Effect of high sucrose diet on insulin secretion and insulin action: a study in the normal rat

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Summary. The effects of chronic high sucrose feeding for 1 month on in vivo and in vitro insulin secretion and on in vivo insulin action were studied in normal male rats. As compared to the standard chow diet, the high sucrose diet induced excess in vivo insulin response to an intravenous glucose load; the high sucrose diet also slightly improved glucose tolerance, as demonstrated by significantly higher rate of glucose disappearance ($p < 0.02$). The increased insulin secretion in response to glucose in vivo seems to be related to an hyper-reactivity of the pancreatic B cell to glucose, since it was still observed in vitro with the isolated perfused pancreas preparation. By contrast, B cells of sucrose-fed rats exhibited in vitro a normal response to arginine and a significantly lowered ($p < 0.05$) response to acetylcholine. The insulin action in the sucrose-fed rats was quantified in vivo with the insulin-glucose clamp technique. The effects of different concentrations of insulin on glucose production and glucose utilization were studied in anaesthetized rats while in the postabsorptive state. The basal glucose utilization was found significantly higher ($p < 0.001$) in sucrose-fed rats. During the clamp studies the glucose utilization induced by submaximal (400 μU/ml) or maximal (7500 μU/ml) insulin levels was significantly more important ($p < 0.02$) in the sucrose-fed rats than in the chow-fed rats. This suggests that insulin-mediated glucose uptake is enhanced over a large range of plasma insulin levels in the sucrose-fed rats. In the basal state hepatic glucose production was significantly higher ($p < 0.001$) in sucrose-fed rats. During the clamp studies, the suppression of glucose production induced by submaximal or maximal insulin levels was significantly less effective ($p < 0.05$) in the sucrose-fed rats as compared to chow-fed rats, thus suggesting that the liver becomes resistant to insulin action after sucrose feeding.

Key words: High sucrose feeding, insulin secretion, perfused pancreas, insulin action, insulin-glucose clamp technique, glucose production, glucose utilization.

Previous studies in man have suggested that a diet high in carbohydrate content leads to an unchanged [22] or significantly improved [25] oral glucose tolerance. In the laboratory rat, some investigators have noticed a decreased glucose tolerance [7, 12, 19, 23, 34] after sucrose diet; others have reported an unchanged or slightly improved glucose tolerance [8, 25, 32, 39]. Further in vitro studies [29, 31] report that basal and/or insulin-stimulated glucose uptake and metabolism are increased in isolated adipocytes of rats fed a high sucrose diet. This would suggest that the improvement of glucose tolerance is mediated, at least in part, by an increased insulin action in target tissues. In fact, there has been no direct demonstration of change of in vivo insulin action in target tissues of animals fed a high glucose diet. Furthermore, in view of the heterogeneity of insulin sensitivity among target tissues, it is clearly desirable to examine in vivo the insulin effect on glu-

cose disposal by the liver and peripheral tissues in order to properly characterise the diet impact. The present study was therefore undertaken to determine the effect of high sucrose feeding on: (1) glucose tolerance, (2) in vivo and in vitro glucose-induced insulin release and (3) basal and insulin-stimulated in vivo glucose production and glucose utilization, using the insulin-clamp technique in conjunction with isotopic measurement of glucose turnover.

Materials and methods

Animals and diets

Male Wistar rats bred in our own colony were weaned 21 days after birth with free access to a commercial pelleted chow (diet No 113, UAR, Villemoisson s/orge, France).

At 6 weeks the rats were randomly divided into two groups: one group was maintained on the standard rat chow containing by

weight (g/100 g) 57% carbohydrate (4% cellulose), 5% lipid and 22% protein, and by calories 61% carbohydrate, 13% lipid and 26% protein. The other group consumed a high sucrose diet containing by weight (g/100 g) 62% carbohydrate (7% cellulose), 4% lipid (maize oil) and 23% protein, and by calories 63% sucrose, 10% lipid and 27% protein. Energy content by 100 g diet was the same (345 cal) in both groups. After being fed the respective diets for 3 weeks, some animals randomly selected in each group were given a glucose tolerance test; 1 week later (at 10 weeks of age) their pancreases were isolated and the insulin secretion tested in vitro. The animals left in each group were used after 4 weeks on the diet (10 weeks of age) for measurement of in vivo insulin action using the glucose-insulin clamp technique.

