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Rat Chromosome 8 Confers Protection against Dyslipidemia Caused by a High Fat/Low Carbohydrate Diet

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Abstract

Background/Aims—Recent studies have highlighted the importance of gene by diet interactions in contributing to risk factors of metabolic syndrome. We used a consomic rat panel, in which a chromosome of the Brown Norway (BN) strain is introgressed onto the background of the Dahl Salt-Sensitive (SS) strain, to test the hypothesis that these animals will be useful for dissecting gene by diet interactions involved in metabolic syndrome.

Methods—We placed the parental SS and BN strains on a low fat/high carbohydrate (LF) or high fat/low carbohydrate (HF) diet for 22 weeks and measured several indices of metabolic syndrome. We then investigated the effect of diet in eight consomic rat strains.

Results—We show that the HF diet resulted in significantly increased levels of fasting plasma cholesterol and triglycerides in the SS strain, with no effect in the BN. Both strains responded to the HF diet with slight increases in body weight. SSBN8 was the only consomic strain that resembled that of the BN, with low levels of fasting cholesterol and triglycerides even on the HF diet.

Conclusions—These results indicate that BN chromosome 8 harbors a gene or genes that confer protection against dyslipidemia caused by the HF diet.

Keywords

Metabolic Syndrome; Consomic Rat Strains; Gene by environment interactions; genetic mapping

Introduction

Multiple metabolic risk factors, such as increased blood pressure, dyslipidemia, raised fasting glucose and abdominal obesity contribute to the development of metabolic syndrome

[1]. Metabolic syndrome is increasing at alarming rates and can lead to increased mortality due to the development of heart disease, type 2 diabetes and stroke [2]. It has been shown that metabolic syndrome results from a combination of poor diet, inactivity and genetics [3]. In addition, recent studies have highlighted the important role of the interaction between genes and diet in aspects of this disorder (i.e., [4-7]).

Rodent models can be useful in further elucidating the role of genes and environment on metabolic syndrome. Rats on a high fat diet can develop several risk factors of metabolic syndrome including increased body weight, increased cholesterol and triglycerides, glucose intolerance and increased blood pressure. Within an outbred Sprague-Dawley population only a subset of rats on a high fat diet develops these risk factors indicating a genetic interaction with diet [8, 9]. Furthermore, several inbred rat and mouse strains become obese on a high fat diet, while others do not, again indicating an interaction of genes with diet [10-13]. Using rodent models, multiple quantitative trait loci (QTL) have been identified for metabolic syndrome and its underlying symptoms (i.e., [14, 15]), and several loci have been shown to interact with diet (i.e., [16-18]).

Our laboratory maintains a panel of consomic rats in which each chromosome from the BN (Brown Norway) rat has been introgressed onto the background of the SS (Dahl Salt Sensitive) rat. This panel is used as a genetic tool to determine chromosomes that harbor genes involved in complex traits [19], and has been used specifically to identify chromosomes involved in hypertension [20-22]. Other panels, such as the Lyon hypertensive (LH) consomic strain (LH-17(BN)) has shown utility for traits involved in metabolic syndrome [23]. The goal of the current study was to employ the use of consomic rats to identify a chromosome or chromosomes that carry a gene(s) that may be responsible for the development of metabolic risk factors after exposure to a high fat/low carbohydrate diet.

Methods

All rats were obtained from the Medical College of Wisconsin in Milwaukee, WI. Consomic rats were derived from inbred normotensive BN/Mcwi rats and salt-sensitive hypertensive Dahl SS/JrHsd/Mcwi rats, referred to herein as BN and SS strains. At weaning (3 weeks of age), male rats of each strain were placed on a high (45% of energy) or low (12% of energy) fat diet (see Tables 1 & 2 for diet information). To maintain a similar percentage of protein between the two diets, amount of carbohydrates significantly decreased in the high fat diet. These diets will be referred to as high fat/low carbohydrate (HF) and low fat/high carbohydrate (LF) diets in the remainder of the manuscript. All diets were purchased from TestDiet, a division of LabDiet (Purina Mills, Richmond, IN). All experiments were approved by either the IACUC committee at the Medical College of Wisconsin or at Beloit College. Three different experiments were performed, as detailed below.

