Effects of the Electric Stress on Insulin Secretion and Glucose Metabolism in Rats Fed with a High Fat Diet

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Synopsis

The synergetic effects of dietary obesity and stress induced by electrical shocks on insulin secretion from the perfused pancreas, the action of insulin on adipose tissues and glucose tolerance were studied in rats.

The rat was fed either a control (C) (50% starch) or a high fat diet (F) (40% butter) for a period of 12 weeks. Half of the rats on each diet received electrical shocks, one hour per day for the last three weeks of the experiment (group C-S, F-S). The remaining rats were not given any stress sessions (group C-N, F-N). The rats on the high fat diet gained a significant amount of weight at the 8th week, and as determined by the adipocytes, were obese at the end of 12 weeks. However, the high fat diet itself did not have any effect on plasma glucose, plasma insulin and insulin release from the perfused pancreas in response to glucose. It caused glucose intolerance and the insensitivity to insulin of adipose tissue. The rats which received electrical shocks stopped gaining weight when the shock sessions began. Moreover, the size of the adipocytes in F-S group was significantly smaller than that in F-N group, but the insensitivity to insulin of adipose tissue remained. In F-S group, glucose-induced insulin release from the perfused pancreas was significantly diminished in the initial phase of release, and glucose tolerance was much impaired by stress, while in C-S group insulin release increased in the late phase of release, and glucose tolerance was unaffected by stress.

These findings indicate that the ability of glucose to stimulate insulin secretion is decreased by the synergetic effects of a high fat diet and stress induced by electric shock. Thus, it may be concluded that the mechanism which mediates the effects of glucose upon insulin secretion, especially in the initial response phase, is modified by such synergetic effects.

It has been said that the development of diabetes increases with the presence of such factors as obesity, pregnancy, infection, psychological stress, pancreatic or other endocrine disorders and so on. However, the mechanism in which these factors accelerate diabetogenic effects remains unknown.

It has been shown that over-eating, especially of foods high in fat content, plays an important role in the regulation of diabetes (Albrink, 1974). In diabetics, a high fat diet causes a further reduction of glucose tolerance. This condition can usually be improved by a reduction of fat in their diet (Brunzell et al., 1974). This can be attributed to several disturbances in the regulation of carbohydrate metabolism in the liver (Eisenstein et al., 1974) and adipose tissue (Zaragoza-Hermans and Felber, 1972), caused by the high fat diet. It has also been shown that obesity produced by a high

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fat diet (Lemonnier, 1972) impairs glucose tolerance and lowers the sensitivity of peripheral tissues to insulin (Stern et al., 1975).

On the other hand, the diabetogenic effects could also be induced by epinephrine, ACTH and glucocorticoids which are released in response to stress (Bates and Garrison, 1973). Endogenous epinephrine released during the periods of stress activates the sympathetic nervous system which suppresses insulin secretion (Wright and Malaisse, 1968) which then stimulates glucose uptake in muscle in vitro (Burns et al., 1953). Glucocorticoids inhibit glucose phosphorylation in muscle and adipose tissue (Morgan et al., 1959; Roth and Livingston, 1976). And when administered to rats, glucocorticoids also increased the levels of blood glucose, plasma insulin (Åkerbloom et al., 1973) and insulin release from the perfused pancreas (Lenzen, 1976). Thus, both a high fat diet and the hormones released during a stress state affect insulin action in the peripheral tissues and insulin secretion, and contribute to the metabolism of glucose.

This study was designed to examine the synergetic effects of dietary obesity and psychological stress on glucose-induced insulin secretion, insulin action on glucose metabolism in adipose tissue and glucose tolerance.

Materials and Methods

Animals and diets

Male Wistar rats weighing approximately 100 g were used. To the rats was assigned either a high fat or control diet, and each condition was further divided into two different stress; Stress and Non-Stress. The rats in Group F were fed the high fat diet (40% butter) while to the rats in Group C was fed the control diet (50% starch) (see Table 1). The rats assigned to Group S were subjected to stress in the form of electrical shocks for the last three weeks, while those in Group N did not receive shocks. Food and water were provided ad libitum. The rats were weighed at the beginning of the experiment and weekly.

