Effect of Linseed Oil on Oxidative Stress Parameters and Lipid Profile in Offspring of Obese Rats

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Abstract

The objective of this study was to determine linseed oil’s effect on oxidative stress parameters and lipid profile in three generations of offspring of obese rats. The initial age of the female rats is two months and of a weight of 100 ± 10 g were fed for two months with a control diet or a cafeteria diet, fortified or not with linseed oil (5%). At the end of this period the female rats are coupled by male rats. After parturition, one third of newborns in each group were scarified. During lactation the mothers continued to follow the same diet as pregnancy. After weaning, the offspring continue to follow the same diet as the mothers. In 30 days after birth the second third in each group of offspring were scarified and the last third in each group were scarified in 90 days after birth. The blood was drawn for determine biochemical parameters (glucose, total cholesterol and triglycerides) and oxidative stress markers (carbonyl proteins, malondialdehyde, nitric oxide, superoxide anion, catalase, superoxide dismutase, reduced glutathione and vitamin C). The offspring of the group which was fed a cafeteria diet showed hyperlipidemia, an imbalance of blood glucose and a high oxidative stress. The addition of linseed oil to cafeteria diet has corrected these complications. In conclusion, Linseed oil in rats during pregnancy has shown beneficial effects in the prevention of obesity and these metabolic disorders and has struggled against oxidative stress in the offspring.

Keywords: Linseed oil, oxidative stress, lipid profile, obesity, rat offspring.
Introduction

Obesity has become a major global health challenge for children and adults, not only in developed countries but also in developing countries (Ng et al., 2014). Worldwide it has nearly doubled since 1980 and it is leading risks for global deaths (WHO, 2014). Overweight and obesity are defined as abnormal or excessive fat accumulation that may impair health (WHO, 2014). Raised BMI is a major risk factor for non-communicable diseases such as cardiovascular diseases, diabetes, oxidative stress, musculoskeletal disorders and some cancers (Ayad et al., 2013; Takeuchi et al., 2014; Fan et al., 2013; Deere et al., 2012).

Mother’s diets play an important role in the regulation of metabolism of its offspring (energetic results, insulin metabolism, blood pressure, etc.) (Desai et al., 2014). Mother overweight during gestational period can lead to gestational diabetes and increase the risk of perinatal morbidity and infant overweight (Dong et al., 2013). Maternal overfeeding can influence the mode of delivery, breastfeeding and the composition of breast milk because of infant gut microbiota disrupting and can also be linked with the risk of predisposition to obesity in children and adult later (Paliy et al., 2014).

Oxidative stress is an imbalance between the production of reactive oxygen species (free radicals) and the ability of the body to counteract or detoxify their harmful effects through antioxidants (Betteridge, 2000). Several studies have demonstrated that obesity during pregnancy is associated with oxidative stress, which may affect embryo development and cause diseases, such as diabetes and atherosclerosis in the offspring of obese mothers (Malti et al., 2014).

Polyunsaturated fatty acids oméga-3 reduce adipogenesis and lipogenesis (Muhlhauser et al., 2011) and help in prevention and treatment of obesity (Goluba et al., 2011). Thanks to the abundance of polyunsaturated fatty acids in linseed oil (73 %) divided in linolenic acid ω6 (around 14. 3%) and α-linolenic acid ω3 (around (58.7%)) and its weakness in saturated fat (9 %). The main objective of this study is to identify the effect of a high calorie diet and high cholesterol diet enriched with linseed oil (5%) over the oxidative stress in offspring from mother obese or not, compared to the same diet but not enriched with linseed oil all this in a strategy of preventing obesity and related disorders.

Materials and Methods

Preparation of the Diet

In this study, we have four different diets:

The control diet [C] (standard laboratory chow: 386 kcal/100g) made by ONAB – Algeria.

