Effects of refined palm oil on glucose metabolism and pro-inflammatory cytokines

ABSTRACT

The data until 2013 shows that around 380 million people suffer from diabetes and about 90% of the patients are with type 2 diabetes (T2D). Insulin resistance is a physiological condition in which cells do not respond to the normal actions of the hormone insulin which leads to high blood sugar. Often this state is undetected and might further progress to the development of T2D or latent autoimmune diabetes of adults. The role of the pro-inflammatory cytokines in the progression of insulin resistance is not well studied. There is insufficient data in the literature about the influence of refined palm oil (RPO) on the pro-inflammatory cytokines and the mechanisms of altering the metabolism including the development of insulin resistance. The aim of this study was to determine the changes in the levels of TNF-α and Il-6 after chronic application of RPO on juvenile and adult Wistar rats and to assess the effect of this high-fat diet on glucose metabolism. The cytokines were determined and the oral glucose tolerance test was performed after 30 and 60 days of oral application of RPO (1 ml/100 g body weight). For the experiment, male juvenile and adult Wistar rats (n=24) were used. They were separated into four groups: group 1 - juvenile control group on standard chow food and saline solution (1 ml/100 g body weight), group 2 - juvenile test group which received standard chow food and refined palm oil (1 ml/100 g body weight), group 3 - adult control group receiving standard chow food and saline solution (1 ml/100 g body weight) and group 4 - adult test group treated with refined palm oil and standard rodent chow food. All rats had free access to tap water. The oral glucose tolerance test was performed at Day 30 and 60 of the experiment. The levels of the cytokines TNF-α and Il-6 in the blood plasma were also measured at Day 30 and 60. The results demonstrate the development of pre-diabetes after chronic treatment with refined palm oil with higher risk for the juvenile animals. This refined plant oil also correlates with increased levels of TNF-α and Il-6. The chronic 60 days application of refined palm oil on juvenile and adult male Wistar rats led to increased levels of TNF-α and Il-6 and impaired the carbohydrate metabolism with data for development of pre-diabetes.

Key words: Refined palm oil, pre-diabetes, TNF-α, Il-6.

INTRODUCTION

The term "diabetes mellitus" describes a metabolic disorder of multiple aetiology characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The effects of diabetes mellitus include long-term damage, dysfunction and failure of various organs (WHO, 1999).
and Hu, 2014) and about 90% of the patients are with type 2 diabetes (T2D) (Vos et al., 2012). Insulin resistance is a physiological condition in which cells do not respond to the normal actions of the hormone insulin which leads to high blood sugar. Often this state is undetected and might further progress to the development of T2D or latent autoimmune diabetes of adults (Chiu et al., 2007). The role of some cytokines is discussed in the development of insulin resistance. These cytokines control both the metabolism and the development of obesity. The role of the pro-inflammatory cytokines in the progression of insulin resistance is not well studied. There is insufficient data in the literature about the influence of refined palm oil (RPO) on the pro-inflammatory cytokines and the mechanisms of altering the metabolism including the development of insulin resistance. In this regard, we performed the oral glucose tolerance test and investigated the changes in the levels of TNF-α and Il-6 after chronic application of RPO on juvenile and adult Wistar rats. The cytokines were determined after 30 and 60 days of oral application of RPO (1 ml/100 g body weight).

MATERIALS AND METHODS

Palm oil

“Del Alma” is plant oil (double fraction of palm oil) which is not oxidized, does not burn at high temperatures and is ideal for frying. It is produced in Italy. The palm oil is applied in a dose of 1 ml/100 g body weight.

Experimental animals

This study was performed according to the Bulgarian ethics legislation governing these experiments.

The Wistar rats were housed in polypropylene cages at temperature-controlled environment (22°C) with a 12 h light: 12 h darkness cycles. All animals had 7 days to acclimatize to the laboratory conditions and they were fed a standard laboratory chow ad libitum, and had free access to tap water. For the purpose of the experiment were used 24 male Wistar rats: 12 juvenile (sexually immature) rats with initial age of 25 days and 12 adult mature male rats which were two months old at the beginning of the trial. To each one of the experimental groups there was a control group which received saline solution.

Oral glucose tolerance test

The test was carried out after the animals were kept without food for 16 h. The blood sugar was determined in the morning by the apparatus One Touch Ultra Easy LifeScan Europe. Then a 20% solution of glucose (2 g/kg body weight) was applied orally with a probe. A drop of blood from the tail vein was used to determine the plasma glucose on the 0, 30, and 60th minute. The test was conducted at days 30 and 60 of the experiment.

ELISA for rat TNF-α and Il-6 (produced by the company DIACLONE)

Principle of the method

A monoclonal antibody specific for rat TNF-α has been coated onto the wells of the microtiter strips provided. Samples, including standards of known TNF-α concentrations and unknowns were pipetted into these wells. During the first incubation, the rat TNF-α antigen and a biotinylated monoclonal antibody specific for rat TNF-α were simultaneously incubated. After washing, the enzyme (streptavidin-peroxdydase) was added. After incubation and washing to remove the unbound enzyme, a substrate solution which is acting on the bound enzyme was added to induce a coloured reaction product. The intensity of this coloured product is directly proportional to the concentration of rat TNF-α present in the samples.

