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Effects of an Olive Oil–Enriched Diet on Plasma GLP-1 Concentration and Intestinal Content, Plasma Insulin Concentration, and Glucose Tolerance in Normal Rats

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The present study aims mainly at measuring, in normal rats, the GLP-1 response to oral intake of an olive oil–enriched diet (OO), and at assessing the long-term effects of such a diet on the GLP-1 content of the intestinal tract, as well as the plasma D-glucose, insulin, and GLP-1 pattern during an oral glucose tolerance test. In meal-trained rats, the mean increment in plasma GLP-1 concentration at min 10 and 20 was 1.39 ± 0.23 ng/mL higher ($p < 0.001$) in the rats given access to the OO diet rather than control diet. Relative to the initial value (d 0), the gain in body weight at d 50 was also higher in the animals fed the OO rather than control diet. At d 50, the GLP-1 content of the jejunum, ileum, colon, and cecum were not significantly different in the two groups of rats. At d 19 and 36, the increment in both plasma insulin concentration and paired ratio between plasma insulin and D-glucose concentrations were again higher, during an oral glucose tolerance test conducted in overnight fasted animals, in the rats otherwise fed the OO, as distinct from control, diet. The intake of an olive oil–enriched diet thus increases, in normal rats, GLP-1 release, this coinciding during long-term exposure to the OO diet with higher body weight gain, increased secretory response of insulin-producing cells to oral glucose administration, and, after 36 d, improved glucose tolerance.

Key Words: Olive oil–enriched diet; GLP-1; insulin; glucose tolerance.

Introduction

Several studies have demonstrated that diets with an increased proportion of monounsaturated fatty acids, compared to higher carbohydrate diets, improve glycemic control and also benefit lipid profiles *(1–7)*. For instance, Garg et al. *(2)* reported that, in patients with non-insulin-dependent diabetes mellitus (NIDDM), a high-monounsaturated-fat diet, provided as the sole nutrient for 6–14 wk, lowers plasma D-glucose values when compared to a high-carbohydrate diet. Likewise, Low et al. *(3)* observed that, in obese patients with NIDDM dieting for 6 wk on a formula diet at a 50% caloric deficit but enriched in monounsaturated fatty acids, both the fasting glucose level and 24-h glycemia decrease significantly more than in patients dieting for the same period on a diet with the same caloric deficit but enriched with carbohydrates. Moreover, in this study, measurements of C-peptide levels in fasting, over a 24-h period and during an oral glucose tolerance test, indicated that the diet enriched in monounsaturated fatty acids increases carbohydrate-induced insulin secretion *(3)*.

The favorable effect of diets rich in monounsaturated fatty acids on glucose homeostasis apparently involves increased secretion of the intestinal and insulinotropic hormone glucagon-like peptide-1 (GLP-1). Monounsaturated fatty acids possessing a free carboxyl group indeed stimulate the secretion of intestinal proglucagon-derived peptides, including GLP-1, from fetal rat intestinal cultures, this effect being lost upon full saturation of the stimulatory fatty acids *(8)*. The postulated role of increased GLP-1 secretion in the improvement of glucose tolerance is supported by several observations. To cite only one example, Joseph et al. *(9)* reported that oral delivery of GLP-1 in a modified polymer preparation normalizes basal glycemia and lowers the glycemic response to an oral glucose challenge in both normal and diabetic db/db mice.

Three previous studies deal specifically with the effects of monounsaturated fatty acid diets on GLP-1 secretion and glycemic tolerance. Thomsen et al. *(10)* first compared the postprandial responses of glucose, insulin, fatty acids, triacylglycerol, gastric inhibitory peptide (GIP), and GLP-1 to test meals rich in saturated and monounsaturated fatty acids in young, lean, healthy subjects. GLP-1 and GIP responses were higher after ingestion of a meal containing 80 g olive oil than after ingestion of either 50 g carbohydrate (control meal) or 100 g butter. This coincided with lower

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average blood D-glucose concentrations after the olive oil meal than after the control meal. The early peak blood D-glucose and plasma insulin concentrations were highest, however, after ingestion of the control meal. In a comparable study later conducted by the same investigators in overweight patients with type 2 diabetes, the GLP-1 response was again highest after the olive oil meal, while no significant difference was seen in the glucose, insulin, and fatty acid responses to the two fat-rich meals *(11)*.

In the third report *(12)*, lean Zucker rats were, for 2 wk, pair-fed a synthetic diet containing 5% fat derived from either olive oil (OO, 74% monounsaturated fatty acids) or coconut oil (CO, 87% saturated fatty acids). The OO group had improved glucose tolerance compared with the CO group in both oral and duodenal glucose tolerance tests. Despite such a difference, the plasma insulin pattern was comparable in the two groups of rats. The secretion of gut glucagon-like immunoreactive material during the duodenal glucose tolerance test was higher (min 10) in the OO rats compared with the CO rats. The benefit in glycemia tolerance conferred by OO feeding was abolished when the GLP-1 receptor antagonist exendin$^{9-39}$ was infused 3 min before the duodenal glucose tolerance test.