The cafeteria diet [CAF] (523 kcal/100 g), his components were groundpaté, cheese, bacon, chips, cookies and chocolate (in a proportion of 2:2:2:1:1:1, by weight) and control diet (mix/control diet) [18].

Standard laboratory chow supplemented with linseed oils (5%) [CL].

The cafeteria diet supplemented with linseed oils (5%) [CAFL].

The composition of the four diets is listed in Table 1. Pure linseed oil is obtained from INRA (INRA, Algeria).

Animals and Experimental Protocol

All aspects of the experiment were conducted according to the guidelines provided by the ethical committee of the experimental animal care at Tlemcen University. Wistar rats were obtained from the Animal Resource Centre (Algeria). Female and male rats were housed in wood chip-bedded plastic cages at a constant temperature (25 °C) and humidity (60 ± 5%) with a 12 h light/dark cycle. The rats had free access to water and were assigned to eight dietary groups of equal average body weight.

The initial age of the female rats is two months and of an initial weight of 100 ± 10 g were fed for two months with a control diet or a cafeteria diet, fortified or not with linseed oil (5%). Four groups of 10 female rats were formed:

One group was fed standard laboratory chow (group 1).

Three groups were fed cafeteria diet (group 1,2 and 3).

At the end of this period the female rats are coupled by male rat (Twenty male rats fed a control diet were used for mating) and during pregnancy:
The group 1 continued to receive the same diet (standard laboratory chow) [control group C].

The group 2 continued to follow the cafeteria diet [CAF].

The group 3 begins to feed on standard laboratory chow supplemented with linseed oils (5%) [CL].

The group 4 begins to feed on cafeteria diet supplemented with linseed oils (5%) [CAFL].

The composition of the four diets is listed in (Table 1). The weight and food consumption of each animal were measured daily.

Table 1: Percentage composition of experimental Diets.

<table>
<thead>
<tr>
<th>Constituting in percentage (%)</th>
<th>Control C</th>
<th>Cafeteria CAF</th>
<th>Control + linseed CL</th>
<th>Cafeteria + linseed oil CAFL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein</td>
<td>19</td>
<td>21,50</td>
<td>18,50</td>
<td>21</td>
</tr>
<tr>
<td>Total Carbohydrate</td>
<td>56</td>
<td>33,50</td>
<td>18,50</td>
<td>32,50</td>
</tr>
<tr>
<td>Total Fat</td>
<td>8,50</td>
<td>30</td>
<td>8,50</td>
<td>32,50</td>
</tr>
<tr>
<td>Fibers</td>
<td>4</td>
<td>2</td>
<td>4</td>
<td>1,50</td>
</tr>
<tr>
<td>Humidity</td>
<td>7,50</td>
<td>9</td>
<td>7,50</td>
<td>8,50</td>
</tr>
<tr>
<td>Minerals</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Vitamins</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><strong>Fatty Acid</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-SFA</td>
<td>27</td>
<td>42</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>-MUFA</td>
<td>24</td>
<td>30</td>
<td>18</td>
<td>24</td>
</tr>
<tr>
<td>-C18:2 n-6</td>
<td>45</td>
<td>27</td>
<td>36</td>
<td>20</td>
</tr>
<tr>
<td>-C18:3 n-3</td>
<td>3</td>
<td>1</td>
<td>25</td>
<td>26</td>
</tr>
<tr>
<td>-C20:4 n-6</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

The composition of the diets was determined in the laboratory of natural product, Department of Biology, Faculty of Nature and Life, Earth and Universe Sciences, University of Tlemcen, Algeria. The fatty acid composition was determined in the laboratory UPRES Lipids and Nutrition, Faculty of Life Sciences, University of Burgundy, Dijon, France.

After parturition, one third of newborns in each group were scarified. During lactation the mothers continued to follow the same diet as pregnancy. After weaning, the offspring continue to follow the same diet as the mothers. In 30 days after birth the second third in each group of offspring were scarified and the last third in each group were scarified in 90 days after birth. They were anaesthetized with chloroform.

