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Comparative Effects of Several Simple Carbohydrates on Erythrocyte Insulin Receptors in Obese Subjects[1]

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RIZKALLA, S. W., F. BAIGTS, F. FUMERON, B. RABILLON, P. BAYN, A. KTORZA, D. SPIELMANN AND M. APFELBAUM. *Comparative effects of several simple carbohydrates on erythrocyte insulin receptors in obese subjects.* PHARMACOL BIOCHEM BEHAV **25**(3) 681–688, 1986.—The effects of simple carbohydrates on erythrocyte insulin receptors, plasma insulin and plasma glucose were studied during four hypocaloric, hyperproteic, diets. One diet contained no carbohydrate; the other three contained 36 g of either glucose, galactose or fructose. These diets were given for a 14-day period to groups of moderately obese subjects. The hypocaloric carbohydrate-free diet produced a decrease in plasma insulin and glucose concentrations concomitant with an increase in the number of insulin receptors. A similar increase in insulin receptor number was found when the diet was supplemented with glucose or galactose, but not with fructose. The presence of fructose in the diet prevented any increase in insulin receptor number.

Erythrocyte insulin receptors Fructose Galactose Glucose Hypocaloric diets Obesity

DIET constituents and caloric content can influence insulin receptors. The increase in caloric level of the diet decreases insulin binding to its receptors both in man [6] and in the rat [40]. Conversely, caloric restriction in obese patients results in an increase in insulin binding [2–4]. Furthermore, the carbohydrate content of the diet has a major effect on insulin receptors, e.g., an isocaloric carbohydrate rich diet decreases the number of insulin binding sites [17, 24, 32, 34, 39], while an isocaloric low carbohydrate diet increases them [29]. Recently, the nature of carbohydrate has appeared to be a major determinant of the postmeal glucose rise [21,31] and to act also on insulin receptors. Insulin binding to monocytes decreases in normal weight subjects, when they are fed a diet rich in fructose, but not when they are fed a diet rich in glucose [5]. Moreover, adding moderate amounts of fructose in usual spontaneous diet, for 5 weeks, cause elevations in glucose and insulin responses [18]. Other authors [9, 36, 49], however, found no effect on either plasma insulin or glucose level by using moderate amounts of fructose for two weeks. However, insulin receptors were not studied by these investigators. The aim of the present study is to compare effects of either glucose, galactose or fructose on insulin binding to erythrocytes. Hypocaloric and hyperproteic diets, containing one of these sugars, or none, were given to groups of moderately obese subjects.

METHOD

The subjects were 53 moderately obese volunteers (19 males and 34 females). They weighed 123.7—8.5% of their ideal body weight. They were 22±2 years old and had approximately the same degree of physical activity. None of the subjects were diabetic or had a family history of diabetes. The subjects were drug-free with the exception of five females who were on progesterone contraceptives.

In the first experiment, 23 volunteers (7 males and 16 females) were studied. They were divided into three groups in a double-blind random method. Each group was given a hypocaloric diet of liquid formula (560 kcal/day) containing protein (70 g), lipids (16 g), and carbohydrate (36 g) as either glucose, galactose or fructose. Each type of diet was given for a period of 14 consecutive days. Blood samples were collected twice after an overnight fast. An initial sample was obtained before diet administration (day 0) and a second immediately after 14 days of the diet (day 14). Plasma aliquots were used for the measurements of glucose, galactose, fructose, insulin and of insulin binding to erythrocytes.

In the second experiment, 25 volunteers (7 males and 18 females) were divided in a double-blind random method into four groups. All groups were given liquid formula diets that contained the same amount of protein (70 g) and lipid (16 g).

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TABLE 1
THE EFFECT OF TWO WEEKS ADMINISTRATION OF SELECTED HYPOCALORIC DIETS ON INTRACELLULAR CREATINE CONCENTRATION

Time	CHO-free Group	Glucose Group	Galactose Group	Fructose Group
Before Diet	4.5 ± 0.4	4.7 ± 0.5	4.5 ± 0.4	4.9 ± 0.5
After Diet	4.2 ± 0.3	5.5 ± 0.3	4.2 ± 0.3	5.3 ± 0.4

Values are for mean ± SEM of creatine concentration expressed as mg% for subjects participating in the second experiment. The hypocaloric diets used contained glucose, galactose or fructose, 36 g each. In addition, a carbohydrate-free (CHO) diet was also used.