The present study aims mainly at measuring, in normal rats, the GLP-1 response to oral intake of an olive oil–enriched diet (OO) and at assessing the long-term effects of such a diet on the GLP-1 content of the intestinal tract, as well as the plasma D-glucose, insulin, and GLP-1 responses during an oral glucose tolerance test.

Results

Intake of the Control and OO Diet by Meal-Trained Rats

The body weight of the meal-trained rats first slightly decreased. For instance, during the second day of this training period, they lost 7.0 ± 1.5 g ($n = 12$; $p < 0.001$). Already during the third day of the training period, however, such a decrease failed to achieve statistical significance (paired fall in body weight: 1.2 ± 1.3 g; $n = 16$). After 1 wk of meal-training, the body weight was no more significantly different ($p > 0.6$) from that measured at the onset of the training period. It averaged 244.4 ± 6.3 g ($n = 8$) in male rats and 190.4 ± 4.8 g ($n = 8$) in female animals ($p < 0.001$ for the gender difference).

On the day of the test (d 7), the rats ingested 2.8 ± 0.3 and 2.9 ± 0.2 g ($n = 4$ in both cases) of the control and OO diet, respectively.

Over the first 60 min of the test, during which period measurements were available in all rats, the incremental area for plasma D-glucose concentrations averaged, in the rats exposed to the OO diet, 67.7 ± 16.0% ($n = 8$) of the mean corresponding values found in rats of the same sex exposed to the control diet (100.0 ± 18.3%; $n = 8$). These two mean values were not significantly different from one another. Likewise, over the same period, the incremental area for plasma insulin concentrations was not significantly different ($p > 0.7$) in the two groups of rats, with an overall mean value of 37.8 ± 4.3 ng·min·mL^{-1} ($n = 16$). The area under the plasma insulin concentration curve (min 0 to 60), when divided by the paired area under the plasma D-glucose concentration curve, yielded, in the rats exposed to the OO diet, a mean value higher than that found in the animals exposed to the control diet. Such a difference only achieved statistical significance ($p < 0.05$), however, after exclusion of one abnormally low individual value found in a male rat exposed to the OO diet and representing no more than 51% of the lower confidence limit for the seven other values found in the same group of rats (i.e., the mean value minus the SD times $t_{0.05}$). The mean increment in plasma GLP-1 concentration at min 10 and 20 was 1.39 ± 0.23 ng/mL higher (d.f. = 6; $p < 0.001$), in the rats given access to the OO diet rather than control diet (Fig. 1).

Body Weight

Figure 2 illustrates the time course for changes in body weight in male and female rats fed the control or OO diet.

When the measurements of body weight at d 0 and d 50 were both available for the same animals, the paired increase in such a variable, expressed relative to the initial value, averaged, in the rats fed the OO diet, 142.1 ± 8.7% ($n = 6$; $p < 0.005$) of that found at the same time in animals of the same sex fed the control diet (100.0 ± 6.6%; $n = 6$).

Likewise, as judged from the paired difference in body weight during the period between d 4 and later times (up to d 50), the increase in such a weight averaged, in the rats fed the OO diet, 150.4 ± 17.2% ($n = 24$; $p < 0.02$) of the mean corresponding values found at the same time in animals of the same sex fed the control diet (100.0 ± 7.1%; $n = 19$). When these paired increases in body weight were divided by the corresponding measurements made at d 4, such a relative increment averaged, in the rats fed the OO diet, 158.2 ± 21.4% ($n = 24$; $p < 0.03$) of that found at the same time in animals of the same sex fed the control diet (100.0 ± 7.9%; $n = 19$). In these several respects, there was no significant difference between male and female rats.

Plasma D-Glucose, Insulin, and GLP-1 Concentrations

The plasma D-glucose concentration in fed rats remained fairly stable between d 4 and d 50 (Table 1). The measurements made in the rats fed the OO diet averaged 98.6 ± 1.0% ($n = 32$; $p > 0.4$) of the mean values recorded at the same time in animals of the same sex fed the control diet. In these fed rats, the plasma insulin concentration also failed to display obvious time-related changes. The values found in rats fed the OO diet averaged 112.4 ± 4.7% ($n = 32$, $p < 0.06$) of the mean corresponding values recorded at the same time in animals of the same sex fed the control diet (100.0 ± 4.5%; $n = 32$). These findings led us to establish the paired ratio between plasma insulin and D-glucose concentration. Such an insulinogenic index averaged, in the