The blood was drawn from the abdominal aorta into heparinized tubes, and plasma was used for biochemical determinations and oxidative stress markers. After removal of plasma, erythrocytes were washed three times with 2 volumes of isotonic saline. Erythrocytes were lysed with ice-cold distilled water (1/4) and stored at 4°C for 15 min. The cell debris was removed by centrifugation (2000g for 15 min). Erythrocyte lysates were assayed for redox marker determinations.

**Statistical Analysis**

Results are expressed as means ± standard deviation. The results were tested for normal distribution using the Shapiro–Wilk test. Data not normally distributed were logarithmically transformed. Significant differences among the groups were analyzed statistically by a one-way analysis of variance (ANOVA). When significant changes were observed in ANOVA tests, Fisher least significant difference tests were applied to locate the source of significant difference. Significant differences between rats in the same group were assessed using a Student’s t test. The significance level was set at P < 0.05. These calculations were performed using STATISTICA version 4.1 (STATSOFT, Tulsa, OK).

**Results**
The cafeteria diet was associated with increased body weight in the three generations of offspring born to obese mothers compared to standard chow-fed ones (Table 2). Supplementation with linseed oil at 5% induced a reduction in body weight in these generations of offspring. Food intake in the rats fed on cafeteria diets were significantly higher than those in the rats fed on the standard diets. The addition of linseed oil induced a decrease in food intake.

Table 2: Body Weights and Biochemical Parameters of the Rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>C</th>
<th>CAF</th>
<th>CL</th>
<th>CAFL</th>
<th>P (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Newborns</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>4.90±0.35</td>
<td>7.97±0.29*</td>
<td>5.41±0.62</td>
<td>5.84±0.28*</td>
<td>0.010</td>
</tr>
<tr>
<td>Glucose (g/L)</td>
<td>1.01±0.02</td>
<td>0.58±0.03*</td>
<td>0.98±0.01</td>
<td>0.96±0.02*</td>
<td>0.004</td>
</tr>
<tr>
<td>Cholesterol (g/L)</td>
<td>0.48±0.01</td>
<td>0.96±0.03*</td>
<td>0.55±0.02</td>
<td>0.72±0.03**</td>
<td>0.006</td>
</tr>
<tr>
<td>Triglyceride (g/L)</td>
<td>0.63±0.01</td>
<td>1.03±0.03*</td>
<td>0.69±0.01</td>
<td>0.75±0.02*</td>
<td>0.002</td>
</tr>
<tr>
<td><strong>30 days</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>57.04±4.00</td>
<td>78.50±1.87*</td>
<td>65.85±5.15</td>
<td>71.19±3.36*</td>
<td>0.004</td>
</tr>
<tr>
<td>Glucose (g/L)</td>
<td>1.06±0.02</td>
<td>2.46±0.05*</td>
<td>0.98±0.03</td>
<td>1.04±0.01*</td>
<td>0.008</td>
</tr>
<tr>
<td>Cholesterol (g/L)</td>
<td>0.61±0.01</td>
<td>1.07±0.02*</td>
<td>0.74±0.03</td>
<td>0.83±0.02**</td>
<td>0.005</td>
</tr>
<tr>
<td>Triglyceride (g/L)</td>
<td>0.73±0.02</td>
<td>1.28±0.04*</td>
<td>0.76±0.02</td>
<td>0.81±0.03*</td>
<td>0.004</td>
</tr>
<tr>
<td><strong>90 days</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>190.48±2.36</td>
<td>276.83±6.49*</td>
<td>201.67±14.61</td>
<td>230.75±18.20*</td>
<td>0.001</td>
</tr>
<tr>
<td>Food intake (g/day/rat)</td>
<td>35.98±2.59</td>
<td>49.63±1.79*</td>
<td>32.51±2.30</td>
<td>37.24±3.01*</td>
<td>0.005</td>
</tr>
<tr>
<td>Glucose (g/L)</td>
<td>1.07±0.02</td>
<td>1.73±0.03*</td>
<td>1.05±0.02</td>
<td>1.12±0.01**</td>
<td>0.001</td>
</tr>
<tr>
<td>Cholesterol (g/L)</td>
<td>0.91±0.01</td>
<td>2.10±0.05*</td>
<td>0.98±0.02</td>
<td>1.05±0.03*</td>
<td>0.004</td>
</tr>
<tr>
<td>Triglyceride (g/L)</td>
<td>0.75±0.02</td>
<td>1.44±0.02*</td>
<td>0.73±0.03</td>
<td>0.84±0.01**</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Note. Values are presented as means±SEM. C: control diet; CL: control diet enriched with linseed oil at 5%; CAF: cafeteria diet; CAFL: cafeteria diet enriched with linseed oil at 5%. *Statistical difference for CL vs. C or CAFL vs. CAF (linseed oil effect). + Statistical difference for CAF vs. C or CAFL vs. CL (diet effect).

