Comparative effects of *Citrullus colocynthis*, sunflower and olive oil-enriched diet in streptozotocin-induced diabetes in rats

N. Sebbagh \textsuperscript{a}, C. Cruciani-Guglielmacci \textsuperscript{b}, F. Ouali \textsuperscript{b}, M.-F. Berthault \textsuperscript{b}, C. Rouch \textsuperscript{b}, D. Chabane Sari \textsuperscript{a}, C. Magnan \textsuperscript{b}, \textsuperscript{*}

\textsuperscript{a} Laboratoire de produits naturels, Department of biology, Hospital of Tlemcen, Tlemcen, Algeria

\textsuperscript{b} Université Denis-Diderot-Paris-7, CNRS, Paris, France

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Abstract

*Citrullus colocynthis* (colocynth) seeds are traditionally used as antidiabetic medication in Mediterranean countries. The present study evaluated the differential effects of diets enriched with *C. colocynthis*, sunflower or olive oils on the pancreatic β-cell mass in streptozotocin (STZ)-induced diabetes in rats. STZ injection induced rapid hyperglycaemia in all animals. However, 2 months later, hyperglycaemia was significantly less pronounced in the rats fed a *C. colocynthis* oil-enriched diet compared with other rat groups (7.9 mM versus 12 mM and 16 mM with colocynth versus olive and sunflower oils, respectively). Assessment of insulin sensitivity using the homoeostasis model assessment (HOMA) method also indicated less insulin resistance in the rats fed a *C. colocynthis* oil-enriched diet versus the other rats. Finally, 2 months after STZ injection, the pancreatic β-cell mass was similar in both the STZ-treated rats fed the colocynth oil-enriched diet and their controls fed the same diet. In contrast, the pancreatic β-cell mass remained lower in the STZ-induced diabetic rats fed with olive oil- and sunflower oil-enriched diets compared with the *C. colocynthis* group. We conclude that *C. colocynthis* oil supplementation may have a beneficial effect by partly preserving or restoring pancreatic β-cell mass in the STZ-induced diabetes rat model.

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Keywords: Type 2 diabetes; Rat; Animal model of diabetes; Streptozotocin; *Citrullus colocynthis* oil; Sunflower oil; Olive oil; Pancreatic β-cell mass

Résumé

*Les graines de colocynthe sont couramment utilisées comme traitement antidiabétique dans la médecine traditionnelle des pays méditerranéens. La présente étude a évalué les effets comparatifs des régimes alimentaires enrichis en huile de colocynth, d’olive ou de tournesol sur l’hypertension artérielle et sur la masse des cellules β-pancréatiques de rats diabétiques de STZ. L’injection de STZ a induit rapidement une hyperglycémie dans tous les groupes. Cependant, 2 mois plus tard, l’hyperglycémie était beaucoup moins sévère chez les rats supplémentés en huile de colocynth (7.9 mM) que chez les rats supplémentés en huile d’olive (12 mM) ou de tournesol (16 mM). L’estimation de l’insulino-résistance par la méthode *homoeostasis model assessment* (HOMA) indiquait une amélioration de la sensibilité à l’insuline dans le groupe « colocynth » par rapport aux deux autres. Finalement, deux mois après l’injection de STZ, la masse des cellules β-pancréatiques était encore significativement diminuée chez les rats supplémentés en huile d’olive ou de tournesol par rapport à leur propre groupe témoin non traité par STZ. Chez les rats traités par STZ mais supplémentés en huile de colocynth, la masse de cellules β-pancréatiques était revenue à la normale. En conclusion, la supplémentation en huile de colocynth pourrait avoir un effet protecteur et/ou régénérant vis-à-vis de la masse des cellules β-pancréatiques des rats rendus diabétiques par la streptozotocine.

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Mots clés : Diabète de type 2 ; Modèle animal de diabète ; Streptozotocine ; Huile de colocynth ; Huile de tournesol ; Huile d’olive ; Masse des cellules β-pancréatiques

* Corresponding author. Université Paris-7, 2, place Jussieu, 75251 Paris cedex 05, France.

