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Hormonal and Lipogenic and Gluconeogenic Enzymatic Responses in LA/N-Corpulent Rats (42079)

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Abstract. Genetically obese normotensive rats, LA/N-corpulent (*cp*), were fed *ad libitum* diets containing either 54% sucrose or cooked corn starch for 12 weeks. Twenty-four rats were used for the study; half were corpulent (*cp/cp*) and half were lean (*cp/+* or +/+). Fasting levels of plasma insulin, glucose, corticosterone, glucagon and growth hormone, and activities of liver and epididymal fat pad glucose-6-phosphate dehydrogenase (G6PD), malic enzyme (ME), and liver and kidney glucose-6-phosphatase (G6Pase), fructose 1,6-diphosphatase (FDPase), and phosphoenolpyruvate carboxykinase (PEPCK) were measured. A significant phenotype effect was observed in insulin, corticosterone, growth hormone, and liver G6PD, ME, FDPase, and kidney PEPCK, G6Pase, FDPase, and epididymal fat pad G6PD and ME (corpulent > lean), and glucagon (lean > corpulent). Diet effect (sucrose > starch) was significant for plasma glucose, liver ME, and kidney G6Pase. Although not significant at the $P < 0.05$ level, insulin, corticosterone, liver G6PD and FDPase and kidney FDPase tended to be higher in sucrose-fed rats. This study suggests that the corpulent rat is more lipogenic and gluconeogenic than the lean, and that the hormones responsible are effective in keeping both the lipogenic and gluconeogenic enzyme activity elevated.

A new congenic rat strain, LA/N-corpulent (*cp*), has been developed (1). Studies conducted in this laboratory have shown corpulent rats to be hyperinsulinemic, normoglycemic, hyperlipogenic, and hyperlipidemic (2, 3). These studies also demonstrated that the feeding of sucrose as compared to starch enhanced weight gain, relative liver size, serum triglyceride, and liver lipogenic enzyme activity in rats fed diets for 4 weeks (2), and after 10.5 months serum insulin was also affected (3).

The LA/N-corpulent rat appears to be metabolically similar to the Zucker fatty rat in its response to dietary carbohydrate. Feeding sucrose as compared to starch to genetically obese Zucker rats has been shown to result in greater liver lipogenic enzyme activity (4). Hypertriglyceridemia was found to be increased in fatty rats fed high-carbohydrate diets (5). Hyperinsulinemia and hyperphagia have also been shown in the obese Zucker rat (6). As with the obese Zucker rat, the LA/N-corpulent rat exhibits relatively normal glucose homeostasis in the presence of hyperinsulinemia (2, 7). However, the magnitude of the insulin levels appears to be greater in the LA/N-corpulent rat as compared to the obese Zucker rat, even though the genetic expression is similar for both (7).

The present study was designed to determine how hormones responsible for either lipogenesis or gluconeogenesis are influenced by phenotype and diet in the LA/N-corpulent rat. Liver and epididymal fat pad lipogenic enzymes, and liver and kidney gluconeogenic enzymes, affected by these hormones, were also evaluated.

Materials and Methods. C. T. Hansen at NIH developed the congenic strain LA/N-corpulent (*cp*) and it is described elsewhere (1). The LA/N-*cp* strain differs from the LA/N strain by the presence of the *cp* gene. Twelve corpulent (*cp/cp*) and 12 lean (*cp/+* or +/+) male rats approximately 4 weeks of age were *ad libitum* fed diets for 12 weeks. The experimental diets consisted of 54% carbohydrate as either sucrose or cooked corn starch (9), 10% casein, 10% lactalbumin, 5.9% cellulose, 4% beef tallow, 4% lard, 4% corn oil, 4% hydrogenated coconut oil, 3.1% AIN salt mix (10) (prepared without sucrose), and 1% vitamin fortification mix (No. 40060, Teklad Test Diets, Madison, Wis.).

Rats were housed individually in stainless-steel wire cages and were allowed free access

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to distilled water throughout the study. Periods of 12 hr of light and 12 hr of dark were controlled by automatic clocks in a room with controlled temperature (21 to 25°C) and humidity (40 to 50%).