Experiment 1

We initially tested the parental male BN and SS strains to determine if the two strains react differently to the HF diet. We measured several indices of metabolic syndrome after 8-10 weeks on the diet and again after 18-20 weeks on the diet. At both time-points, we measured fasting plasma cholesterol (8 and 18 weeks on diet), glucose tolerance (9 and 19 weeks on

diet) and blood pressure (10 and 20 weeks on diet). Fasting plasma triglycerides were only measured after 18 weeks on the diet. Body weight and food intake were measured weekly. Animals were sacrificed after 22 weeks on the diet and retroperitoneal, epididymal and mesenteric fat pads were dissected and weighed.

To measure glucose tolerance levels, animals were fasted overnight for 16 ± 1 hours. In the morning, a basal sample of blood was taken from the tail and subsequent samples were collected 15, 30, 60, 90 and 120 minutes after a glucose (1 g per kg body weight) injection into the peritoneal cavity. We used the Ascensia Elite system for reading blood glucose values (Bayer Corp., Elkhart, IN).

Systolic blood pressure was measured by the tail-cuff plethysmograph in awake, but restrained animals. Temperature of the plethysmograph chamber was 83 degrees F at the time measurements were taken. Animals were placed in a restrainer within the chamber and a rubber cuff was placed around the base of the rat's tail. The cuff was inflated to approximately 250 mmHg to briefly occlude blood flow to the tail and the cuff pressure was slowly reduced over approximately 20 seconds. When the pressure in the cuff reached the systolic blood pressure of the rat, pulsatile blood flow occurred through the tail artery, which was detected by a photoelectric sensor. The cuff pressure at which the flow was detected was recorded as the systolic blood pressure. Animals were trained for this protocol by exposing them to the restraint for approximately 20 minutes the day prior to data collection. Blood pressure measurements were collected over three days. On each day, the animals were subjected to 20 repeats of the cuff inflating. The blood pressure readings were averaged to obtain the systolic blood pressure for each animal.

Experiment 2

Upon establishing that BN and SS strains respond differently to the HF diet, we tested the effect of HF diet on body weight and fasting plasma cholesterol and triglycerides in eight different consomic strains. The consomic strains included: SSBN4, 5, 8, 10, 16, 17, 18, 20. Consomic strains were chosen based on location of candidate genes or QTL previously identified in human or rat for obesity and/or other metabolic related traits [14, 15, 24, 25]. To quickly assess if one or more of these chromosomes harbors a gene that is protective against the effects of the HF diet, we ran only a small number of animals in each group (two to three). While more animals are required for statistical confidence, the small number of animals was used simply as a means of determining which chromosome(s) to follow-up using additional animals in experiment 3 (see below). Consomic rats were placed on either the LF or HF diet at the time of weaning. Fasted plasma cholesterol and triglyceride levels were measured after 20 weeks on the diet. As no differences were seen in the parental strains, we did not measure glucose tolerance or blood pressure in the consomic strains. Body weight and food intake were measured weekly. Animals were sacrificed at 22 weeks of age and fat pads were dissected and weighed.

Experiment 3

Results from experiment 2 suggested that BN chromosome 8 may be protective against high levels of cholesterol and triglycerides caused by the HF diet. To confirm these results, we

increased the number of animals from this consomic strain and repeated Experiment 2, detailed above.

Statistics—Using the SPSS statistical package, we ran a repeated-measures two-way ANOVA to determine effect of strain and diet on body weight and Kcal consumption over time. We used a two-way ANOVA to determine the effects of strain and diet on all other phenotypes (fasting plasma levels of cholesterol and triglycerides, glucose tolerance levels, blood pressure and percentage of fat pad weights). Where significant interactions were detected, a follow-up one-way ANOVA was conducted separately by strain.

