, and hypertension. These disorders frequently cluster together in an individual; in this case, they are referred to as metabolic syndrome X. In addition, they also cosegregate in families, suggesting that they may be determined by genes with pleiotropic effects on adiposity, glucose tolerance, lipid metabolism, and blood pressure (BP). Alternatively, the cosegregation of these disorders may be due to the effects of several closely linked genes that are transmitted together from generation to generation within families. The prevalence of obesity, diabetes, dyslipidemia, and hypertension is rising in most modern human societies. Much of the increase has been attributed to changes in our lifestyle involving both less physical activity (decreased energy expenditure) and greater caloric intake (increased energy intake). Although environmental influences are important for the development of obesity, genetic makeup plays an essential role, in that it can modify the response of an individual to such influences. It has been demonstrated that overfeeding identical twins with an extra 1000 kcal per day for a period of 3 months resulted in an increase of body weight, which was significantly more similar within twins than between twins. Several forms of gene-environment interactions exist; most typically, they are understood as effects of gene variants that become evident only when exposed to a particular environment. Considering the current trend in our lifestyle, we believe that gene-environment interactions involving environmental factors, such as diet, play an important role in the pathogenesis of obesity and associated comorbidities.

Several pathophysiological mechanisms have been implicated in obesity-associated glucose intolerance, dyslipidemia, and hypertension. Most of them are related to excess body fat. This is because the development of adipocyte hypertrophy and hyperplasia is associated with altered production of endocrine and paracrine/autocrine signals that contribute, in
to the regulation of adipogenesis, glucose tolerance, sympathetic nervous activity, renal sodium reabsorption, and hemostasis.\(^7\)–\(^9\) These signals include, for example, tumor necrosis factor (TNF)-\(^\alpha\), leptin, adiponectin, resistin, and plasminogen activator inhibitor-1.\(^9\)–\(^14\)

Previous linkage and association studies suggest that a region of human chromosome 6, which contains the TNF-\(^\alpha\) gene, plays a significant role in the development of obesity and obesity-associated insulin resistance and hypertension.\(^15\)–\(^18\) The current investigation was designed to further our understanding of these effects. Based on the above studies, it appears that the relationships between the TNF-\(^\alpha\) gene locus and body weight, insulin sensitivity, and BP exist mainly in overweight and/or obese individuals.\(^15,17,18\) Therefore, we hypothesized that the TNF-\(^\alpha\) gene demonstrates its effects on adiposity, insulin sensitivity, and BP mainly in the environment of excess body fat accumulation. Several lines of evidence support such a possibility. It has been demonstrated that adipocyte and muscle production of TNF-\(^\alpha\) is related to the degree of adiposity.\(^10,19\) It has been also shown that TNF-\(^\alpha\) is involved in the regulation of lipogenesis and lipolysis\(^20\)–\(^22\) and in the induction of obesity-associated insulin resistance.\(^10,23,24\) In addition, TNF-\(^\alpha\) regulates the adipocyte expression and secretion of leptin\(^23\) that, in turn, has been shown to influence BP.\(^7,26,27\)

**Methods**

**Strains**

Two inbred strains of rats, namely, the spontaneously hypertensive rat (SHR/Ola) and a congenic strain, SHR.1N, were studied. Both strains have been maintained at the Centre de recherche, Centre hospitalier de l’Université de Montréal (Montréal, Canada) since 1996, when they were transferred from the Czech Academy of Sciences in Prague (Czech Republic).\(^28\) The congenic strain SHR.1N was derived from SHR/Ola (termed SHR from here onwards) and normotensive Brown Norway (BN.Lx/Cub) rats. It differs from SHR by a segment RNO20 that is of BN.Lx/Cub (called BN from here onwards) origin and includes the TNF-\(^\alpha\) gene.\(^29\) A total genome scan with 255 informative markers, carried out in the present study, confirmed the congenic status of SHR.1N.
Experimental Protocol

Sixteen-week-old male SHR and SHR.1N rats were placed on either a normal diet (ND; 5P14, Prolab 2500; 3.4 kcal/g) or HFD (F3282, Bio-Serv; 5.3 kcal/g) for 12 weeks. During this period, body weight (BW) and food consumption were monitored weekly. Glucose tolerance was assessed by the intraperitoneal glucose tolerance test at weeks 0, 6, and 12 of the dietary intervention. Systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR), and locomotor activity (ACT) were measured by radiotelemetry also at weeks 0, 6, and 12 of the intervention for 3 to 14 consecutive days.

