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Journal of Nutritional Biochemistry 14 (2003) 32–39

THE JOURNAL OF Nutritional Biochemistry

Effects of dietary carbohydrate on the development of obesity in heterozygous Zucker rats

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Received 24 May 2002; received in revised form 5 August 2002; accepted 10 September 2002

Abstract

Rats carrying one copy of the *fa* allele are predisposed to diet-induced metabolic disturbances which contribute to hyperinsulinemia, obesity and dyslipidemia. To investigate the role of dietary carbohydrate and fat in the development of these conditions, we fed 6-week old male heterozygous (*fa*/+) lean rats carbohydrate-free diets containing primarily saturated fat either ad libitum or pair-fed. These diets were compared to standard chow and to a high saturated fat mixed diet containing 10% energy from sucrose for 4 weeks. The carbohydrate-free diet resulted in significantly lower circulating glucose levels compared to all other groups ($p = 0.006$). Weight gain was negligible in the carbohydrate free groups compared to standard diet and 10% sucrose diet ($p = 0.03$). This was reflected in energy efficiency which was markedly reduced (90%) in the carbohydrate-free groups compared to the other groups ($p = 0.04$). Corresponding changes were noted in fat pad mass. The subscapular and epididymal fat pads were increased 42% and 44%, respectively, in animals consuming the 10% sucrose diet compared to all other groups ($p < 0.01$). Comparable changes in fatty acid synthase (FAS) mRNA were observed in response to the carbohydrate-free diet, which resulted in a 53% decrease in adipocyte FAS mRNA ($p < 0.001$). Addition of 10% sucrose to the diet completely reversed this effect resulting in a 69% increase in adipocyte FAS mRNA compared to the carbohydrate-free groups ($p = 0.01$). Similarly, hepatic FAS mRNA was elevated by 51% and 66% in the 10% sucrose and standard diet groups respectively, compared to the carbohydrate-free groups. Therefore, diets that contain minimal carbohydrate may minimize net lipid storage and adiposity.

Keywords: fa; Dietary fat; Fatty acid synthase; Insulin; Lipogenesis; Adiposity

1. Introduction

Strategies designed to reduce body weight often rely on the consumption of low-fat diets. Dietary fat reduction is often accomplished via replacement with dietary carbohydrate. However, carbohydrate-induced hypertriglyceridemia is a well documented consequence of the partial substitution of dietary carbohydrate for dietary fat [1–4]. Current dietary recommendations suggest that less than 30% of energy be supplied by dietary fat. However, significant hypertriglyceridemia has been observed at this level of dietary fat [2–5]. Weisweiler et al. demonstrated that substituting carbohydrate for as little as 10% of total dietary fat profoundly alters lipoprotein metabolism by enhancing the assembly, secretion, and composition of very-low-density lipoprotein (VLDL) particles [6,7]. Such an effect has been independently confirmed by other investigators [8]. Consistent with this, Knopp et al. suggest that greater increases in dietary carbohydrate, at the expense of dietary fat, will lead to greater increases in circulating triglyceride levels [9].

Long term weight maintenance requires that average daily energy intake be commensurate with average daily energy expenditure. Furthermore, to prevent overaccumulation of energy-yielding substrates, macronutrient oxidation must occur in proportion to the contribution of protein, carbohydrate and fat to the energy content of the diet. It is well accepted that protein oxidation is rapidly and efficiently adjusted to dietary protein intake [10]. Protein oxidation is greater during the day than at night, which is consistent with a relationship between the use of amino acids for energy production and their availability [11]. The same is true for dietary carbohydrate. Following the ingestion of a carbohydrate-containing meal, there is a prompt increase in the rate of glucose oxidation [12]. Typically

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0955-2863/03/$ – see front matter
PII: S0955-2863(02)00249-8

Table 1
Composition of AIN-93 based semi-purified diets

Component	Standard		Carbohydrate free		10% sucrose	
	g/kg	% energy	g/kg	% energy	g/kg	% energy
Cornstarch	550	57	0	0	0	0
Corn oil	50	12	77	11	71.5	11
Coconut oil	0	0	482	69	383.5	59
Casein	200	21	314	20	292.5	20
Sucrose	100	10	0	0	146.25	10
AIN mineral mix[a]	35		35		35	
AIN vitamin mix[b]	10		10		10	
Cellulose[c]	50		77		56.25	
DL-methionine	3		3		3	
Choline bitartrate	2		2		2	