E-mail address: christophe.magnan@univ-paris-diderot.fr (C. Magnan).
1. Introduction

Type 2 diabetes mellitus (T2D) is one of the fastest growing epidemics of our time [1–3]. This disease affected nearly 150 million adults worldwide in 2000. T2D is characterized by decreased insulin sensitivity leading to insulin resistance in its target tissues (mainly liver, skeletal muscle and adipose tissues) [4–6]. On the other hand, impaired glucose-induced insulin secretion (GIIS) with a decrease in pancreatic β-cell mass will eventually lead to chronic hyperglycaemia [7]. Both genetic and environmental factors are involved in the aetiology of T2D and dysfunction of fatty-acid (FA) metabolism appears to be an early key event leading to insulin resistance [6]. However, on the basis of experimental, clinical and epidemiological studies so far, it is clear that the relationship between the type of dietary FA and its effect on insulin sensitivity is still a matter of debate [4,8]. One population-based study, for example, found that the risk of raised insulin resistance was significantly lower in people who consumed olive oil compared with those who consumed only sunflower oil, thus indicating an association between the intake of oleic acid and the composition of oleic acid in plasma phospholipids and peripheral insulin action [9,10]. In animal models, it has been shown that the intake of garlic oil improves glycaemic control in diabetic rats through increased insulin secretion and increased insulin sensitivity [11]. In yet another study, spontaneously hypertriacylglycerolaemic obese and diabetic inbred idiopathic inflammatory myopathy (IIM) beta rats treated with oleoylestrone for 10 days displayed decreased plasma total cholesterol and triglyceride (TG) concentrations compared with controls [12]. This suggests that some types of FA may have protective effects in terms of both insulin secretion and action, thus highlighting the importance of dietary lipid composition.

<table>
<thead>
<tr>
<th>Constituents (g/100 g)</th>
<th>Sunflower oil (diet 1)</th>
<th>Olive oil (diet 2)</th>
<th>Colocynth oil (diet 3)</th>
<th>Energy values (kcal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>64</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>1.2</td>
</tr>
<tr>
<td>Starch</td>
<td>55.7</td>
<td>55.7</td>
<td>55.7</td>
<td>222.8</td>
</tr>
<tr>
<td>Saccharose</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td>Cellulose</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>–</td>
</tr>
<tr>
<td>Mineral mix</td>
<td>7.37</td>
<td>7.37</td>
<td>7.37</td>
<td>–</td>
</tr>
<tr>
<td>Vitamin mix</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>–</td>
</tr>
<tr>
<td>Oil</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>72</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
<td>380</td>
<td></td>
</tr>
</tbody>
</table>

In this case, the antidiabetic effects of some traditional plants could be related to their FA composition. Of these plants, it is well-known that infusions of *Citrullus colocynthis* Schrad (colocynth; Cucurbitaceae) fruit are traditionally used as antidiabetic medication in Mediterranean countries [13,14]. Nmilta et al. reported that different extracts of seeds have an insulinotropic effect — evaluated in vitro in the isolated rat pancreas and isolated rat islets in the presence of 8.3 mM glucose — which may at least partly account for their antidiabetic actions [15]. Another study in rabbits has suggested that oral administration of the aqueous extract of colocynth rind has a hypoglycaemic effect that could be attributed to the presence of saponins as well as glycosidic components [16]. The effect of the aqueous extract of *C. colocynthis* seeds was also studied in a rat model of streptozotocin (STZ)-induced diabetes [17]. Oral administration of the plant extract reduced plasma concentrations of both aspartate aminotransferase and lactate dehydrogenase, but failed to reduce the rats’ hyperglycaemia.

Nevertheless, the effect of *C. colocynthis* oil extract remains poorly documented and so the present study was aimed at testing whether or not it has an antidiabetic effect, especially in terms of preservation of the pancreatic β-cell mass. To that end, STZ-induced diabetic rats were fed a diet enriched with either sunflower, olive or *C. colocynthis* oil and the effects on glucose homoeostasis, pancreatic β-cell mass and atherogenic parameters studied.

