Influence of reversible obesity on eating behavior, blood glucose, and insulin in the rat

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Steffens, A. B. Influence of reversible obesity on eating behavior, blood glucose, and insulin in the rat. Am. J. Physiol. 228(6): 1738–1744. 1975. Excessive food intake, resulting in extreme obesity, was induced in rats by electrical stimulation (three 30-min sessions per day for 3 wk) of the lateral hypothalamus (LH). Outside the stimulation sessions, no voluntary food intake occurred during these weeks. In the subsequent recovery period, accurate records of spontaneous feeding were made. Blood levels of glucose, insulin, and free fatty acids (FFA) were determined during the stimulation period as well as the recovery period. During feeding elicited by electrical stimulation of the LH in the obese phase, there was a large increase in the glucose level. The insulin level, very high in the intervals between the stimulation sessions, showed no change, a small decline, or a large decrease, respectively, during stimulation in the morning, the afternoon, or the night. If food was withheld during stimulation, there was a large increase in the glucose level and a large decrease in the insulin level. The first spontaneous meal after the termination of stimulation was extremely postponed, viz., until the glucose and insulin levels returned to normal for the first time. The insulin response to the first spontaneous meal was exaggerated. Although in the following period (about 10 days) a rapid decrease in body weight occurred, food intake was only minimal. The insulin level was high and the glucose level subnormal during that period. The insulin and glucose levels were normal again only after recovery of normal body weight and food intake. It is tentatively concluded that the regulation of body energy stores (body weight) is achieved through the control of food intake by circulating insulin and glucose levels.

Food intake; satiety mechanism; blood glucose levels; blood FFA levels; blood insulin levels; electrical stimulation; lateral hypothalamic area

IN PREVIOUS PUBLICATIONS (23) it has been shown that in free-feeding rats, in a condition of complete nutritional equilibrium, there is a strong correlation between the time pattern of feeding and that of the fluctuation of glucose availability (as indicated by the combination of glucose and insulin levels in the blood). It has been argued that this supports the “glucostatic theory” of regulation of food intake (23).

The question now arises whether the same control mechanism might be operating in animals which, having first been subjected to treatment leading to obesity, are subsequently left free to feed as they wish. The point seems worth investigating for, as pointed out by Hoebel in 1971, “in spite of twenty years of searching, no one yet has managed to specify the stimulus which is correlated with body weight and serves such an important role in the control of food intake” (11).

In the present experiment, therefore, rats were made hyperphagic and obese by frequent stimulation of the lateral hypothalamus (LH) over a period of weeks. After this, they were kept under normal ad libitum conditions. Blood levels of insulin and glucose were measured in a period beginning during the final phase of stimulation and continuing during the gradual recovery of normal body weight and normal feeding behavior.

METHODS

Subjects and maintenance. Male Wistar rats weighing 250–300 g were maintained in individual Plexiglas chambers (25 cm x 25 cm x 30 cm) at a room temperature of 20°C. Lights were on from 6 A.M. until 6 P.M. Food and water were allowed ad libitum unless stated otherwise. A standard diet, providing 20% protein, 53.5% carbohydrate, 4.5% fat, and 22% water, with added minerals and vitamins, was available ad libitum. The diet was presented in the form of a bar, which could slide easily through a dispensing tube attached to one of the walls of the cage. The bar could be removed from the dispenser after a meal and weighted without disturbing the animal. Practically no food was spilled. Body weight and food intake of the animals were measured every day.