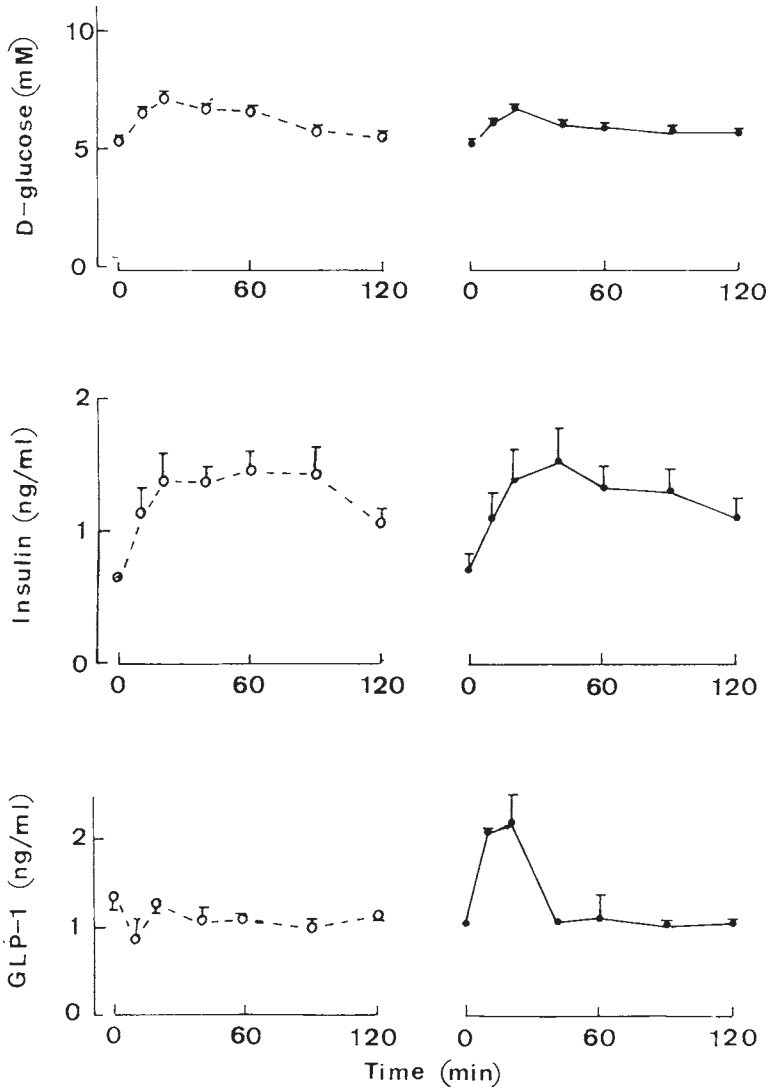

Fig. 1. Time course for the changes in plasma D-glucose, insulin, and GLP-1 concentrations in meal-trained rats given access to the control (left) or OO (right) diet. Mean values (± SEM) refer to eight individual experiments (D-glucose and insulin) or two measurements made in pooled plasma samples (GLP-1).

rats fed the OO diet, 113.1 ± 4.5% ($n = 32$; $p < 0.05$) of the corresponding mean values found at the same day in animals of the same sex fed the control diet (100.0 ± 4.4%; $n = 32$). In this respect, the difference between the rats fed the control and OO diet remained significant ($p < 0.05$) when all values recorded in each group of rats were pooled together, independently of the age and sex of the animals (comparison of geometric means).

The GLP-1 plasma concentration remained fairly stable between d 4 and d 50, the measurements made in pooled plasma samples averaged, in the rats fed the OO diet, 109.3 ± 3.7% ($n = 8$; $p < 0.05$) of those made at the same time in animals of the same sex fed the control diet (Table 1). Taken as a whole, these findings indicate that, in the fed state, the animals fed the OO diet display higher plasma GLP-1 and insulin concentrations and a higher insulinogenic index than the rats fed the control diet, despite the virtually identical plasma D-glucose concentrations in these two groups of animals.

Oral Glucose Tolerance Test

An oral glucose tolerance test was performed at d 19 and 36 in overnight fasted rats. As expected from the results already mentioned, the body weight of these fasted rats was higher in the animals otherwise fed the OO diet, rather than control diet. In the former rats, it indeed averaged 105.2 ± 1.9% ($n = 16$; $p < 0.05$) of the mean corresponding values found at the same time in animals of the same sex otherwise fed the control diet (100.0 ± 1.5%; $n = 17$).