Erythrocyte catalase and superoxide dismutase activities were increased in the CAF group of older offspring of 30 and 90 days compared to controls (Figure 1). Linseed oil supplementation induced a reduction in catalase activities in CAFL group but had no effects on the CL group. However, in the newborns, there were no significant differences at erythrocyte catalase and SOD activities between groups. Cafeteria diet induced a decrease in erythrocyte reduced significantly glutathione levels (GSH) in the three generations of offspring born to obese mothers (CAF) compared to standard chow-fed ones (C) (Figure 1). Linseed oil supplementation increased GSH levels in CAFL group, but not in CL group exceptionally in offspring older 30 days.

Plasma vitamin C levels were reduced in the CAF group of older offspring of 30 and 90 days compared to C group (Figure 1). Linseed oil supplementation induced an increase in vitamin C amounts in these generations, but in the newborns there were no significant differences between groups.

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Plasma vitamin C levels were reduced in the CAF group of older offspring of 30 and 90 days compared to C group (Figure 1). Linseed oil supplementation induced an increase in vitamin C amounts in these generations, but in the newborns there were no significant differences between groups.
Fig. 1: Blood antioxidant status in offspring. Values are presented as means±SEM. C: control diet, CL: control diet enriched with linseed oil at 5%, CAF: cafeteria diet, CAFL: cafeteria diet enriched with linseed oil at 5%. GSH: reduced glutathione, SOD: superoxide dismutase. *Statistical difference for CL vs. C or CAFL vs. CAF (linseed oil effect). + Statistical difference for CAF vs. C or CAFL vs. CL (diet effect).
Plasma carbonyl proteins, malondialdehyde, nitric oxide and superoxide anion levels were increased in the three generations of offspring born to obese mothers in the CAF group compared to the C group (Figure 2). Linseed oils supplementation induced a reduction of these oxidative stress markers in the CAFL group but had no effects on the CL group exceptionally on plasma malondialdehyde level in offspring older 90 days which showed an increase compared to the C group. However, plasma nitric oxide level in the three generations of offspring and the others oxidative stress markers in offspring older 90 days remained higher in the CAFL group compared to the CL group.

Fig. 2: Blood oxidant status in offspring. Values are presented as means±SEM. C: control diet, CL: control diet enriched with linseed oil at 5%, CAF: cafeteria diet, CAFL: cafeteria diet enriched with linseed oil at 5%. CP: carbonyl proteins, MDA: malondialdehyde, NO: nitric oxide, O2-: superoxide anion. *Statistical difference for CL vs. C or CAFL vs. CAF (linseed oil effect). + Statistical difference for CAF vs. C or CAFL vs. CL (diet effect).
Discussion

Overeating during pregnancy has a considerable influence on fetal development and on the health of the newborns with potential long-term consequences (Bouanane et al., 2010; Benkalfat et al., 2011). Numerous studies have shown the beneficial effects of linseed oil on health, its wealth in polyunsaturated fatty acids n-3 can fight against the risk of stroke, cardiovascular disease and oxidative stress cancers (Ayad et al., 2013; Benaissa et al., 2015; Rasmy, 2007).