### Apparatus and procedure for the electric shock

Clear acrylic plastic chambers with iron grids imbedded in the floor were used. 200 V (ac) of scrambled electric shock were delivered, through a 300 Kohm current-limiting resister to the subject. The rats received 100 shocks during each session (1 hr/day) and the duration of each shock was one sec. The inter-shock intervals were randomly assigned from 8 to 108 sec with a mean of 36 sec. The stress sessions were continued for 3 weeks.

### Glucocorticoids assay

Twenty-four hr after the end of the last stress session, rats from each group were decapitated and blood samples were collected in heparinized tubes. Plasma 11-OHCS levels were determined by a slight modification (Usui et al., 1970) of the fluorometric method described by DeMoor et al. (1960).

### Intravenous glucose tolerance test (iv GTT)

The intravenous glucose tolerance test was performed by injecting glucose (0.5 g/kg, in 50% w/v solution) into rats which had been deprived of food for 18 hr, under anesthesia with sodium pentobarbital (40-50 mg/kg) intraperitoneally. Blood samples were drawn from the tail vein immediately before (0), and at 3,10, 20, 30, 40 and 60 min after the injection of glucose, and were placed in tubes containing sodium fluoride. Plasma glucose was determined by o-toluidine method using a commercial kit (Kokusai-shiyaku K.). Glucose disappearance rates (K) were estimated from the decline of plasma glucose levels after the injection of glucose (Amatsuzaio et al., 1953).

### Perfusion system

The pancreas was isolated from the food-deprived rats for 18 hr and perfused using the procedure described by Grodsky et al. (1963). The preparation of pancreas was placed into a perfusion vessel. The

<table>
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<tr>
<th>Table 1. Composition of diets</th>
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<td></td>
</tr>
<tr>
<td>Control</td>
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<tr>
<td>-------------------------</td>
</tr>
<tr>
<td>Corn starch</td>
</tr>
<tr>
<td>Milk casein</td>
</tr>
<tr>
<td>a-Starch</td>
</tr>
<tr>
<td>Sucrose</td>
</tr>
<tr>
<td>Butter</td>
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<tr>
<td>Cellulose powder</td>
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<tr>
<td>Mineral mixture</td>
</tr>
<tr>
<td>Vitamin mixture</td>
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<tr>
<td>Calories</td>
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<tr>
<td>% of Calories</td>
</tr>
<tr>
<td>Protein</td>
</tr>
<tr>
<td>Carbohydrate</td>
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<tr>
<td>Fat</td>
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perfusion experiments were performed at 37°C with Krebs-Henseleit bicarbonate buffer (pH 7.4) containing 4% (w/v) dextran and equilibrated with a mixture of 95% O_2 and CO_2. The flow rate was adjusted to 1 ml/min. After an equilibration period of 10 min with the basal medium containing 2.8 mm glucose, the isolated pancreas was perfused for 60 min with a medium containing 16.7 mm glucose. Effluents were collected at 60 sec intervals and frozen at -60°C until assayed. Immunoreactive insulin (IRI) was measured using the double antibody radioimmunoassay (Dinabot RI Labs. Ltd., RIAKIT) with pig insulin as a standard.

Glucose metabolism in adipose tissue
The epididymal fat pads of the rats decapitated after being fed were removed immediately, cut into small pieces (approx. 100 mg each) and placed into vials containing 1 ml of incubation medium. The medium for the incubation was Krebs-Ringer bicarbonate buffer (pH 7.4) containing 3% (w/v) bovine serum albumin, 5 mm [U-14C]-glucose, with or without 1000 μU/ml insulin and saturated with a mixture of 95% O_2 and 5% CO_2. The incubation period continued for 2 hr at 37°C. After incubation, 0.4 ml of Hyamine was injected into the inner vessel and ^14CO_2 was trapped into Hyamine by the injection of 0.8 ml of 1 N H_2SO_4 into the outer vial. After another hour of incubation, the level of radioactive CO_2 was measured. Total lipids were extracted in an aliquot of tissue with chloroform: methanol (w/v, 2:1) by the method of Folch (1957). The radioactivity was measured with a liquid-scintillation spectrometer (Packard, model 3385).

Results

Body weight
The effects of a high fat diet and stress on body weight are shown in Fig. 1. The initial weight of the four groups was identical, about 100 g. Rats in the F-N group were significantly heavier after 8 weeks, and were approximately 50 g heavier than the rats in the C-N group at the end of the experiment (see Table 3). During the 3 weeks of stress sessions neither the F-S group nor the C-S group gained weight. After 3 weeks of shock sessions rats in the S groups weight about 50 g less than their counterparts in the N groups. There was a significant difference of weight between the C-S group and the F-S group (Table 3).