By choosing the above protocol, we have compared rats fed either a commercial rat chow with a semi-synthetic sucrose diet. Moreover, while these two diets have a similar gross composition of protein and fat, one cannot ascertain that they do not differ with regard to the vitamin and mineral content. In order to take into consideration the possibility that the chow diet should not be the correct control diet for the sucrose diet, we performed pilot experiments with a batch of rats fed a semi-synthetic diet, similar to that used, in which the sucrose was replaced by starch. That control semi-synthetic diet contained by weight (g/100 g) 59% starch (4% cellulose), 4% lipid (maize oil) and 18% protein, and by calories 67% carbohydrate, 11% lipid and 22% protein. Both semi-synthetic diets contained the same salt mixture (3.5 g/100 g) and vitamin mixture (2.2 g/100 g).

In mg/g of vitamin mixture there were folic acid 0.094, biotin 0.047, vitamin A 469 UI, cholecalciferol 94 UI, menadione 0.469, thiamin 0.703, riboflavin 0.703, pyridoxine 0.469, calcium pantothenate 2.344, niacine 2.344, p-aminobenzoic acid 14.062, inositol 23.437, α-tocopherol 14.1 UI, choline 93.747, vitamin B-12 0.0023 and cellulose to make 1 g. In g/kg of salt mixture, there were $CaHPO_4$ 500; NaCl 74; $K_3C_6H_5O_7$, H_2O 220; K_2SO_4 52; MgO 24; $MnCO_3$ 3.5; $FeC_6H_5O_7$, H_2O 6; ZnO 1.6; $CuCO_3$, $Cu(OH_2)$ 0.3; KIO_3 0.01; $NaSeO_3$, $5H_2O$ 0.01; $CrK(SO_4)_2$, $12H_2O$ 0.55 and sucrose to make 1 kg.

After feeding the semi-synthetic control diet for 3 weeks, the rats were given a glucose tolerance test. Results obtained in that group were not significantly different than those obtained in the group maintained on the commercial chow, for daily food intake, weight gain over the 3-week period, basal plasma glucose and insulin, rate of glucose disappearance (K) during the glucose tolerance test, incremental plasma glucose values and incremental plasma insulin values (results not shown). These results provide evidence that rats fed the chow diet behave similarly to rats maintained on a semi-synthetic starch diet. Thus, for the sake of simplification, the results presented in the present paper and obtained with rats fed the high sucrose diet were directly compared to results obtained in rats fed the chow diet.

Glucose tolerance tests

Intravenous glucose tolerance tests (2.8 mmol glucose/kg body weight) were performed in the non-fasted state under pentobarbital anaesthesia (4 mg/100 g body weight intraperitoneally). Blood was withdrawn from the tail vein. Blood samples (300 μl) were immediately centrifuged at 4 °C, and plasma was stored at −20 °C until assayed.

Euglycaemic insulin clamp studies

These studies were performed at 14.00 hours in rats fasted from 09.00 hours according to a previously detailed procedure [20]. In these conditions, the rats were considered to be in the post-absorptive period and the rate of glucose production was a measure of endogenous glucose production. Rats were anaesthetized with pentobarbital. Body temperature was maintained at 37–38 °C with heating lamps. One carotid artery was catheterized for blood sampling, and a tracheotomy was systematically performed to avoid respiratory problems during anaesthesia. A 150 μl blood sample was collected