At d 19, the mean plasma D-glucose concentration in overnight fasted rats was identical in the two groups of rats (Fig. 3). Likewise, the incremental area in plasma D-glu-

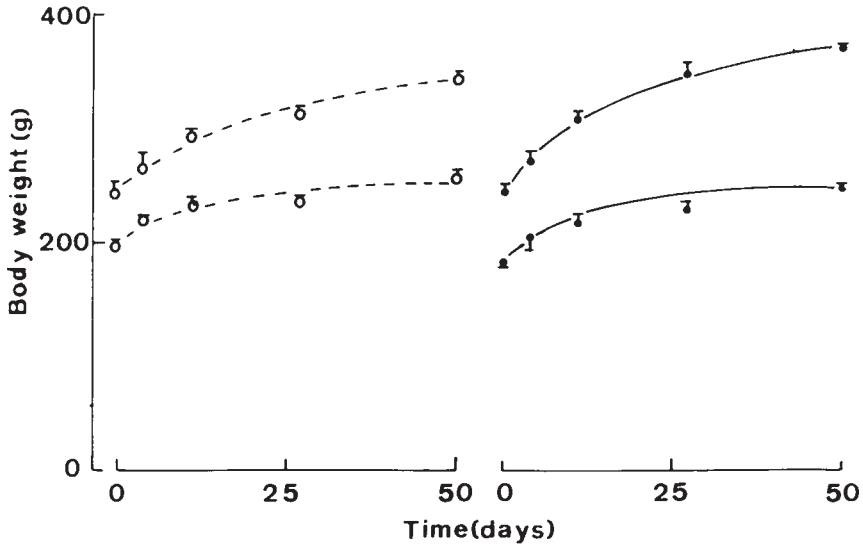

Fig. 2. Time course for the changes in body weight of male (upper curves) and female (lower curves) rats fed the control (left) or OO (right) diet. Mean values (± SEM) refer to four individual observations.

Table 1
Time Course for the Changes in Plasma D-Glucose and Insulin Concentrations, Insulinogenic Index, and Plasma GLP-1 Concentration in Male and Female Rats Fed the Control or OO Diet

		d4	d11	d27	d50
Plasma D-glucose (m*M*)					
Control diet	Male rats	6.78 ± 0.29 (4)	6.47 ± 0.21 (4)	7.19 ± 0.34 (4)	6.83 ± 0.39 (3)
	Female rats	6.53 ± 0.39 (5)	6.82 ± 0.18 (5)	6.64 ± 0.11 (5)	7.10 ± 0.29 (5)
OO diet	Male rats	6.82 ± 0.15 (4)	6.68 ± 0.11 (4)	7.00 ± 0.04 (4)	6.40 ± 0.24 (4)
	Female rats	6.65 ± 0.27 (4)	6.44 ± 0.16 (4)	6.74 ± 0.23 (4)	6.82 ± 0.09 (4)
Plasma insulin (ng/mL)					
Control diet	Male rats	1.03 ± 0.13 (4)	1.33 ± 0.20 (4)	1.37 ± 0.07 (3)	1.13 ± 0.12 (3)
	Female rats	0.87 ± 0.07 (3)	1.34 ± 0.21 (5)	1.20 ± 0.11 (5)	1.18 ± 0.23 (5)
OO diet	Male rats	1.38 ± 0.18 (4)	1.35 ± 0.10 (4)	1.43 ± 0.09 (4)	1.18 ± 0.12 (4)
	Female rats	1.18 ± 0.10 (4)	1.10 ± 0.04 (4)	1 23 ± 0.17 (4)	1.60 ± 0.04 (4)
Insulinogenic index (ng/mmol)					
Control diet	Male rats	151 ± 17 (4)	206 ± 35 (4)	197 ± 1 (3)	165 ± 9 (3)
	Female rats	139 ± 18 (3)	194 ± 25 (5)	181 ± 17 (5)	168 ± 35 (5)
OO diet	Male rats	200 ± 23 (4)	202 ± 14 (4)	207 ± 13 (4)	185 ± 23 (4)
	Female rats	177 ± 15 (4)	172 ± 11 (4)	182 ± 25 (4)	235 ± 9 (4)
Plasma GLP-1 (ng/mL)					
Control diet	Male rats	1.02	0.79	0.97	0.84
	Female rats	1.19	1.08	1.12	1.07
OO diet	Male rats	1.12	0.98	1.03	1.04
	Female rats	1.21	1.01	1.22	1.14

cose concentration during the oral glucose tolerance test (min 0 to 120) was not significantly different ($p > 0.27$) in the control animals (273.3 ± 46.0 m*M*·min; $n = 9$) and the rats fed the OO diet (347.6 ± 46.1 m*M*·min; $n = 8$). The trend toward higher increments in plasma D-glucose concentration in the latter animals was borne out by the finding that the increase in such a concentration above paired basal value averaged, in the rats fed the OO diet, 131.2 ± 10.0% ($n = 40$; $p < 0.025$) of the mean corresponding values found at the same sampling time in the control rats (100.0 ± 8.7%; $n = 45$).

