Reversibility of Chronic Experimental Syndrome X by Diet Modification

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Abstract—This study was designed to examine whether abnormalities that comprise the metabolic syndrome, including insulin resistance, hyperinsulinemia, hypertension, hyperlipidemia, and obesity, are reversible by diet. Female Fischer rats were placed on either a high-fat, refined-carbohydrate (HFS) diet or low-fat, complex-carbohydrate (LFCC) diet for a period of 20 months. After 20 months, a group of HFS rats were switched to the LFCC diet (HFS/LFCC) for a period of 2 months. Skeletal muscle glucose transport, plasma insulin, systolic blood pressure, and plasma lipids were measured in all groups after 22 months. Energy intake and body weight were measured weekly. In the HFS group, insulin-stimulated glucose transport was significantly reduced (67±4 versus 98±4 pmol·mg⁻¹·15 s⁻¹), whereas plasma insulin (300±49 versus 82±8 pmol/L), blood pressure (147±4 versus 123±4 mm Hg), plasma triglycerides (2.58±0.31 versus 0.39±0.04 mmol/L), LDL cholesterol (C) (3.45±0.40 versus 0.89±0.06 mmol/L), LDL-C to HDL-C ratio (2.9±0.1 versus 2.2±0.1), VLDL-C (1.53±0.23 versus 0.37±0.07 mmol/L), Total-C (5.56±0.58 versus 1.49±0.10 mmol/L), and body weight (360±11 versus 260±5 g) were all significantly elevated compared with the LFCC. Energy intake did not differ significantly; however, the LFCC had a much poorer feed efficiency. Conversion to a LFCC diet for 2 months led to normalization of glucose transport, blood pressure, plasma insulin, and VLDL-C and significant amelioration of obesity and other lipid abnormalities. These results demonstrate that syndrome X induced by an inappropriate diet is reversed with implementation of a low-fat, unrefined-carbohydrate diet without caloric restriction and suggest that diet may be a possible treatment for multiple simultaneous cardiovascular risk factors.

(Key Words: blood pressure • insulin • lipids • obesity • syndrome X)

The metabolic syndrome, ie, "syndrome X,"¹ refers to the aggregation of atherosclerotic risk factors, including insulin resistance/hyperinsulinemia, dyslipidemia (including small, dense LDL particles, hypertriglyceridemia, and increased VLDL particles), and hypertension. This syndrome is also characterized by additional abnormalities, including obesity, endothelial dysfunction, depressed HDL cholesterol (C), and a hypercoagulable state.² Three The syndrome has also been named the "Insulin-Resistance Syndrome" to stress the presumed role of insulin resistance as an underlying defect.⁴,⁵ We previously reported that diet can induce several characteristics of the metabolic syndrome.⁶,⁷ When female Fischer rats were raised for 2 years on a high-fat (primarily saturated fat), refined-carbohydrate (sucrose) diet (HFS), similar to the typical diet in the United States, the animals developed skeletal muscle insulin resistance, hyperinsulinemia, hypertriglyceridemia, hypertension, enhanced blood coagulation, and obesity, marked by disproportionately severe abdominal obesity.⁶−⁸ Insulin resistance has been demonstrated in animal and human studies to precede other manifestations of the syndrome.⁴,⁹,¹⁰

The prevalence of the metabolic syndrome is extremely high in Westernized societies; it has been estimated that it affects between 25% to 35% of the population.¹¹ Accordingly, the examination of modifiable variables (ie, diet and lifestyle) is of major importance, because this abnormal profile greatly increases the risk for coronary artery disease, diabetes, and other chronic disorders.¹ Consequenly, we set out to assess whether it is possible to reverse any of the manifestations of the syndrome once they had been chronically established, because long-term inappropriate diet consumption may lead to irreversible metabolic and/or structural changes, prohibiting amelioration of the risk profile. To this end, the present study was designed to test the reversibility of abnormalities associated with long-term metabolic syndrome induction by diet in female Fischer rats raised on the HFS diet for 20 months by switching them to the low-fat, complex-carbohydrate (LFCC) diet for a 2-month period.