Results

Experiment 1

There was a significant main effect of diet ($F_{1, 18} = 5.7$, $p < 0.05$), with the HF diet slightly increasing body weight in both BN and SS strains (see Figure 1a). The weight gain was likely due to the fact that both BN and SS rats on a high fat diet consumed more daily Kcal than those on a LF diet ($F_{1, 18} = 33.1$, $p < 0.001$) (see Figure 1b). Independent of diet, SS rats weighed more than BN rats ($F_{1, 18} = 209.7$, $p < 0.001$), as previously shown [26], and consumed significantly more daily Kcal than BN rats ($F_{1, 18} = 621.8$, $p < 0.001$). There was a significant effect of both diet ($F_{1, 18} = 7.7$, $p < 0.05$) and strain ($F_{1, 18} = 20.1$, $p < 0.001$) on percent body fat of retroperitoneal fat pads (see Figure 2 and Table 3). The increase in retroperitoneal fat pad weight on a HF diet was seen in both SS and BN strains; however, a larger difference was seen in the SS strain. Upon running a one-way ANOVA separately in SS and BN animals, the effect of diet on retroperitoneal fat pad weight was only significant in the SS strain ($F_{1, 8} = 5.3$, $p = 0.05$). While there was a significant effect of strain ($F_{1, 18} = 9.2$, $p < 0.01$) on percent epididymal fat pad weight, the effect of diet did not reach significance levels ($F_{1, 18} = 3.8$, $p = 0.066$). The SS strain exhibited increased epididymal fat pad weight relative to BN, and a greater difference in fat pad weight based on diet (see Table 3). A significant effect of strain was also found for percent of mesenteric fat pad weight ($F_{1, 18} = 33.1$, $p < 0.001$), with SS rat showing a larger percent fat pad weight than BN. No effect was seen for diet on percent mesenteric fat pad weight (see Table 3).

Diet differentially affected fasting levels of plasma cholesterol and triglycerides in the two strains after being on the diet for 18 weeks (see Figure 3). At the early time-point, after only eight weeks on the diet, an effect of strain was found for fasting plasma levels of cholesterol ($F_{1, 23} = 17.8$, $p < 0.001$), with SS animals exhibiting higher levels of cholesterol, as expected [27] (data not shown). No effects of diet or interaction were noted at this time-point. After another 10 weeks on the diet, the strain effect became more pronounced ($F_{1, 45} = 92.8$, $p < 0.001$) and a significant strain x diet interaction was found ($F_{1, 45} = 4.1$, $p < 0.05$). To determine the effect of diet on each strain, we ran a oneway ANOVA separately in BN and SS strains. While diet had no effect in the BN strain, a significant effect was found in the SS strain ($F_{1, 35} = 12.3$, $p < 0.01$). For fasting plasma triglyceride levels (only measured after 18 weeks on the diet), there was also a main effect of strain ($F_{1, 44} = 67.9$, $p < 0.001$), again with SS animals showing significantly higher triglyceride levels than BN animals. There was also a significant effect of diet ($F_{1, 44} = 6.4$, $p = 0.015$), with animals on

the HF diet showing increased triglyceride levels. While there was not a statistically significant interaction between strain and diet ($F_{1,44} = 2.6$, $p = 0.11$), most of the diet effect appeared to be due to the SS strain. To confirm this, we again ran a one-way ANOVA to test the effect of diet separately in the BN and SS strains. While a significant effect of diet was not found in the BN strain, a highly significant effect was found in the SS strain ($F_{1,35} = 15.6$, $p < 0.001$).

While diet did not have a statistically significant effect on blood pressure levels at either time-point, SS animals on the HF diet showed a trend toward increased blood pressure relative to SS animals on the LF diet after 20 weeks when using a one-way ANOVA ($F_{1,11} = 4.4$, $p = 0.062$). There was a main effect of strain at both 10 weeks ($F_{1,23} = 64.5$, $p < 0.001$) and 20 weeks ($F_{1,23} = 110.0$, $p < 0.001$) with higher blood pressure in SS animals relative to BN, as expected [20] (data not shown). There was no effect of diet or strain on glucose tolerance levels at either time-point (data not shown).