In our study, we explored the metabolic effects of a cafeteria diet enriched with 5% linseed oil consumed by pregnant mothers on their offspring. The cafeteria diet-fed was strongly associated with the increase in adipose lipid depots. The offspring of these dams had an increase in food intakes and were heavier than rats fed control standard diet; but the addition of linseed oil to the regime cafeteria corrected these increases, in agreement with others previous studies (Ayad et al., 2013; Benaissa et al., 2015). An obese rat has a high risk of encountering metabolic diseases such as gestational diabetes which subsequently increase the risk of hypoglycemia in their newborns (Aasa et al., 2013; Ducarme et al., 2007). This may explain the decrease in the content of plasma glucose in the newborns of rats that have fed on cafeteria diets. However, the offspring aged 30 and 90 days presented an increase in plasma glucose compared to control offspring. These findings were compatible with metabolic dysfunctions linked to obesity leading to altered glucose metabolism (Katarzyna et al., 2016). It has been demonstrated that the increase of omega 3 fatty acids in the diet can balance glycemic (Stene et al., 2003). This may explain our results in CAFL group.

The cafeteria diet in expectant mothers induced an increased hepatic synthesis of lipoproteins as well as an increase in adipose lipid accumulation in their offspring (Benaissa et al., 2015). This explains the increased levels of plasma triglycerides and total cholesterol in the offspring of rats fed a diet cafeteria. However, linseed oil protects against these metabolic complications (Ayad et al., 2013; Benaissa et al., 2015).

Oxidative stress is characterized by an imbalance between pro-oxidants and antioxidant, it is potentially involved in the development of metabolic and neurodegenerative diseases, as well as aging (Haleng et al., 2007). Obesity increases oxidative stress by elevations in lipid peroxidation (Vincent et al., 2007) and during pregnancy, it causes an oxidation-reduction imbalance in newborns (Malti et al., 2014).

In this study, the offspring of obese rats (CAF) showed high levels of pro-oxidant markers (carbonyl proteins, malondialdehyde, superoxide anion, nitric oxide) in agreement with our previous study (Malti et al., 2014). The low rate of plasma vitamin C in offspring aged 30 and 90 days of obese rats (CAF) compared to control (C) is due to the use of this vitamin to reduce oxidative stress linked to obesity (Ayad et al., 2013). The antioxidant enzymes may also be consumed or inactivated in high oxidative conditions (Malti et al., 2014). This may explain the decrease of reduced glutathione level in CAF group. The offspring of obese rats showed also high catalase and SOD activities, this might be an adaptive response against the higher production of reactive oxygen species (Ayad et al., 2013; Malti et al., 2014). The linseed oil addition to a high-fat diet in rats decreases the accumulation of lipids which reduces lipid peroxidation and free radical synthesis (Xu et al., 2013), this explains the reduction of pro-oxidant markers (carbonyl proteins, malondialdehyde, superoxide anion, nitric oxide) in CAFL group compared to obese rats (CAF). The increase of vitamin C and GSH levels in the CAFL group could be due to a decrease in use of these two antioxidant related to low oxidative stress after linseed oil supplementation (Ayad et al., 2013; Xu et al., 2013). The reduction of the activities of superoxide dismutase and catalase in the CAFL group is probably due to a low rate of pro-oxidants compared to CAF group (Ayad et al., 2013).

Conclusion

Linseed oil in rats has shown beneficial effects in the prevention of obesity and these metabolic disorders and has struggled against oxidative stress in the offspring babies, youth and adults. The
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integration of this oil in the human diet can participate in improving the metabolic profile and reduce the incidence of obesity and its complications.

Acknowledgments

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References