One group was given a carbohydrate (CHO)-free diet containing 70 g protein and 16 g lipid (424 kcal/day). The remaining three groups were given 36 g of either glucose, galactose, or fructose in addition to the protein and the lipid (560 kcal/day). Each type of diet was administered for a period of 14 days. To ensure the double-blind random method, a caloric-free sweetener was added to the CHO-free diet.

Oral glucose tolerance tests were performed using 75 g glucose at day 0 and day 14. Blood samples were obtained at time 0, 60, and 180 min for measurements of plasma glucose and insulin. Blood collected at time 0 was also used for measurements of plasma galactose, plasma fructose and insulin binding to erythrocytes. Samples were collected after an overnight fast.

Compliance to the different diets was checked through the nutritional diary by assessment of weight loss 4 to 6.5 kg (see Table 2 and by the determination of the decrease in the glycosylated haemoglobin.

In a separate experiment, fructose tolerance tests were performed on five male volunteers. After an overnight fast, the subjects were given fructose (36 g) dissolved in water. Blood samples were drawn at time 0, 15, 30, 60, 90, and 120 min after the beginning of fructose consumption for measurements of plasma glucose, insulin and fructose.

Biochemical Methods

Plasma insulin was determined by a radioimmunoassay [50] and the plasma glucose was measured by a glucose oxidase method (Beckman Glucose Analyzer). Plasma galactose was assayed after deproteinization of the plasma with 0.33 N perchloric acid [26]. Fructose determination was made by the method of Bergmeyer *et al.* [7], using glucose oxidase and catalase to avoid interference in the fructose determination due to the large ratio of glucose to fructose in the sample. Glycosylated haemoglobin was measured according to the method of Kynoch and Lehman [27].

Erythrocyte insulin receptor binding assay was determined by an established procedure [14,15]. The cells were incubated with monoiodinated porcine ^{125}I-insulin (specific activity 150–196 μCi/μg). Binding analysis was performed by means of competition curves and Scatchard plots. The competition curve was considered as the curve in which the specific cell binding fraction was plotted as a function of insulin concentration. The maximum specific binding was the binding at tracer insulin concentration after subtraction of the non-specific binding (binding in the presence of 10^5 ng/ml unlabeled insulin). Specific insulin binding was expressed as the percentage of binding to 3.5×10^9 cells/ml. The receptor's affinity was considered as the concentration of native insulin required to reduce by 50% the specific cell bound fraction of ^{125}I-insulin. The Scatchard plot [44] was the curve in which bound/free insulin was plotted as a function of bound insulin. The highest insulin concentration used for calculation in Scatchard plots was 100 ng/ml. The receptor number (Ro) was obtained by extrapolation of each curve to the intercept on the abscissa as described by De Meyts and Roth [11]. The intracellular creatine content, of the blood samples used for the binding assays in the second experiment, was measured according to the method of Griffiths [16], to overcome variations due to cell age [8, 12, 30].

Statistical analysis was performed by paired *t*-test, and by analysis of variance.