Experiment 2

Similar to the inbred strains, after 20 weeks on the diet, consomic strains on the HF diet weighed significantly more than those on the LF diet ($F_{1,26} = 32.4$, $p < 0.001$) (see Figure 4). There was also a main effect of strain ($F_{1,26} = 43.2$, $p < 0.001$), with some strains weighing close to the BN strain and others weighing close to or more than the SS strain. There was an effect of both strain and diet for percentage of fat pad weight, with fat pads in animals on a HF diet weighing significantly more than those from animals on a LF diet (see Table 3). The effect of both strain and diet was seen in percentage of retroperitoneal fat pads ($F_{1,25} = 5.3$, $p < 0.01$ and $F_{1,25} = 17.6$, $p < 0.001$, respectively), epididymal fat pads ($F_{1,26} = 11.9$, $p < 0.001$ and $F_{1,26} = 23.2$, $p < 0.001$, respectively) and mesenteric fat pads ($F_{1,26} = 11.7$, $p < 0.001$ and $F_{1,26} = 10.7$, $p = 0.003$, respectively). No interaction was found between strain and diet for body weight or fat pad weights.

Although it appears that the HF diet had little or no effect on fat pad weight in some strains (SSBN5 and SSBN17; see Table 3), we did not run an ANOVA on individual consomic strains because the number of animals in each group was so small (2-3 animals per group).

For fasting plasma cholesterol levels, there was a significant main effect of both strain ($F_{1,26} = 15.5$, $p < 0.001$) and diet ($F_{1,26} = 13.3$, $p = 0.001$), as well as a strain x diet interaction ($F_{1,26} = 3.2$, $p = 0.01$). The interaction was due to the fact that cholesterol levels increased on the HF diet in some strains (SSBN5 and 20), while no effect was seen in other strains (SSBN 4, 8, 16 and 17). Importantly, SSBN8 was the only strain to show similar fasting plasma cholesterol levels to the parent BN strain, suggesting a gene or genes protecting the animal from dyslipidemia levels lies on rat chromosome 8 (see Figure 5). For fasting plasma triglyceride levels, there was also a significant main effect of both strain ($F_{1,26} = 9.9$, $p < 0.001$) and diet ($F_{1,26} = 6.0$, $p = 0.02$), with no significant interaction. Again, SSBN8 was the only strain to show fasting plasma triglyceride levels similar to the BN parent strain.

Experiment 3

In a follow-up study on SSBN8, while animals on the HF diet weighed slightly more than animals on the LF diet, this difference was not significant ($p = 0.33$). In contrast, a significant effect was found on both percentage retroperitoneal fat pads ($F_{1, 12} = 5.0$, $p = 0.047$) and epididymal fat pads ($F_{1, 12} = 21.0$, $p = 0.001$) (see Figure 6).

There were no significant differences in fasting plasma cholesterol ($p=0.21$) or triglyceride ($p = 0.58$) levels between SSBN8 animals on either HF or LF diets (see Figure 7). SSBN8 cholesterol and triglyceride levels fell in between levels of the parent strains. To determine if these levels differed significantly from the parent strains, we ran a two-way ANOVA on the effect of diet and strain on all three strains: BN, SS and SSBN8. For fasting plasma cholesterol, there is a significant effect of strain ($F_{2, 56} = 61.1$, $p < 0.001$) and diet ($F_{1, 56} = 4.6$, $p = 0.037$), with the SSBN8 strain exhibiting intermediate levels of cholesterol. Cholesterol levels in the SSBN8 were significantly different from both SS (Tukey HSD, $p < 0.001$) and BN (Tukey HSD, $p = 0.01$) with a larger difference relative to the SS (see Figure 7). For fasting plasma triglycerides, there was a significant effect of strain ($F_{2, 55} = 43.1$, $p < 0.001$) and a strain x diet interaction ($F_{2, 55} = 4.16$, $p = 0.02$), with SSBN8 exhibiting intermediate triglyceride levels. These levels were again significantly different from both SS (Tukey HSD, $p < 0.001$) and BN (Tukey HSD, $p = 0.033$), with a larger difference seen relative to the SS (see Figure 7). These results indicate that BN chromosome 8 is protective against high levels of cholesterol and triglycerides resulting from a HF diet.

