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# Genetic Obesity and Dietary Sucrose Decrease Hepatic Glucagon and Insulin Receptors in LA/N-Corpulent Rats (42226)

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*Abstract.* A catabolic and hypolipemic effect of glucagon has been described in normal animals. We therefore studied the role of glucagon in genetically obese, hyperlipemic rats. Twelve genetically obese hyperlipemic LA/N-*cp/cp* (corpulent) rats and 12 lean littermates were fed either 54% starch or 54% sucrose for 12 weeks. Plasma glucagon and insulin levels and glucagon and insulin binding to liver membranes were measured. Comparing all corpulent and lean animals regardless of diet, a significant ($P < 0.0001$) phenotypical effect (*cp/cp* > lean) was observed in plasma insulin levels (464 ± 54 vs 70.3 ± 7.6 μu/ml, mean ± SEM). Plasma glucagon levels were lower in *cp/cp* rats (99.6 ± 5.4 vs 127.1 ± 8.6 pg/ml, mean ± SEM). Insulin binding (2.68 vs 16.1%/50 μg protein) and glucagon binding (25.6 vs 47.3%/50 μg protein) were both significantly lower ($P < 0.0001$) in corpulent rats as compared to their lean littermates. Sucrose feeding had marginal effect on plasma insulin or insulin binding. It, however, decreased glucagon binding in corpulent rats but not in their controls. A significant negative correlation was observed between plasma insulin and insulin binding, while a positive correlation was seen for plasma glucagon and glucagon binding. A significant negative correlation was observed between plasma glucagon and lipogenic enzymes (glucose-6-phosphate dehydrogenase and malic enzyme) in liver and between glucagon binding and these enzymes. We propose that in these genetically obese rats, in addition to hyperinsulinemia, impaired glucagon activity as manifested by decreased glucagon binding to target cells may be an important contributor to the hyperlipemia and obesity. A further decrease in glucagon binding in rats fed sucrose indicates that sucrose, per se, may be an additional contributory factor.


A new animal model of genetic obesity, the LA/N-corpulent rat, has been recently developed by Hansen at NIH (1) by inserting a corpulent gene from the Koletsky rat (2) in the LA/N rat. The latter was produced by cross breeding Albany strain and a hooded strain of rat (3). The LA/N-corpulent rats are obese, hyperlipemic, and hyperinsulinemic but are nondiabetic (normoglycemic) and normotensive. Thus, they differ from other models of genetic obesity which are usually hyperglycemic. The LA/N-corpulent rat has been suggested as a useful model for studying hyperlipemia (4). Some of the biochemical parameters such as plasma lipids (4, 5) and lipogenic enzymes (6) have been recently studied in these animals. However, the molecular mechanism underlying the very prominent hyperlipemia is not understood.

In normal human subjects (7, 8) and animals (9, 10) glucagon has been demonstrated to be catabolic and hypolipemic. Eaton *et al.* (11) have "proposed that a reduction in net glucagon activity may be the final common pathway mediating or participating in the development and/or maintenance of endogenous hyperlipemia in man. It is suggested that this hormonal mechanism may be initiated by diets, drug, or genetic influences." With this possibility in mind, we examined circulating levels of glucagon as well as hepatic glucagon receptors in this genetically obese rat and its lean littermates. We also measured plasma insulin and hepatic insulin receptors.

Sucrose feeding has been demonstrated to produce hyperlipemia in normal as well as obese humans (12–14) and animals (4–6, 15, 16). Beck-Nielsen *et al.* (17, 18) have fur-

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ther shown that sucrose feeding decreases insulin receptors on circulating mononuclear cells while low sucrose feeding increases insulin receptors (19). However, to date the effect of feeding sucrose on glucagon receptors has not been described. It has been reported, however, that the nutritional state of the animal, namely, fed vs fasted, affects glucagon receptors (20–22). Gill and Hart (23) reported that high protein feeding (which should increase plasma glucagon levels) increases glucagon binding to goat hepatocytes as compared to high carbohydrate feeding (which should decrease plasma glucagon). It therefore seemed pertinent to investigate the effect of dietary sucrose on glucagon receptors in this newly described model of genetic obesity and hyperlipemia.