2. Materials and methods

*C. colocynthis* oil was extracted using the following method. Fresh or dry ripe *C. colocynthis* fruits were collected from Mecheria (western Algeria), sliced in half and the seeds removed by hand. Mature black seeds were selected, the pulp removed and

<table>
<thead>
<tr>
<th>Oil composition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myristate</td>
</tr>
<tr>
<td>Palmitate</td>
</tr>
<tr>
<td>Stearate</td>
</tr>
<tr>
<td>Oleate</td>
</tr>
<tr>
<td>Linoleate</td>
</tr>
<tr>
<td>Linoleate</td>
</tr>
<tr>
<td>Arachidonate</td>
</tr>
</tbody>
</table>

Mineral mix composition (g/100 g of dry diet): Ca²⁺, 4; K⁺, 2.4; Na⁺, 1.6; Mg²⁺, 0.4 Fe²⁺, 0.12; trace elements: manganese, 0.032; copper, 0.05; zinc, 0.018.
Vitamin mix composition (mg/kg of dry diet): retinol, 1.8; cholic acid, 0.019; thiamine, 6; riboflavin, 4.5; pantothenic acid, 21; inositol, 5; ascorbic acid, 240; α-tocopherol, 51; nicotinic acid, 30; folic acid, 1.5; biotin, 0.09.
the seeds ground into powder using the Ultraturrax method. The lipid fraction was extracted using petroleum ether (40–60°C) in a Soxhlet apparatus for 2 hours by the Natural Products laboratory in Tlemcen, Algeria. The solvent was then evaporated and the lipid fraction residues weighed; the oil content of the seeds was 17% and the FA composition was analyzed by gas chromatography, using a Triathlon autosampler (Spark Holland, Emmen, The Netherlands), a model 218 quaternary pump (Beckman), a fluorescence detector (Shimazu RF-551, Tokyo, Japan) and a recorder (BD112, Becton Dickinson). Separation of FA was achieved using a reversed-phase Kromasil C18 column (5 μm, 250 × 4.6 mm ID). Composition of the mobile phase changed along a gradient from MeOH-H2O (92:8) to MeOH-H2O (100:0) in 25 minutes at 1.7 mL/min. The selected excitation and emission wavelengths were 325 nm and 395 nm, respectively. Both the sunflower and olive oils were commercially available products (Cevital, Tlemcen, Algeria) and their FA compositions were also analyzed as described above. As shown in Table 1, three different diets were prepared: diet 1: 8% sunflower oil-enriched diet; diet 2: 8% olive oil-enriched diet; diet 3: 8% C. colocynthis oil-enriched diet. The percentages of the main FA in each diet are presented in Table 2. Those in the sunflower and olive oils are similar whereas the omega-6 linoleic acid (linoleate) is the major FA found in coloycynth oil (C_CO rats). Two weeks after the beginning of the study, 50% of the rats in each group were randomly made diabetic by a single, intraperitoneal injection of streptozotocin (STZ; Sigma-Aldrich, Steinheim, Germany), dissolved in 0.1 M of saline buffer at a dose of 65 mg/kg body weight (bw). These animals were referred to as STZSFO rats, STZOLO rats and STZCO rats in the sunflower, olive and coloycynth oil-enriched groups, respectively. Both glycaemia and insulinaemia were measured once daily at 0, 4, 8, 12 and 16 days following STZ injection.

At the end of the experiment, all the rats were given a lethal, intraperitoneal dose of pentobarbital 10% (500 μL/100 g bw). Blood was immediately collected from the abdominal aorta and plasma obtained by low-speed (2000 rpm) centrifugation. Blood glucose was determined using a glucose analyzer (Boehringer Mannheim, Mannheim, Germany).