Discussion

Metabolic syndrome results from a combination of poor diet, inactivity and genetics. We have shown here that consomic rat strains may be useful in uncovering the interplay between genes and diet. Specifically, we have shown that rat chromosome 8 likely harbors a gene or genes that are protective against dyslipidemia caused by a high fat/low carbohydrate diet. This is also the first paper to demonstrate clear differences between parent strains, SS and BN, in their responses to a high fat diet, thus demonstrating the utility of the consomic rat panel for dissecting diet by chromosome interactions.

In the current study, SS rats exhibited significantly higher levels of both fasting plasma cholesterol and triglycerides relative to BN rats, a finding that has previously been reported [27]. This difference became even more pronounced after being fed a high fat/low carbohydrate diet, as the diet significantly increased cholesterol and triglycerides in the SS strain, while it had no effect in the BN strain. Studies in humans have found that both high fat and high cholesterol diets can increase triglyceride levels, (see [28] for review). A previous study in SS rats shows that triglyceride levels increase under both high fat and high sucrose diets relative to a low fat/high complex carbohydrate diet, with the largest increase in plasma triglycerides on a high fat/high sucrose diet (similar to the one used in the current study) [29]. Our study does not specifically look at differences in type of carbohydrates, but based on this previous finding, one could hypothesize that triglyceride levels in the SS strain would be higher on a low fat diet high in sugars, such as fructose or sucrose, compared to a low fat diet high in complex carbohydrates.

In humans the change in lipid levels in response to diet can vary depending on sex, ethnicity, glucose tolerance, body mass index (see [28] as well as genetics (see [6]. For example, one study found that triglyceride levels do not increase in response to a low fat/high carbohydrate diet in individuals with a relatively low body mass index [30]. Similarly, triglyceride levels in response to either a low or high fat diet varies depending on APOA5 genotype [31]. While neither the SS nor BN strain completely mimics the general response of humans, we believe this study may help to elucidate the genetic basis for protection against lipidemia under high fat and high carbohydrate conditions. Our study demonstrates that in contrast to the SS strain, levels of plasma triglyceride and cholesterol levels remain consistently low in the BN rat strain on both a high fat/low carbohydrate diet and a low fat/high carbohydrate diet. Identification of the genes that protect against increased plasma cholesterol and triglycerides under either diet condition may prove useful as potential therapeutic targets for humans.

SS animals also had significantly higher percent retroperitoneal fat pad weight on the high fat diet, while again no effect was seen in the BN strain. Despite this effect on fat pad weight, the high fat/low carbohydrate diet increased body weight only slightly in both SS and BN animals. Surprisingly, diet had no effect on glucose tolerance nor blood pressure levels in either parent strain. Studies of incretin knockout mice in which you would clearly expect to see changes in glucose homeostasis, surprisingly show no effect on glucose homeostasis, even when fed a high fat diet, suggesting compensatory mechanisms, such as regulation of adipose deposition, changes in insulin sensitivity, and/or increased energy expenditure are occurring [32]. High fat diets have previously been shown to increase systolic blood pressure in Dahl salt-sensitive rats [29, 33, 34]. In contrast, a high fat diet was not shown to effect blood pressure in the spontaneously hypertensive SHR rat strain [35]. Although blood pressure increased in SS rats on high fat diet in our study, the increase only approached significance ($p = 0.062$). It is possible that our results would show a significant effect of high fat diet on blood pressure with an increased sample size.