The reduced weight in S groups was a result of decreased food consumption: 18% in the C-S group and 31% in the F-S group. The mean daily caloric intake during the shock sessions was not significantly different between the C-S group (55.4±3.9 Cal/day, n=14) and the F-S group (53.5±7.9 Cal/day, n=13).

Plasma 11-OHCS levels
Seligman (1968) stated that electrical shocks administered at random intervals produced a chronic fear state in the subjects. Therefore, it would be expected that the degree of psychological stress (Weiss, 1972) was much greater in the random interval schedule, used in this experiment, than in the fixed interval schedule.

In a preliminary experiment, plasma 11-OHCS was measured as an indicator of stress. Rats received electrical shocks in the random or fixed interval schedule.
Both conditions were the same physical state, but not the same psychological state. It was found that the level of plasma 11-OHCS was higher in the former condition (39 ± 5 µg/100 ml, n = 7) than in the latter (26 ± 6 µg/100 ml, n = 6) (p < 0.02).

Table 2 indicates the levels in plasma 11-OHCS of the present experiment. There is no difference between two diet conditions, but a significant difference is seen between the stress and nonstress conditions (p < 0.001).

**Table 2. Effects of a high fat diet and stress on plasma corticosterone (11-OHCS) levels**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>High fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Stress</td>
<td>19.9 ± 1.5 (9)</td>
<td>19.1 ± 3.6 (7)</td>
</tr>
<tr>
<td>Stress</td>
<td>34.9 ± 3.8 (8)†</td>
<td>39.1 ± 5.3 (7)†</td>
</tr>
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</table>

* µg/100 ml. Values are means ± SE. The number of rats is indicated in parentheses.
† Significantly different from non-stress group in each diet group, p < 0.001.

This shows that stress-induced hormones such as glucocorticoids play a role in the diabetogenic effects.

**Plasma glucose, insulin and adipose cellularity**

The fasting level of plasma glucose was not affected by the high fat diet or stress alone, but was slightly elevated in the F-S group. Plasma IRI was not changed by the high fat diet. The plasma IRI level rose significantly in the C-S group (p < 0.02) but not in the F-S group.

The mean diameter per 200 adipocytes from fed rats in the F-N group was greater than that of those from the C-N group (p < 0.005). The mean diameter of adipocytes in the F-S group was smaller than that from the F-N group (p < 0.005), and was similar to that in the C-S group (Table 3).

![Fig. 2. Changes in blood glucose during the intravenous glucose tolerance test in rats fed with a control (A) and high fat diet (B). Glucose, 0.5 g per kilogram body weight, was administered intravenously into rats fasted for 18 hr under anesthesia. Values are means ± SE, and the number of rats is indicated in parentheses. k: the disappearance rates of glucose from blood after the injection of glucose. NS: Non-Stress group, S: Stress group.](image_url)
Intravenous glucose tolerance tests

The results of ivGTT are shown in Fig. 2. The fasting blood glucose levels of the four groups are listed in Table 3. Three min after the injection of glucose, the blood glucose level of the F-N group (243 ± 100 mg/1000 ml) had risen higher than that of the C-N group (199 ± 8 mg/100 ml) (p < 0.005), while the blood glucose levels in the S groups had increased much more than those in the N groups (259 ± 13 mg/100 ml in the C-S group and 305 ± 13 mg/100 ml in the F-S group). The fall from the peak values of the C groups was more rapid than that of the F groups. 60 min after the injection of glucose, the blood glucose levels were restored to the original levels in all groups except the F-S group.

When compared with the glucose disappearance rates (K), the mean k values in the C-S group were identical to those in the C-S group, 5.0 and 4.9 respectively. The mean k values in the F-N group (k = 3.0) were much lower than those in the F-N group (k = 3.5). Thus, the reduction of glucose tolerance was the greatest in the F-S group.