Methods

Animals and Diet

All protocols were conducted in accordance with the University of California, Los Angeles, Animal Research Committee. Inbred fe...
male Fischer rats were obtained at 2 months of age from Harlan Sprague Dawley, Inc (San Diego, Calif). We have used this rat model in our previous studies because the female Fischer rat shows little growth after its maturation phase.6,8 The rats were randomly assigned to either the LFCC or HFS diet. The animals were housed 4 per cage, with a 12-hour light cycle starting at 7:00 AM at 75°F to 76°F. The diets, fed ad libitum and with large bowls placed in the cages to assure that all animals had access to the food, were prepared in powder form by Purina Test Diets Inc and contained a standard vitamin and mineral mix and all essential nutrients. The LFCC diet is low in saturated fat and contains mostly complex carbohydrates (starch), whereas the HFS diet is high in saturated and monounsaturated fat (primarily from lard plus a small amount of corn oil) and high in refined sugar (sucrose). The diet components are listed in Table 1. The LFCC diet is ground up standard rat chow (Purina 5001), which we have documented to elicit no differences in plasma insulin and glucose transport (R.J.B. et al, unpublished data, 2000) compared with the LFCC diet used in our previous studies,6,7 thus we have adopted this diet as the LFCC diet. After 20 months on the HFS diet, there was a reduction in food intake for the first few days more energy. When the HFS rats were switched to the LFCC diet, there was no statistical difference in energy intake among any of the subgroups of HFS rats designated for continuation on the LFCC diet or conversion to the LFCC diet at the time of randomization. After the 2-month switch to the LFCC diet, body weight significantly decreased, but it did not reach the value found in the LFCC rats at 22 months (P<0.05, ANOVA, Figure 1).

The energy intake for the HFS and LFCC groups for the first year was published previously.8 We documented a slight, nonsignificant increase in total energy intake in the HFS group. During the final 2-month period when some of the HFS rats were switched to the LFCC diet, there was no statistical difference in energy intake among any of the groups; however, once again, the HFS rats consumed slightly more energy. When the HFS rats were switched to the LFCC diet, there was a reduction in food intake for the first few days followed by an increase in food intake. The average daily energy intake for the final 2-month period were as follows, respectively: LFCC, 173.2±2.36 kJ/d; HFS, 182.2±6.63 kJ/d; and HFS/LFCC, 171.0±5.01 kJ/d.

The energy intake per gram of body weight gained, termed the FE, was calculated as a digestive and metabolic indicator of the ease that energy consumed was added as body weight. The FE was much poorer in the LFCC group compared with the LFCC diet, body weight significantly decreased, and at 96 weeks, all 3 groups were significantly different from each other (P<0.05, ANOVA). Values are mean±SE from 16 rats in each group.

Values reported are mean±SE, with 7 to 8 rats per group unless otherwise indicated.

**Results**

**Body Weight, Caloric Intake, and FE**

By the end of the 20-month period, the HFS group weighed significantly more than the LFCC group (P<0.01, Figure 1). No significant difference was found in body weight between the subgroups of HFS rats designated for continuation on the HFS diet or conversion to the LFCC diet at the time of randomization. After the 2-month switch to the LFCC diet, body weight significantly decreased, but it did not reach the value found in the LFCC rats at 22 months (P<0.05, ANOVA, Figure 1).

**Blood Chemistry**

After an overnight fast, the rats were anesthetized with chloral hydrate (250 mg/kg, IP), and blood was obtained via cardiac puncture. Blood samples were centrifuged, and the plasma was frozen at −70°C until insulin determination. Insulin was quantified in the plasma using a double-antibody radioimmunoassay with materials obtained from Ventrex Laboratories. Plasma concentrations of total cholesterol (Total-C), LDL-C, HDL-C, VLDL-C, and triglycerides (TG) were determined using standard laboratory techniques.

**Blood Pressure**

Systolic blood pressure was measured by the tail-cuff method, as previously described.14

**Statistical Analysis**

Data from the experiments were analyzed using ANOVA when 3 groups were compared and a t test when 2 groups were compared. When significant F values were noted, post hoc analyses were performed using the Newman-Keuls Multiple Comparison Test. Differences were considered statistically significant at P<0.05.
months, skeletal muscle glucose transport increased significantly. We did not measure fasting blood glucose because this was measured previously and because the HFS rats exhibited a nonsignificant elevation compared with the LFCC rats.

**Plasma Insulin**

The fasting plasma insulin levels of all groups of rats were measured after 22 months on the diets. Consistent with the decrease in insulin-stimulated glucose transport in the HFS rats, the fasting plasma insulin levels were significantly elevated in the HFS rats compared with the LFCC rats ($P<0.05$, Figure 2). When the HFS rats were switched to the LFCC diet, there was a decrease in the fasting plasma insulin level, which is consistent with the increase in glucose transport.

**Plasma Lipids**

Fasting plasma Total-C, LDL-C, VLDL-C, and TG and the LDL-C/HDL-C ratio were markedly increased in the HFS group as compared with the other groups ($P<0.01$, ANOVA, Table 2). These abnormalities were significantly alleviated in the HFS/LFCC group compared with the HFS group ($P<0.01$); however, aside from VLDL-C, the plasma lipid levels did not fully normalize to the level of the LFCC group ($P<0.05$). The Total-C/HDL-C ratios for the 3 groups were as follows: LFCC, 4.10; HFS, 5.06; and HFS/LFCC, 4.52 ($P<0.05$).