Based on our study and others [16, 36, 37], BN rats express a gene(s) that provides metabolic protection from metabolic syndrome when exposed to high fat or high carbohydrate diets. We used consomic strains as a tool to identify chromosome(s) that harbor gene(s) that may provide a better understanding of gene(s) that may be involved in fighting obesity and its associated maladies. Of the eight consomic strains tested, SSBN8 was the only strain that exhibited a profile similar to the BN with low levels of cholesterol and triglycerides even after exposure to a high fat/low carbohydrate diet. These results suggest that BN chromosome 8 harbors genes that are protective against dyslipidemia caused by a high fat/low carbohydrate diet and thus are involved in diet by gene interactions in the development of dyslipidemia. Dyslipidemia is one of the main risk factors of cardiovascular disease and is known to have both dietary and genetic causes [38]. While ours is the first study in the rat to show a relationship between chromosome 8 and a high fat/low carbohydrate diet, previous studies have identified an interaction between diet and rat chromosome 8 in which a high sucrose diet resulted in increased triglyceride levels [16, 36]. Independent of diet, QTLs for cholesterol have also been identified on rat chromosome 8 in a GK x BN F2 intercross [37]. Using congenic strains in which portions of chromosome 8 from the GK rat were transferred to the BN background, this group found that different

regions regulate cholesterol from those that have an effect on triglyceride levels, indicating there are likely multiple genes on rat chromosome 8 that regulate lipid metabolism [37]. .

Human studies, particularly recent human genome-wide association studies, have identified several genes that are associated with lipid levels [39, 40]. Interestingly, four of these genes (LDLR, LIPC, APOA4 and APOA5) reside on rat chromosome 8 and are therefore potential candidates for playing a protective role against dyslipidemia in the BN rat. APOA5, a protein that is expressed only in the liver and plays an important role in triglyceride metabolism (see [41]), is a particularly strong candidate because it has previously been found to interact with dietary fat to alter fasting plasma triglyceride levels in humans [42]. .

Despite potential dyslipidemia protective genes associated with BN chromosome 8, this chromosome did not offer protection against increased fat pad weight, as levels of retroperitoneal and epididymal fat pad weights were significantly increased in both SS and SSBN8 animals on a high fat/low carbohydrate diet. These results suggest that in contrast to other studies [43, 44], visceral fat may not be a good predictor of dyslipidemia in SSBN8 animals. These results also demonstrate that increased fat pad weight in SSBN8 is independent from increased lipid levels, which is in contrast to other studies as previously demonstrated in humans (see [45]). Many studies have shown a relationship between visceral adiposity and multiple risk factors of metabolic syndrome, such as insulin resistance [44], elevated blood pressure [34], fatty liver [43] and cardiovascular disease [46]. While it is clear that the SSBN8 strain does not mimic human response in this regard, further studies could be used to investigate the disconnect between fat pad weight and lipid levels in this strain. A previous study examining the anti-obesity effects of eicosapentaenoic acid in mice fed a high fat/high sucrose diet, Sato et al. [47] observed increased fat pad weights, hepatic lipogenesis and triglyceride secretion, but reduced plasma triglyceride concentrations. The authors suggest the activation of lipoprotein lipase in white adipose tissue as a potential cause of the apparent disconnect between plasma triglycerides and increased triglyceride secretion. Conclusions put forth by Sato et al. [47] are consistent with increased hepatic triglyceride secretion and decreased removal of plasma triglycerides observed in Dahl salt-sensitive rats [48] and increased production and reduced clearance of very low density lipoprotein particles in humans (see [28]). The scope of our study is not sufficient to elucidate the disconnect between fat pad weight and plasma levels of triglycerides, but the genes associated with metabolism, lipogenesis and accumulation of lipoproteins may be located on SSBN8. The potential connection between dyslipidemia and visceral fat, at least in their relationship to interactions between genetic and dietary factors deserves further study.

It is also interesting to note that after exposure to a high fat diet, fasting levels of cholesterol and triglycerides significantly worsen in SSBN20 relative to the SS rat. SSBN20 is also much heavier than many of the other strains, with a larger body weight and percentage of fat pad weights, both before and after exposure to the high fat diet. Previous work has found that transfer of a segment of BN rat chromosome 20 onto the SHR background significantly increased adiposity levels after high-fat feeding [25]. These data indicate that BN chromosome 20 may contain a gene or genes that lead to increased body weight and fasting lipid levels when exposed to a high fat diet. Further work is needed to dissect which gene(s)

are involved. It is interesting to note that when transferred to the FHH genome, chromosome 20 does not lead to an increase in body weight or percentage of fat pad weight [26], indicating likely interactions of chromosome 20 with the background genome. Our work also demonstrates several other strains that may be interesting strains for follow-up regarding obesity (SSBN20, 18, 16) or increased lipid levels (SSBN5, 17, 20).

