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ORIGINAL PAPER

Effects of diet supplementation with olive oil and guar upon fructose-induced insulin resistance in normal rats

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Abstract Exposure of normal rats to fructose-containing drinking water represents a current model of insulin resistance. The major aim of the present study was to assess the possible effect of diet supplementation with either olive oil or guar upon the metabolic consequences of exposure to exogenous fructose. For this purpose, the changes in body weight, plasma D-glucose and insulin concentrations, and D-glucose infusion rate during a hyperinsulinemic-euglycemic clamp were measured after 65 days exposure to exogenous fructose and either olive oil- or guar-enriched diet. The results were compared to those previously collected in control animals exposed for the same period to either tap water or the fructose-containing drinking water and a standard diet. Diet supplementation with olive oil or guar failed to affect the increase in the insulinogenic index and the decrease in insulin sensitivity and fasted/fed ratio for plasma insulin concentration caused by exogenous fructose. In the rats exposed to exogenous fructose, the olive oil-fed rats differed from other animals by the absence of a decrease in food intake and body weight gain, whilst the guar-fed rats differed from other animals in a lower plasma D-glucose concentration in fed state and an absence, at day 65, of a higher plasma D-glucose concentration than that at day 0 measured in after overnight fasting state. These findings argue in favour of guar, rather than olive oil, to oppose the effect of exogenous fructose on glucose homeostasis.

Keywords Fructose-enriched diet · HOMA · Euglycemic-hyperinsulinemic clamp · Insulin secretion · Insulin resistance

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Introduction

To improve glucose homeostasis, exposure to diets supplemented with several specific nutrients such as olive oil [1–3], long-chain polyunsaturated ω3 fatty acids [4–7] or guar [8–11], amongst others, is under current investigation.

It was proposed that diets containing an increased proportion of monounsaturated fatty acids, compared to carbohydrates, improve glycemic control and lipid metabolism [12–14]. Recently, it has been reported that an olive oil-enriched diet improves insulin-response to oral glucose and glucose tolerance [15].

Guar gum intake immediately decreases postprandial plasma glucose and insulin concentrations in either normal human subjects [16–21] or patients with type 2 diabetes [9]; it was also reported that in normal subjects [22] or patients with type 1 or type 2 diabetes [23–26], long-term ingestion of guar-supplemented diet lowers fasting blood glucose, haemoglobin A1C, cholesterol and triglycerides, decreases systolic and diastolic blood pressure, increases insulin sensitivity and improves postprandial glucose tolerance. Guar gum-enriched diet improves glucose tolerance and insulin sensitivity in normal [27, 28] and streptozotocin-induced diabetic [29] rats, or in rats rendered glucose intolerant and hypertriglyceridemic by fructose feeding [10], and lowers postprandial insulinemia in either normal animals or rats exposed to fructose-based diet.

The aim of the present study was to assess the effect of olive oil- or gum guar-enriched diet upon the metabolic consequences of exposure to exogenous fructose.

Results

The results collected in rats fed the control diet and given access to either fructose-free or fructose-enriched tap water were already reported elsewhere [30]. They are, therefore, only mentioned in the text for purpose of comparison with those recorded in rats exposed to the fructose-enriched drinking water and fed a diet supplemented with olive oil or guar.

Body weight and food intake

Despite comparable body weight ($P > 0.3$) at day 0 after overnight starvation, the gain in body weight of fasted animals averaged after 65 days 138.1 ± 3.4 g ($n = 9$) in control animals, as compared ($P < 0.01$) to only 115.3 ± 6.1 g ($n = 13$) in the rats exposed to the fructose-enriched drinking water and fed the control diet (FC rats). Likewise, despite comparable body weight ($P > 0.5$) at day 0 after overnight starvation, the gain in body weight of fasted rats over 65 days was significantly lower ($P < 0.03$) in the rats exposed to the fructose-containing drinking water and fed the guar-enriched diet (FG rats: 109.3 ± 5.8 g; $n = 8$) than in the animals also given access to the fructose-containing water but fed the olive oil-enriched diet (FO rats: 142.0 ± 10.2 g; $n = 12$). The low value found in the former rats was not significantly different ($P > 0.5$) from that recorded in FC rats, the initial body weight in fed rats (day –5) being also comparable ($P > 0.3$) in these two groups of rats. When examined in the fed state, the gain in body weight over 65 days was again lower ($P < 0.005$) in FG rats (107.3 ± 9.5 g; $n = 8$) than in FO rats (148.3 ± 8.3 g; $n = 12$), despite comparable body weight on day –5 ($P > 0.1$). This situation coincided with the fact that the food intake was also significantly lower ($P < 0.001$) in FC rats (15.8 ± 0.5 g/day; $n = 6$) than in the control animals (25.9 ± 0.7 g/day; $n = 7$). Likewise, the food intake was significantly lower ($P < 0.005$) in the FG rats (13.5 ± 1.9 g/day; $n = 8$) than in the FO rats (24.7 ± 2.1 g/day; $n = 8$).