RESULTS

The First Experiment

Figure 1 shows the effect of insulin binding to erythrocytes of the first experiment. The insulin binding to erythrocytes was increased in the three groups studied after 14 days of the hypocaloric diet with either glucose, galactose or fructose. The increase in insulin binding was from 6.2±0.4 to 7.2±0.4% ($p<0.01$) in the glucose group; from 6.6±0.3 to 7.6±0.3% ($p<0.005$) in the galactose group; and from 6.3±0.4 to 7.1±0.5% ($p<0.01$) in the fructose group. In the glucose and galactose groups, the increase in binding was significant at most of the insulin concentrations tested (except at 10^3, 10^4, 10^5 ng/ml), whereas, in the fructose group, the increase in binding was only significant at the four lowest insulin concentrations. There were no significant changes in the ED_{50} in the glucose (6.6±1.2 vs. 8.1±1.5 ng/ml) or the galactose (4.9±1.4 vs. 6.5±1.2 ng/ml) groups suggesting no change in the apparent receptor affinity. In the fructose group, however, the ED_{50} was significantly decreased (4.9±1.1 vs. 3.8±1.1 ng/ml; $p<0.05$), suggesting an increase in the apparent receptor affinity. By Scatchard analysis, receptor number increased in the glucose group from 51±8 to 86±10 receptor/cell ($p<0.01$), and in the galactose group from 37±4 to 82±8 receptor/cell ($p<0.005$), but not in the fructose group (from 45±6 to 57±7 receptor/cell).

Plasma insulin and glucose. There was a decrease in fasting plasma insulin and glucose in the three groups studied after 14 days of the hypocaloric diet with either glucose, galactose or fructose. Plasma insulin decreased from 12.6±2.1 to 5.1±0.9 ($p<0.05$) in the glucose group, from 10±1.3 to 6.7±1.1 μU/ml ($p<0.05$) in the galactose group and from 12.7±2.0 to 6.1±0.7 μU/ml ($p<0.05$) in the fructose group. Plasma glucose decreased from 0.82±0.03 to 0.66±0.02 g/l ($p<0.005$) in the glucose group, from 0.79±0.03 to 0.64±0.04 g/l ($p<0.05$) in the galactose group, and from 0.82±0.03 to 0.64±0.02 g/l ($p<0.001$) in the fructose group.

There was no difference between the change in plasma insulin and plasma glucose in the three groups studied. Likewise, there were no changes in plasma galactose or fructose after 14 days diet intake in all groups studied.

The Second Experiment

Figure 2 shows insulin binding to erythrocytes in the second experiment. Insulin binding to erythrocytes increased in the four groups studied using either a CHO-free diet, a glucose diet, a galactose diet or a fructose diet. The increase in insulin binding was from 5.8±0.4 to 7.9±0.6% ($p<0.001$) in