20 min after the end of the surgery for the determination of basal blood glucose and plasma insulin concentrations. An insulin solution was then infused at a constant rate of 20 μl/min in a saphenous vein, and the blood glucose level was clamped at the level measured in the basal state by a variable infusion of glucose through the other saphenous vein with a Precidor pump (Infors, Basel, Switzerland). The insulin dose-response curves were obtained by infusing various amounts of insulin (0.4–3.2 $U \cdot h^{-1} \cdot kg^{-1}$) to raise the plasma insulin levels to 400–7500 μU/ml values. Insulin (porcine monocomponent insulin, Actrapid, Novo, Copenhagen, Denmark) was dissolved in 0.9% NaCl containing 0.2% bovine serum albumin (Sigma, St. Louis, Mo, USA). The infusion of exogenous glucose (4%–15% solution) was begun 5 min after the initiation of insulin. 25 μl of blood were then sampled from the carotid artery every 5 min, and plasma glucose concentrations were determined within 60s with a glucose analyzer (Beckman, Palo Alto, Calif, USA).

The coefficient of variation in blood glucose concentration during the first 30 min of the clamp was less than 10%. The steady-state plasma insulin level was reached 30 min after the beginning of insulin infusion, and the steady-state blood glucose level was reached 45–50 min after the begining of insulin infusion. Two hundred-microlitre blood samples were collected at 50, 55 and 60 min to determine blood glucose specific activity and plasma insulin concentration.

Endogenous glucose production

Endogenous glucose production in the basal state as well as during each of the glucose clamp studies was assessed by a primed continuous infusion of [3-³H] glucose (New England Nuclear, Boston, Mass, USA). The labelled glucose was administered as an initial intravenous priming dose (4 μCi) followed immediately by a continuous intravenous infusion at a rate of 0.2 μCi/min. A steady-state of glucose specific activity was established by 40 min both in the basal state studies and in the clamp studies. The rate of glucose appearance (Ra) was then equal to the rate of glucose disappearance (Rd). These 2 parameters were calculated by dividing the [3-³H] glucose infusion rate (dpm/min) by the steady-state value of glucose specific activity (dpm/g). In the basal state the rate of endogenous glucose production is equal to the rate of glucose appearance (Ra). In the clamp studies, the rate of endogenous glucose production was calculated by substracting the exogenous glucose infusion rate from the rate of glucose appearance (Ra).

Isolated pancreas perfusion technique

The animals were anaesthetized with pentobarbital (4 mg/100 g body wt. intraperitoneally). Isolation and perfusion of the pancreas were performed as previously described (11). The perfusate was a Krebs-Ringer bicarbonate buffer with the following components: NaCl, 118 mmol/l; Kcl, 4 mmol/l; $Cacl_2$, 2.5 mmol/l; Mg SO_4, 1.2 mmol/l; KH_2PO_4, 1.2 mmol/l, $NaHCO_3$, 25 mmol/l; fatty acid-free bovine serum-albumin, 1.25 g/l (Sigma Co, St. Louis, Mo, USA); dextran T70, 40 g/l (Pharmacia, Uppsala, Sweden). When needed, L-arginine (Sigma), D-glucose (Merck, Darmstadt, FRG) and acetyl-β-methylcholine bromide (Sigma) were administered through a side-arm syringe. The complete effluent was collected from the cannula in the portal vein at 1 min intervals in chilled tubes and frozen for storage at −20 °C until assay.

Analytical techniques and calculations

Plasma glucose was determined using a glucose analyzer (Beckman). Blood samples for determination of glucose specific activity were deproteinized with $Ba(OH)_2$-$ZnSO_4$ and immediately centrifuged. An aliquot of the supernatant was used for the determination of glucose concentration using a glucose oxidase method. Another aliquot of the supernatant was evaporated at dryness at 60 °C to remove tritiat-

Table 1. Characteristics of chow- or high sucrose-fed male rats

	Body weight (g)	Plasma		K (%/min)	Δ Glucose (mmol·min^{-1}·l^{-1})	Δ Insulin (mU·min^{-1}·l^{-1})
		Glucose (mmol/l)	Insulin (mU/l)			
Chow-fed	246±4	8.4±0.5	78±6	2.03±0.10	19.5±3.6	412±43
n=	(6)	(6)	(9)	(6)	(6)	(6)
High sucrose-fed	265±6	8.7±0.2	106±12	2.67±0.18[a]	15.2±1.1	905±73[b]
n=	(9)	(9)	(9)	(9)	(9)	(9)