**Methods.** Four-week-old 12 genetically obese homozygous LA/N-*cp/cp* rats (corpulent) and their 12 lean homozygous LA/N-+/+ and heterozygous LA/N-*cp*/+ littermates (lean) were used in the present investigation. The rats were housed in individual cages with reverse dark–light cycle. They were fed *ad libitum* for 12 weeks a diet containing 54% starch or 54% sucrose along with 10% casein, 10% lactalbumin, 4% corn oil, 4% cocoanut oil, 4% lard, 4% beef tallow, 5.9% cellulose, 3.1% AIN-76 salt mix (prepared without sucrose), and 1% vitamin mix (40060, Teklad Test Diets, Madison, Wis.). The rats were sacrificed by decapitation after an overnight fast. Blood was collected in tubes containing 1000 units of Trasylol (FBA Pharmaceutical, N.Y.) and 10.5 mg EDTA. Plasma was separated and stored at −70°C for hormone analysis. Immunoreactive glucagon was measured using Unger's 04A antibody by the method previously described (24). Immunoreactive insulin was determined using a kit from Immunonuclear Corporation, Stillwater, Minnesota, (Cat No. 0600). Pancreatic porcine insulin and pancreatic porcine glucagon which were utilized as assay standards were gifts from Eli Lilly & Company. Plasma membranes were prepared according to the method of Neville (25) and stored at −70°C. Plasma membrane protein was assayed according to Lowry *et al.* (26).

To measure glucagon binding, membranes (80–120 μg protein) were incubated for 30 min at 30°C in 0.5 ml of Tris buffer, pH 7.6, with 50 pg $^{125}$I-glucagon in presence of 0–10 μg/ml native porcine pancreatic glucagon. Insulin binding was measured by incubating membranes for 60 min at 30°C in 0.5 ml Tris buffer, pH 7.6, with 50 pg $^{125}$I-insulin in presence of 0–10 μg/ml native porcine pancreatic insulin. The composition of the buffer used and the method of separating bound from free hormones have been previously described (24, 27, 28). Monoiodinated $^{125}$I-insulin and monoiodinated $^{125}$I-glucagon were purchased from New England Nuclear Corp., Boston, Massachusetts. The data were analyzed by Scatchard plots (29) and competition–inhibition plots (28). Statistical analysis was carried out by randomized complete block analysis of variance to see the effect of phenotype, diet, and phenotype × diet interactions (30).

**Results.** There were significant differences in weight gain, liver weight, and adipose tissue weight between lean and corpulent rats (Table I). Dietary sucrose had no significant effect on weight gain or adipose tissue weight but did increase liver weight. There were no significant differences in plasma glucose between lean and corpulent rats, but sucrose feeding increased plasma glucose significantly in all rats, especially in corpulent rats (6). A significant phenotypical effect was observed for plasma insulin and glucagon levels, in that the corpulent rats had higher insulin and lower glucagon levels as compared to their lean littermates (Table II). Feeding sucrose had no statistically significant effect on the levels of these hormones though it tended to increase plasma insulin and decrease plasma glucagon levels.

Insulin binding to liver plasma membranes was significantly decreased in corpulent rats as compared to lean rats. However diet had no effect on insulin binding. Glucagon binding was markedly decreased in corpulent rats as compared to their lean littermates despite lower plasma glucagon levels. In addition, there was also a significant diet effect on glucagon binding in that high sucrose feeding further decreased glucagon binding. This effect was more pronounced in corpulent rats. There were no significant interactions between diet and phenotype on any of these parameters (Table II).