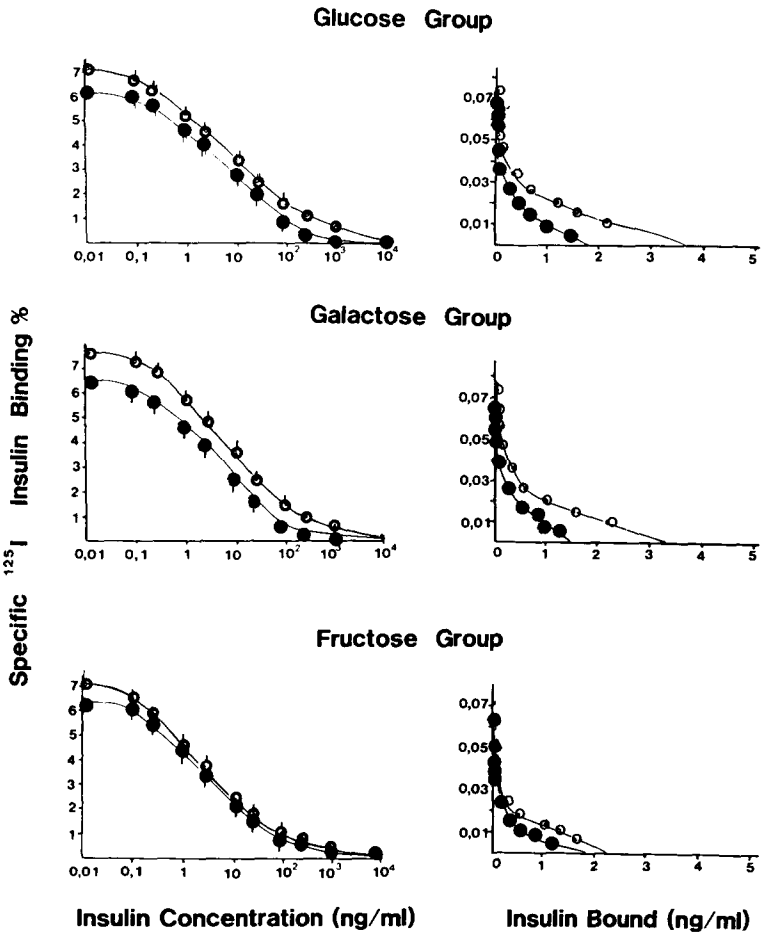

FIG. 1. The effect of two weeks of hypocaloric diet consumption on the number of erythrocytes insulin receptors. The diets contained equal amounts of glucose, galactose or fructose and were given to three groups of moderately obese volunteers. The left panel shows Competition curves of insulin binding studies before diet (●) and after 14 days of the diet (○) in the three groups studied in the first experiment. The binding was expressed per 3.52×10^9 erythrocytes/ml and per 4.9 mg% creatine (mean±SEM). Scatchard plots of the binding curves of the results in the left panel are shown in the right panel.

the CHO-free group, from 5.7±0.5 to 6.9±0.4% ($p<0.01$) in the glucose group, from 5.6±0.3 to 6.9±0.5% ($p<0.001$) in the galactose group and from 5.1±0.2 to 6.2±0.3% ($p<0.01$) in the fructose group. The ED_{50} was not significantly changed in the CHO-free (4.2±1.1 vs. 3.8±0.8 ng/ml), in the glucose (4.1±1.5 vs. 4.8×1.4 ng/ml) or in the glactose (10.3±2.4 vs. 7.9±2.5 ng/ml) group suggesting no change in the apparent affinity. This is compared to the fructose group whose ED_{50} was significantly decreased from 5.9±1.0 to 2.6±0.4 ng/ml ($p<0.05$), after the 14 days diet, suggesting an increase in the apparent affinity. By Scatchard analysis, the receptor number per cell was increased in the first three groups but not in the fructose group. The increase in receptor number was from 45±3.3 to 109±10.2 receptor/cell ($p<0.001$) in the CHO-free group, from 64±9 to 122±13 receptor/cell ($p<0.001$) in the glucose group and from 51±6 to 112±9 receptor/cell ($p<0.001$) in the galactose group.

The blood samples used for all the binding assays had a mean creatine concentration of 4.9 mg%. All the binding values were corrected to the same mean creatine concentration. There were no significant differences between the mean creatine concentration at the basal state, before consuming the diet, and values after 14 days of diet administration in the four groups studied (Table 1).

The fasting plasma insulin and glucose levels were decreased in the four groups studied. Fasting plasma insulin decreased from 16.5±3.1 to 5.8±1.3 μU/ml ($p<0.01$) in the CHO-free group, from 10.5±1.1 to 6.2±0.5 μU/ml ($p<0.01$) in the glucose group, from 14.8±4.9 to 7.2±2.4 μU/ml ($p<0.05$) in the galactose group and from 12.7±1.0 to 7.3±1.5 μU/ml ($p<0.05$) in the fructose group. Fasting plasma glucose decreased from 0.75±0.05 to 0.61±0.06 g/l ($p<0.01$) in the CHO-free group, from 0.75±0.01 to 0.64±0.04 g/l ($p<0.01$) in the glucose group, from 0.77±0.04 to 0.60±0.04 g/l ($p<0.01$) in the galactose group and from 0.75±0.03 to 0.65±0.03 g/l ($p<0.05$) in the fructose group. There were no differences between the changes in fasting plasma insulin and plasma glucose in the four groups studied.