Electrical stimulation of lateral hypothalamus. Rats were anesthetized with ether and placed in a stereotaxic apparatus. Bipolar electrodes (Plastic Products Co., Roanoke, Va.) were implanted unilaterally in the lateral hypothalamus according to a procedure previously described (13). The coordinates used according to the De Groot atlas (3) were A: 5.4 to 5.8; L: 1.7; V: −2.4. Ten days after recovery the animals were tested for food intake in response to stimuli delivered by a constant-current, 50-cycles/wave stimulator. In the first test session of 15 min, stimuli of gradually increasing strength (starting with 2 μA and increased in steps of 2 μA) were applied by the experimenter each time the head of the animal was in the neighborhood of the food jar, which was filled with a mixture of powdered food (50%) and water (50%). Some animals manifested increased attention for the food, and even performed food intake, when the current was switched on (provided that it was of sufficient intensity). Increasing the current above a certain optimal level (ranging from 15 to 60 μA in different individuals) caused restlessness and less responsiveness to food. Animals that ate during the first session...
showed more eating behavior, and more stimulus-bound eating in later trials, until a plateau was reached after a few days. Rats that ate less than 10 g during 30 min after 5 days of training were discarded. Eight out of forty operated animals fulfilled this criterion and were used for the experiments.

To obtain obese rats (a procedure starting 14 days after the last test session), the animals were given a stimulation session 3 times a day for a period of 30 min, viz., at 9.25 A.M., 4.15 P.M., and 9.30 P.M. During such a session, the current was switched on as soon as the animal came in the neighborhood of the food dish, and the stimulus was continued as long as the animal was eating (ranging from 4 to 10 s). As soon as it stopped eating and turned away from the food dish, the current was switched off. This procedure was repeated each time that the animal turned its head to the food dish (which occurred nearly always within 10 s). In this way, the total duration of all stimulations during one session of 30 min was more than 15 min. All eight animals in the experiment had three stimulation sessions a day, and this was continued for 21 days without interruption.

The food offered during these sessions consisted of a mixture of 30% powdered standard diet, 25% water, and 25% vegetable oil.

**Implantation of heart cannulas.** Two weeks after the start of the daily three-stimulation periods, cannulas were inserted into the heart of the animals through the jugular vein by the technique described by Steffens (19). The implantation of the cannulas disturbed the animals so little that it was not necessary to interrupt the stimulation schedule. Three days after implantation of the cannulas, blood sampling was begun. It was carried out as described previously (17). For the determination of blood glucose and insulin levels, samples of 0.25 ml were withdrawn; if glucose-insulin and free fatty acid (FFA) levels were determined, samples of 0.6 ml were taken. Blood was replaced immediately after sampling with the same quantity of donor blood. This procedure influenced neither behavior nor the level of blood constituents as described previously (17).

**Experimental procedure.** On the 18th day of stimulation for each animal, blood samples were withdrawn during the morning, afternoon, and night sessions. Blood samples were taken 25 and 5 min before the start of each stimulation session. During the session blood samples were taken 5, 15, and 25 min after the start. Finally, samples were taken 5, 25, and 60 min after termination of the session. In the morning additional samples were taken 15 min before stimulation, and 15 and 35 min after the end of the sessions. During the periods between the sessions, blood samples were withdrawn at 30-min intervals. The time pattern of sampling of the morning session was repeated on the 19th day during an intercalated stimulation session in which no food was offered. The first prestimulation sample of this session was taken 60 min after the normal morning session, during which food was present and eaten. This nonfood session was given only once to all animals.

From the end of the final stimulation session, the subjects were kept under continuous direct visual observation for 84 hr. Twelve hours after the last stimulation session, blood sampling was resumed. A sample was taken every 60 min until the start of the first voluntary meal; then the blood samples were taken 5, 15, 25, and 35 min after the start of that meal, and then again every 60 min. This sampling procedure was continued during 50 h. Samples were taken again 84 h after the last stimulation, seven samples being withdrawn in all, with 30-min intervals. The sampling during a voluntary meal was repeated after 3 wk, when food intake and body weight had regained prestimulation levels.

**Chemical determinations.** The blood samples were immediately chilled and centrifuged at 4°C. Glucose was measured by the ferricyanide method of Hoffman (12) in a Technicon AutoAnalyzer on 0.035 ml blood samples.