Values are expressed as mean±SEM; the number of observations is shown in parentheses. K, rate of glucose disappearance. [a]$p<0.02$; [b]$p<0.01$
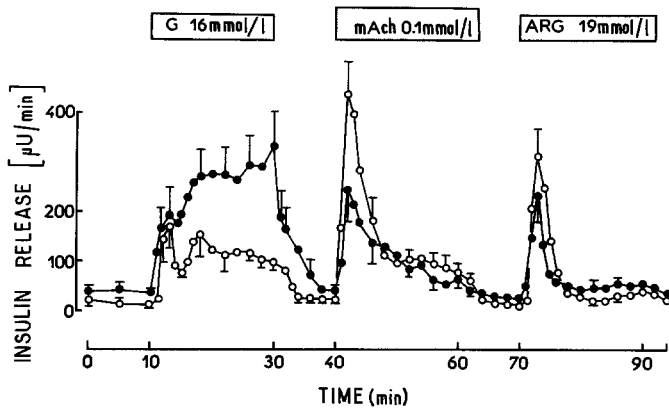
Fig. 1. Effect of 16 mmol/l glucose (G), 10^{-4} mol/l methylacetyl-choline (mAch) and 19 mmol/l arginine (ARG) on insulin release from the perfused pancreas of chow-fed (O—O) and sucrose-fed (●—●) male rats. These experiments were carried out in the absence of glucose during the pre- and post-stimulatory periods. Each point is the mean±SEM of 6 observations in each group

ed water. The dry residue was dissolved in 0.2 ml distilled water and counted with 10 ml of Ready Solv-MP scintillation solution (Beckman). Plasma immunoreactive insulin was estimated using purified rat (studies in the basal state) or porcine (clamp studies) insulin as standards (Novo), antibody to mixed (porcine + bovine) insulin cross-reacting similarly with pork and rat insulin standards and porcine mono-iodinated ^{125}I-insulin [9]. Charcoal was used to separate free from bound hormone. The method allows the determination of 6 μU/ml (0.25 ng/ml) with a coefficient of variation within and between assays of 10%.

The insulin and glucose response during the glucose tolerance test were calculated as the incremental plasma insulin values integrated over the period (30 min) following the injection of glucose (ΔI, mU·min^{-1}. l^{-1}) and the corresponding incremental integrated plasma glucose values (ΔG, mmol·min^{-1}. l^{-1}). The rate of glucose disappearance (K) was calculated from the slope of the regression line obtained with the log-transformed plasma glucose values between 5 and 30 min after glucose administration, and was expressed as %/min.

Insulin secretion rate per total pancreas was calculated by multiplying the insulin concentration in the samples by the flow rate and expressed as μU/min. Total insulin response to glucose, arginine and acetylcholine was obtained by planimetry of the individual perfusion profiles and expressed as the difference in hormonal secretion rate (Δinsulin, μU/min) relative to the mean hormonal output recorded at the end of the prestimulation period.

Statistical analysis

Results are given as mean±SEM. Statistical analysis were performed using the unpaired Student's t-test. A p value of <0.05 was considered statistically significant.

Results

Effect of sucrose diet on glucose tolerance

Feeding of sucrose or standard diet over a 3-week period resulted in no significant difference of total body weight (Table 1) with similar weight gain after 3 weeks in the sucrose-fed group (119±5 g) and in the chow-fed group (111±4 g). The daily food intake was similar for sucrose-fed rats (25.6±1.6 g/rat) and for chow-fed rats (24.4±0.6 g/rat). In rats maintained on sucrose diet, the basal plasma glucose levels were not significantly altered; in response to intravenous glucose load the mean incremental glucose areas, though lowered, were not significantly different from those in control rats. The K values in sucrose-fed rats were, however, significantly increased ($p<0.02$).