Plasma D-glucose concentration

After overnight starvation, the plasma D-glucose concentration was comparable, in the control rats, at day 0 (5.31 ± 0.27 mM) and day 65 (5.47 ± 0.28 mM), with a paired (day 65–day 0) difference of +0.16 ± 0.13 mM ($n = 9$ in all cases). In the FC rats, however, also examined after overnight fasting, the plasma D-glucose concentration was 0.72 ± 0.21 mM higher ($n = 13$; $P < 0.01$) at day 65 than at day 0. Likewise, in the overnight fasted FO rats, the plasma D-glucose concentration was 0.68 ± 0.29 mM higher ($n = 12$; $P < 0.05$) at day 65 than at day 0 (Table 1). These observations suggest that prolonged exposure to the fructose-enriched drinking water opposed the lowering of plasma D-glucose concentration, otherwise recorded after overnight fasting.

Somewhat unexpectedly, in the FG rats, always examined after overnight starvation, the plasma D-glucose concentration was virtually identical at day 0 (5.53 ± 0.17 mM) and day 65 (5.49 ± 0.29 mM), with a paired (day 65–day 0) difference of –0.04 ± 0.30 mM ($n = 8$ in all cases). This situation may be related to the effect of the fibre upon postprandial glycemia (see below).

Plasma insulin concentration

The plasma insulin concentration averaged in the control rats examined after overnight fasting 200 ± 26 and 186 ± 20 pM on day 0 and 65, with a paired (day 65–day 0) difference –15 ± 9 pM ($n = 9$ in all cases). In the overnight fasted FC, FO and FG rats, however, the mean plasma insulin concentration was higher on day 65 than on day 0, with a mean increase of 64 ± 15 pM ($n = 33$; $P < 0.001$). In this respect, there was no significant difference between FC, FO and FG rats.

Insulinogenic index

In the control rats, the insulinogenic index, i.e. the ratio between plasma insulin and D-glucose concentration, failed to differ significantly ($P > 0.09$) after overnight fasting at day 0 (37.9 ± 4.6 pM/mM) and day 65 (34.1 ± 3.3 pM/mM) with a paired (day 65–day 0) difference of –3.8 ± 2.0 pM/mM ($n = 9$ in all cases). However, in the FC, FO or FG rats, the mean value for such an insulinogenic index was always higher at day 65 than at day 0. In this respect, there was no significant difference ($P > 0.15$ or more) between these three groups of rats, with an overall paired (day 65–day 0) difference of +9.4 ± 2.8 pM/mM ($n = 33$). The latter value was significantly different from both 0 ($P < 0.005$) and the corresponding mean value recorded in the control rats ($P < 0.02$).

These findings document that, in the rats exposed to the fructose-enriched drinking water, the secretory responsiveness to D-glucose of insulin-secreting cells is increased, possibly to compensate for insulin resistance.

HOMA and euglycemic-hyperinsulinemic clamp

In the control rats, the HOMA, as measured after overnight fasting, failed to differ significantly at day 0 and day 65,