### Table 2

<table>
<thead>
<tr>
<th>n</th>
<th>Control diet 1</th>
<th>STZ diet 1</th>
<th>Control diet 2</th>
<th>STZ diet 2</th>
<th>Control diet 3</th>
<th>STZ diet 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial bw (g)</td>
<td>85 ± 4</td>
<td>82 ± 3</td>
<td>78 ± 2</td>
<td>77 ± 4</td>
<td>85 ± 4</td>
<td>77 ± 3</td>
</tr>
<tr>
<td>Final bw (g)</td>
<td>255 ± 4</td>
<td>217 ± 5a</td>
<td>261 ± 3</td>
<td>212 ± 5a</td>
<td>178 ± 6b</td>
<td>168 ± 8c</td>
</tr>
<tr>
<td>Glycaemia (mM)</td>
<td>4.3 ± 0.1</td>
<td>13.6 ± 0.1b</td>
<td>5.4 ± 0.05</td>
<td>11.6 ± 0.2a</td>
<td>5.4 ± 0.1</td>
<td>7.6 ± 0.6bc</td>
</tr>
<tr>
<td>Insulinaemia (pM)</td>
<td>1308 ± 80</td>
<td>906 ± 30a</td>
<td>1140 ± 36</td>
<td>782 ± 17b</td>
<td>864 ± 82b</td>
<td>906 ± 41b</td>
</tr>
<tr>
<td>HOMA</td>
<td>269 ± 70</td>
<td>546 ± 10b</td>
<td>273 ± 13</td>
<td>918 ± 11b</td>
<td>286 ± 10</td>
<td>282 ± 9e</td>
</tr>
<tr>
<td>Triglycerides (g/L)</td>
<td>0.39 ± 0.1</td>
<td>0.53 ± 0.3</td>
<td>0.61 ± 0.15</td>
<td>0.70 ± 0.32</td>
<td>0.43 ± 0.1</td>
<td>0.63 ± 0.2</td>
</tr>
<tr>
<td>Cholesterol (g/L)</td>
<td>0.63 ± 0.1</td>
<td>0.76 ± 0.1</td>
<td>0.60 ± 0.11</td>
<td>0.65 ± 0.29</td>
<td>0.36 ± 0.8</td>
<td>0.47 ± 0.1a</td>
</tr>
<tr>
<td>LDL-C (g/L)</td>
<td>0.18 ± 0.5</td>
<td>0.30 ± 0.14a</td>
<td>0.18 ± 0.3</td>
<td>0.36 ± 0.2a</td>
<td>0.16 ± 0.3</td>
<td>0.15 ± 0.3</td>
</tr>
<tr>
<td>HDL-C (g/L)</td>
<td>0.42 ± 0.9</td>
<td>0.38 ± 0.2</td>
<td>0.30 ± 0.11</td>
<td>0.20 ± 0.8a</td>
<td>0.24 ± 0.1</td>
<td>0.26 ± 0.3</td>
</tr>
<tr>
<td>FFA (g/L)</td>
<td>0.51 ± 0.7</td>
<td>0.35 ± 0.5</td>
<td>0.67 ± 0.4</td>
<td>0.63 ± 0.5</td>
<td>0.20 ± 0.3</td>
<td>0.40 ± 0.11a</td>
</tr>
</tbody>
</table>

Results are expressed as means ± standard error.

FFA: free fatty acids.

a P < 0.05 versus controls fed the same diet.

b P < 0.05 versus control rats fed diet 1 or 2.
c P < 0.05 versus STZ-treated rats fed diet 1 or 2.

### 2.1. Animals

The experimental protocol was approved by the Animal Care and Use Committee of the University of Tlemcen. One-month-old male Wistar rats were housed in stainless-steel cages with a 12-hour light/dark cycle. Food and water were available ad libitum. Food was replaced daily with any uneaten portions discarded. Room temperature was maintained at 24°C and the humidity kept constant at 60%. Each group of rats was randomly divided into three groups, each of which was fed with one of the three diets for 2 months: diet 1: 16% casein + 8% sunflower (C_SFO rats); diet 2: 16% casein + 8% olive oil (control rats, C_OLO rats); diet 3: 16% casein + 8% coloycynth oil (C_CO rats). Two weeks after the beginning of the study, 50% of the rats in each group were randomly made diabetic by a single, intraperitoneal injection of streptozotocin (STZ; Sigma-Aldrich, Steinheim, Germany), dissolved in 0.1 M of saline buffer at a dose of 65 mg/kg body weight (bw). These animals were referred to as STZSFO rats, STZOLO rats and STZCO rats in the sunflower, olive and coloycynth oil-enriched groups, respectively. Both glycaemia and insulinaemia were measured once daily at 0, 4, 8, 12 and 16 days following STZ injection.