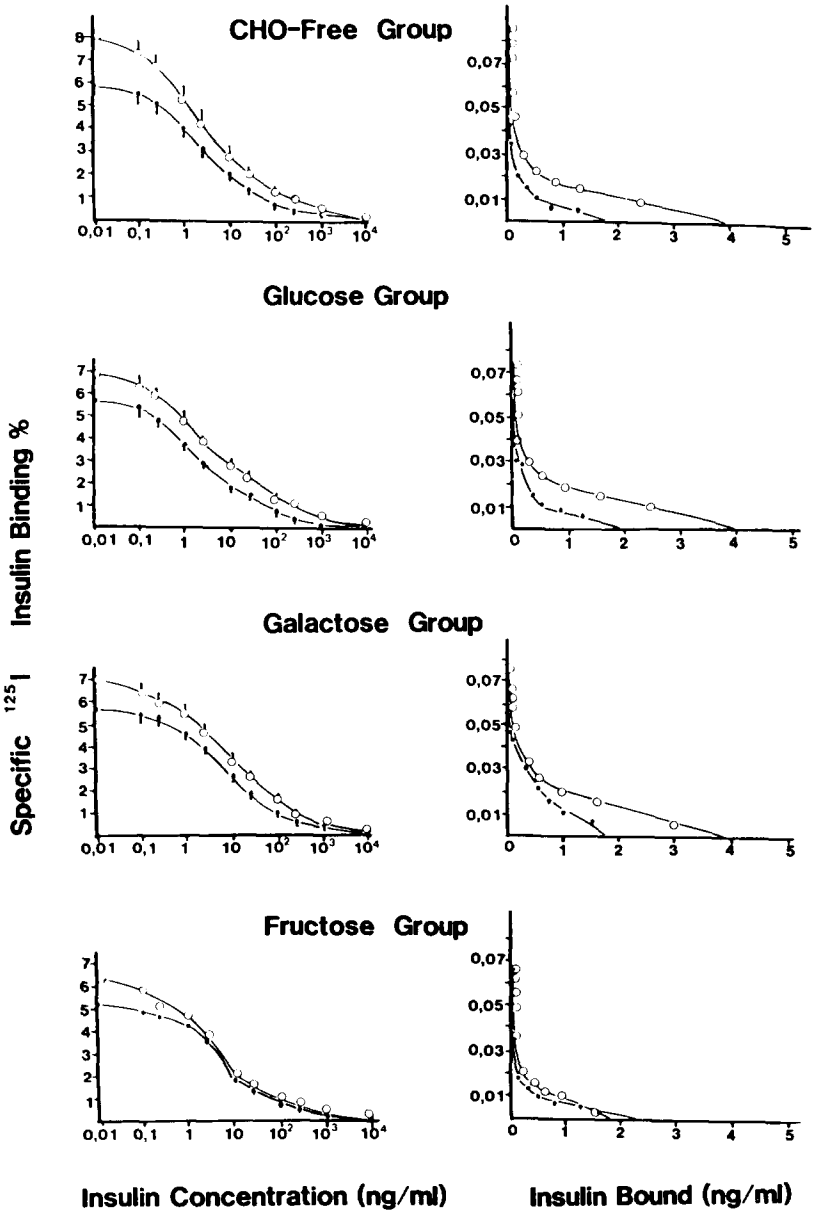

FIG. 2. The effect of two weeks of hypocaloric diet consumption on the number of insulin receptors. The diets contained equal amounts of glucose, galactose, fructose or were carbohydrate free (CHO), and were given to four groups of moderately obese volunteers. The left panel illustrates Competition curves of insulin binding studies before diet (●) and after 14 days of the diet (○) in the four groups studied in the second experiment. The binding was expressed per 3.5×10^9 erythrocytes/ml and per 4.9% creatine (mean±SEM) [Corrected binding per 3.5×10^9 cells/ml = binding per 3.5×10^9 cells/ml (average creatine)/(subject's creatine)]. Scatchard plots of the binding curves of the results in the left panel are shown in the right panel.

Figure 3 shows the changes in plasma glucose and insulin responses to a glucose load in the four groups studied. It can be seen that the plasma glucose responses were significantly decreased in the galactose ($p<0.001$) and the fructose group ($p<0.01$) at 60 minutes, whereas, in the CHO-free ($p<0.05$) and galactose group ($p<0.05$) at 120 minutes. The plasma insulin responses were decreased only significantly in the galactose group at 60 minutes ($p<0.001$).