At d 36, the mean plasma D-glucose concentration in overnight fasted rats was not significantly different ($p > 0.3$) in control rats (5.38 ± 0.26 m*M*; $n = 8$) and animals fed the OO diet (5.09 ± 0.08 m*M*; $n = 8$). The mean incremental area in plasma D-glucose concentration during the glucose toler-

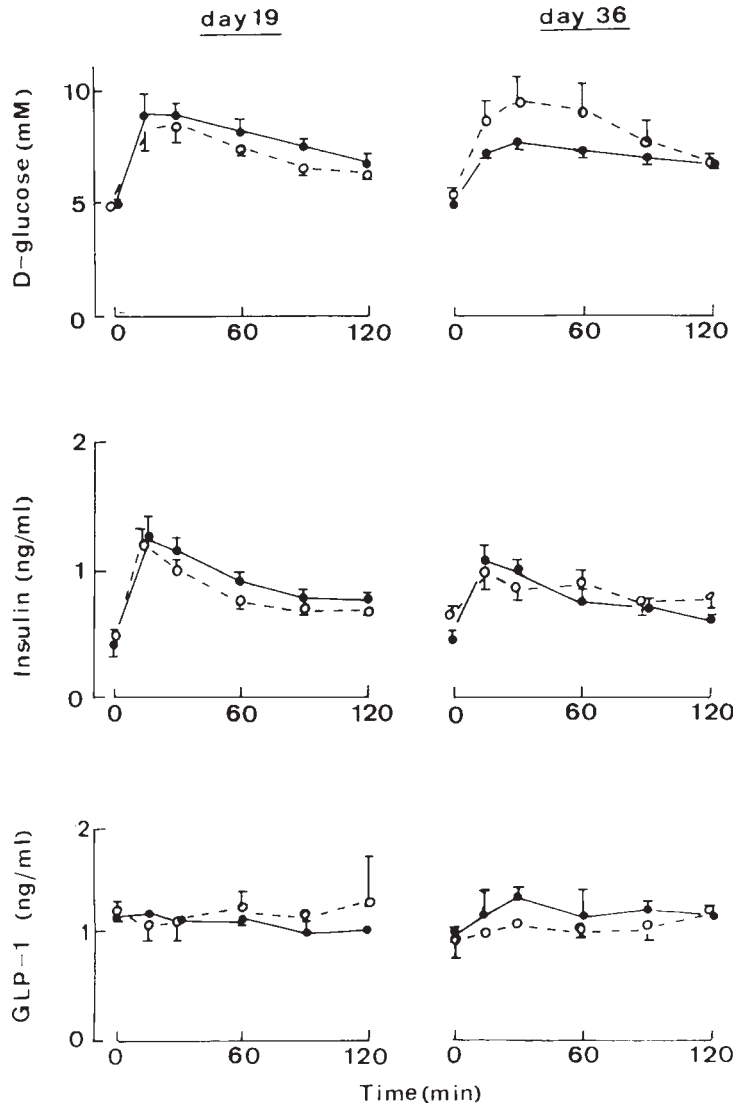

Fig. 3. Time course for the changes in plasma D-glucose, insulin, and GLP-1 concentrations during an oral glucose tolerance test performed at d 19 (left) or 36 (right) in overnight fasted rats otherwise fed the control diet (open circles and dashed line) or OO diet (closed circles and solid line). Mean values (± SEM) refer to eight or nine individual experiments (D-glucose and insulin) or two measurements made in pooled plasma samples (GLP-1).

ance test (min 0 to 120) was higher, albeit not significantly so ($p > 0.2$), in the control rats (336.8 ± 82.1 mM·min; $n = 8$) than in the animals fed the OO diet (229.8 ± 27.6 mM·min; $n = 8$). The trend toward an improved glucose tolerance in the latter animals was supported by the observation that, in the three first samples (min 15, 30, and 60), the increment in plasma D-glucose concentration above paired basal value averaged, in the rats fed the OO diet, only 60.8 ± 5.0% ($n = 24$; $p < 0.01$) of the mean corresponding values found at the same sampling time in the control animals (100.0 ± 13.6%; $n = 24$). Even when the measurements made at all sampling times (min 15 to 120 inclusive) were taken in to account, such a paired increment in plasma D-glucose concentration represented, in the rats fed the OO diet, no more than 75.5 ± 5.8% ($n = 40$; $p < 0.07$) of the mean corresponding control values (100.0 ± 11.4%; $n = 40$). The normalized percentage found in the rats fed the OO diet for 36 d was much lower ($p < 0.001$) than that recorded in the same animals at d 19 (Fig. 4).