Blood FFA were determined according to the method of Antonis (2) based on the measurements of the chloroform-soluble copper salts of the long-chain fatty acids. The method was adapted to 0.2 ml plasma in order to avoid large blood samples. Everything was done as described by Antonis with the exception that after the evaporation of the diisopropyl ether the residue was dissolved in 2.5 ml instead of 5 ml chloroform. For every sample two blanks were run in which the 0.2 ml plasma was replaced by 0.2 ml water. The extinction of the blanks measured in the AutoAnalyzer was subtracted from the plasma determination. The plasmas for FFA determination were extracted immediately, and the chloroform extracts were stored at −30°C until the determination. For insulin determination the plasma samples were stored at −30°C. Plasma insulin was determined according to the Hales-Randle method (9) with use of a rat insulin standard. The assays were performed using a radioimmunoassay kit (Radiochemical Centre, Amersham, England). Duplicate assays were performed on 25-μl samples of plasma from experimental animals. In LH-stimulated obese animals the assays were performed on 12.5-μl samples. To each of the standards were added 25 μl (or in the case of LH-stimulated obese, 12.5 μl) of plasma from alloxan-diabetic rats that had been deprived of food for 36 h.

**Histology.** All animals were sacrificed with ether and perfused with 10% Formalin. The brains were removed and embedded in paraffin and sectioned at 50 μm. The sections were stained with haemalum. On microscopic examination the electrode tip proved to be located in the desired area in the lateral hypothalamus in all eight animals.

**RESULTS.**

**Effects of stimulation on behavior and body weight.** After the start of the stimulation session, a rapid increase in body weight occurred, as also described by Steinbaum and Miller (22). Figure 1 presents the average weight of the eight experimental animals and four controls and some data on the spontaneous food intake of the experimental animals. The average weight before stimulation was 359 ± 9.3 g (range 337–378 g). The average weight gain was 195 ± 21.1 g (range 103–262 g) in 21 days. The average weight gain of controls during these days was 41 ± 8.0 g (range 22–57 g), whereas their body weight was 359 ± 11.3 g (range 321–378 g). Three points deserve mention: 1) during the sessions excessive quantities of food were eaten. During the morning, afternoon, and night sessions the
animals ate, respectively, 14.8 ± 1.2, 14.0 ± 1.3, and 12.9 ± 1.8 g. During the intervals between the sessions the animals did not eat at all. (Normal animals eat about 23 g during 24 h in about 14 meals). 2) During LII stimulation in the absence of food, the animals were running around in an agitated manner. 3) The first voluntarily taken meal after the last stimulation was postponed indefinitely. The first meal was taken on the average 28 h and 15 min (range 18 h and 35 min to 40 h and 34 min) after the last stimulation. This meal was very small: 0.68 ± 0.11 g (range 0.2-1.2 g) as compared to meals taken in the normal rat in the ad libitum situation (1.6 ± 0.07 g). Seven days after the last stimulation session, when the animals ate still only a small quantity of food in 24 h (4.8 ± 1.3 g), meal size remained small (0.77 ± 0.09 g). Sixteen days after the last stimulation, when body weight was nearly normal again, meal size (1.7 ± 0.17 g) was normal. Also the 24-h intake is approaching its normal level (15.4 ± 1.6 g).

**Blood parameters during stimulation sessions (18th day).** For a detailed survey of the average levels of insulin, glucose, and FFA at various times under the different experimental conditions, see Figs. 2 and 3.

**Morning session.** Five minutes before the start of stimulation (Fig. 2), the glucose level is high (150 ± 8.8 mg/100 ml) as compared to normal animals (115 mg/100 ml).
The insulin level (150 ± 17.5 μU/ml plasma) is relatively low as compared to the average intermeal levels for animals in this obese condition. However, one should bear in mind that the normal level between meals in the ad libitum situation is in the range from 23 to 50 μU/ml plasma. The FFA level is about normal (0.35 ± 0.09 μeq/ml plasma). However, the FFA levels 25 and 15 min before stimulation are unusually high (0.64 ± 0.17 and 0.55 ± 0.12 μeq/ml plasma, respectively). During stimulation there is a rapid increase in the glucose level. The peak value of 211 ± 23.2 mg/100 ml is reached in 5 min after the end of stimulation. There is a nonsignificant decline in the insulin level to 130 ± 19.5 μU/ml plasma after the start of stimulation, followed by a rapid increase to a peak value of 470 ± 54 μU/ml plasma 75 min after the termination of stimulation. The FFA level does not change much during or after stimulation.