At the end of the intervention, the animals were killed by decapitation under 4% isoflurane gas anesthesia, and their epididymal fat, retroperitoneal fat, soleus muscle, and liver were removed and snap-frozen. Their sera were collected for the determination of leptin by radioimmunoassay (Linco Research, Inc) and of total cholesterol, triglycerides (TG), and free fatty acid (FFA) levels by enzymatic assays, as described previously.30 The animals were killed in a nonfasted state (after regular overnight feeding, between 8 AM and 12 noon), because fasting has been shown to reduce circulating leptin levels.31

The intraperitoneal glucose tolerance test was carried out in conscious rats after an overnight fast at 12 noon. Two grams per kilogram of body weight (30% glucose in 0.9% NaCl) was injected. Blood samples were drawn from the tail vein, and glucose concentration was measured with a glucometer (One Touch Basic) at 0, 15, 30, 60, 120, and 180 minutes after the injection (Bayer, Inc). Glucose tolerance was assessed as the area under the glycemia curve (glucose AUC).

SBP, DBP, HR, and ACT were measured in unanesthetized and unrestrained rats with radiotelemetry transducers placed in the lower
abdominal cavity and connected to catheters implanted in the lower abdominal aorta (Data Sciences International, Inc). Before the dietary intervention, the animals were allowed to recover for 3 weeks after implantation of the radiotelemetry transducers. SBP, DBP, HR, and ACT were recorded in 10-second bursts every 1.5 minutes. From these data, 24-hour averages were calculated for each rat, and these values were subjected to statistical analyses.

The data presented in this article have been collected in 3 separate experiments. Adiposity and glucose tolerance were assessed in all 3 experiments (24 SHR and 24 SHR.1N rats fed HFD and in 16 SHR and 14 SHR.1N rats fed ND). Serum and gene expression analyses were carried out in 2 of the 3 experiments (15 SHR and 15 SHR.1N rats fed HFD and 11 SHR and 9 SHR.1N fed ND). Telemetry recording was performed in randomly selected animals from the latter subset (9 SHR and 9 SHR.1N rats fed HFD and 6 SHR and 6 SHR.1N rats fed ND).

Sequence Analysis of the TNF-α Gene in SHR and SHR.1N
The whole TNF-α gene and 600 bp of the promoter region were sequenced directly from three overlapping polymerase chain reaction products (Laboratory of Nucleic Acid Analysis and Synthesis, Université Laval, Quebec). Each product was sequenced in at least 3 SHR.1N and 2 SHR rats selected at random. The promoter and 5′-untranslated region (UTR) were sequenced in 9 SHR.1N and 6 SHR rats. In these animals, the promoter, 5′-UTR, and 3′-UTR were sequenced twice to verify nucleotide differences between the SHR and SHR.1N strains. In addition, 5 of these differences were verified with the following restriction enzymes: MspI, BsaAI, AcII, and BssSI in 9 SHR.1N and 6 SHR.

Gene Expression Analyses
Total RNA was extracted from retroperitoneal fat, epididymal fat, skeletal muscle (soleus muscle), and liver. Expressions of the TNF-α gene was determined by semiquantitative reverse transcription–polymerase chain reaction (RT-PCR, QuantumRNA Classic II Internal Standards kit, Ambion, Inc). TNF-α cDNA was amplified with primers designed with the aid of Primer3 software (www-genome.wi.mit.edu/cgi-bin/primer/primer3.cgi) from GenBank sequences (L00981). All reactions were carried out in duplicate.

Statistics
The data were analyzed by 2-way ANOVA with strain (SHR vs SHR.1N) and diet (ND vs HFD) as the main factors (SPSS for Microsoft Windows, v 11.5); simple main effects of strain within each diet group were assessed.

Results
Comparison of SHR and SHR.1N Before, During, and at the End of the 12-Week Dietary Intervention
Before the 12-week dietary intervention, BW did not differ between SHR and SHR.1N (SHR, 349.8 ± 3.3 g and SHR.1N, 349.2 ± 3.3 g). During the intervention, cumulative BW increments were significantly higher in both strains fed HFD than in those fed ND. Importantly, the BW gain in animals fed HFD was greater in SHR.1N than in SHR, whereas in animals fed ND, it did not differ between the 2 strains (Figure 2A). Consistently, the weight of epididymal fat pads measured at sacrifice was higher in both strains fed HFD than in those fed ND. In animals fed HFD, the weight was greater in SHR.1N than in SHR, and in animals fed ND, it did not differ between the 2 strains (Figure 2B). No significant strain differences on either diet were observed in energy intake (SHR/ND, 75.5 ± 2.9 kcal/d; SHR.1N/ND, 70.6 ± 2.9 kcal/d; SHR/HFD, 109.3 ± 4.3 kcal/d; and SHR.1N/HFD, 113.6 ± 5.4 kcal/d).