Scatchard analysis of insulin binding (Fig. 1A) demonstrated that there was a significant decrease in the number of receptors as judged by the intercepts on the abscissa in corpulent

TABLE I. EFFECT OF PHENOTYPE AND DIET ON WEIGHT GAIN, LIVER, AND ADIPOSE TISSUE WEIGHTS

| Phenotype | Diet | Weight gain[a] (g) | Liver weight (g) | Adipose tissue weight (g) |
|---|---|---|---|---|
| Lean | Starch | 252 ± 7[b,c] | 7.04 ± 0.19[c] | 3.07 ± 0.10[c] |
| Lean | Sucrose | 282 ± 12[c] | 8.42 ± 0.40[c] | 4.26 ± 0.38[c] |
| Corpulent | Starch | 501 ± 21[d] | 12.19 ± 0.79[d] | 14.78 ± 0.62[d] |
| Corpulent | Sucrose | 518 ± 16[d] | 17.42 ± 0.99[d] | 14.94 ± 0.39[d] |
| | | | ANOVA | |
| Phenotype | | S | S | S |
| Diet | | NS | S | NS |
| Phenotype × diet | | NS | S | NS |

[a] Final weight at sacrifice − initial weight when the rats were placed on the diets.
[b] Means ± SEM of six rats. Means within a column not sharing a common superscript letter are statistically different according to Duncan's multiple range test at $P < 0.05$.

rats as compared to their lean littermates. Sucrose feeding further decreased the number of insulin receptors in corpulent rats. No such effect was observed in lean rats. Competition–inhibition plots of insulin binding are shown in Fig. 1B. Affinity of the receptors was measured by the amount of native hormone required to displace 50% of the bound tracer. There were no differences in the affinity of insulin receptors between lean and corpulent rats fed starch. However, sucrose feeding increased the affinity in corpulent rats compared to those fed starch. (Note the leftward shift of the curve).

Scatchard analysis of glucagon binding (Fig. 2A) to liver plasma membranes showed that corpulent rats had a 50% decrease in number of glucagon receptors, $P < 0.05$. There was a small decrease in the number of receptors in corpulent rats fed sucrose as compared to those fed starch which was not statistically significant. There were no significant phenotype or diet effects on the affinity of glucagon receptors as seen from competition–inhibition plots (Fig. 2B).

When insulin binding to liver plasma membranes was plotted against plasma insulin levels (Fig. 3), a significant negative correlation was observed ($r = -0.806$, $P < 0.0001$). In contrast when glucagon binding to liver plasma membranes was plotted against plasma glucagon levels (Fig. 4) a weak but significant

TABLE II. EFFECT OF PHENOTYPE AND DIET ON PLASMA INSULIN AND GLUCAGON AND ON INSULIN AND GLUCAGON BINDING TO LIVER PLASMA MEMBRANES

| Phenotype | Diet | Plasma insulin (μU/ml) | Plasma glucagon (pg/ml) | Insulin binding (%)[a] | Glucagon binding (%)[a] |
|---|---|---|---|---|---|
| Lean | Starch | 65.4 ± 7.7[b,c] | 130.2 ± 10.3[c] | 16.1 ± 0.8[c] | 48.5 ± 2.3[c] |
| Lean | Sucrose | 75.2 ± 13.7[c] | 124.0 ± 14.7[c,d] | 16.0 ± 1.9[c] | 46.1 ± 2.6[c] |
| Corpulent | Starch | 433.4 ± 86.6[d] | 105.5 ± 8.8[d,e] | 3.1 ± 0.6[d] | 30.4 ± 2.7[d] |
| Corpulent | Sucrose | 496.5 ± 71.3[d] | 93.7 ± 6.1[e] | 2.6 ± 0.3[d] | 20.8 ± 1.1[e] |
| | | | ANOVA | | |
| Phenotype | | $P < 0.0001$ | $P < 0.05$ | $P < 0.0001$ | $P < 0.001$ |
| Diet | | NS | NS | NS | $P < 0.05$ |
| Phenotype × diet | | NS | NS | NS | NS |

[a] Percentage specific binding/50 μg protein. Nonspecific binding (binding in presence of 10 μg/ml hormone) was subtracted from total binding to obtain specific binding.
[b] Means ± SEM of six rats. Means within a column not sharing a common superscript letter are statistically different according to Duncan's multiple range test at $P < 0.05$.