In the rats examined at d 19, the increment in plasma insulin concentration above paired basal value was, at all sampling times, higher in the OO group than in the control animals, the values recorded in the former group averaging 160.6 ± 14.4% ($n = 40$; $p < 0.001$) of the mean corresponding values found at the same sampling time in the control rats (100.0 ± 8.0%; $n = 45$). Even at the earliest sampling times (min 15 and 30), the paired increment in plasma insulin concentration already averaged, in the OO group, 130.2

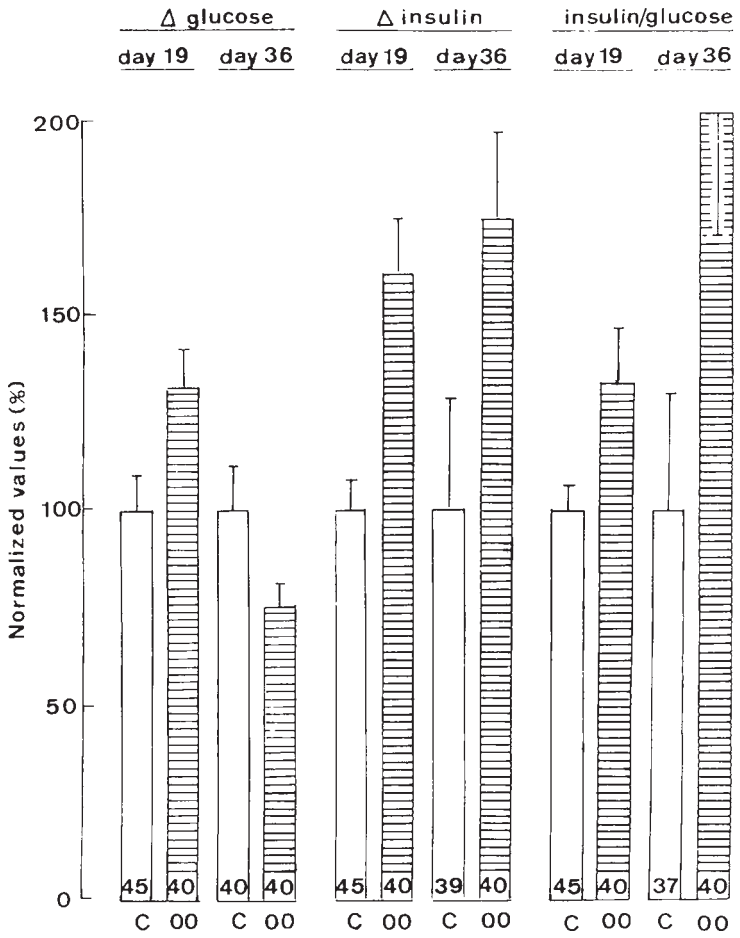

Fig. 4. Increments in plasma D-glucose and insulin concentrations above paired basal value during an oral glucose tolerance test conducted at d 19 or 36 in overnight fasted rats otherwise fed the control diet (C; open columns) or OO diet (OO; hatched columns). Also shown are the paired ratios between the increment in plasma insulin concentration and the corresponding increment in plasma D-glucose concentration. Mean values (± SEM) refer to the number of measurements indicated at the bottom of each column, and are expressed relative to the mean corresponding values found in the animals fed the control diet.

± 10.7% ($n = 16$; $p < 0.07$) of the mean corresponding control values (100.0 ± 11.5%; $n = 18$).

Likewise, at d 36, the paired increment in plasma insulin concentration in the rats fed the OO diet averaged, at min 15 and 30, 216.2 ± 27.7% ($n = 16$; $p < 0.02$) of the mean corresponding values found at the same sampling time in the control rats. Taking into account the measurements made at all sampling times (up to min 120 inclusive), the paired increment in plasma insulin concentrations above basal value found at d 36 in the rats fed the OO diet averaged 174.9 ± 22.3% ($n = 40$; $p < 0.05$) of the mean corresponding values found at the same sampling time in the control rats (100.0 ± 28.9%; $n = 39$).

The diet-induced changes in insulin-producing cell responsiveness to D-glucose and/or sensitivity to insulin in its target tissues were borne out by the finding that, at d 19, the paired ratios between the increments in plasma insulin and D-glucose concentrations averaged, in the rats fed the OO diet, 132.9 ± 13.8% ($n = 40$; $p < 0.03$) of the mean corresponding values found at the same sampling time (min 15 to 120 inclusive) in control rats (100.0 ± 7.2%; $n = 45$) of the same sex. Likewise, at d 36, the same ratio averaged, in the rats fed the OO diet, 201.9 ± 30.9% ($n = 40$; $p < 0.025$) of the mean corresponding value found in the control animals (100.0 ± 30.0%; $n = 37$). In the rats fed the OO diet, such normalized ratios were thus significantly higher ($p < 0.05$) at d 36 than at d 19 (Fig. 4).