Effect of sucrose diet on insulin secretion in vivo and in vitro

In sucrose-fed rats the basal plasma insulin values were increased, but the difference did not reach statistical significance. Values of the mean; incremental insulin areas were multiplied by 2.2 ($p<0.01$) (Table 1), thus clearly indicating that glucose-induced insulin secretion was increased in vivo in sucrose-fed rats as compared to chow-fed rats. Concerning the insulin release in vitro from the isolated perfused pancreas under the basal condition (i.e. when perfusate did not contain glucose), the basal insulin release in the sucrose-fed rats was increased, although not significantly, as compared to that measured in the chow-fed rats. Exposure of the pancreases to glucose elicited in both groups a typical biphasic increase of the insulin output; there was no significant difference in the altitude of the first peak between the 2 groups. The latter response was markedly elevated in the sucrose-fed rats, thus leading to a pronounced hyperinsulinism compared to chow-fed animals (Fig. 1 and Table 2). Higher insulin secretory response in sucrose-fed rats seems to be related specifically to stimulation by glucose, since it was not evoked by arginine or acetylcholine. More precisely the insulin responses to arginine and to acetylcholine were, respectively, similarly and significantly decreased ($p<0.05$) in the sucrose-fed rats as compared to control rats (Fig. 1 and Table 2).

Table 2. Insulin secretory rates from the perfused pancreas of chow- or high sucrose-fed rats. Basal release values were expressed as absolute values. Stimulated release values were calculated as the mean increase above basal release values.

	Addition	Insulin output (μU/min)	
		Chow-fed	High sucrose-fed
Basal release	0 mmol/l glucose	25 ± 9 (6)	41 ± 6 (6)
Stimulated release (increase above basal release)	16 mmol/l glucose	101 ± 20 (6)	193 ± 40 (6)[a]
	10^{-4} mol/l methylacetyl- choline	133 ± 27 (6)	64 ± 15 (6)[a]
	19 mmol/l arginine	67 ± 12 (6)	54 ± 10 (5)

Values are mean ± SEM. The number of observations is shown in parentheses. [a] $p < 0.05$ as related to respective chow-fed groups
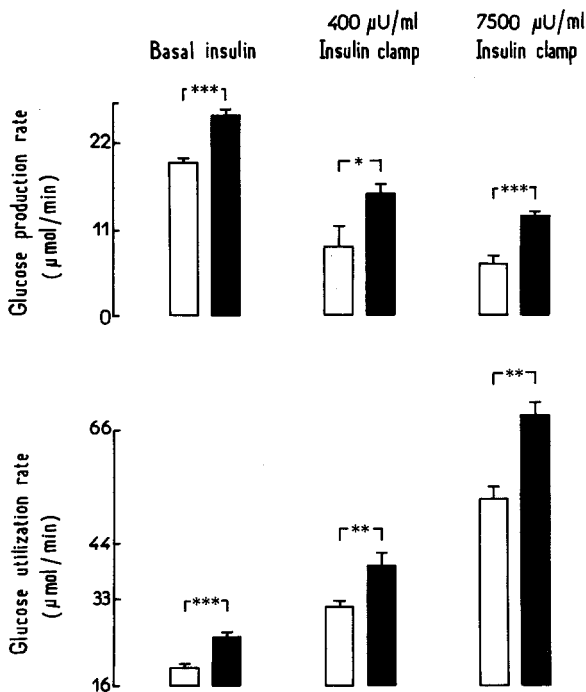
Fig. 2. Glucose production and glucose uptake during euglycaemic insulin clamps in chow-fed (□) and in sucrose-fed (■) male rats. These experiments were performed at basal (150 μU/ml) submaximal (400 μU/ml) and maximal (7500 μU/ml) steady-state plasma insulin concentrations. The height of the bar represents the mean ± SEM for each group. The number of observations in the groups was from 4 to 8. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