At the end of the experiment, all the rats were given a lethal, intraperitoneal dose of pentobarbital 10% (500 μL/100 g bw). Blood was immediately collected from the abdominal aorta and plasma obtained by low-speed (2000 rpm) centrifugation. Blood glucose was determined using a glucose analyzer (Boehringer Mannheim, Mannheim, Germany).

### 2.2. Morphometric analysis of pancreatic β-cell mass

Pancreata were fixed in 4% paraformaldehyde (PFA) for 2 hours at room temperature, then embedded in paraffin. The pancreatic β-cell mass was evaluated after immunostaining of deparaffinized, rehydrated sections for insulin as above and incubated for 1 hour at 37°C; detection was made by streptavidin–biotin–peroxidase complex developed with aminoethylcarbazole. Each pancreatic block was serially sectioned (7 μm) along its entire length to avoid bias due to changes in islet distribution or cellular composition and mounted on slides. For each pancreas, six to eight sections were presented in Table 2. Those in the sunflower and olive oils are similar whereas the omega-6 linoleic acid (linoleate) is the major FA found in coloycynth oil (Table 1).
standard concentration of haematoxylin was added as a counterstain. After staining, sections were mounted in Eukitt™ medium (O. Kindler GmbH, Freiburg, Germany). Islet cells stained very light brown to dark brown were considered insulin-positive cells, which meant that even highly degranulated cells could be counted as insulin-positive and included in the calculation of β-cell mass. Quantitative evaluation was performed using an Olympus BH2 microscope connected via a colour video camera to a computer, using Imagenia 2000 software (Biocom, Les Ulis, France). The areas of the insulin-positive cells and of the total pancreatic sections were found for each section and the β-cell area determined by calculating the ratio between these areas using stereology. The β-cell mass was calculated by multiplying the relative β-cell area by pancreatic weight.

2.3. Analytical methods

Plasma insulin concentration was measured by radioimmunoassay. The homeostasis model assessment (HOMA) score of insulin resistance was calculated from the fasting glucose and insulin concentration as described by Matthews et al. [18]. Plasma TG, HDL cholesterol (HDL-C), total cholesterol (TC) and FA concentrations were determined enzymatically using specific kits (Biomérieux, Lyon, France). Plasma LDL cholesterol (LDL-C) concentration was calculated using the Friedewald–Levy–Fredrickson formula [19].

2.4. Statistical analysis

Results are expressed as means ± standard error of mean (SEM). The significance of differences between the mean of STZ-treated rats and the controls was established by unpaired Student’s t test as well as the significance of differences between the mean of diet 3-fed rats group versus the diet 1 and diet 2 groups. \( P < 0.05 \) was considered significant.

3. Results

3.1. Glycaemia and insulinaemia

As shown in Fig. 1A, STZ injection rapidly induced hyperglycaemia in all rat groups. However, in the colocynth oil-fed rats, hyperglycaemia remained lower compared with both the sunflower oil and olive oil-fed rats (Fig. 1A). At day 16, glycaemia was reverted to 7.9 ± 0.2 mM in the STZCO group whereas it was higher in both the STZOLO and STZSFO rats (13.9 ± 0.5 mM and 16.0 ± 0.2 mM, respectively). At the end of experiment (Table 2), glycaemia was significantly higher in both the STZOLO and STZSFO rats compared with their respective controls. Also, HOMA scores were much higher in the STZOLO than in the STZSFO rats, thus suggesting marked insulin resistance in the former group. In STZCO rats, the HOMA score was similar to that of the CCO (Table 2).

At the end of the experiment, insulin sensitivity was assessed by HOMA in all groups (Table 2) and was found to be significantly increased in both the STZOLO and STZSFO rats compared with their respective controls. Also, HOMA scores were much higher in the STZOLO than in the STZSFO rats, thus suggesting marked insulin resistance in the former group. In STZCO rats, the HOMA score was similar to that of the CCO (Table 2).