The total areas under the glucose and insulin response curves were not significantly altered after 14 days of the diet in the four groups studied. The glucose responses were in the CHO-free group: 105±9 vs. 85±6 g/l min; in the glucose group: 106±5 vs. 111±6 g/l min; and in the fructose group: 105±6 vs. 93±7 g/l min. The insulin responses for values before diet vs. values after diet were as follows: in the CHO-free group, it amounted to 5760±690 vs. 3440±430 μU/ml

TABLE 2

THE EFFECT OF TWO WEEKS ADMINISTRATION OF SELECTED HYPOCALORIC DIETS ON BODY WEIGHT AND GLYCOSYLATED HAEMOGLOBIN (HB_{A1c}%)

Experiment and Treatment	Body Weight (kg)		% Glycosylated Haemoglobin	
	Before Diet	After Diet	Before Diet	After Diet
First Experiment				
Glucose Group (8)	75.7 ± 3.2	70.4 ± 3.2*	6.9 ± 0.6	5.8 ± 0.3*
Galactose Group (7)	64.0 ± 2.7	59.9 ± 2.3*	6.6 ± 0.4	5.8 ± 0.3*
Fructose Group (8)	69.8 ± 5.3	65.0 ± 4.8*	6.1 ± 0.5	5.3 ± 0.4*
Second Experiment				
CHO-Free Group (7)	75.8 ± 5.6	69.3 ± 4.9*	6.7 ± 0.2	5.7 ± 0.3*
Glucose Group (6)	71.9 ± 5.4	67.2 ± 5.1*	6.5 ± 0.3	5.3 ± 0.2*
Galactose Group (6)	69.5 ± 2.4	63.5 ± 2.3*	6.9 ± 0.2	5.0 ± 0.3*
Fructose Group (6)	70.4 ± 5.3	65.4 ± 4.7*	6.9 ± 0.3	5.1 ± 0.6*

Values represent the mean ± SEM of measurements made for the number of subjects given between parenthesis. The hypocaloric diets used contained 36 g of glucose, galactose or fructose. A hypocaloric carbohydrate (CHO) free diet was used in the second experiment.
*$p<0.001$.

min; in the glucose group: 5050±1290 vs. 4675±759 μU/ml min; in the galactose group: 4420±955 vs. 3115±202 μU/ml min; and in the fructose group: 5447±500 vs. 4525±800 μU/ml min.

There were no changes in plasma galactose or fructose after 14 days of the diet in all groups. The plasma fructose, glucose and insulin responses to a fructose load are summarized in Fig. 4. Plasma fructose increased with a peak of 1.85 g/l at 60 minutes, whereas, plasma glucose and insulin showed flattened curves.

DISCUSSION

Very restrictive diets are known to provoke an increase in insulin binding to membrane receptors in obese subjects. Total fasting has been studied in man [35,46] as well as in rat [13,33]. In both species the increase in binding is very rapid (a few days). Several authors [10,25] reported that during an initial 1 to 3 day phase this increase is due to an increase in the affinity. Later, after 10 to 14 days, the increase in binding persists, the affinity returns to initial levels, but the number of receptors is increased. However, Spanheimer *et al.* [46] reported an increase in receptor affinity, in erythrocytes, with no change in receptor number after 14 day fast in 4 hyperinsulinemic massively obese subjects. When the total fast is supplemented with protein to form a protein sparing diet [1], the number of erythrocyte insulin receptors increases after 3 weeks [38].