Table 1 Metabolic data in FO and FG rats

Day	–5 (fed)	0 (fasted)		60 (fed)	65 (fasted)
Body weight (g)					
FO rats	173 ± 6 (12)	187 ± 6 (12)		321 ± 7 (12)	329 ± 6 (12)
FG rats	187 ± 5 (8)	192 ± 6 (8)		293 ± 11 (8)	301 ± 10 (8)
Food intake (g day^{-1} rat^{-1})					
FO rats			24.7 ± 2.1 (8)		
FG rats			13.5 ± 1.9 (8)		
Plasma D-glucose (mM)					
FO rats	7.1 ± 0.2 (12)	4.3 ± 0.2 (12)		7.9 ± 0.2 (12)	5.0 ± 0.2 (12)
FG rats	6.9 ± 0.3 (8)	5.5 ± 0.2 (8)		7.1 ± 0.3 (8)	5.5 ± 0.3 (8)
Plasma insulin (pM)					
FO rats	218 ± 17 (12)	94 ± 19 (12)		223 ± 17 (12)	168 ± 27 (12)
FG rats	317 ± 39 (8)	123 ± 27 (8)		287 ± 38 (8)	203 ± 27 (8)
Insulinogenic index (pM/mM)					
FO rats	30.8 ± 2.4 (12)	23.0 ± 4.9 (12)		28.3 ± 2.5 (12)	34.1 ± 5.2 (12)
FG rats	45.2 ± 4.5 (8)	22.3 ± 5.0 (8)		41.4 ± 6.1 (8)	36.8 ± 4.2 (8)
HOMA (pM mM)					
FO rats	69.5 ± 6.0 (12)	17.6 ± 3.4 (12)		78.3 ± 6.1 (12)	37.7 ± 7.0 (12)
FG rats	100.5 ± 15.2 (8)	30.3 ± 6.8 (8)		88.7 ± 10.7 (8)	50.4 ± 8.2 (8)

whether judged from the paired (day 65–day 0) difference (–2.0 ± 1.7 pM mM; $n = 9$; $P > 0.25$) or paired (day 65/day 0) ratio (98.3 ± 4.5%; $n = 9$; $P > 0.7$). In the rats given access to the fructose-enriched drinking water, however, the mean HOMA at day 65 was always higher than at day 0. In this respect there was no significant difference ($P > 0.2$ or more) between FC, FO and FG rats, with overall mean values for the paired (day 65–day 0) difference of +18.1 ± 4.0 pM mM ($n = 33$; $P < 0.001$) and for the paired (day 65/day 0) ratio of 179.8 ± 23.2% (geometric mean; $n = 33$; $P < 0.001$).

Likewise, the glucose infusion rate during the euglycemic-hyperinsulinemic clamp was not significantly different ($P > 0.15$ or more) in FC, FO and FG rats, with an overall mean value of 5.68 ± 0.18 μmol h^{-1} g^{-1} ($n = 33$) significantly lower ($P < 0.005$) than that recorded in the control animals (6.92 ± 0.34 μ mol h^{-1} g^{-1}; $n = 9$).

It should be underlined that the difference between control and FC rats ($P < 0.03$) remained significant ($P < 0.05$) when the glucose infusion rate was taken at a time when the mean glycemia was closely comparable ($P > 0.65$) in control animals (63.3 ± 4.4 mg/dl; $n = 9$) and FC rats (64.9 ± 1.0 mg/dl; $n = 13$), with mean values of 7.01 ± 0.42 ($n = 9$) and 5.87 ± 0.34 ($n = 13$) μmol h^{-1} g^{-1} in the former animals and latter rats, respectively. The plasma insulin concentration reached during the euglycemic-hyperinsulinemic clamp was also comparable in these two groups of rats. For instance, the measurements of plasma insulin concentration at min 30, 45 and 60 yielded in the FC rats mean values averaging 101.7 ± 4.7% ($n = 38$; $P > 0.8$) of those reached at the same time in the control animals (100.0 ± 6.4%; $n = 27$).

Likewise, when comparing FO to FG rats, the plasma insulin concentration at min 30, 45 and 60, averaged in the latter rats 95.8 ± 5.6% ($n = 24$; $P > 0.6$) of those reached at the same time in the former rats (100.0 ± 6.9%; $n = 31$). As shown in Table 2, in these animals, the glucose infusion rate remained comparable ($P > 0.5$) in FO rats (5.56 ± 0.32 μ mol h^{-1} g^{-1}; $n = 12$) and FG rats (5.88 ± 0.37 μmol h^{-1} g^{-1}; $n = 8$), even when measured at virtually identical mean glycemia ($P > 0.8$), i.e. 63.3 ± 2.5 mg/dl ($n = 12$) and 62.6 ± 1.3 mg/dl ($n = 8$). The mean time at which these glycemic levels were reached failed to differ significantly ($P > 0.9$) in the FO rats (min 60.4 ± 6.0; $n = 12$) and FG rats (min 59.4 ± 4.5; $n = 8$).

Response to overnight starvation

In the rats given access to the fructose solution, the metabolic response due to starvation was assessed by the paired (day 0/day –5) ratio before the study and the paired (day 65/day 60) ratio at the end of the study.