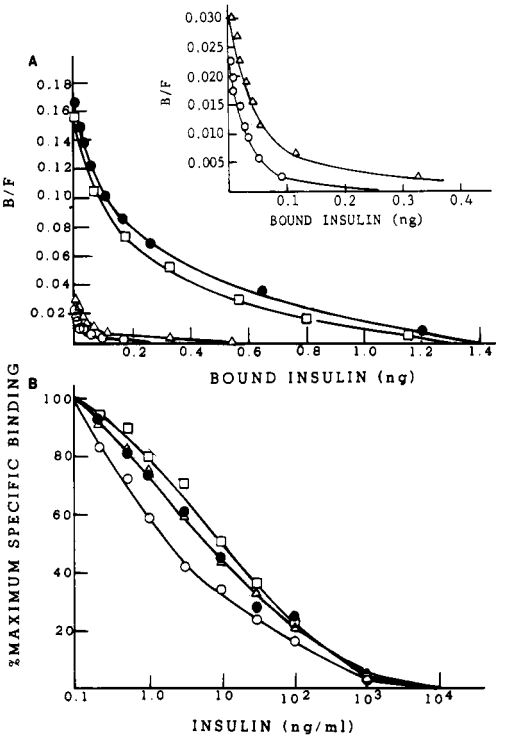


FIG. 1. Scatchard analysis (A) and competition-inhibition plots (B) of insulin binding to liver plasma membranes. The data are means of six separate determinations. ●, Lean, starch fed; □, Lean, sucrose fed; △, corpulent starch fed; and ○, corpulent, sucrose fed. Nonspecific binding (i.e., binding observed in presence of 10 μg/ml insulin) was subtracted from total binding to obtain specific binding. Inset represents a magnification of the binding curves for corpulent rats.

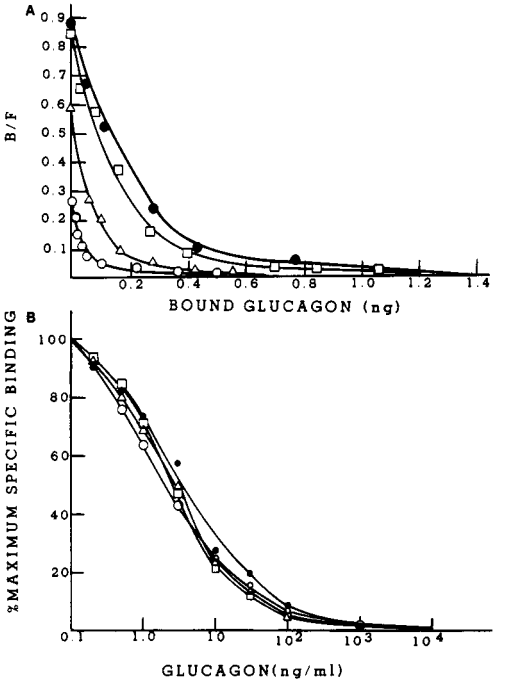


FIG. 2. Scatchard analysis (A) and competition-inhibition plots (B) of glucagon binding to liver plasma membranes. For other details refer to Fig. 1 legend.

positive correlation was observed ($r = 0.648$, $P < 0.001$). Significant negative correlations were observed between glucagon binding and the lipogenic enzymes in these animals: liver glucose-6-phosphate dehydrogenase (G6PDH; $r = -0.705$, $P < 0.0002$) and malic enzyme (ME) ($r = -0.780$, $P < 0.0001$; Fig. 5), and adipose tissue G6PDH ($r = -0.513$, $P < 0.015$) and ME ($r = -0.427$, $P < 0.05$; data not shown). The lipogenic enzyme values were previously reported by our laboratory (6).