Both at d 19 and 36, the basal GLP-1 plasma concentration in the overnight fasted rats was not significantly different in the animals otherwise fed the control or OO diet, with mean respective values of 1.08 ± 0.11 and 1.08 ± 0.07 ng/mL ($n = 4$ in both cases). At d 19, no sizable increase in GLP-1 plasma concentration was recorded during the oral glucose tolerance test, whether in the control or OO group of rats. At d 36, however, and whether judged from the measurements made at min 15 and 30 or at all sampling times,

Table 2
GLP-1 Content of the Intestinal Tract

	Control		OO	
	Male	Female	Male	Female
Jejunum				
Weight (g)	4.5 ± 0.3 (3)	3.6 ± 0.3 (5)	5.1 ± 0.2 (4)	3.8 ± 0.3 (4)
Protein (mg)	35.1 ± 0.9 (3)	28.4 ± 2.3 (5)	38.5 ± 1.1 (4)	28.4 ± 2.4 (4)
(mg/g weight)	7.94 ± 0.46 (3)	7.80 ± 0.34 (5)	7.51 ± 0.15 (4)	7.58 ± 0.33 (4)
GLP-1 (ng)	202 ± 100 (3)	285 ± 60 (5)	237 ± 32 (4)	213 ± 49 (4)
(ng/g weight)	45.3 ± 23.3 (3)	77.5 ± 15.3 (5)	46.7 ± 6.8 (4)	58.4 ± 14.4 (4)
(ng/mg protein)	5.9 ± 3.0 (3)	10.1 ± 2.3 (5)	6.2 ± 0.8 (4)	7.5 ± 1.6 (4)
Ileum				
Weight (g)	3.7 ± 0.4 (3)	2.8 ± 0.1 (5)	3.6 ± 0.2 (4)	2.9 ± 0.1 (4)
Protein (mg)	28.0 ± 1.1 (3)	23.2 ± 1.4 (5)	27.0 ± 4.1 (4)	24.1 ± 0.9 (4)
(mg/g weight)	7.72 ± 0.59 (3)	8.25 ± 0.37 (5)	7.32 ± 0.79 (4)	8.26 ± 0.55 (4)
GLP-1 (ng)	453 ± 219 (3)	497 ± 47 (5)	792 ± 164 (4)	406 ± 63 (4)
(ng/g weight)	129.1 ± 68.3 (3)	177.2 ± 15.6 (5)	221.5 ± 46.5 (4)	138.3 ± 21.3 (4)
(ng/mg protein)	16.8 ± 8.8 (3)	21.7 ± 2.2 (5)	33.6 ± 10.3 (4)	16.8 ± 2.5 (4)
Colon				
Weight (g)	1.4 ± 0.1 (3)	1.4 ± 0.1 (5)	1.6 ± 0.2 (4)	1.3 ± 0.1 (4)
Protein (mg)	8.1 ± 3.4 (3)	5.5 ± 0.3 (5)	7.6 ± 1.6 (4)	8.4 ± 1.3 (4)
(mg/g weight)	5.68 ± 2.13 (3)	3.98 ± 0.18 (5)	4.68 ± 0.64 (4)	6.35 ± 0.57 (4)
GLP-1 (ng)	223 ± 127 (3)	219 ± 30 (5)	245 ± 31 (4)	179 ± 20 (4)
(ng/g weight)	169.9 ± 81.6 (3)	152.9 ± 12.8 (5)	153.2 ± 6.1 (4)	137.1 ± 11.1 (4)
(ng/mg protein)	42.5 ± 21.5 (3)	39.0 ± 4.1 (5)	34.8 ± 5.3 (4)	22.3 ± 3.0 (4)
Cecum				
Weight (g)	1.4 ± 0.3 (3)	0.9 ± 0.1 (5)	1.0 ± 0.1 (4)	0.9 ± 0.1 (4)
Protein (mg)	9.9 ± 2.8 (3)	7.6 ± 0.9 (5)	7.4 ± 1.4 (4)	8.4 ± 0.6 (4)
(mg/g weight)	8.31 ± 2.99 (3)	8.10 ± 0.48 (5)	7.01 ± 1.09 (4)	9.36 ± 0.48 (4)
GLP-1 (ng)	126 ± 51 (3)	128 ± 21 (5)	115 ± 18 (4)	121 ± 27 (4)
(ng/g weight)	101.0 ± 52.5 (3)	134.8 ± 13.1 (5)	109.6 ± 16.9 (4)	133.8 ± 24.7 (4)
(ng/mg protein)	17.3 ± 8.0 (3)	16.5 ± 0.9 (5)	16.8 ± 4.0 (4)	14.7 ± 3.1 (4)

the paired increment in plasma GLP-1 concentration above basal value, which failed to achieve statistical significance in the rats otherwise fed the control diet ($p > 0.1$ or more), averaged, in the rats otherwise fed the OO diet, 0.26 ± 0.07 ng/mL ($n = 4$; $p < 0.05$) at min 15 and 30 and 0.23 ± 0.06 ng/mL ($n = 10$; $p < 0.005$) throughout the test.