The glucose AUC, as an index of glucose tolerance, did not differ among the 4 experimental groups at week 0 of the dietary intervention (Figure 3). At weeks 6 and 12, in
contrast, the glucose AUC was higher in both strains fed HFD than in those fed ND. Furthermore, in animals fed HFD, the glucose AUC was significantly higher in SHR.1N than in SHR, and in animals fed ND, it did not differ between the 2 strains (Figure 3). The strain differences on HFD were mainly due to significant dissimilarities in serum glucose levels at 30, 60, and 120 minutes after glucose administration (Figure 3).

Before the dietary intervention, SBP and DBP were higher in SHR than in SHR.1N (Figures 4A and 4B). At weeks 6 and 12, SBP and DBP became similar in SHR and SHR.1N fed HFD and remained different in SHR and SHR.1N fed ND (Figures 4A and 4B). In contrast, no strain differences in HR and ACT were observed before or during the dietary intervention (Figures 4C and 4D). In both strains, though, a significant effect of diet was present at weeks 6 and 12 for HR and at week 6 only for ACT; at these time points, HR and ACT were higher in the HFD groups than in the ND groups (Figures 4C and 4D). These data suggest that the strain differences in adiposity cannot be attributed to differences in locomotor activity.

Nonfasted serum leptin levels rose in both strains fed HFD compared with both strains fed ND (Figure 5A). In animals fed HFD, the levels were significantly higher in SHR.1N than in SHR, and in animals fed ND, they did not differ between the 2 strains (Figure 5A). Serum TGs and FFAs were also increased in both strains fed HFD compared with those fed ND. In contrast, however, no strain differences were observed on either HFD or ND; neither diet nor strain showed a significant influence on serum levels of total cholesterol (Figures 5B to 5D). Taken together, these results suggest that the investigated segment of RNO20 determines HFD-induced augmentations in adiposity, glucose intolerance, circulating leptin, and BP but not in HR, ACT, and serum levels of FFAs and TGs.

Sequence Analysis of the TNF-α Gene in SHR and SHR.1N
Because TNF-α has been previously implicated in the regulation of adipogenesis, insulin resistance, and leptin production,10,20,21,24,25,33 we sequenced the TNF-α gene in SHR and SHR.1N. A total of 26 sequence differences in the TNF-α gene were identified between SHR and SHR.1N (Figure 6). From these, 2 variants were found in the promotor region, 3 in the coding region, 8 in the 3'-UTR, and 14 in intronic sequences. The 3 sequence differences in the coding region were synonymous, suggesting that TNF-α does not differ structurally between the 2 strains. On the other hand, the observed sequence differences in the noncoding regions might have an influence on gene expression and mRNA stability and processing. For example, a nucleotide change at −308 of the human TNF-α gene promotor has been associated with transcriptional activation of the cytokine.34 In addition, 1 of the nucleotide changes in the 3'-UTR (at position 6507) is located within an imperfect “TTATTTAT” element, which is characteristic of cytokine genes and makes
their mRNAs more prone to degradation. Furthermore, most of the intronic differences were identified in intron 3. Interestingly, this intron is the most evolutionarily conserved of the TNF-α and TNF-β genes. This indicates a possible functional importance of the intron. In addition, a relatively high number of sequence differences have been identified within the gene between SHR and SHR.1N. This is consistent with the fact that the TNF-α gene is located within the RT1 complex that is characterized by a high degree of genetic variability.

**TNF-α Gene Expression in Retroperitoneal Fat, Epididymal Fat, Skeletal Muscle, and Liver**

The strain differences in the TNF-α gene observed in the noncoding regions might have an influence on gene expression and mRNA stability and processing. Therefore, we examined mRNA levels in metabolically active tissues. These analyses showed that, in retroperitoneal fat and liver, HFD feeding compared with ND feeding is associated with a decrease in TNF-α gene expression and that this decrease is of a similar degree in both strains (Figure 7). In epididymal fat and muscle, no significant strain and diet effects were observed (Figure 7). Thus, these results do not provide support for the involvement of the TNF-α gene in the development of the observed strain differences in HFD-induced augmentations in adiposity, glucose intolerance, and BP.