Effect of sucrose diet on in vivo insulin action

Basal rate of glucose production was significantly greater ($p < 0.001$) in sucrose-fed rats than in control rats (Fig. 2 and Table 3). Following a submaximal hyperinsulinaemia (406 ± 69 μU/ml in the sucrose-fed group, 432 ± 31 in the control group) or following very high plasma insulin levels (7500 μU/ml as a mean) endogenous glucose production was gradually sup-

pressed in both animal groups, but the values in the sucrose-fed rats still remained significantly higher ($p < 0.05$ or $p < 0.01$) than the corresponding values in the chow-fed rats (Fig. 2 and Table 3). Basal rate of glucose utilization was significantly greater ($p < 0.001$) in sucrose-fed rats than in control rats (Fig. 2 and Table 3). Following a submaximal hyperinsulinaemia (400 μU/ ml) or a maximal hyperinsulinaemia (7500 μU/ml) the glucose utilization in the sucrose-fed rats remained significantly greater ($p < 0.02$) than the corresponding utilization in the control rats (Fig. 2 and Table 3).

Discussion

In the normal male rats the high sucrose diet used in the present study did not lead to greater weight gain, but increased the in vivo insulin response to an intravenous glucose load; it also modestly improved glucose tolerance, as attested by K values significantly higher than control values. This beneficial long-term (1 month) effect of a high carbohydrate diet is in accordance with previous observations by ourselves [32] and others [1, 8, 25, 39], indicating that glucose tolerance was ameliorated with a high carbohydrate diet in the normal rat as well as in the normal human. However, such a conclusion is not shared by all the investigators; some have reported that rats given sucrose or fructose diet displayed a relative inability to tolerate a glucose load [12, 19, 24, 33]. Several factors may be responsible for the differences seen across studies. As previously pointed out [2], differences among experiments in the strain, sex or age of the animals used, or the way the sucrose is offered to the animals, may lead to conflicting results. For example, it has been reported that, although a high sucrose diet rather than a high starch diet was associated with greater weight gain in some strains of rats, the high sucrose diet did not lead to greater weight gain in carbohydrate-sensitive BHE rats [3]. Previous studies also have shown that the age of the animals could lead to different interpretations about the effect of sucrose on weight gain. Adult rats given sucrose displayed increased body weight within 2 weeks of exposure to the diet as compared to controls, while weanling rats given sucrose did not [18]. Concerning the way the diet is offered, it has been reported that rats given a single granulated diet containing the sucrose did not take in more calories than animals given the standard diet, while they did when the sugar was given as a sucrose solution in addition to the standard diet [19].

Our results indicate that the increased insulin secretion in response to glucose is in fact related to an hyper-reactivity of the pancreatic B cell to glucose per se, since it is still observed with the isolated perfused pancreas preparation. This point is an accordance with previous observation by Laube et al. [23]. The mechanism involved in the high insulin output by the pan-

Table 3. Blood glucose, plasma insulin, glucose and insulin infusion rates and glucose kinetics during hyperinsulinaemic euglycaemic clamps in chow- or high sucrose-fed male rats

	IIR $(U \cdot h^{-1} \cdot kg^{-1})$	SSPI (mU/l)	BBG (mmol/l)	SSBG (mmol/l)	SSGIR (μmol/min)	Ra (μmol/min)	Rd (μmol/min)
Chow-fed							
$n=8$	0	145± 9	6.3±0.3	6.2±0.4		20.0±0.3	20.0±0.3
$n=6$	0.4	432± 31	6.5±0.2	6.6±0.2	22.6±2.8	9.0±2.5	31.8±0.9
$n=4$	3.2	9123±692	6.9±0.2	7.1±0.2	41.3±0.6	6.7±0.9	46.9±2.5
High sucrose-fed							
$n=4$	0	148± 22	6.9±0.6	6.5±0.6		25.9±0.7	25.9±0.7
$n=6$	0.4	406± 69	6.8±0.6	6.6±0.3	23.7±3.3	15.7±1.3	39.7±2.3
$n=4$	3.2	6116±437	7.3±0.3	7.4±0.3	50.6±2.8	12.8±0.6	63.4±2.6