[a] American Institute of Nutrition Mineral Mixture 76 (ICN Pharmaceuticals, Costa Mesa, CA).
[b] American Institute of Nutrition Vitamin Mixture 76 (ICN Pharmaceuticals, Costa Mesa, CA).
[c] Fiber used was Alphacel non-nutritive bulk (ICN Pharmaceuticals, Costa Mesa, CA).
Diets were isonitrogenous for percent total energy contribution. Standard diet contained 16.15 kJ/g, carbohydrate-free diet contained 26.32 kJ/g and 10% sucrose diet contained 24.48 kJ/g.

consumed mixed meals provide carbohydrate in excess of the amount of glucose that can be oxidized in the immediate post-prandial period. Consequently, most of the ingested glucose is used to maintain the body's relatively small glycogen stores [13–15]. This tight regulation of fuel utilization assures that carbohydrate oxidation closely approximates carbohydrate intake so that neither overaccumulation nor exhaustion of glycogen stores occurs [14,16]. In contrast to protein and carbohydrate, fat balance is poorly regulated [17]. Consequently, positive energy balance is accompanied by expansion of adipose tissue mass [18]. Maintenance of fat balance would be possible if adjustments in fat oxidation occurred in response to variations in dietary fat intake, as is the case for carbohydrate and protein. On the other hand, if fat oxidation can not be adjusted to fat intake, fat oxidation appears to play a more passive role in energy balance than do carbohydrate and protein balance. Under these circumstances, fat oxidation is merely the means by which the body compensates for any imbalance between total energy expenditure and carbohydrate and protein oxidation [19]. Thus, when faced with a decrease in energy expenditure and a simultaneous increase in energy intake, fat oxidation would decrease simply because immediate energy needs could be readily met by the preferential oxidation of excess dietary carbohydrate and/or protein [20]. Flatt has proposed two mechanisms by which fat oxidation can be increased in response to increases in dietary fat intake: 1) maintenance of glycogen stores in the lower range, which would be accompanied by lower inter-meal blood glucose and insulin levels as well as increased levels of circulating fatty acids and consequently, higher rates of fat oxidation, or 2) an expanded adipose tissue mass, which necessarily leads to greater fat oxidation [14,16,17,20].

In addition to dietary fat, the typical Western diet often contains excessive amounts of refined sugars, particularly sucrose [21,22]. Rats fed a high sucrose diet develop hepatic and skeletal muscle insulin resistance, independent of any changes in body weight or composition, and chronic consumption of a high sucrose diet decreases both hepatic and peripheral insulin sensitivity in rodents [23,24].

While it is well accepted that dietary macronutrient composition can influence the development of obesity, the contribution of carbohydrate or fat remains controversial [25–29]. Consequently, the purpose of this study was to examine the role of the level of dietary carbohydrate on the development and treatment of obesity in lean rats susceptible to diet-induced obesity. Specifically, we assessed the metabolic effects of a high-fat diet devoid of dietary carbohydrate to a high-fat diet containing a minimal (10 en%) amount of sucrose on body weight regulation, glucose tolerance, de novo lipogenesis and lipid metabolism. We propose that the effects of dietary fat in conjunction with a minimal amount of sucrose will exert synergistic effects on adiposity and that their concomitant ingestion may significantly increase total body weight.

2. Materials and methods

Forty heterozygous Zucker lean rats (*fa*/+) of 6 to 8 weeks in age were adapted to handling for use in this study. Animals were housed in individual stainless steel hanging cages in a climate-controlled environment with a 12-h light-dark cycle. Prior to this study, animals had been maintained on standard rodent chow. All diets were semi-purified and were based on AIN-93 guidelines for rodent diets as shown in Table 1 [30]. Animals were randomly assigned to one of three dietary treatment groups: standard diet, carbohydrate free diet, 10% sucrose diet. Animals were allowed ad libitum access to food and water throughout the feeding period. A fourth group was included as a pair fed control group. These animals consumed the carbohydrate-free diet ad-

justed for the energy intake of the standard diet group on the previous day. This group also had ad libitum access to water throughout the feeding period. Animals spilled only minimal amounts of food; all spilled food was collected and recorded. Food intake was measured and replaced daily. Body weight was recorded every two days. This protocol was approved by the University of Tennessee Institutional Animal Care and Use Committee. At the completion of the study, animals were anesthetized with sodium pentobarbital (50 mg/kg body weight) and killed by exsanguination while under anesthesia.