**Discussion**

The results of the present investigation demonstrate that 12-week HFD feeding leads to augmentations in adiposity, glucose intolerance, serum leptin levels, and BP that are all greater in SHR.1N than in SHR and to elevations in HR and serum TGs, FFAs, and cholesterol levels that do not differ between the 2 strains. The results also demonstrate that 12-week ND feeding does not induce any strain differences in any of the above-mentioned variables. These results suggest that the investigated RNO20 segment contains 1 or more gene variants that affect adiposity, glucose tolerance, serum leptin levels, and BP, but only when they are exposed to a particular environment. This pattern of genetic and environmental effects represents a typical example of gene-environment interactions.

The present investigation supports the findings of previous candidate-gene studies demonstrating that the region of human chromosome 6, which contains the TNF-α gene, may contribute to the pathogenesis of obesity and obesity-associated hypertension and insulin resistance. Based on the known functional properties of TNF-α, namely, its involvement in the regulation of lipolysis/lipogenesis, insulin resistance, and leptin production, the TNF-α gene has been considered the most likely candidate within the chromosomal region (www.ensembl.org, AF129756.1.1.184666). However, the present investigation does not provide support for the involvement of the TNF-α gene. Although a number of sequence differences between SHR and SHR.1N were identified in the regulatory regions of the TNF-α gene, no significant gene-diet interactions in its mRNA expression were observed. This could be due to the fact that the observed HFD-induced strain differences in adiposity, glucose tolerance, and BP develop as a result of a cumulative effect of small changes in TNF-α expression, which are difficult to detect in a cross-sectional manner. Alternatively, it could be due to the fact that the animals were sacrificed in a nonfasted state, which might not be optimal for the measurement of...
dietary alterations in TNF-α gene expression. In addition, the investigated segment of RNO20 is ≈30 cM long, and, as such, may contain other genes that determine the observed effects. Among others, these include the peroxisome proliferator-activated receptors (PPAR)-δ and retinoid X receptor (RXR)-β genes. PPAR-δ is a transcription factor that is activated by fatty acids and controls the initial stages of adipocyte differentiation. RXR-β is a molecule that forms a heterodimer with PPAR-δ. Further studies are needed to explore these possibilities.

In the present investigation, it was the BN variant of the investigated chromosomal segment that, when compared with the SHR variant, determined greater adiposity, glucose intolerance, and BP responses to HFD. Several previous investigations have demonstrated that the BN strain is more environmentally sensitive than the SHR strain. Most of these studies examined the impact of a high-fat/high-cholesterol diet on serum lipids. To the best of our knowledge, however, the effect of HFD on adiposity and BP has not been compared between SHR and BN. It has been demonstrated, though, that SHR compared with other normotensive strains, namely, Wistar-Kyoto or Sprague-Dawley rats, are resistant to weight gain in response to HFD feeding; they either do not increase BW or increase it less than does the normotensive strain. Regarding a BP response to HFD, conflicting results have been obtained in SHR. Although some studies showed that chronic feeding with HFD elevates BP, others demonstrated that it decreases BP.

Finally, the data of the present study also suggest that the investigated segment of rat chromosome 20 contains 1 or more genes that are involved in the regulation of BP under standard dietary conditions. The effect of the segment on BP under standard dietary conditions is consistent with some but not all previous studies. A BP quantitative trait locus (QTL) was identified within the differential chromosomal segment of SHR.1N in a set of recombinant inbred strains derived from the same progenitors (SHR and BN.Lx/Cub) as the congenic strain studied here. In addition, the existence of the QTL was confirmed by generating a congenic strain. A BP QTL within the same region of rat chromosome 20 was also identified in a cross involving New Zealand genetically hypertensive and BN rats. No significant effect of the differential segment was found in some other studies, however.

Perspectives
The results of this study suggest that a specific segment of RNO20 contains 1 or more genes that are involved in the regulation of adiposity, glucose tolerance, circulating leptin levels, and BP in response to overfeeding. As such, the current study supports the findings of previous human investigations demonstrating that a homologous region in the human genome includes genes that determine susceptibility...
to the development of obesity and associated comorbidities, namely, insulin resistance and hypertension.

In light of the current changes in our lifestyle involving greater calorie and fat intake and decreased physical activity, gene-environment interactions, such as those described here, might be the common causes of obesity and associated comorbidities. Further research into identifying specific genes might uncover involved pathophysiological pathways and thus, provide scope for developing drugs that would prevent or ameliorate the pathological consequences of overfeeding.

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13. Berg AH, Combs TP, Du X, Brownlee M, Scherer PE. The adipocyte-

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References


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