The ELISA kit for Il-6 was produced by the same company. The ELISAs were carried at days 30 and 60 of the experiment.

After days 60 of the experiment, the animals were decapitated under Nembutal (55 mg/kg BW, i.p.), blood plasma was collected and the ELISA for TNF-α and Il-6 were performed.

Data management

Data was made using EXCEL platform, and statistical evaluations were performed by INSTAT program package, using one-way ANOVA with Bonferoni post-test, and Bartlett test for the Standard deviations.

RESULTS AND DISCUSSION

The results of the study are shown in Table 1 and in Figures 1 to 4. In both age groups the treatment with RPO led to statistically significant increase of TNF-α in the blood. The strength of this effect is not influenced by the age or by the duration of application of RPO (p>0.05). It is well known that TNF-α is a pro-inflammatory cytokine. For the first time the presented results demonstrate increase of this cytokine in the blood plasma after chronic application of RPO on Wistar rats at different age. The results give a reason to assume that the treatment with RPO in both juvenile and adult animals increases the risk of inflammation.

The application of RPO also leads to statistically
Table 1. Nutritional value of the used palm oil per 100 grams.

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy value</td>
<td>3700kJ/900 kcal</td>
</tr>
<tr>
<td>Fats</td>
<td>100 g</td>
</tr>
<tr>
<td>Saturated fatty acids</td>
<td>40 g</td>
</tr>
<tr>
<td>Monounsaturated fatty acids</td>
<td>46 g</td>
</tr>
<tr>
<td>Polyunsaturated fatty acids</td>
<td>14 g</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>0 g</td>
</tr>
<tr>
<td>Proteins</td>
<td>0 g</td>
</tr>
<tr>
<td>Fibers</td>
<td>0 g</td>
</tr>
<tr>
<td>Sodium</td>
<td>0 g</td>
</tr>
</tbody>
</table>

Figure 1. Effect of refined palm oil (1 ml/100 g body weight) on TNF-α in blood plasma. The increase of this pro-inflammatory cytokine in the blood of adult (2 months) and juvenile (25 days) rats compared to the control groups is statistically significant. (*p<0.05; **p<0.001). Statistical analysis: One way Anova: p<0.0001. Variations among column means are significantly different than expected by chance. Bonferroni: t_0=2.697; H0: if t>t_0, then p<0.05.

Figure 2. Effect of refined palm oil (1 ml/100 grams body weight) on IL-6 in blood plasma. The increase of this pro-inflammatory cytokine in the blood of adult (2 months) and juvenile (25 days) rats compared to the control groups is statistically significant. (*p<0.05; **p<0.001). Statistical analysis: One way Anova: p<0.0001. Variations among column means are significantly different than expected by chance. Barlett: The SD's belong to the same population. Bonferroni: t_0=3.418.
Figure 3. Influence of the treatment with refined palm oil for 30 and 60 days on the oral glucose tolerance test (OGTT) in male adult rats. There is no statistically significant change in the results from OGTT in the adult rats.

Figure 4. Influence of the treatment with refined palm oil for 30 and 60 days on the oral glucose tolerance test (OGTT) in juvenile Wistar rats. The results demonstrate changes in OGTT which are statistically significant on the 60th day compared to the 30th day of application of RPO.

significant (p<0.001) increase in the levels of IL-6 in both age groups of animals. The increase of IL-6 is present both on the 30 and the 60th day of the experiment. Taken together, the results demonstrate development of pre-diabetes after chronic treatment with RPO. The risk is higher for the sexually immature animals. Also the RPO increases the levels of TNF-α and IL-6 in the blood plasma and this increase correlates with the pre-diabetic state.
The current study demonstrates for the first time alterations in the level of TNF-α and IL-6 due to chronic application of RPO. It is well documented that these cytokines are involved in the process of inflammation and its complications (Michaud et al., 2013; Koleva-Georgieva et al., 2011). Large volume of research reveals the role of inflammation in the pathogenesis of diabetes mellitus (Akash et al., 2013; Marselli et al., 2013; Ehses et al., 2008; Apostolopoulos et al., 2015). In addition, there is convincing data which demonstrates the connection between obesity and the risk of inflammation in some patients (Ramsay et al., 2002; Ramsay, 2013; Sobieska et al., 2013; Grant et al., 2013; Nguyen et al., 2015; Apostolopoulos et al., 2015). The present experiment and the results from it demonstrate that the refined palm oil increases the levels of pro-inflammatory cytokine in the blood plasma. These results give us the basis to assume that the pathogenesis of pre-diabetes is due to increased levels of TNF-α and IL-6. The differences in the OGTT between the two age groups after chronic application of RPO reveal greater risk for the juvenile animals.

Conclusions

The chronic 60 days application of refined palm oil on juvenile and adult male Wistar rats led to increased levels of TNF-α and IL-6 and impaired the carbohydrate metabolism with data for development of pre-diabetes. The risk is higher for the juvenile (sexually immature) animals.

REFERENCES


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