Values are mean ± SEM; n = number of rats. IIR, insulin infusion rate; SSPI, steady-state plasma insulin; BBG, basal blood glucose; SSBG, steady-state blood glucose; SSGIR, steady-state glucose infusion rate; Ra, glucose production rate; Rd, glucose utilization rate

creases of sucrose-fed rats remains presently unknown, as the insulin biosynthesis by isolated islets was not found elevated [23], and the islet insulin content [23] or the total pancreatic insulin content [32] remained unaffected as compared to controls. Nevertheless, it appears characteristic that the hypersecretion of insulin is found only after glucose stimulation, since the same pancreases of sucrose-fed rats exhibited a normal response to arginine and a significantly lowered response to acetylcholine. In the light of data presently available concerning the respective mechanisms of action of glucose and arginine on insulin release [26] and in the framework of the metabolic theory of stimulated insulin secretion, one may speculate that it is the metabolism of glucose within the B cell of sucrose-fed rats which is specifically affected by the diet. Such a proposal, however, suggests further studies. Concerning the decreased insulin response to acetylcholine in sucrose-fed rats, we are not aware of any previous observation on this topic. From a variety of experimental studies it is acknowledged that the cholinergic system plays an important role in the regulation of insulin secretion [review in 27]. More closely related to our present observations in the sucrose-fed rat, should be the results of Campfield et al. [6] suggesting that interruption of vagal input to pancreatic B cell by vagotomy in the rat induces a decreased sensitivity to acetylcholine and an increased glucose responsiveness. Thus, one may speculate that sucrose feeding determines changes in parasympathetic neural input to the pancreas which are similar to those observed after subdiaphragmatic vagotomy.

Our present data indicate that in the normal rat, after a 1 month sucrose diet, the K value was significantly higher as compared to control values, suggesting enhanced insulin action. However, the relationship between the K value and the efficiency of insulin on glucose uptake is not a direct one. In order to obviate interpretation difficulties, we have investigated this question of insulin action in sucrose-fed rats using the insulin-glucose clamp technique in conjunction with isotopic measurement of glucose turnover. During the clamp experiments performed at similar blood glucose

levels in both groups, the rates of exogenous glucose infusion (SSGIR, Table 3) required to maintained blood glucose level at euglycaemia, at steady-state plasma insulin, are taken as a measure of the effect of insulin on total-body glucose metabolism. Under the present experimental conditions these infusion rates are equal to the sum of the decrement in glucose production and the increment of glucose utilization caused by insulin. As shown by data in Table 2, total body glucose metabolism in sucrose-fed rats is not significantly different from that in controls at submaximal plasma insulin levels, but it is significantly higher at maximal insulin levels. This last observation indicates that the total body glucose metabolism is hyper-responsive to insulin in sucrose-fed rats as compared to controls. However, such a parameter does not show whether the insulin responsiveness of glucose-producing or glucose-utilizating tissues is modified in sucrose-fed rats.

The basal glucose utilization was found significantly higher in sucrose-fed rats. During the clamp studies the glucose utilization induced by submaximal or maximal insulin levels was significantly more important in the sucrose-fed rats than in the chow fed rats. These data suggest that insulin-mediated glucose uptake is enhanced over a very large range of plasma insulin levels in the sucrose-fed rats. Our data are in agreement with observation in normal human subjects receiving high carbohydrate diet, indicating that the efficiency of insulin on glucose uptake was increased [22]. The mechanism of the enhanced insulin action was not elucidated in the data herein, but in isolated adipocytes from rats fed a high carbohydrate diet, an enhanced activity of both the glucose transport system and the intracellular pathways of glucose metabolism was found [15, 16, 29, 31]. In these last experiments the increased insulin action was evidenced in the face of decreased insulin binding, indicating a post-receptor modification of glucose metabolism induced by high carbohydrate diet in the adipocytes.