Blood was obtained by cardiac puncture of the left ventricle and collected into an EDTA vacutainer blood tube. Blood samples were kept on ice prior to centrifugation to collect plasma. Plasma samples were subsequently stored at −80°C until time of analysis. Liver, epididymal adipose tissue and subscapular adipose tissue samples were collected, immediately weighed, snap frozen in liquid nitrogen and stored at −80°C until time of analysis.

2.1. RNA extraction

Total RNA was extracted from the medial lobe of the liver by using a standard phenol chloroform extraction protocol, and adipocyte total RNA was extracted via cesium chloride density gradient. Samples were then electrophoresed, subjected to Northern blot analysis, and hybridized with a radiolabeled rat cDNA probe for fatty acid synthase using standard methods [31,32]. Autoradiograms were quantitated using the Ultra Lum Electronic UV Transilluminator system (Ultra Lum, Inc., Paramount, CA) and ZERO-Dscan image analysis software (Scanalytics, Inc., Fairfax, VA) to perform volume densitometry on bands in the gel images. Results are reported as FAS:β-actin ratio.

2.2. Biochemical measures

Circulating glucose levels were measured by the glucose oxidase method (Sigma Aldrich Company, St. Louis MO) [33]. Serum triglycerides were determined by hydrolysis to glycerol and free fatty acids via lipoprotein lipase in a modification of the method previously described by Bucolo and David (Sigma Diagnostics, St. Louis, MO) [34]. Total cholesterol was determined by enzymatic hydrolysis of cholesterol esters and quantitated spectrophotometrically (Sigma Diagnostics, St. Louis, MO) [35]. HDL cholesterol was determined by a precipitation followed by spectrophotometric quantitation, and LDL- cholesterol was derived by the formula using total and HDL-cholesterol (Sigma Diagnostics, St. Louis, MO) [34–37]. Plasma insulin and leptin levels were determined using commercial rat radioimmunoassay (RIA) kits (Linco Research Inc., St. Charles, MO) which utilize rat antibodies and standards.

Table 2
Cumulative four-week food intake by diet group

Dietary group	Total (grams)	Energy (kJ)
Standard	1388	22407
Carbohydrate free	1157	30463
Carbohydrate free pair fed	884	22719
10% sucrose	1207	29538

2.3. Statistics

Data were analyzed by analysis of variance (ANOVA) to compare overall group means with subsequent analysis via least significant difference test to identify significantly different group means using the SPSS Statistics Package.

3. Results

There were no significant differences in food or energy consumption among groups during the four week feeding period, although animals consuming the carbohydrate-free diet and 10 en% sucrose diets ad libitum consumed 25% more energy than did other animals (Table 2).

Animals consuming the standard or mixed (10 en% sucrose) diets gained 90% more weight ($p = 0.03$) than animals consuming the carbohydrate-free diet ad libitum (Fig. 1, upper panel). Changes in body weight were reflected in energy efficiency (weight gain/energy intake ratio) which was 90% lower ($p = 0.04$) in the carbohydrate-free groups compared to standard and mixed diet (10 en% sucrose) groups (Fig. 1, lower panel).

As shown in Fig. 2, changes in body weight are reflected in fat pad mass. Animals consuming the 10% sucrose diet exhibited increases of 42% and 44% in the subscapular (Fig. 2, upper panel) and epididymal fat pads (Fig. 2, lower panel) respectively, compared to all other groups ($p < 0.01$ for both). These increases were reflected in an approximately two-fold increase in circulating leptin (Table 3, $p = 0.052$).

Adipose tissue fatty acid synthase (FAS) mRNA levels were 53% lower in animals consuming the carbohydrate-free diet compared to those consuming the semi-purified standard diet (Fig. 3, upper panel, $p < 0.001$). This effect was completely reversed upon addition of 10 en% sucrose to the diet, which instead resulted in a 69% increase in epididymal fatty acid synthase mRNA compared to the carbohydrate-free diet (Fig. 3, upper panel, $p < 0.01$). Similarly, hepatic FAS mRNA levels were 51% and 66% higher in animals consuming the mixed diet (10 en% sucrose) and standard diet, respectively, compared to animals receiving the carbohydrate-free diet (Fig. 3, lower panel, $p < 0.01$).