Afternoon session. The glucose level rises gradually in the interval between the morning and afternoon sessions from 110 to 130 mg/100 ml (see Fig. 2). Conversely the insulin level decreases gradually from 470 ± 51 μU/ml plasma to 240 ± 94 μU/ml plasma. Then an unexplained nonsignificant increase occurs just before the start of the afternoon session. During stimulation first a transitory nonsignificant drop is observed in the insulin level is reached. After the termination of stimulation, the insulin level rises strongly to a peak value of 552 ± 75 μU/ml plasma after 85 min. The insulin level remains high with relatively small fluctuation until the night stimulation session.

Night session. The insulin curve shows a picture completely different from that during the other sessions (Fig. 2). During the stimulation the level declines significantly from 412 ± 91 to 156 ± 30 μU/ml plasma (P < 0.025). Immediately after stimulation the level increases again to 430 ± 52 μU/ml plasma.

Stimulation without food. This stimulation session started 1 h after the morning session during which the animals could eat. The glucose level 15 min before the session is 145 ± 8 mg/100 ml and the insulin level is 410 ± 58 μU/ml plasma (Fig. 3). In contrast to the situation during the sessions with food, there is no increase in the glucose level 5 min after the start of the stimulation, but subsequently there is a pronounced glucose peak. The insulin level decreases nonsignificantly during stimulation from 364 ± 60 to 181 ± 30 μU/ml plasma (P < 0.005) and remains low during stimulation. After the termination of stimulation the insulin level rises immediately, and 15 min later the premeal level is reached again. The FFA level is, here too, unusually high, 25, 15, and 5 min before stimulation (1.06 ± 0.19, 0.77 ± 0.16, and 0.60 ± 0.13 μeq/ml plasma, respectively). During stimulation it remains at the same level, then it declines slightly.

Blood parameters in period surrounding first spontaneous meal of the obese animal. As already stated above, it took on the average 28 h and 15 min after the last stimulation session with food for the animals to resume spontaneous-food intake. Figure 4 presents the insulin and glucose levels in the 9 h before the meal (every hour a blood sample was taken) and at 5, 15, 25, 35, and 60 min after the beginning of that meal. As the start of the meal could not be predicted, the interval between the last premeal blood sample and the start of eating ranged from 5 to 45 min. In Fig. 4 the glucose and insulin levels of these samples were pooled and presented as the average level at 30 min. All earlier samples were treated in an analogous manner. Insulin remains at a high level of about 150–190 μU/ml plasma until 5 h before the meal. Then a steep decline of the insulin level follows. The last premeal insulin level measured is 62 ± 10 μU/ml plasma. Figure 5 presents the decline in insulin level in all eight animals in the last two samples before spontaneous eating. The dashed lines present an
extrapolation of the insulin level from the last sample taken to the moment of eating.

In spite of the very small meal (0.68 ± 0.11 g), the response of the insulin level is very pronounced and reaches a peak value of 185 ± 38 μU/ml plasma 25 min after the initiation of the meal. In contrast the insulin level in a normal animal taking a normal meal (1.6 g) reaches a level of 83 ± 9 μU/ml plasma. The glucose level is normal in the 9 h before the spontaneous meal. Only in the last hour a nonsignificant decline occurs from 111 ± 5.9 to 102 ± 4.7 mg/100 ml.