**Blood Pressure**

Systolic blood pressure measured after 22 months was significantly elevated in the HFS rats compared with the LFCC rats ($P<0.05$, Figure 2). In fact, systolic blood pressure exceeded 140 mm Hg in all HFS rats but in none of the LFCC rats. After 2 months of switching to the LFCC diet, blood pressure in the formerly hypertensive, HFS group returned to a normotensive state.

**Discussion**

There is evidence that high-fat and/or refined-carbohydrate diets can induce features of the metabolic syndrome in rats,\textsuperscript{6,7,15,16} and we recently documented that diet-induced insulin resistance precedes the other characteristics of the metabolic syndrome.\textsuperscript{6} Human studies have also revealed that insulin resistance is present before other metabolic syndrome abnormalities.\textsuperscript{4,9,10} Furthermore, individuals in Westernized societies typically consume diets high in saturated fat and processed carbohydrates throughout their life span. Accordingly, the present study was designed to investigate whether chronic metabolic syndrome abnormalities induced by the HFS diet could be reversed by diet modification.

The present data are the first to demonstrate, using an ad libitum LFCC diet for 2 months, simultaneous reversibility of numerous metabolic syndrome-associated abnormalities despite long-term HFS-diet consumption. Furthermore, it is important to note that the changes observed in the HFS rats in the present study are due to diet, not aging per se, because in the LFCC group (control) none of the parameters studied appreciably changed during the course of the study (except body weight).

The present study, demonstrating diet-induced insulin resistance using the HFS diet, agrees with our previous work\textsuperscript{17} as well as that of others.\textsuperscript{15} A key finding in the present study, however, is reversal of long-term insulin resistance and hyperinsulinemia within 2 months of switching from the HFS diet to the LFCC diet. These data suggest that despite prolonged impairment of carbohydrate metabolism, diet therapy can potentially reverse insulin resistance and hyperinsulinemia. The induction of hyperinsulinemia with the HFS diet may be due to the high sucrose, which is a disaccharide, and the low-fiber content,
which would elicit different insulin kinetics compared with the
high-fiber LFCC diet, despite the lower carbohydrate content of the HFS diet (40% versus 59% of energy). Amelioration of the defects in insulin-receptor autophosphorylation and tyrosine kinase activity may govern the reversal of diet-induced insulin resistance in this animal model.18

Previously, we demonstrated that changes in blood pressure are delayed and are one of the final abnormalities to manifest in the metabolic syndrome.6 In the present study, hypertension was present in the HFS animals after 22 months on the diet, and 2 months after switching to the LFCC diet, the blood pressure was normalized. It was recently noted in the DASH (Dietary Approaches to Stop Hypertension) clinical trial19 that a diet low in refined sugar and with reduced saturated fat and high fruit and vegetable intake (sources of antioxidants) rapidly decreased blood pressure in both hypertensive and normo-
tensive individuals. We have recently demonstrated that long-term HFS-diet consumption induces oxidative stress, which promotes inactivation and sequestration of nitric oxide by reactive oxygen intermediates, thereby decreasing endothelium-dependent relaxation via a reduction in nitric oxide availability.14 We also recently reported reversal of HFS diet–induced endothelial dysfunction and hyp-
tension in male rats when they were switched from the HFS diet to a LFCC diet.20

The present study also demonstrates that a HFS diet induces hypertriglyceridemia and an increase in VLDL-C, which is in agreement with our previous studies reporting elevated TG concentrations on a HFS diet.6–8 The me-
chanism by which the HFS diet increases plasma TG is thought to include increased hepatic TG production and VLDL secretion.21,22 The high-fat content of the diet (∼39% of energy) combined with the hyperinsulinemia resulting from the high-refined sugar content (∼40% of energy) increase the production of apoprotein B-100 and leads to hypertriglyceridemia.23 Increased adipocyte hormone-sensitive lipase activity may also contribute by increasing free fatty acid availability for hepatic TG formation and subsequent VLDL secretion.24 In addition, the hypertriglyceridemia occurs despite an increase in adipocyte lipoprotein lipase activity that increases TG storage, probably caused, in part, by a concomitant decrease in muscle lipoprotein lipase activity.8 When the HFS animals were switched to the LFCC diet, the hyper-
triglyceridemia was ameliorated. This is in agreement with data in humans25 and suggests that when saturated fat and refined sugar in the diet are substituted with natural, unrefined carbohydrates high in fiber, there is a reduction in the production and/or an increase in the clearance of TG-rich particles. Conversely, several studies note a rise in insulin and/or TG with low-fat diets,26,27 which is due to the use of refined carbohydrates and/or inappropriate isocaloric diets.