Plasma metabolites are shown in Table 3. Consumption of the carbohydrate-free diet lowered circulating glucose levels by 15% ($p = 0.006$) compared to animals consuming

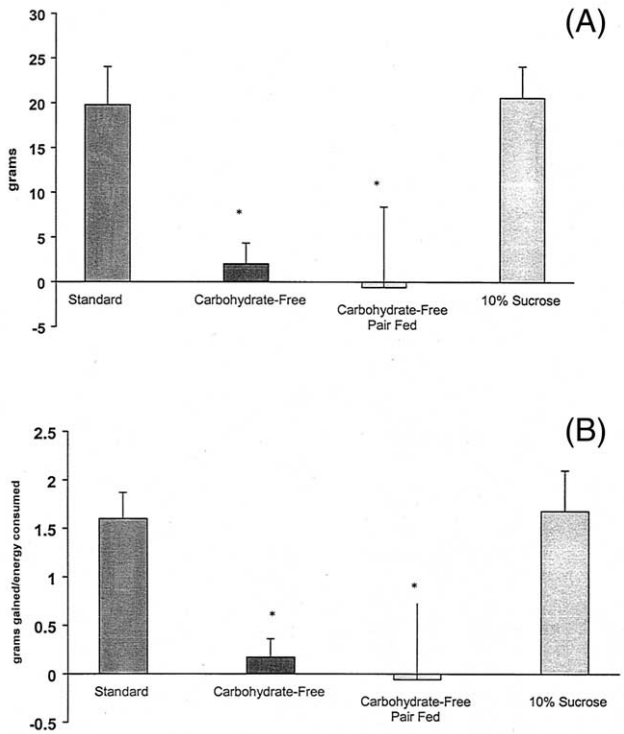

Fig. 1. A: Change in body weight by dietary treatment. Expressed in total grams gained (mean ± standard error) following the 4 week feeding period. *$p < 0.01$. B: Effect of diet on energy efficiency ratio. Energy efficiency is defined as total grams gained/total calories consumed during the feeding study and expressed as mean ± standard error. *$p = 0.04$.

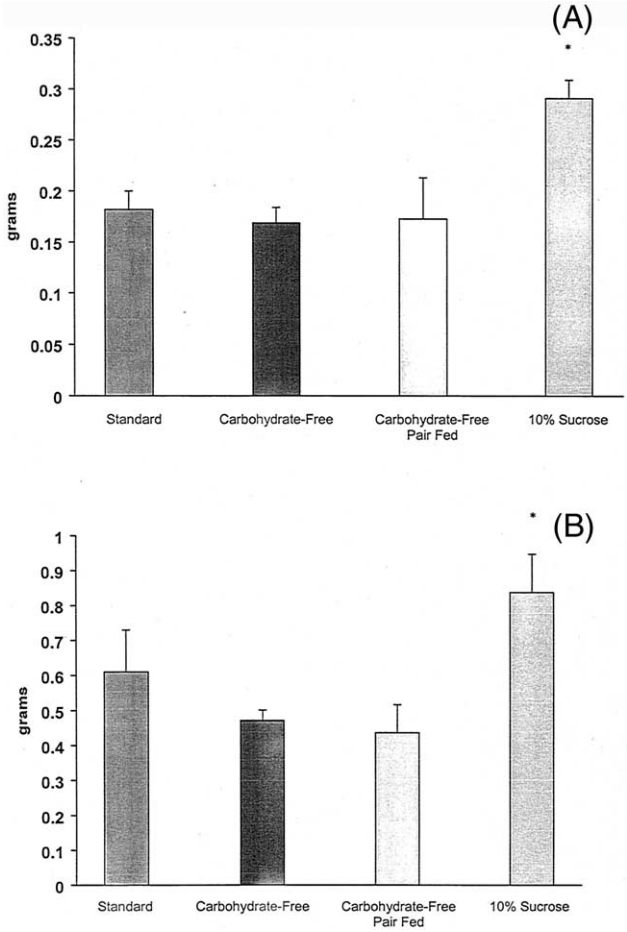

Fig. 2. A: Total subscapular fat pad mass (in grams) by dietary treatment. Data is expressed as mean ± standard error. *$p < 0.01$. B: Total epididymal fat pad mass (in grams) by dietary treatment. Data is expressed as mean ± standard error. *$p < 0.01$.

the mixed (10 en%) sucrose diet and 26% compared to animals consuming the standard diet (Table 2, $p = 0.006$,), while there were no significant effects of the diets on circulating insulin, total cholesterol, or LDL-cholesterol. There was a tendency for lower circulating levels of leptin in animals consuming the carbohydrate-free diets, although this did not reach significance (Table 3, $p = 0.052$).