From this study and others, it is clear that consomic rat strains as animal models have been important in understanding the interaction of genes and food intake behavior. However, the bulk of consomic animal models have been used to understand salt intake and hypertension [19, 49-51]. With obesity and associated metabolic syndrome reaching epidemic levels [52] the power of the consomic model to better understand the interplay of genetics and diet as it relates to diet-induced obesity is critical. Our study has used the consomic model to show that BN chromosome 8 harbors a gene or genes that protect against dislipidemia when on a high fat/low cholesterol diet. This study is the first step in identifying genes that may provide a tool in the fight to reduce obesity and its associated maladies.

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Figure 1.

Changes in body weight and average Kcal consumption on the high fat/low carbohydrate (HF) and low fat/high carbohydrate (LF) diets. A) Body weight was increased in both strains after 20 weeks of treatment, with SS strain significantly heavier ($p < 0.001$) than BN strain and both strains were significantly heavier when fed HF diet ($p < 0.05$). B) Average kcal consumption was increased in both BN and SS rats on the HF diet ($p < 0.001$). $n = 6$ in each group.

Figure 2.

Percentage retroperitoneal fat pad weights in BN and SS on on the high fat/low carbohydrate (HF) and low fat/high carbohydrate (LF) diets for 22 weeks. * $p = 0.05$ relative to SS on low fat diet using a one-way ANOVA. Difference between low and high fat diet was not significant in BN strain using a one-way ANOVA ($p = 0.19$). $n = 6$ in each group.

Figure 3.

Changes in fasting levels of plasma cholesterol and triglycerides in SS and BN rats fed high fat/low carbohydrate (HF) or low fat/high carbohydrate (LF) diets for 18 weeks. A)

Cholesterol levels were significantly higher in SS rats fed high fat diet. B) Triglycerides levels increased in both strains, but were only significantly higher in high fat fed SS rats. * $p < 0.01$, ** $p < 0.001$. $n = 6$ in each group.

Figure 4. Changes in body weight in parental and consomic strains after 20 weeks on high fat/low carbohydrate (HF) or low fat/high carbohydrate (LF) diets: Rats fed a HF diet were significantly heavier than the LF group. $p < 0.001$. $n = 2-3$ animals in each group of consomic animals.

Figure 5.

Changes in fasting levels of plasma cholesterol and triglycerides in SS, BN and eight consomic strains (BN chromosome 4,5,8,10,16,17,18, and 20) fed high fat/low carbohydrate (HF) or low fat/high carbohydrate (LF) diets for 18 weeks. A) Fasting plasma cholesterol levels. There was a significant strain x diet interaction on cholesterol levels with increased cholesterol in some strains (SSBN5 and SSBN20), but not others (SSBN4, 8, 16 and 17) after 18 weeks on HF diet. Only SSBN8 had cholesterol levels similar to the BN parent strain. B) Fasting plasma triglycerides levels. Significant effect of diet and strain were found, but no interaction (see text). Again, only SSBN8 had values similar to parent BN strain triglyceride levels. n = 2-3 animals in each group of consomic animals

Figure 6.

Changes in percentage fat pad weight in SS, BN and SSBN8 animals after 22 weeks on high fat/low carbohydrate (HF) or low fat/high carbohydrate (LF) diets. A) Percentage retroperitoneal fat pad weights were higher in SS and SSBN8 rats fed HF diet compared to same strain on LF diet. B) Percentage epididymal fat pad weights were only significantly higher in SSBN8 on HF diet compared to LF diet. * $p < 0.05$, ** $p < 0.01$. $n = 6-7$ in each group.

Figure 7.

Fasting plasma levels of cholesterol and triglyceride SS, BN and SSBN8 animals after 20 weeks on high fat/low carbohydrate (HF) or low fat/high carbohydrate (LF) diets. A) Cholesterol levels were higher in SS fed HF diet compared to same strain on LF diet. B) Triglyceride levels were higher in SS rats fed a HF diet. * $p < 0.05$, ** $p < 0.01$. $n = 6-7$ in each group.