Under conditions of high sucrose-feeding in rats, therefore, increased plasma insulin concentrations (at least during the meals) are associated with enhanced

insulin action, a relationship between plasma insulin level and insulin action which is the inverse of that found in obesity [30, 42], acromegaly [28] and glucocorticoid excess [17], but which is the same as that described in rats made chronically hyperinsulinaemic by insulin injection [21, 41] or insulin infusion [38, 40]. In these last studies, the observed increase in glucose metabolism in adipose cells and muscle is related by the authors to the chronic hyperinsulinaemia. However, it is also possible that the lower plasma glucose concentrations in the insulin-treated rats could have affected insulin action in adipose tissue and muscle, although the mechanism by which this might occur is unknown. Further information which may clarify the relationship between high sucrose diet, hyperinsulinaemia and enhanced insulin action in adipocytes and muscles is the report that dietary sucrose supplementation increases sympathic activity in rats [43, 44]; one possible explanation is that increased insulin-stimulated glucose metabolism could be secondary to chronic stimulation of the sympatho-adrenal system. It has been shown previously that epinephrine results in an increase of the rate of glucose transport in rat muscles [4], and that chronic administration of epinephrine to rats results in an apparent adaptation so that there is less inhibition of insulin release and increased sensitivity to exogenous insulin in vivo [35]. These effects appear to be mediated by β-adrenergic receptors. In humans, using the clamp technique, chronic administration of a β_2-adrenergic agonist terbutaline has been shown to result in significant increase in both total and nonoxidative glucose disposal rate during insulin infusion without any change in basal rates of glucose turnover [36]. These data suggest that chronic stimulation of the sympatho-adrenal system may lead to decreased β-receptor sensitivity and that this may, in turn, lead to increased insulin action in peripheral tissues.

One of the aims of the present study was to evaluate the effect of insulin on endogenous glucose production in sucrose-fed rats. As rats from both groups were in the post-absorptive state, one can assume that the rate of glucose production measured in the present studies represents hepatic glucose production. In the basal state, hepatic glucose production was higher in sucrose-fed rats than in chow-fed rats. The factors responsible for maintaining this elevated rate of basal hepatic glucose production remain to be identified. This could, at first glance, be a reflection of a hepatic insulin resistance, since the liver of sucrose-fed rats was in fact resistant to submaximal and maximal insulin levels. This last notion is based on the finding that, during the clamp studies, the suppression of glucose production induced by submaximal or maximal insulin levels was significantly less effective in the sucrose-fed rats than in the controls (Fig. 2 and Table 3). Nevertheless, it has been observed in sucrose-fed animals that glucose-6-phosphatase activity was substantially elevated, as in the case on fructose diet [10]. This pattern

was also associated with simultaneous stimulation of glycolytic and lipogenic enzymes. In contrast to glucose, the bulk of dietary fructose must be metabolized by the liver [13]; the metabolism of fructose through fructose-1-phosphate increases the flow of triose phosphate intermediates to glycolysis [5] and augments the flow of hexose phosphate in the opposite pathway (gluconeogenesis). Moreover, the increased activity of glucose-6-phosphatase would be expected to reduce the overall rate of glucose phosphorylation and thus interfere with the hepatic glucose uptake. Indeed, deficient hepatic glucose utilization was demonstrated in fructose-fed rats [14], and more recently it was reported that the ability of insulin to suppress glucose outflow was less in perfused livers from fructose-fed rats at submaximal and maximal insulin levels [37].

In conclusion, the current results confirm our previous observation [32] that chronic sucrose-feeding in the normal rat does not lead to a deterioration of glucose tolerance. Furthermore, they provide direct evidence that the modest improvement of the K value results from a complex balance between two opposite changes in insulin's effect upon target tissues; i.e. the insulin-mediated glucose uptake by peripheral tissues is enhanced, while the liver is resistant to insulin action due to a diminished ability of insulin to suppress hepatic glucose output.

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