4. Discussion

De novo lipogenesis plays a significant role in increasing adiposity in response to carbohydrate feeding in rodents [23,24,38–42]. However, until recently, this pathway was thought to be of relatively minor importance in adult humans [43–45]. Humans demonstrate significantly lower levels of hepatic lipogenesis than rodents. This may be partially due to the high fat content of the human diet [21,22]. Inhibition of lipogenesis in both liver and adipose tissues has been demonstrated to be a function of dietary fat intake and independent of a concomitant decrease in dietary carbohydrate [46–48]. The effects of a high fat diet may be dependent, in part, on the type of dietary fat ingested, as the degree of saturation has been shown to alter lipogenic enzyme activity [48,49]. In the liver, de novo lipogenesis was reduced by polyunsaturated, but not saturated fats [49]. Data on the effects of dietary fats in adipose tissue are less conclusive. Human adipocyte lipogenesis has been shown to be regulated by nutritional factors, including carbohydrates and insulin [50–54]. Fasting inhibits the lipogenic effects of insulin by mechanisms involving glucose transport and metabolism [50]. In vitro data had previously estimated that less than 1% of dietary carbohydrate was directed to synthesis of lipids by adipose tissue over a 24-hr period, leading to the conclusion that de novo lipogenesis by adipose tissue is of little consequence in humans [52]. The notion that human lipogenesis occurs primarily in the liver

Table 3
Effects of diet on serum measures

Dietary group	Glucose (mmol/L)	Insulin (pM)	Leptin (nM)
Standard	19.68 ± 1.21	85.08 ± 36.77	0.22 ± 0.06
Carbohydrate free	14.46 ± 0.01	128.60 ± 42.45	0.25 ± 0.04
Carbohydrate free pair fed	13.89 ± 1.68	88.16 ± 15.78	0.20 ± 0.04
10% sucrose	16.95 ± 0.53	179.89 ± 51.60	0.37 ± 0.08

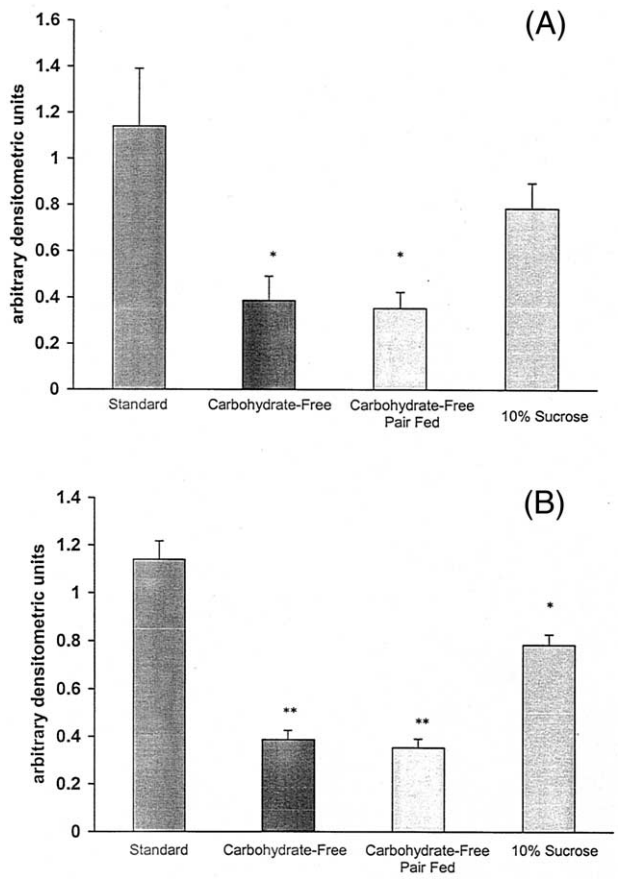

Fig. 3. A: Relative levels of fatty acid synthase (FAS) mRNA in rat epididymal fat pads. Total RNA was extracted from the epididymal fat pad as described. Data are for corrected for β-actin as a loading control. $*p < 0.01$, $**p < 0.001$. B: Relative levels of hepatic FAS mRNA in the medial lobe of the liver. Total RNA was extracted from the liver as described. Data are corrected for β-actin as a loading control. $*p < 0.01$.