In the present study, a small amount of lipids was added to the protein diet resulting in a very calorie restricted, CHO-free, diet and again the receptor number increased. A further series of subjects received a similar diet supplemented with a small amount of a simple carbohydrate. When the sugar used was glucose or galactose the increase in receptor number occurred, and these increases were not significantly different from results obtained with the CHO-free diet. A similar increase in the number of insulin receptors during balanced restricted diets has already been reported but in other types of cells (monocytes and lymphocytes). The carbohydrates used, when specified, were mixed. In obese subjects, Archer *et al.* [2] found that a hypocaloric diet (600 kcal/day: 45% carbohydrate, 20% protein and 35% lipid) results in an increase in insulin receptor number of lymphocytes after 7–10 days; Bar *et al.* [3], using a similar diet, also reported an increase in receptor number of monocytes after 6 weeks. They measured insulin receptors only once. In obese diabetic subjects, Beck-Neilsen *et al.* [4] showed that 1200 to 1500 calorie diet (43% carbohydrate, 27% protein and 30% lipid) increases insulin binding to monocytes by an increase in binding affinity in 10 days and an increase in receptor number in 4 weeks. Similarly Savage *et al.* [43] found an increase in receptor number of monocytes in 4 weeks using 500 calorie diet. However, in extremely insulin resistant diabetics a hypocaloric diet (1000 kcal/day) failed to increase insulin binding in 3 months [41]. Thus, apart from this study [41], there is a general agreement that in obese subjects, whether diabetics or not, hypocaloric diets (with or without small amounts of carbohydrates) provoke an increase in the number of receptors, whether in lymphocytes, monocytes or erythrocytes, within approximately 2 weeks.

The most intriguing finding of the present study is that a small amount of fructose inhibits such an increase in receptor number. However, this result may be an artifact. Therefore we designed and performed another study which closely confirmed the initial results. There is only one relevant reference in the literature: Beck-Nielsen *et al.* [5] has reported that a one week fructose rich diet (usual spontaneous diet + 250 g fructose/day, i.e., 32.6% fructose), in normal man, decreases both insulin sensitivity and insulin binding to monocytes, while an equivalent (250 g=32.6%) glucose rich diet does not. Thus, both 36 g fructose in a hypocaloric diet for 2 weeks and 250 g fructose in a hypercaloric diet for one week have inhibitory effects on insulin receptors.

Mechanisms involved in such a fructose-induced inhibition need to be elucidated. Changes in plasma insulin and/or glucose may be involved: Sleder *et al.* [45] found that rats consuming a high fructose (66%) diet for one week had a post prandial hyperinsulinemia, two-fold higher than that produced by the same glucose percentage. In a shorter period of time (3 days), Hill *et al.* [19] found that feeding rats 58% fructose diet resulted in decreased glucose utilization due to impaired liver glucokinase activity, but this did not