After 12 weeks, the rats were fasted for 12 to 14 hr, then killed by decapitation. The blood was collected in centrifuge tubes containing heparin and trasylol and kept on ice. Plasma was obtained by centrifugation at 0 to 5°C at 3000*g* for 30 min. Plasma samples were assayed for insulin (11), glucose (12), corticosterone (13), glucagon (14), and growth hormone (15). Commercially available materials were used for all assays except glucagon.[1]

After decapitation, the livers, epididymal fat pads, and kidneys were quickly removed, weighed, and then frozen. A 10% liver homogenate in 0.01 *M* Tris buffer (pH 7.4)[2] was prepared with a Potter-Elvehjem homogenizer for the determination of G6PD (EC 1.1.1.49) (16), ME (EC 1.1.1.40) (16), FDPase (EC 3.1.1.11) (17), and PEPCK (EC 4.1.1.32) (18). For the determination of liver G6Pase (EC 3.1.3.9) (19), 0.2 *M* potassium citrate buffer was used. A 10% kidney homogenate in 0.01 *M* Tris buffer (pH 7.4)[2] was prepared with a mechanical high frequency homogenizer (Polytron PT10, Brinkmann Instruments, Westbury, New York) for determining FDPase (17), PEPCK (18), and G6Pase (19). For the determination of kidney G6Pase, 0.2 *M* potassium citrate buffer was used. A 10% epididymal fat homogenate in 0.01 *M* Tris buffer (pH 7.4)[2] was prepared as previously described for the determination of G6PD and ME (16). Enzyme activity is expressed as units per gram of soluble liver, kidney, or epididymal protein and one unit is defined as that amount of enzyme producing 1 μmole of measured product per minute under the conditions of the assay. Soluble protein solution was determined by the method of Lowry *et al.* (20).

A randomized complete block ANOVA with replication was used to test for phenotype, diet, and phenotype × diet effects. Differences with P values less than 0.05 were considered statistically significant.

Results. Weight gain, relative liver size, and relative epididymal fat pad size were greater in the corpulent rats than in lean rats (Table I). Sucrose-fed rats had a greater relative liver size than those fed starch.

Insulin levels were higher in corpulent rats as compared to lean; whereas, glucose levels were not different between phenotypes (Table II). Glucose levels were different between diets (sucrose > starch).

Corpulent rats had greater levels of corticosterone and growth hormone than did the lean rats (Table III). Glucagon levels were higher in the lean rats as compared to the corpulent rats. Levels of corticosterone, growth hormone, and glucagon did not show a significant diet effect.

Liver and epididymal fat G6PD and ME had greater activity in corpulent as compared

TABLE I. EFFECT OF PHENOTYPE AND DIET ON WEIGHT GAIN, RELATIVE LIVER SIZE, AND RELATIVE EPIDIDYMAL FAT PAD SIZE

Diet	Weight gain[a] (g)	Relative liver size[b] (%)	Relative epididymal fat pad size[c] (%)
Sucrose			
Corpulent	518 ± 16.0[d]	2.75 ± 0.131	2.37 ± 0.066
Lean	282 ± 11.9	2.28 ± 0.087	1.15 ± 0.076
Starch			
Corpulent	501 ± 20.8	2.12 ± 0.110	2.43 ± 0.092
Lean	252 ± 7.0	2.08 ± 0.050	0.91 ± 0.027
ANOVA[e]			
Phenotype	S	S	S
Diet	NS	S	NS
Interaction	NS	S	S

[a] Mean body weights before carbohydrate diets were fed: 110 g for obese rats and 86 g for lean rats. Mean body weights after carbohydrate diets were fed: 619 g for obese rats 353 g for lean rats.

[b] Relative liver size = (liver weight/body wt) × 100.

[c] Relative epididymal fat pad size = (epididymal fat pad weight/body wt) × 100.

[d] Each mean represents the average of six rats ± SEM.

[e] Effect significant S ($P < 0.05$) or not significant NS.

[1] The commercial sources of the assays were glucose (from Union Carbide, Pleasantville, N.Y.); insulin (from Amersham Corporation, Arlington Heights, Ill.); corticosterone (from Radioassay Systems Laboratories, Inc., Carson, Calif.); growth hormone (from Cambridge Medical Diagnostics, Inc., Billerica, Mass.). (Mention of a trademark does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.)