Glucose-induced insulin secretion from the perfused pancreas

The typical biphasic glucose-induced insulin release from the perfused pancreas, as described by Grodsky et al. (1968), was observed in all four groups (Fig. 3). Following the equilibration period with 2.8 mm glucose for 10 min, the first peak of insulin release was reached about 2 min after the elevation to 16.7 mm glucose. The insulin release rate of the first peak in the F-S group (126 ± 39 μU/ml/min), which was significantly lower than that of the F-N group (187 ± 26 μU/ml/min) (p < 0.02). However, the insulin release rates in the C groups were the same in both stress and non-stress conditions. The insulin-releasing patterns to glucose in both the C-N and the F-N groups were similar. As shown in Table 4, in the F-S group the first phase (t₀ to t₁₀) above the basal secretion was significantly decreased (p < 0.02), and the second phase of release (t₁₀ to t₆₀) was the same as that in the F-N group. In the C-S group, the second phase of release was increased (p < 0.01).

Table 3. Effects of a high fat diet and stress on fasting plasma glucose and insulin, and adipose cellularity

<table>
<thead>
<tr>
<th></th>
<th>Body weight (g)</th>
<th>Plasma glucose (mg/100 ml)</th>
<th>Insulin# (ng/ml)</th>
<th>Diameter of adipocytes## (µ per 200 cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Stress</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>369 ± 5 (13)</td>
<td>101 ± 4 (17)</td>
<td>1.2 ± 0.2 (6)</td>
<td>110 ± 1</td>
</tr>
<tr>
<td>High fat</td>
<td>407 ± 9 (17)†††</td>
<td>103 ± 5 (17)</td>
<td>1.7 ± 0.3 (7)</td>
<td>119 ± 2†††</td>
</tr>
<tr>
<td>Stress</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>309 ± 9 (7)****</td>
<td>107 ± 7 (7)</td>
<td>2.1 ± 0.3 (8)*</td>
<td>109 ± 2</td>
</tr>
<tr>
<td>High fat</td>
<td>352 ± 11 (7)****, oo</td>
<td>121 ± 7 (7)</td>
<td>1.8 ± 0.3 (7)</td>
<td>107 ± 1***</td>
</tr>
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</table>

# Insulin was measured with rat insulin as a standard.
## Adipocytes were obtained from fed rats used for the experiment on glucose metabolism in adipose tissues (in Fig. 4).

Values are means ± SE, and the number of rats is indicated in parentheses.

††† Significantly different from controls in the non-stress group, p < 0.005.

* Significantly different from the non-stress group in each diet group, p < 0.02, ** p < 0.005, **** p < 0.001.

oo Significantly different from the control in the stress group, p < 0.01.
Fig. 3. Insulin release from the isolated perfused pancreas in rats fed with a control (A) and high fat diet (B). Each perfusion system was equilibrated with the basal medium (pH 7.4) containing 2.8 mM glucose from t-10 to t0, and then perfused with a medium containing 16.7 mM glucose from t0 to t60. Values are means±SE, and the number of rate is indicated in parentheses. NS: Non-Stress group, S: Stress group.

Table 4. Effects of a high fat diet and stress on insulin release in the first and second phase stimulated with 16.7 mM glucose in the isolated perfused rat pancreas

<table>
<thead>
<tr>
<th></th>
<th>First phase</th>
<th>Second phase</th>
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<tbody>
<tr>
<td></td>
<td>t0–t10</td>
<td>t10–t60</td>
</tr>
<tr>
<td>Control (Non-Stress)</td>
<td>8</td>
<td>1001±141</td>
</tr>
<tr>
<td>High fat</td>
<td>9</td>
<td>1115±114</td>
</tr>
<tr>
<td>Stress (Control)</td>
<td>4</td>
<td>1257±321</td>
</tr>
<tr>
<td>High fat</td>
<td>5</td>
<td>670±145*</td>
</tr>
</tbody>
</table>

Values are means ± SE of the integrated amount of insulin released above the base secretion seen in Fig. 3, during the periods of t0–t10 and t10–t60, respectively.

* Significantly different from the non-stress group in each diet group, p<0.02, ** p<0.01.