GLP-1 Content of the Intestinal Tract

At d 50, the GLP-1 content of the jejunum, ileum, colon, and cecum was, as a rule, not significantly different in rats of the same sex fed either the control or OO diet, whether such a content was expressed per g wet weight or per mg protein. The sole exception, among 16 comparisons, consisted in a lower GLP-1 content of the colon ($p < 0.02$) when expressed per mg protein, in female rats fed the OO diet rather than control diet. Pooling all available data, the GLP-1 content (ng/mg protein) averaged, in the rats fed the OO diet, 97.7 ± 11.0% ($n = 32$; $p > 0.8$) of the mean corresponding value found at the same level of the intestinal tract in animals of the same sex fed the control diet (100.0 ± 8.7%; $n = 32$) (Table 2).

Discussion

The major findings in this study can be summarized as follows.

First, in meal-trained rats, exposure to the OO diet provoked a marked increase in plasma GLP-1 concentration, a phenomenon not observed in animals given access to the control diet. Over a period of 50 d, the plasma GLP-1 concentration was also higher in fed rats exposed to the OO, as distinct from control, diet. No significant difference between the two groups of rats was observed, however, in the GLP-1 content of the jejunum, ileum, colon and cecum.

Second, in both male and female rats, the gain in body weight over the same period of 50 d was higher in the rats exposed to the OO diet rather than control diet.

Finally, when an oral glucose tolerance test was performed at d 19 and 36 in overnight fasted rats, the increment in plasma insulin concentration was higher in the rats fed the OO diet than in the control group of animals. This coincided with a higher value, during the glucose tolerance test, for the paired ratio between the increments in plasma insulin and glucose concentration in the rats fed the OO diet. At d 36, these rats also displayed a higher GLP-1 response and improved glucose tolerance, when compared to the animals fed the control diet.

Thus, although long-term exposure to a food rich in monosaturated fatty acids may offer the disadvantage of a higher increase in body weight, it results in a better tolerance to D-glucose. The latter phenomenon appears attributable, in part at least, to a higher insulin response to D-glucose enteral intake, which may itself result, among other factors, from a higher level of circulating GLP-1 in the rats fed the OO diet. On the sole basis of the present results, it cannot be decided whether the improved tolerance to D-glucose eventually present in the rats fed the OO diet also entails increased sensitivity to insulin of its target cells.

Taken as a whole, the present findings argue in support of the proposal that a diet enriched in triglycerides containing a high percentage of monounsaturated fatty acids may favor glucose homeostasis *(1–7)*. A comparable study is presently being conducted in an animal model of type 2 diabetes in order to find out whether this proposal is also pertinent in the latter disease.

Materials and Methods

Experimental Procedure

A standard diet (UAR; Panlab, Barcelona, Spain) was placed for 2–4 h in an olive oil (13% saturated fatty acids, 79% monounsaturated fatty acids, and 8% polyunsaturated fatty acids) bath. This procedure resulted in 6.7 ± 0.1% (n = 7) increase in weight of the diet.

Groups of four to six male and female Wistar rats, from a colony maintained at the Fundacíon Jiménez Diaz (Madrid, Spain), were first meal-trained over a 1-wk period. For such a purpose, the rats were deprived of food from 6.00 PM to 2.00 PM on the next day over seven successive days. They were given access to food, however, for 15 min at about 10.00 to 11.00 AM. After this week of meal-training, during which only the standard diet was used, the rats were exposed for 15 min at the same time to either the standard diet (control) or the olive oil–enriched (OO) diet. The plasma D-glucose, insulin, and GLP-1 concentrations were measured before and 10, 20, 40, 60, 90, and 120 min after the onset of this test.

The body weight, plasma D-glucose, insulin, and GLP-1 concentrations were also measured after 4, 11, 27, and 50 d of exposure to the control or OO diet in fed rats. After 19 and 36 d of exposure to these diets, an oral glucose tolerance test was conducted in overnight fasted animals. A solution of D-glucose (30%, w/v) was administered intragastrically, the amount of the sugar given to each animal being 1.2 mg per g body wt. The plasma D-glucose, insulin, and GLP-1 concentrations were measured before and 15, 30, 60, 90, and 120 min after delivery of the solution of D-glucose.

Finally, at d 50, the animals were sacrificed and the protein and GLP-1 content measured in jejunum, ileum, colon, and cecum extracts.

Analytical Methods

The plasma concentrations of D-glucose *(13)*, insulin *(14)*, and GLP-1 *(15)* were measured by methods described in the cited references. Because of the larger volume of plasma required for the GLP-1 measurements, the samples obtained under identical conditions from three to five rats were pooled together. The protein and GLP-1 content of intestinal extracts was measured as indicated elsewhere *(16)*. In the assay of plasma and intestinal GLP-1, a specific N-terminal directed antibody (98302), kindly supplied by Drs. C.F. Deacon and J.J. Holst (University of Copenhagen, Copenhagen, Denmark) was used at a final 1:120,000 dilution.

Presentation of Results

All results, including those mentioned above, are presented as mean values (± SEM) together with the number of individual observations (n) or degree of freedom (d.f.). The statistical significance of differences between mean values was assessed by use of Student's t-test.

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