is largely based on studies comparing the lipogenic enzyme activities in human and adipose tissue [53,54]. However, it is important to note that normal laboratory rodent chow is a high carbohydrate, low fat formulation [30]. On the other hand, the macronutrient composition of the typical Western diet is much different, containing significantly greater amounts of fat [21,22]. Therefore, it seems likely that a different lipogenic profile may be observed in rodents fed a diet with a macronutrient content more similar to the typical human diet. Under comparable dietary conditions, Swierczynski et al. have shown that the activities of lipogenic enzymes, including FAS, are comparable to those observed in adipose tissue from rodents fed a diet containing 45 en% lard. Results from this study indicate that on a per-milligram protein basis, the lipogenic capacity of human liver and adipose tissue are very similar. On a wet weight basis, Swierczynski et al. estimate the activities of liver lipogenic enzymes to be approximately 10-fold higher due to the higher protein content (per gram of tissue) of the liver. However, considering that whole body adipose tissue mass in a non-obese human is as much as 10-fold greater than liver mass, the lipogenic capacity of human adipose tissue is comparable to that observed in the liver [53]. This is corroborated by in vitro studies which demonstrate the induction of de novo lipogenesis in response to dietary carbohydrate [55]. Cultured human adipose tissue incubated for one week in glucose-supplemented media with insulin alone or in combination with dexamethasone increased the incorporation of ^{14}C-glucose into total lipids by 75 $\pm$ 58% and 166 $\pm$ 56%, respectively [55]. The results of this study demonstrate that lipogenesis occurs in human adipose tissue and can be induced by insulin and glucocorticoids in vitro. These findings are supported by human studies [56,57]. Aarsland demonstrated marked induction of adipocyte lipogenic enzymes by hypercaloric high carbohydrate feeding in obese women, thereby challenging the notion that excess dietary carbohydrate only passively influences the development of obesity by a fat sparing effect [56]. Using indirect calorimetry, Aarsland demonstrated that adipose tissue lipid accumulation is significantly increased following consumption of a hyperenergetic, high carbohydrate diet for one day. After four days of high carbohydrate feeding, whole body net fat synthesis was approximately 170 g/day, of which only 3 g/day could be attributed to hepatic lipogenesis. Although this level of hepatic lipid synthesis was 50 fold greater than in the basal state, hepatic de novo lipogenesis accounted for only 2% of total lipid synthesized de novo over 4 days [56]. Further evidence that adipose tissue lipogenesis contributes significantly to whole body lipid biosynthesis can be found in studies of undernourished patients fed hyperenergetic high carbohydrate diets [57]. Chascione et al. measured de novo lipid synthesis in adipose tissue biopsies prior to and following the administration of a hyperenergetic high carbohydrate diet for 6–10 days. There was a significantly greater rate of lipid synthesis following the high carbohydrate feeding. When extrapolated to whole body adipose tissue mass, adipose tissue lipid synthesis could account for as much as 40% of whole body lipogenesis [57].

The effect of dietary carbohydrate on accelerating fat synthesis reflects an increase in the capacity of the lipogenic pathway. The amount of lipogenic enzyme is determined by interplay between dietary carbohydrate and hormonal signals generated in response to their consumption [57,58]. In vivo and in vitro studies demonstrate that the effects of a high carbohydrate diet on increasing lipogenic enzymes require the presence of dietary carbohydrate and insulin [59–62]. Fatty acid synthase is a multi-enzyme complex which catalyzes all of the reactions in the synthesis of long chain saturated fatty acids from malonyl-CoA and acetyl CoA. Furthermore, the regulation of FAS in response to dietary manipulation occurs predominantly at the level of transcription, making this the rate-limiting step in the long term regulation of de novo fatty acid synthesis [59–67]. An increase in glucose metabolism has been shown to be necessary for insulin-mediated induction of both FAS and acetyl coA carboxylase mRNA in liver and white adipose

tissue in the rat [61,62]. The half-life of the FAS protein has been determined to be roughly 7 hr and protein turnover is sufficiently rapid so that changes in the level of FAS mRNA are reflected in corresponding changes in enzyme activity [61]. Claycombe et al. demonstrated that the insulin induced an approximately five-fold increase in FAS mRNA by increasing the rate of gene transcription in human adipocytes [62]. In vitro studies have further shown that glucose acts independently of insulin to stabilize FAS mRNA [59,61, 62]. Therefore, the induction of FAS appears to require the presence of carbohydrate or a carbohydrate metabolite and the appropriate hormonal stimuli.