3.2. Body weight and plasma lipids

By the end of the study, bw was significantly decreased in both STZOLO and STZSFO rats compared with the CCO rats (Fig. 1B). Two months later (Table 2), the STZSFO rats were still hypoinsulinaemic (Table 2) whereas insulinaemia in the STZCO was similar to that of the CCO (Table 2).

Plasma TG concentrations remained similar in all groups. Plasma LDL-C concentration was significantly lower in both the STZOLO and STZSFO rats compared with the CCO rats (Table 2). However, bw in both groups fed the colocynth oil-enriched diet was lower than in animals fed with either the sunflower or olive oil-enriched diet (Table 2).
fed the same diet; mass was similar in both the C CO and STZCO groups, and was not with STZ. As for plasma cholesterol concentrations, it also significantly increased in both the STZOLO and STZSFO rats whereas HDL-C did not change in the STZSFO (Table 2). Plasma FFA was higher in the STZCO compared with the C CO, although it was still lower than in rats fed with diets 1 and 2, treated or not with STZ. As for plasma cholesterol concentrations, it also increased in the STZCO versus C CO, but remained lower than the values obtained in the diet 1 and 2 rats.

3.3. Pancreatic β-cell mass

Pancreatic β-cell mass was decreased at the end of the study in STZSFO compared with C SFO rats (2.9 ± 0.05 mg/g versus 5.0 ± 0.4 mg/g, respectively; P < 0.05) but, in the STZOLO rats, the decrease was not significant (4.5 ± 0.5 versus 3.5 ± 0.5). In animals fed the colocynth oil-enriched diet, pancreatic β-cell mass was similar in both the C CO and STZCO groups, and was significantly larger than in either the STZOLO or STZSFO rats. Indeed, by day 16, hyperglycaemia was back to 7.9 mM in the STZCO whereas it remained significantly higher in the STZOLO and STZSFO. In parallel, plasma insulin was significantly decreased in the STZOLO and STZSFO compared with the STZCO animals, suggesting a deficit in pancreatic β-cell function and/or a decreased β-cell mass.

Finally, at the end of the experiment, hyperglycaemia was still elevated in the STZOLO and STZSFO, thereby underscoring the less protective effect of the olive- and sunflower-oil supplementation compared with C. colocynthis oil. Furthermore, the HOMA scores appear to support an effect of C. colocynthis to increase insulin action.

Taken altogether, our data suggest the partial preservation of functional β-cell mass in the latter diet group, emphasizing the specific effect of C. colocynthis compared with both olive and sunflower oils. However, it must be pointed out that STZ treatment appears to induce insulin resistance in the STZOLO and STZSFO, so we cannot exclude that such a phenomenon might be due to supplementation with these oils. In normal rats (with intact pancreas), there is no detectable effect of either diet on insulin sensitivity. In contrast, in the presence of insulin-secretion deficiency (following STZ treatment), a lack of insulin sensitivity is revealed. A less oil-enriched diet (4%) closer to the standard chow pellet may avoid the induction of such impaired insulin sensitivity.

To our knowledge, this is the first time that a protective effect of colocynthis oil extract against STZ-induced diabetes has been seen. However, the plant has long been used as a traditional antidiabetic medicine by the local populations of western Algeria, south-eastern Morocco and Jordan [13,20,21] and the ability of C. colocynthis to control glucose homeostasis has previously been shown using an aqueous extract in rabbits [16] and in STZ-induced diabetic rats [17]. In these studies, oral administration of the plant extract reduced plasma levels of aspartate aminotransferase and lactate dehydrogenase, suggesting improved hepatic function. The glycosidic content of aqueous extracts as a possible mediator of the C. colocynthis effect has been reported elsewhere [22–24]. As regards pancreatic β-cell function, one study has found an insulinotropic effect of colocynthis fruit extract [15]. In that study, different extracts of C. colocynthis seed components were studied and their insulin secretory effects evaluated in vitro in isolated rat pancreas and isolated rat islets in the presence of 8.3 mM of glucose. All of the tested extracts significantly stimulated insulin secretion [15]. However, to our knowledge, there is no data concerning the pancreatic β-cell mass in any of these studies.