Additionally, this study demonstrates that the HFS diet increases the Total-C and LDL-C concentration as well as the LDL-C/HDL-C ratio. Previous studies have reported a downregulation of the hepatic LDL-receptor (LDL-R) gene expression on a high-fat diet. Saturated fat, in the form of coconut oil28 or lard,29 has been shown to decrease hepatic LDL-R mRNA levels in baboons. Woollett et al30 showed that saturated fat decreases LDL-R activity compared with a control, high-carbohydrate diet. Hara et al31 using 125I-labeled LDL-C, demonstrated that substituting satu-
rated fat with complex carbohydrates decreased LDL-C levels and increased the binding affinity of LDL for its receptor. From these data, it appears that the increase in VLDL, which is the precursor of LDL, and a decrease in hepatic clearance of LDL, because of an associated down-
regulation of LDL-R expression, are responsible for the increase in plasma LDL-C seen on a HFS diet. Interest-
ingly, we have recently documented a decrease in both adipose tissue and skeletal muscle VLDL-R and hepatic LDL-R with long-term HFS-diet consumption (C.K.R. et al, unpublished data, 2000). Elevation of plasma Total-C and LDL-C concentrations were coupled with a marked increase in the LDL-C/HDL-C ratio in the HFS group, pointing to a deleterious effect of the HFS diet on the cardiovascular system, which was reversible when switched to the LFCC diet.

The present data suggest that obesity can be partially reversed by implementation of a low-fat, unrefined-carbohydrate diet, without caloric restriction. We measured food consumption in all groups, which was provided ad libitum to the rats. Except for the first few days when the HFS/LFCC group consumed less food compared with the LFCC group, the caloric intake was similar between the LFCC and the HFS/LFCC groups, indicat-
ing that the animals did not have an aversion for the LFCC diet, and thus caloric restriction cannot be responsible for the changes noted, for example, in insulin sensitivity.32 However, the non-
significant decrease in caloric intake between the HFS and the HFS/LFCC groups can explain a portion of the weight loss noted in the HFS/LFCC group. We cannot unequivocally attribute our overall findings to the diet per se rather than the resultant weight loss. However, it is evident from the findings that the dietary changes, not caloric restriction, induced weight loss, as well as the other changes noted. In addition, it has been demonstrated that humans spontaneously decrease caloric intake once adopting a low-fat, high-fiber diet.33,34 Furthermore, the LFCC diet contained significantly more fiber than the HFS diet; thus, the reduced digestible and metabolizable energy content of the LFCC diet probably contributed to the weight loss, because it has been shown that the digestibility of the diet affects FE and weight gain.35 The better FE in the HFS diet despite similar energy consumption supports this contention.

We previously reported that the HFS diet resulted in severe obesity, with 38% of body weight as fat, and significant abdominal obesity.7 The HFS group lost significant body weight after implementation of the LFCC diet for 2 months. Although body fat per se was not measured in the HFS/LFCC group, we observed that this group not only had significantly less fat, but also much less in the abdominal cavity. Furthermore, the change in body weight may have been caused by increased physical activity and energy expenditure associated with improved biological condition of the LFCC animals. However, it should be noted that we have measured body temperature and daily
activity in the HFS and LFCC animals and found no difference between diet groups for either parameter (R.J.B. et al, unpublished data, 2000).

Although several investigators have suggested that obesity is the cause of the metabolic syndrome, these studies indicate an association rather than direct causality. There is evidence that insulin resistance and hyperinsulinemia precede the development of obesity. In addition, others have documented that there is a relationship between insulin levels and other metabolic syndrome factors (ie, blood pressure), independent of obesity. Conversely, it has been demonstrated that severe caloric restriction and weight loss is associated with restoration of insulin respon- siveness in humans and improvement of insulin resistance. It should be noted that calorically restricted diets cannot be sustained for long periods, as evidenced by the failure of weight-reducing programs in the United States. However, in the present study, the animals were fed ad libitum, a feeding schedule that could be sustained for long periods. Finally, the present data do not allow conclusions to be drawn as to which aspect of the diet is responsible for the changes noted. However, our goal was to formulate a diet that was representative of a typical Western-type diet. Previously, we documented that refined carbohydrates had a more deleterious effect than saturated fat alone on glucose tolerance, but their combined effect was even more detrimental.

Overall, the present study indicates that a low-fat, high-fiber diet is an effective strategy for prevention and reversal of several of the abnormalities of the metabolic syndrome. In fact, every abnormality measured in the present study was reversed or significantly ameliorated on adoption of an ad libitum LFCC diet. The results obtained with the LFCC diet in this study may help explain the previously documented success in humans in controlling aspects of the metabolic syndrome. Future studies are necessary to assess whether weight loss per se contributes to the changes observed in this animal model.

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