[2] Contained 0.01 *M* Tris, 0.005 *M* $MgCl_2$, 0.005 *M* mercaptoethanol, and 0.001 *M* EDTA.

TABLE II. EFFECT OF PHENOTYPE AND DIET ON FASTING LEVELS OF PLASMA INSULIN AND GLUCOSE

Diet	Insulin (μunit/ml)	Glucose (mg/dl)
Sucrose		
Corpulent	496 ± 71.3[a]	111 ± 2.7
Lean	75 ± 13.7	97 ± 6.6
Starch		
Corpulent	433 ± 86.6	63 ± 8.7
Lean	65 ± 7.7	81 ± 10.6
ANOVA[b]		
Phenotype	S	NS
Diet	NS	S
Interaction	NS	S

[a] Each mean represents the average of six rats ± SEM.
[b] Each significant S ($P < 0.05$) or not significant NS.

to the lean rats (Table IV). Liver ME activity was greater in the sucrose-fed rats than in the ones fed starch.

Liver and kidney FDPase, kidney G6Pase, and kidney PEPCK activity was greater in the corpulent rat than in the lean rat (Table V). Liver G6Pase activity was higher in the lean rat than in the corpulent rat. Kidney G6Pase activity was greater in rats fed sucrose as compared to those fed starch.

Discussion. Differences between phenotypes (corpulent > lean) were more apparent than differences between the diets (Tables I–V). Corpulent rats produced more body fat,

TABLE III. EFFECT OF PHENOTYPE AND DIET ON FASTING LEVELS OF PLASMA CORTICOSTERONE, GLUCAGON, AND GROWTH HORMONE

Diet	Corticosterone (ng/ml)	Glucagon (pg/ml)	Growth hormone (ng/ml)
Sucrose			
Corpulent	359 ± 25.9[a]	94 ± 6.1	3.1 ± 0.16
Lean	268 ± 25.0	124 ± 14.7	3.1 ± 0.21
Starch			
Corpulent	316 ± 25.5	106 ± 8.8	3.4 ± 0.34
Lean	245 ± 25.8	130 ± 10.3	2.6 ± 0.06
ANOVA[b]			
Phenotype	S	S	S
Diet	NS	NS	NS
Interaction	NS	NS	NS

[a] Each mean represents the average of six rats ± SEM.
[b] Each significant S ($P < 0.05$) or not significant NS.

TABLE IV. EFFECT OF PHENOTYPE AND DIET ON FASTING LEVELS OF LIVER AND EPIDIDYMAL FAT PAD GLUCOSE-6-PHOSPHATE DEHYDROGENASE AND MALIC ENZYME

	Liver		Epididymal fat pad	
Diet	G6PD	ME	G6PD	ME
	(U/g protein)			
Sucrose				
Corpulent	50 ± 6.9[a]	26 ± 3.5	24 ± 4.2	24 ± 6.6
Lean	22 ± 2.3	9 ± 0.8	10 ± 1.8	10 ± 3.0
Starch				
Corpulent	43 ± 9.4	16 ± 2.8	36 ± 5.3	36 ± 6.8
Lean	11 ± 1.1	4 ± 0.5	10 ± 2.0	11 ± 1.9
ANOVA[b]				
Phenotype	S	S	S	S
Diet	NS	S	NS	NS
Interaction	NS	NS	NS	NS

[a] Each mean represents the average of six rats ± SEM.
[b] Each significant S ($P < 0.05$) or not significant NS.

which is partly due to their ability to be more efficient than lean rats in converting equivalent amount of food into fat (2). Differences between diets might be attributed to differences in food intake, which was not measured in this study. However, in previous studies with genetically obese rats[3] (2, 4) differences between sucrose or starch were not observed when food intake was expressed as g/100 g body wt. Previous studies in this laboratory have shown the corpulent rat to be hyperinsulinemic and normoglycemic when fed either sucrose or starch diets for 4 weeks and up to 10.5 months (2, 3). This was also true in the present study (Table II). It has been reported for the fatty Zucker rat that hyperinsulinemia contributes to hyperphagia, which enhances fat deposition (6, 21). The LA/N-corpulent rat is also observed to be hyperphagic (2). High corticosterone levels combined with the high insulin levels in corpulent rats could lead to hyperphagia. This has also been shown in other genetically obese animals (6, 22).