In three of the eight animals, a cluster of very small meals was observed in the first few hours after the first meal. Then they stopped eating for several days. The insulin level is in wild oscillation during the outburst of small meals. At the start of each individual meal the insulin level is at its lowest point. In the other five animals very small meals were spread regularly in time. Just before the start of such a meal, the insulin level was at its lowest point.

Blood parameters 84 h after last stimulation. During this period the animals eat scarcely, and this results in a rapid reduction in body weight. In spite of the prolonged voluntary deprivation, the glucose level stabilizes at 100 mg/100 ml (Fig. 6). This is well above the deprivation level in normal animals (85 mg/100 ml), but below the glucose level in normal ad libitum fed animals (115 mg/100 ml). The insulin level fluctuates around 70 ± 18 μU/ml plasma. The FFA level fluctuates a little around 0.47 ± 0.04 μeq/ml plasma, which is significantly higher than the level in normal ad libitum fed animals (0.34 ± 0.025 μeq/ml plasma).

Blood parameters 3 wk after last stimulation. The picture here obtained (Fig. 7) resembled the situation described for normal animals fed ad libitum (20), although the glucose peak is rather low and the insulin peak is broadened.

**DISCUSSION**

**General increase of insulin level.** In this set of experiments reversible hyperphagia and obesity were induced. In a previous paper it was already shown that during excessive food intake, caused in a nonobese rat by LH stimulation, insulin rises to a high level (21), but declines thereafter to normal levels again. The insulin level remains high all the time in the rats described in this paper, which were very obese already. This high-insulin level can be understood partly because of the glucose levels, which are higher than in normal rats. This hyperglycemia can be explained by the huge quantities of food in the alimentary tract and presumably also, a reduced turnover capacity for glucose due to insulin resistance (15). In addition, insulin release will have been stimulated by factors other than glucose, such as hormones from the alimentary tract (pancreozymin, secretin, etc. (4, 24)) and amino acids (6).

**Diurnal variation of insulin level.** Nevertheless, there was some daily variation in insulin levels, the lowest levels being reached just before the morning sessions. This can be understood in part from differences in duration of voluntary fasting preceding morning, afternoon, and night sessions (670, 300, and 300 min, respectively). The longer the interval, the more the insulin level will decline due to further progress of food digestion and absorption. For another part, the diurnal rhythmicity in insulin-release capacity of the β-cells of the endocrine pancreas (7) may explain the lower level in the morning.

**Short-term effects of LH stimulation on blood glucose and insulin.** In the stimulation session without food, the insulin level starts to decline within 5 min, i.e., at a time when the subsequent rise of the glucose level has not yet begun. This chain of events no doubt is primarily attributable to suppression of insulin release by catecholamines put into circulation due to enhancement of sympathetic activity by the LH stimulation. Low-insulin levels, of course, will entail a rise of blood glucose. In addition it cannot be excluded that the catecholamines exert direct effects on muscle (and liver) cells that also contribute to this hyperglycemia (10).

During the stimulation with food, the glucose peaks resemble those during stimulation without food. Probably the same sympathetic mechanisms are acting here as described above. The only difference, with regard to glucose, is the immediate increase (without a delay of at least 5 min). This can be due only to the ingestion of food. As appears from previous experiments (18, 25), glucose originating from newly ingested food is already present in the 3rd min after the start of eating. It is remarkable that, in spite of the fact that the alimentary tract is full of food at all times due to the forced overeating, apparently the new meal results in a sudden increase in rate of glucose absorption. Regarding the insulin level, the first point to mention is that this manifests a much more transient decline during stimulation with than without food. Moreover, there are some notable differences among the different sessions. During the morning session there is only a suggestion of a decrease in insulin level during stimulation (Fig. 2); during the afternoon session there is a transient nonsignificant decrease (Fig. 2); during the night session, however, there is a significant decrease during the whole stimulation session (Fig. 2). The more transient nature of the decrease in insulin levels during stimulation with food may be due partly to the ingestion of food resulting in an early glucose absorption (as we have just seen) and possibly also release of gut hormones. These two fact in increase the insulin release of the β-cells. The variations or the decrease of insulin during the sessions can be explained by a circadian rhythmicity either in the sympathetic tone (5) or in the response to electrical stimulation of neurons in the LII (16), which act directly or indirectly (via the syn-