**Discussion.** Previous studies by several of the present authors (4–6) have demonstrated that LA/N corpulent obese rats are hyperlipemic as well as hyperinsulinemic and that sucrose feeding intensified the hyperlipemia and increased insulin levels as well as the activity of several lipogenic enzymes. Similar effects of sucrose feeding on plasma lipids and insulin have also been observed in Zucker fatty rats (15, 16).

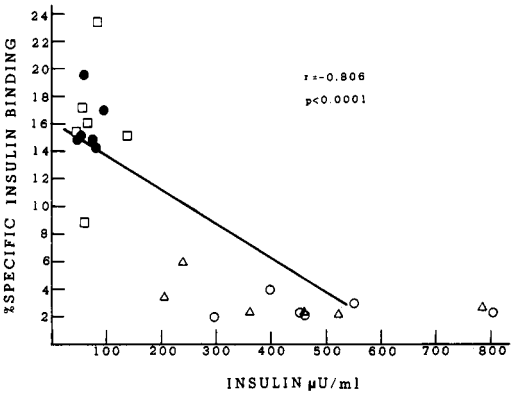


FIG. 3. Relationship between plasma insulin level and insulin binding to liver plasma membranes. ●, Lean, starch fed; □, lean, sucrose fed; △, corpulent, starch fed; ○, corpulent, sucrose fed. $r = -0.806$, $P < 0.0001$. When the data for the means ± SEM for each group are plotted, the correlation was $r = -0.996$, $P < 0.0001$.

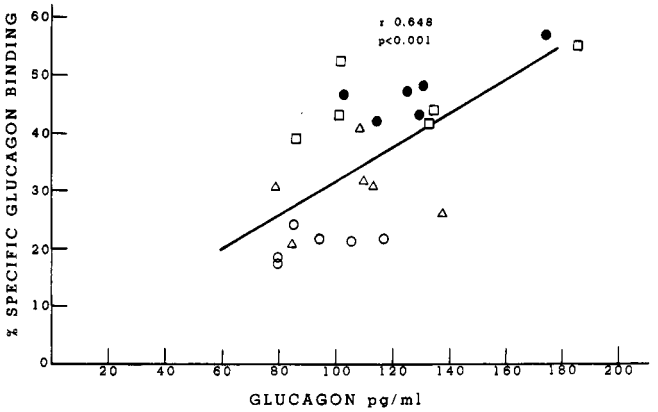


FIG. 4. Relationship between plasma glucagon and glucagon binding to liver plasma membranes. $r = 0.648$, $P < 0.001$. Symbols are same as in Fig. 3. When the data for the means ± SEM for each group are plotted the correlation was $r = 0.997$, $P < 0.0001$.

In the present investigation we have shown that the obese *cp/cp* rats have a 45% decrease in glucagon binding to receptors in the liver, an organ which is a major target for glucagon action. This binding change is due to a reduction in the number of receptors, and was further magnified by sucrose feeding. This reduction in glucagon receptors was accompanied by a decrease in plasma glucagon levels in the corpulent animals. Such a combination, must represent a state of significantly diminished biologically active glucagon. Previous studies from some but not all (31) laboratories indicate that glucagon receptors are controlled by plasma glucagon levels, in a manner similar to the control of insulin receptors by plasma insulin levels. Increased plasma levels of glucagon, which are observed in diabetes (24, 31) and starvation (20, 21) or as a consequence of acute or chronic glucagon injections (21, 24) decrease glucagon receptor number. However the present study differs in that we observed a positive correlation between plasma glucagon levels and glucagon binding to target tissue. These findings suggest that normal control of glucagon receptors is altered in these animals, and this may be a primary defect responsible for glucagon resistance.