It has been reported that serum growth hormone levels are decreased in obese Zucker rats when compared to lean rats. Martin *et al.* (21) found that in lean Zucker rats, growth

[3] Personal communication, Dr. O. E. Michaelis, Carbohydrate Nutrition Laboratory, Beltsville Human Nutrition Research Center, Beltsville, Md.

TABLE V. EFFECT OF PHENOTYPE AND DIET ON FASTING LEVELS OF LIVER AND KIDNEY FRUCTOSE 1,6-DIPHOSPHATASE, GLUCOSE-6-PHOSPHATASE, AND PHOSPHOENOLPYRUVATE CARBOXYKINASE

	Liver			Kidney		
Diet	FDPase	G6Pase	PEPCK	FDPase	G6Pase	PEPCK
	(U/g protein)					
Sucrose						
Corpulent	52 ± 5.0[a]	201 ± 28.6	5.2 ± 0.77	16 ± 1.6	688 ± 29.1	11.9 ± 2.95
Lean	40 ± 10.0	369 ± 47.9	8.4 ± 1.21	10 ± 1.8	487 ± 48.5	7.6 ± 1.71
Starch						
Corpulent	43 ± 6.7	258 ± 49.3	6.5 ± 0.82	12 ± 1.6	500 ± 53.9	11.7 ± 1.34
Lean	22 ± 5.4	303 ± 27.9	7.1 ± 0.94	8 ± 1.8	461 ± 40.3	7.0 ± 1.08
ANOVA[b]						
Phenotype	S	S	NS	S	S	S
Diet	NS	NS	NS	NS	S	NS
Interaction	NS	NS	NS	NS	NS	NS

[a] Each mean represents the average of six rats ± SEM.
[b] Each significant S ($P < 0.05$) or not significant NS.

hormone levels increased dramatically up to 11 weeks of age, but this increase was marginal in obese rats. Decreased levels of growth hormone were not observed in corpulent when compared to lean rats (Table III). In contrast to other hormones studied, glucagon levels were higher in lean than obese rats. This may be due to extremely elevated insulin levels in the corpulent as compared to the lean rat, which could suppress glucagon. Another factor which may be influencing the suppression of glucagon in the corpulent rat is elevated levels of free fatty acid (2). In a study on the obese Zucker rat conducted by Eaton *et al.* (23), they found the secretion of glucagon suppressed and attributed this to increased levels of insulin, free fatty acid and glucose. The combined hormonal abnormality of excess insulin and reduced glucagon in the corpulent rat may contribute to the hyperlipidemic characteristic in this animal model (2, 3).

Liver and epididymal fat lipogenic enzyme activity was greater in corpulent than in lean rats (Table IV). Increased lipogenic enzyme activity could explain the increased fat deposition observed in corpulent rats. It is interesting to find that in addition to increased lipogenic enzyme activity there is also increased gluconeogenic enzyme activity in the corpulent rat. Increased hepatic and kidney gluconeogenic enzyme activity did not affect fasting plasma glucose levels (Table II). The increase in liver G6Pase activity in the lean rat as compared to the corpulent rat was unexpected. One would postulate that increased levels of corticosterone and growth hormone in the corpulent rat would increase liver G6Pase activity. These findings suggest that in the corpulent rat the kidney may be a more important organ for gluconeogenesis than in other strains of rats (24, 25).

Although there are similarities in metabolic responses between the Zucker and the LA/N-corpulent strains (2–4, 6, 26–28), the LA/N-*cp* rat appears to be more insulin resistant and exhibits differences in the pattern of gluconeogenic enzyme activity. The differences observed between the two strains of rats could be explained by differences in genetic background (29), as well as in experimental design. Hormonal and enzymatic alterations observed in this rat suggest that the corpulent rat is a useful model for the study of obesity.

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