The differential effects of oil-enriched diets on pancreatic β-cell mass could be related to their compositions. Both olive and sunflower oils are not very different in terms of their FA composition (Table 1). In contrast, omega-6 linoleic acid is the main component of C. colocynthis oil (12% and 10.8% versus 72%, respectively) whereas its oleate concentration is lower (60.8%...
and 63% versus 6.8%, respectively). Myristate is also poorly represented in C. colocynthis oil compared with olive and sunflower oils (0.8% versus 14.4% and 18.5%, respectively).

It is now well established that FA composition is crucial to explaining the differential effects of oils on parameters such as the lipid profile [25–27] and cardiac cell and vascular function [28]. It has been shown that 2-week treatments with evening primrose oil lowered lipid and haemostatic risk factors for cardiovascular disease in STZ-induced diabetic rats [29]. The beneficial effects of gamma-linoleic supplementation on nerve conduction velocity, Na⁺/K⁺ ATPase activity and membrane FA composition in the sciatic nerve of diabetic rats have also been demonstrated [30]. FA are also clearly identified as insulin-secretion modulators, depending on their chain length and degree of saturation [31]. Thus, linoleic acid — the major FA in C. colocynthis oil as mentioned above — may be involved in the modulation of pancreatic β-cell function, as recently reported by Feng et al. [32]. Other authors have demonstrated that linoleate reduced the voltage-gated K⁺ current in rat β-cells through GPR40 and the cAMP–protein kinase A system, leading to an increase in [Ca²⁺]i and insulin secretion [32]. Similar data were found in vivo in mice [33] in which dietary supplementation of conjugated linoleic acid and omega-3 polyunsaturated FA augmented insulin secretion partly because of increased islet glucose oxidation, although this was not sufficient to reverse the insulin resistance induced by high-fat feeds [33]. Yet, besides the effect of linoleate on insulin secretion, little is known of its effects on endocrine pancreas preservation and β-cell mass.

A recent study by Noto et al. showed that dietary conjugated linoleate preserved pancreatic function and reduced inflammatory markers in obese insulin-resistant rats [34]. In that study, obese Zucker rats were fed a linoleate-supplemented diet for 8 weeks, which led to smaller islet cells, improved oral glucose tolerance and insulinemia, and attenuated serum haptoglobin levels compared with control-fed fa/fa Zucker rats, despite no differences in bw and a slightly higher visceral adipose mass [34]. Thus, it may be postulated in our study that supplementation with C. colocynthis oil, which provided linoleic acid, also had a protective or regenerative effect on the endocrine pancreas compared with both olive and sunflower oils against the toxic effects of STZ. However, the molecular mechanisms involved in either C. colocynthis oil or the linoleate effect are still unknown.

Nevertheless, it has been shown in pigs that conjugated linoleic acid and omega-3 polyunsaturated FA were important pharmaconutrients for modulating inflammatory bowel disease through activation of keratinocyte growth factor (KGF) expression [35]. Interestingly, Movassat and Portha recently found that early administration of KGF improved β-cell regeneration in rats with STZ-induced diabetes [36]. Thus, it may be that C. colocynthis supplementation could lead to increased KGF expression, thus contributing to pancreatic regeneration and/or preservation. The differential effects of oil supplementation also suggest that other factors such as peroxisome proliferator-activated receptors (PPARs) could play a role in pancreatic β-cell function, as shown in different experimental models [37,38]. Also, the variable properties of co-activator recruitment as regards the composition of oil extracts might explain their differential effects — also described with PPAR agonists [39,40].

In conclusion, our data suggest that supplementing with C. colocynthis oil extract partly preserved pancreatic function together with an improved peripheral glucose in STZ-induced diabetic rats. Identification of the active components in such plants traditionally used in folk medicine to treat arterial hypertension and/or diabetes in Mediterranean countries may well contribute to our knowledge of their precise molecular effects.

References