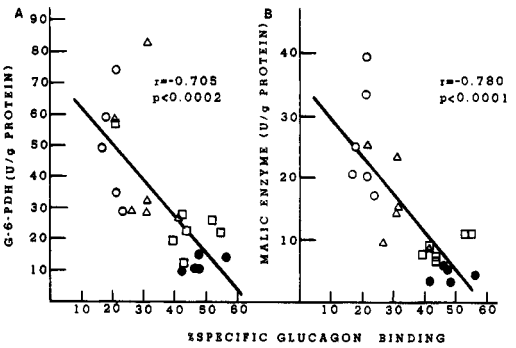


FIG. 5. Relationship between glucagon binding to liver membranes and liver G6PDH (A; $r = -0.705$, $P < 0.0002$) and ME (B; $r = -0.780$, $P < 0.0001$).

Increased plasma insulin has been universally observed in obesity but there is no consensus on the plasma glucagon level either in man or animals. In analyzing the glucagon levels in animals, it may be pertinent to note that obese mice are not hyperlipemic while Zucker and corpulent rats are. Eaton *et al.* (11, 32) and others (33, 34) observed decreased levels of plasma glucagon in obese Zucker rats, and similar findings have been reported in hypothalamic obese rats (35) and hypothalamic obesity in monkeys (31).

In general obese mice have not shown (36–38) decreased plasma glucagon levels. However, to our knowledge, glucagon binding studies have not been reported in these animals.

What is the potential significance of the reduced glucagon receptors in these *cp/cp* obese rats? We have seen that there is a significant negative correlation between glucagon receptors and the activity of lipogenic enzymes (Fig. 5). Unfortunately we did not examine the

present experimental animals for plasma lipids. However, utilizing data from LA/N corpulent rats of ages, weights, and diets similar to those in the present experiment, we have also observed a significant negative correlation between plasma glucagon levels and triglycerides ($r = -0.990$, $P < 0.01$) as well as cholesterol ($r = -0.993$, $P < 0.01$; data not shown).

Martin and Gahagan (39) observed that the inhibitory effect of glucagon on liver lipogenesis was blunted in obese rats. Lombardo *et al.* (40) showed that rats fed sucrose are less sensitive to the lipolytic effects of exogenous glucagon. They interpreted these phenomena as associations of hyperinsulinism. It is possible, however, that glucagon resistance (as a consequence of decreased glucagon receptors) as observed in these corpulent rats could be acting in concert with increased insulin levels to intensify the hyperlipemic state.

Dietary sucrose produces higher fasting plasma insulin levels in human subjects (14, 41, 42) and animals (40, 43). In the present study there was an increase in plasma insulin, in both lean and corpulent rats, but the increases were not significant. The effect of sucrose on plasma glucagon has not been well documented. In the present study we observed no significant effect of sucrose feeding on plasma glucagon either in lean or corpulent rats. It thus appears that plasma insulin is more sensitive than plasma glucagon to dietary sucrose although plasma glucagon is certainly increased by a high protein diet (23).

It is well documented that the nutritional status of normal as well as obese humans and animals alters plasma insulin levels and inversely alters insulin binding to target and nontarget tissues (17–19, 44), but only recently has it been shown that changes in nutritional state in humans and animals also alter glucagon binding (20, 21). We now show that dietary carbohydrate also alters glucagon receptors in that sucrose feeding decreases glucagon binding to liver plasma membranes and this decrease is due to the decrease in the number of glucagon receptors. The effect of sucrose is more prominent in corpulent than lean rats.

In conclusion, we have shown that obese LA/N-*cp* rats have hyperinsulinism and hypoglucagonemia but significantly decreased hepatic receptors for both hormones. We postulate that this combination is important in the development of the hyperlipemia documented in these obese rats. Definitive studies regarding this postulate are in process.

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