The present studies employed dietary extremes as a first step in delineating the role of dietary carbohydrate structure and quantity in the development of obesity and were designed to minimize post-prandial hyperglycemia. Interestingly, this did not necessarily lead to reductions in circulating insulin, although animals consuming the carbohydrate-free diet had significantly lower blood glucose levels. The most striking observations from the present study relate to adiposity. We observed significant changes in both adiposity and energy efficiency, specifically that consumption of the carbohydrate-free diet resulted in substantially less weight gain compared to the standard or the mixed (10 en%) sucrose diet. These results further challenge the view that high fat diets typically result in greater adipose tissue mass than high carbohydrate diets because of increased energy density or overconsumption [17,25,26,69,70]. Furthermore, these findings suggest that the obesity promoting effects of a high fat diet may depend on the concomitant presence of a modest amount of dietary carbohydrate. This is consistent with our current understanding of post-prandial metabolism. Ingestion of carbohydrate stimulates insulin release and thus promotes the immediate utilization of diet-derived glucose and the concomitant suppression of release of non-esterified fatty acids (NEFAs) from adipose tissue with a resulting decrease in fat oxidation. In the fasting state, NEFAs are the principle substrates for fat oxidation. Following the consumption of a mixed meal, glucose becomes the dominant fuel substrate and NEFA release from adipose tissue is almost completely suppressed within 60–90 min of food intake [71]. At the same time, insulin stimulates the activity of LPL to further reverse whole-body fuel economy and begin the net uptake, esterification and storage of fatty acids in adipose tissue. The effect of dietary carbohydrate ingestion to promote fat storage may be most prominent following consumption of a mixed meal. The findings of the present study suggest that a carbohydrate-derived metabolic cue mediates the preferential storage of dietary fat in adipose tissue. Furthermore, these studies suggest that the capacity of fat oxidation more closely matches the fat content of the carbohydrate free diet. Regardless, the substitution of a very modest amount of sucrose for dietary fat completely reversed the effect of the carbohydrate-free diet on energy efficiency and in fact facilitated the storage of excess energy as fat. Consistent with this, the expression of FAS, the rate limiting enzyme in synthesis of long chain saturated fatty acids, was significantly lower in animals consuming the carbohydrate-free diet, while the addition of only a modest amount of sucrose completely reversed this effect.

Insulin is an established lipogenic and antilipolytic hormone [50,55,58,59]. Therefore, it is possible that manipulations of dietary macronutrient content and structure designed to minimize the insulin response would also minimize the anabolic effects of insulin in adipose tissue [70,73–78]. Consistent with this, Pawlak et al. recently demonstrated the chronic consumption of a low glycemic index starch by rats decreased first-phase insulin secretion during an intravenous glucose tolerance test and decreased epididymal fat pad mass without affecting peripheral or hepatic insulin sensitivity compared to rats consuming a high glycemic index starch diet [76]. Furthermore, Kabir et al. demonstrated that carbohydrate structure may have profound effects on glucose and lipid metabolism. Replacing a low glycemic index starch (mung bean starch) with a high glycemic index starch (waxy corn starch) in a mixed diet decreased insulin-stimulated glucose oxidation and increased glucose incorporation into total lipids and adipocytes diameter in both normal and diabetic rats [77]. Thus, dietary starches are not metabolically interchangeable. Consequently, alterations in dietary carbohydrate source may profoundly alter post-prandial metabolism. These findings were substantiated in a subsequent study by Kabir et al. in which replacement of low glycemic index mung bean starch with waxy corn starch resulted in significant elevations in adipose tissue FAS activity and mRNA as well as enhanced adipose tissue Glut4 gene expression in non-diabetic rats [78]. Furthermore, this same diet reduced hepatic phosphoenoylpyruvate carboxykinase (PEPCK) mRNA in normal and diabetic rats. These data suggest that manipulation of dietary carbohydrate may modulate the development of metabolic abnormalities associated with diet-induced obesity. Thus, the metabolic fuel mix and hormonal milieu in the post-prandial state may influence the deposition of substrates to a greater degree than a single macronutrient.

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