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**Fig. 6 (left):** Insulin-glucose and FFA levels in period ranging from 84 until 87 h after last stimulation session. **Fig. 7 (right):** Insulin and glucose levels during a spontaneous meal after recovery of normal weight in formerly LH-stimulated animals.
pathetic system) on the β-cells or in the release capacity of the β-cell itself.

**Exaggerated insulin responses during recovery.** The insulin response after the first spontaneous meal is very exaggerated as compared to an animal eating a normal meal (185 vs. 83 μU/ml plasma) in spite of a normal increase in glucose level (from 102 to 118 mg/100 ml vs. 114–127 mg/100 ml). The exaggerated insulin response may be due to an extreme hyperplasia and activity of the β-cells induced by the huge quantities eaten in the preceding weeks (8).

Control of meal pattern during return to normal body weight. Finally with regard to the question raised in the introduction, the following comments are pertinent. A first point of interest is the long interval between the last stimulation with food and the first spontaneous meal. Most striking is the small size of this first meal (0.68 ± 0.11 g), particularly in comparison to the huge quantities of food eaten during stimulation in the preceding weeks. The levels of blood glucose and insulin in the time before that meal and immediately thereafter indicate what may be the reason why spontaneous eating is so long postponed and why the meal is so small. In the 5 h before the meal, the insulin level declines rapidly from 180 to 60 μU/ml plasma. As already described in a previous paper (23), a low-insulin level (25 μU/ml plasma) is reached just (i.e., 4–8 min) before the onset of a meal in the normal rat under ad libitum conditions. The level just before the first spontaneous meal in this experiment is 60 μU/ml plasma. The discrepancy between these values may be attributable in part to the fact that the last insulin sample taken before the first spontaneous meal was not taken a couple of minutes but in the range from 5 to 45 min before that meal. An attempt was made to bridge this discrepancy by the extrapolations presented in Fig. 5. The insulin sample taken at the moment that the animal started eating cannot be used because the insulin level goes up strongly as soon as a rat eats (unpublished data).

It is clear as reported in several studies (1, 14) that glucose and insulin play an important role in the activity of neurons in the LI and ventromedial hypothalamus (VMH). The discharge frequency of some neurons in the VMH increases considerably when a mixture of insulin and glucose is injected near these neurons (14). It seems plausible that a high-insulin and approximately normal glucose level in the rat’s circulation causes an increased activity in these satiety neurons in the VMH, which then suppress food intake (20).

In the experiments here described, a high insulin level and nearly normal or high-glucose level is present during the stimulation intervals, but little can be concluded from the fact that during these intervals the rats do not show spontaneous feeding, for other satiety signals will be coming in, e.g., from the over-filled digestive tract. More telling is the fact that in the recovery period, when the gut is no longer full due to the prolonged voluntary fast, spontaneous feeding starts only when the insulin and glucose levels return to about that point that is observed in normal rats when they start eating. Also the rats eat only minor quantities in the days after the first spontaneous meal. The glucose and insulin levels are stabilized at 100 mg/100 ml and 70 μU/ml plasma during these days as appears from Fig. 6. The high-insulin level is probably caused by the insulin insensitivity of peripheral tissues in the still-obese animal (15). It is a tempting hypothesis that it is this combination of high insulin and normal glucose that is responsible for the low level of food intake, as discussed above. This view, of course, implies the improved assumption that the satiety neurons mentioned above are exempt from the insulin insensitivity manifested by ordinary body cells in obesity.

Therefore, the high-insulin level in this type of obesity may be a major contribution to the regulation of body weight. Further data are necessary, in particular records of activity of VMH and LH neurons during obesity caused by forced feeding, and would be of great value.

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