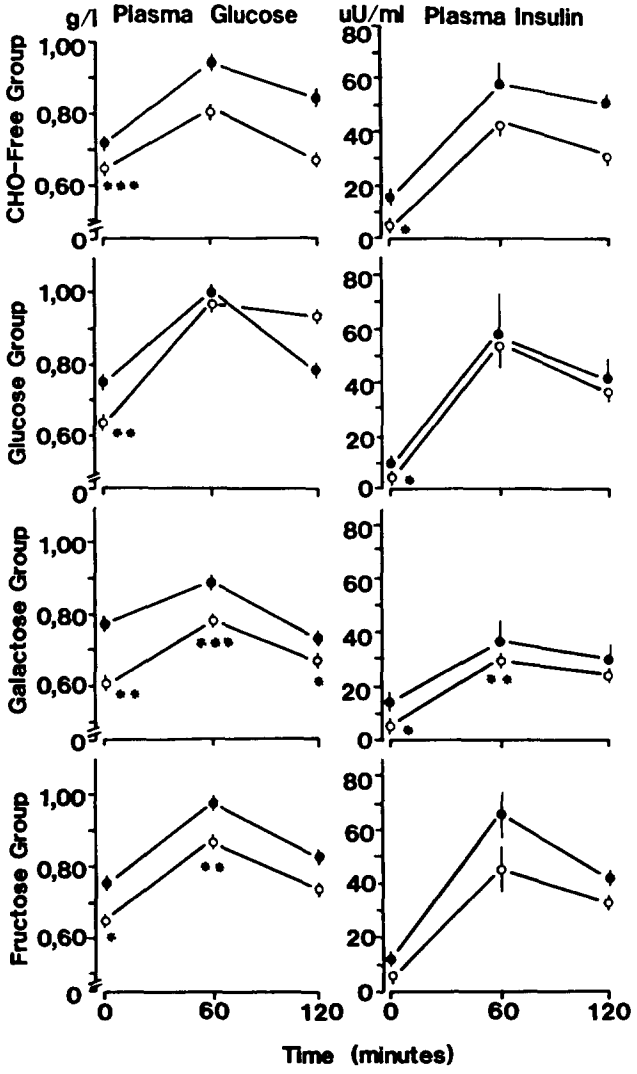

FIG. 3. The effect of glucose load on the subject's plasma levels of glucose and insulin as a function of four selected diets. Values represent mean±SEM of plasma glucose (left panel) and insulin (right panel) responses to a glucose load before (●) and after (○) 14 days of the experimental diets in the four groups studied.

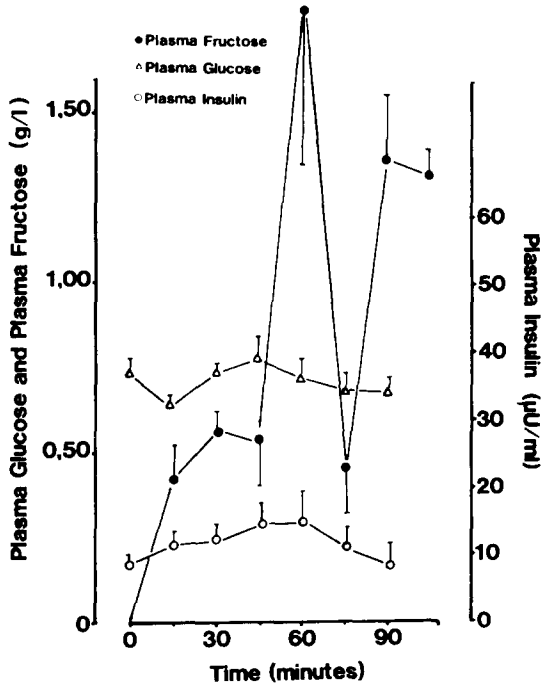

FIG. 4. The effect of fructose load on plasma glucose, fructose and insulin concentrations. Values represent the mean±SEM of plasma glucose (△), fructose (●) and insulin (○) responses to an oral fructose load (36 g).

occur with a 58% glucose diet. However, the use of 36 g fructose (25% of total calories) in the present study, did not alter the levels of fasting plasma insulin and glucose as well as the plasma insulin and glucose responses to a glucose load compared to those obtained with glucose or galactose. Thus, the effect on insulin receptors is unlikely to be related to particular changes in circulating insulin or in glucose utilization. However, the marked increase in circulating plasma fructose, after each fructose intake, might influence directly the insulin receptors by altering intracellular ATP and cyclic AMP which may provoke an effect on the receptors. The effects of fructose on intracellular ATP and cyclic AMP have been documented both in vivo and in vitro: in vivo, high fructose feeding decreases intracellular ATP of human hepatocytes [20]. Also, fructose when administered as a single large intravenous dose decreases intracellular ATP of rat hepatocytes [37]. In vitro, high concentrations of fructose decrease ATP and increase cyclic AMP content of isolated perfused liver or hepatocytes of rat [22,28]. Moreover, cyclic AMP [47] as well as ATP [48] are found to have an insulin binding regulatory effect on different cells and membrane preparations in vitro. Recently Roth *et al.* [42] and Kahn *et al.* [23] found that the insulin receptor occupancy is closely related to the kinetics of autophosphorylation of the insulin receptors which depends mainly on a protein-kinase activity and the presence of ATP. The experimental evidence reviewed suggests that fructose acts on intracellular ATP both in vivo and in vitro and that ATP exerts an effect on insulin receptors only in vitro. Obviously further investigations are needed to verify if such changes also occur totally in vivo.

In conclusion, it has been shown that the addition of fructose in small amounts to the hypocaloric diet, inhibits the

expected increase in receptor number. This effect is not found with an equal amount of glucose or galactose. This finding calls for excercising caution in prescribing fructose-rich diet, either in quantity or percentage of fructose, to the obese subjects, till the mechanisms and meaning of such a phenomenon are clarified.

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