Fig. 4. Metabolism of [U-14C] glucose in the adipose tissue from rats fed a control (C) and high fat diet (F). Incorporation of 14C-glucose into (A) CO₂ and (B) total lipids. Adipose tissues from the fed rats were incubated for 2 hr at 37°C in 1 ml of Krebs-Ringer bicarbonate buffer (pH 7.4) containing 3% albumin, 5 mM [U-14C] glucose, with or without 1000 µU/ml insulin and saturated with 95% O₂ and 5% CO₂. Vertical lines indicate ±SE. The number of rats is indicated in parentheses.
Glucose metabolism in adipose tissue

The basal conversion of \([U-^{14}C]\) glucose into \(^{14}CO_2\) (Fig. 4, A) and into total lipids-\(^{14}C\) (Fig. 4, B) in adipose tissue were not significantly affected by the combination of the high fat diet and stress. The effect of insulin on the conversion of \([U-^{14}C]\) glucose to \(^{14}CO_2\) and lipids-\(^{14}C\) was less in the F groups than the C groups. The decreased action of insulin on glucose metabolism in adipose tissue was no greater in the F-S group than in the F-N group and was independent of stress as in the C groups. It appears that the stimulatory effect of insulin on glucose metabolism in adipose tissue is greatly dependent on the high fat diet.

Discussion

This experiment demonstrates that the high fat diet produces obesity, as shown by the enlarged adipocytes, and described by Lemonnier (1972). This state had no effect upon glucose and insulin levels in plasma at the fasting state. These findings are in agreement with the observations of Stern et al. (1975) and Malaisse et al. (1969). The concomitant effects of a high fat diet seem to be reflected by the enlarged adipocytes which induced a relative obese state without a markedly increased body weight as proposed by Zaragoza and Felber (1970), and the size of the adipocytes play an important role in the response to insulin (Lemonnier et al., 1974).

But the fact that adipose tissue from such obese rats showed a diminished stimulatory effects of insulin on glucose metabolism (Fig. 4). Salans et al. (1969) suggested that the insulin resistance to obesity could be explained by the enlarged adipocytes. However, the small adipocytes obtained from the obese rats in the stress group were just as insensitive to insulin as the enlarged adipocytes from the obese rats in the non-stress group. Therefore, the effect of insulin on adipose tissue in vitro may be influenced by the diet composition rather than the size of the fat cells, as suggested in the observation of experimental obesity in human subjects by Sims et al. (1973).

It has been suggested that the hyper-insulin response to glucose in genetic obesity is due to a greater quantity of insulin available for release rather than to an increased sensitivity of the beta cell to glucose (Karam et al., 1974). The insulin response to glucose from the perfused pancreas was not affected by the high fat diet (Fig. 3). It follows that glucose tolerance in the obese rats contributes to a relative lack of insulin which causes the insensitivity to insulin in peripheral tissues.

The introduction of stress by electrical shock elevated the plasma 11-OHCS level, but the high fat diet had no effect on the plasma 11-OHCS level (Table 2). Roth and Livingston (1976) have proposed that the exposure of adipocytes to glucocorticoid hormones in vitro causes inhibition of glucose transport and metabolism. However, the glucose metabolism in adipose tissue was not influenced by stress (Fig. 4). Thus, glucose tolerance impaired by stress in obese rats cannot account for the insensitivity to insulin in peripheral tissues.

It has been suggested that basal insulin may be normal or elevated by prolonged stress (Porte and Robertson, 1973). In normal rats, the insulin release from the perfused pancreas in response to glucose and the plasma insulin level were increased by stress. This findings show that the electrical shock stress directly affects the pancreatic islets. But in obese rats insulin release, especially in the initial phase of release was markedly reduced by stress, which show a similar pattern to that of prediabetics (Cerasi et al., 1972) and alloxan-induced diabetics in dogs (Pupo et al., 1976). As suggested in diabetes by Cerasi et al. (1972), the decreased insulin response to glucose in
obese rats subjected to stress may be ascribed to the decreased sensitivity to glucose in the pancreatic beta cell. Hedeskov and Capito (1975) who used mouse pancreatic islets during its starvation have suggested that the decreased sensitivity of insulin secretory mechanism may be caused by an ability to increase the concentration of cyclic AMP upon stimulation and a disturbance of the distribution of calcium in the pancreatic beta cell. Thus, some factors which are associated with the initial insulin response to glucose in the pancreatic beta cell may be modified by stress.

In conclusion, the results of this experiment indicate that the synergistic effects of obesity produced by a high fat diet and stress induced by electrical shock play an important role in the development of the diabetic syndrome. This was observed in the decreased initial insulin response to glucose from the perfused pancreas, an impaired glucose tolerance and a slightly elevated blood glucose level at the fasting state.

Acknowledgements

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References