The plasma D-glucose concentration in fed rats was not significantly different ($P > 0.15$ or more) in FC, FO and FG rats examined on day –5, with an overall mean value of 7.18 ± 1.27 mM ($n = 33$). On day 60, however, it was significantly lower ($P < 0.02$ or less) in the FG rats (7.09 ± 0.25 mM; $n = 8$) than in either the FC rats (7.98 ± 0.17 mM; $n = 13$) or FO rats (7.95 ± 0.22 mM;

Table 2 Euglycemic-hyperinsulinemic clamp

Rats	FO (olive oil)	FG (guar)
Glucose infusion rate (μmol h^{-1} g^{-1})		
Steady-state value	5.46 ± 0.25 (12)	6.01 ± 0.32 (8)
At comparable glycemia	5.56 ± 0.32 (12)	5.88 ± 0.37 (8)
Plasma D-glucose (mM)(min)		
0	5.0 ± 0.2 (11)	5.5 ± 0.3 (8)
15	4.6 ± 0.4 (11)	4.9 ± 0.3 (8)
30	4.7 ± 0.4 (10)	5.0 ± 0.5 (8)
45	5.6 ± 0.3 (10)	5.5 ± 0.3 (8)
60	6.3 ± 0.4 (11)	5.5 ± 0.4 (8)
75	6.2 ± 0.5 (11)	5.5 ± 0.4 (8)
90	7.3 ± 0.8 (11)	5.9 ± 0.4 (8)
Plasma insulin (nM) (min)		
0	0.19 ± 0.03 (11)	0.21 ± 0.03 (8)
15	4.63 ± 0.63 (11)	5.87 ± 0.69 (8)
30	5.32 ± 0.78 (10)	5.45 ± 0.40 (8)
45	6.56 ± 0.73 (11)	5.09 ± 0.47 (8)
60	5.40 ± 0.63 (10)	5.81 ± 0.61 (8)
75	7.28 ± 0.87 (10)	5.26 ± 0.62 (8)
90	10.81 ± 2.70 (10)	5.96 ± 0.86 (8)

$n = 12$). This finding is consistent with the knowledge that guar lowers the postprandial glycemia.

The starved/fed ratio in plasma D-glucose concentration was not significantly different ($P > 0.1$ or more) before the onset of the study or at its end, whether in FC, FO or FG rats. It averaged 66.4 ± 2.5 and 68.4 ± 2.4% ($n = 33$ in both cases) before the study and at its end.

The starved/fed ratio in plasma insulin concentration was much higher ($P < 0.005$), however, at the end of the study (70.6 ± 5.6%; $n = 33$; $P < 0.001$ versus unity) than before its onset (46.9 ± 4.7%; $n = 33$). In this respect, there was no significant difference between FC, FO and FG rats.

Likewise, the starved/fed ratio for the insulinogenic index averaged before the onset of the study 60.7 ± 7.1% ($n = 33$; $P < 0.001$ versus unity). At the end of the study, however, it failed to be significantly different from unity ($P > 0.2$ or more) whether in FC, FO or FG rats with an overall mean value of 94.1 ± 8.7% ($n = 33$). In the latter respect, there was no significantly difference between FC, FO and FG rats. These findings indicate that prolonged exposure to the fructose-containing solution abolished the impairment of the secretory response of insulin-producing cells to glucose, otherwise resulting from overnight fasting.

Discussion

The results concerning the comparison between control animals and rats exposed to the fructose-enriched solution, whilst being maintained on a normal diet, were already reported and commented upon in the framework of a more extensive study dealing with such items as the modality of exogenous D-fructose supplementation by incorporation of the ketohexose in either solid food or drinking water and the reversibility of fructose-induced insulin resistance [30]. In the present discussion, therefore, emphasis will be placed on the effects of diet supplementation with olive oil and guar in the rats exposed to exogenous D-fructose.

For several metabolic variables, no significant difference was found between FC, FO and FG rats. Such was the case for the increase in plasma insulin concentration, insulinogenic index and HOMA observed in these rats at day 65 after overnight starvation. Likewise, the glucose infusion rate during the euglycemic-hyperinsulinemic clamp was not significantly different in FC, FO and FG rats. Moreover, the decrease in the fasted/fed ratio for either the plasma insulin concentration or insulinogenic index found at the end of the study in the rats exposed to exogenous D-fructose, but not so in the control animals, also failed to differ significantly in FC, FO and FG rats.

The FO rats differed from the FC and FG rats in only the following respect. Both the daily food intake and gain in body weight were higher in the FO rats than in the FG rats which, in the same respect, failed to differ significantly from the FC rats. A comparable situation was already observed when comparing normal rats exposed to fructose-free tap water and fed either a control diet or the olive oil-enriched diet [15].