Table 1

Dietary Composition (in % kcal)

	LF	HF	Source
Fat	12	45	Corn oil/lard
Protein	18.7	18.7	Casein
Carbohydrate	69.3	36.4	Sucrose/dextrose
Salt	0.2	0.24	

Custom made diets (5TX1 control and 5TU2 high fat) purchased from TestDiet, division of LabDiet (Purina Mills). LF is low fat/high carbohydrate and HF is high fat/low carbohydrate.

Table 2

Composition of Fat

	LF	HF
Cholesterol, ppm	4	168
Linoleic Acid, %	2.75	4.99
Linolenic Acid, %	0.04	0.13
Arachidonic Acid, %	0.00	0.03
Omega-3 Fatty Acids, %	0.04	0.13
Total Saturated Fatty Acids, %	0.78	8.12
Total Monounsaturated Fatty Acids, %	1.32	8.85
Total Fat %	5.2	23.5

Custom made diets (5TX1 control and 5TU2 high fat) purchased from TestDiet, division of LabDiet (Purina Mills). LF is low fat/high carbohydrate and HF is high fat/low carbohydrate. % is percentage of ration on an As Fed basis.

Table 3

Percentage of fat pad weight in SS and BN parent strains and eight different consomic strains after 22 weeks on a high or low fat diet.

		% Retroperitoneal	%Epididymal	%Mesenteric
SS	LF	0.91 ± 0.14	1.14 ± 0.13	0.18 ± 0.02
	HF	1.35 ± 0.10*	1.42 ± 0.03	0.24 ± 0.03
BN	LF	0.55 ± 0.07	0.96 ± 0.09	0.10 ± 0.01
	HF	0.72 ± 0.10	1.04 ± 0.06	0.11 ± 0.01
SSBN4	LF	1.17 ± 0.14	1.28 ± 0.02	0.20 ± 0.02
	HF	1.76 ± 0.13	1.83 ± 0.13	0.23 ± 0.04
SSBN5	LF	1.14 ± 0.26	1.29 ± 0.29	0.21 ± 0.03
	HF	1.10 ± 0.67	1.36 ± 0.24	0.21 ± 0.03
SSBN8	LF	1.41 ± 0.06	1.45 ± 0.09	0.29 ± 0.05
	HF	1.91 ± 0.04	1.69 ± 0.14	0.34 ± 0.01
SSBN10	LF	0.83 ± 0.07	0.96 ± 0.11	0.19 ± 0.03
	HF	1.18 ± 0.11	1.22 ± 0.09	0.19 ± 0.03
SSBN16	LF	1.31 ± 0.33	1.39 ± 0.20	0.25 ± 0.11
	HF	2.20 ± 0.40	1.95 ± 0.28	0.32 ± 0.08
SSBN17	LF	1.21 ± 0.36	1.04 ± 0.08	0.18 ± 0.01
	HF	1.21 ± 0.00	1.19 ± 0.00	0.44 ± 0.00
SSBN18	LF	1.49 ± 0.05	1.65 ± 0.04	0.36 ± 0.03
	HF	2.47 ± 0.12	2.43 ± 0.11	0.47 ± 0.00
SSBN20	LF	1.53 ± 0.06	1.88 ± 0.08	0.45 ± 0.02
	HF	2.44 ± 0.11	2.38 ± 0.05	0.50 ± 0.04

SS rats consistently had significantly higher percentage of fat pad weights than BN rats (see text for statistics). High fat/low carbohydrate (HF) diet led to significantly increased retroperitoneal fat pads in the SS strain only (*p=0.05). Need to check one-way ANOVA in SS to determine if diet effected epididymal fat pads! The HF diet led to slightly increased percentage fat pad weight of all three fat pad types in the consomic rat strains (see text for statistics). However, because the number of animals in each consomic group were so low (2-3), ANOVA's were not run for each individual consomic strain, so it is not possible to determine if these differences are statistically significant.