The FG rats differed from the FC and FO rats in two respects. First, on day 60, the plasma D-glucose concentration of fed rats was significantly lower in FG rats than in either FC or FO rats. This finding is consistent with the lowering of postprandial glycemia by guar, as attributable to decreased glucose absorption [11]. Second, in the FG rats, the plasma D-glucose concentration found at day 65 after overnight starvation failed to differ from that recorded, in the same animals, before the exposure to exogenous D-fructose, whilst in both the FC and FO rats, the plasma D-glucose concentration in overnight-fasted rats was significantly higher at day 65 than at day 0. Such a difference could also be somehow related to the lowering of postprandial glycemia by guar, such an antidiabetic effect opposing the diabetogenic action of exogenous D-fructose supplementation.

Taken into account both the daily food intake and the composition of either the standard diet or those diets enriched with guar or olive oil, the calculated daily caloric intake was significantly higher in the FO rats (102.6 ± 8.7 kcal/day, $n = 8$) and significantly lower in the FC rats (52.1 ± 1.6 kcal/day, $n = 6$) and FG rats (35.6 ± 5.0 kcal/day, $n = 8$) than in the control animals (85.4 ± 2.3 kcal/day, $n = 7$). These differences remained

significant, even when allowance was made for the estimated intake of fructose through the drinking water.

In conclusion, the present results indicate that, on one hand, in a rat model of fructose-induced insulin resistance, the long-term administration of an olive oil-enriched diet opposes the lowering effect of the ketohexose on food intake and body weight gain. On the other hand, the long-term administration of a guar-enriched diet prevents the fructose-induced increase in glycemia otherwise recorded after overnight starvation, whilst maintaining a low-food intake. These findings argue in favour of guar, rather than olive oil, to oppose the effect of exogenous fructose on glucose homeostasis.

Materials and methods

The experiments were approved by the Animal Use Committee of the Fundación Jiménez Díaz (Madrid, Spain).

All experiments were conducted in normal male Wistar rats, maintained at the Fundación Jiménez Díaz (Madrid, Spain) and kept on a standard pellet diet (UAR—containing: 15.4% protein, 2.9% fat, 60.5% carbohydrates, 3.9% fibre, 5.3% minerals and 12% water; Panlab, Barcelona, Spain).

Experimental procedures

Groups of rats were given free access to standard diet alone –FC–, or enriched with powdered guar (20% w/w) –FG– or olive oil (15% v/w: 13% saturated fatty acids, 79% monounsaturated fatty acids and 8% polyunsaturated fatty acids) –FO–, and D-fructose (20%, w/v) dissolved in tap water. The body weight, plasma D-glucose and insulin concentrations were measured in rats before and after exposure to the diets. The food intake was measured every other week during 65 days in groups of 4–5 rats and over periods of 3–5 days. After 65 days, a hyperinsulinemic-euglycemic clamp [31] was performed over 90 min in rats in overnight starved condition. For such a purpose, rats were anaesthetized with pentobarbital administered intraperitoneally (60 μg per g body wt.; Pentothal®, Abbott Laboratories, Madrid, Spain). At time zero, insulin (40 U/ml; pork insulin, Novo Biolabs, Copenhagen, Denmark) dissolved in saline (NaCl 0.9%) containing 0.2% bovine serum albumin (Sigma Aldrich, St Louis, MO, USA) was appropriately diluted to deliver 0.4 mU/h/g body wt. (1.2 ml/h/rat) intravenously through a catheter inserted in the femoral vein, together with D-glucose (20%, w/v, in NaCl 0.9%) administered at an initial rate of 0.5 ml/h. Blood samples (5 μl) were collected every 5 min, to measure the glycemia (Accutrend strips; Boehringer Mannheim, Mannheim, Germany), from a catheter inserted in a carotid artery. The amount of glucose infused was changed in order to maintain glycemia close to basal value. The rate of glucose infusion required to achieve a stable glycemia value was considered as an indication of insulin resistance. Blood samples (500 μl) were also collected every 15 min to measure plasma D-glucose and insulin during this hyperinsulinemic-euglycemic clamp.

Analytical methods

The plasma concentration of D-glucose [32] and insulin [33] was measured by methods described in the cited references.

Presentation of results

All results are presented as mean values (±SEM) together with the number of individual observations (*n*). The statistical significance of differences between mean values was assessed by use of Student's *t*-test, and confirmed by ANOVA.

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