HISTOPATHOLOGICAL EXAMINATION
OF SELECTED INTERNAL ORGANS OF PASTEL MINKS
IN RELATION TO OXIDATIVE STATE PARAMETERS

HANNA BIS-WENCEL, WOJCIECH ŁOPUSZYŃSKI¹, LEON SABA,
MONIKA BRYL, AND AGNIESZKA ROWICKA

Department of Animal and Environment Hygiene, ¹Department of Pathological Anatomy,
Faculty of Veterinary Medicine, University of Life Science in Lublin, 20-950 Lublin, Poland
hanna.biswencel@up.lublin.pl

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Abstract

The aim of the study was histopathological characteristics of mink females showing a tendency to decrease body condition in a perinatal period against a background of selected parameters of antioxidant state. The minks were divided into two groups according to body condition scoring system (BCS). The blood was collected twice: after weaning and at the end of the production cycle. The serum activity of oxidative state enzymes was determined. Anatomic and histopathological examinations were conducted after the production cycle. Samples of the liver, kidneys, small intestine, and ovaries were fixed in 10% buffered formalin and stained with haematoxylin-eosin and Sudan IV. The histopathological examinations revealed hyperaemia of the liver and kidneys, with large content of blood in the capillary and central vessels of obese females. The kidney structure was normal in animals of experimental group. On the other hand, kidneys of control group animals showed degenerative changes in the epithelial cells of the tubules of the excretory part, especially of the proximal tubules. Against the background of the obtained results, it should be stated that lower activities of selected enzymes with anatomical and histopathological changes were obtained in the animals with worse body condition.

Key words: female mink, obesity, oxidative state.

Oxidative stress has been implicated in a number of chronic diseases, usually grouped under the umbrella of the metabolic syndrome and is thought to contribute to the aging process. It is also involved in the progression of chronic diseases including obesity and type 2 diabetes mellitus (3, 4, 15). Maternal obesity is increasing in prevalence and is associated with adverse perinatal outcomes. It has been suggested that pregnancy in obese females has an in utero influence on offspring, leading to a cycling of risk factors through the generations. Obese offspring are more likely to suffer from metabolic diseases such as type 2 diabetes and cardiovascular disease later in their life (5).

The present paper deals with the results of the histopathological characteristics of females minks, showing a tendency to decrease body condition in perinatal period, against a background of selected parameters of antioxidant state.

Material and Methods

Two groups of 40 female Pastel minks were chosen in farm of 1,200 minks. The minks were divided into two groups according to body condition scoring system (BCS), which was developed to assist in evaluating the amount of body fat and lean body mass. BCS contains of five different scores: score 1 - very thin, score 2- thin, score 3 - ideal, score 4 - heavy, and score 5 - obese (7). In control group K, the weight of females was 1.3 kg (score 4-5), while in experimental group E, it ranged from 1.1 to 1.2 kg (score 3).

The animals were fed once a day, usually before noon, and throughout the study all animals were given free access to tap water by means of an automatic system. In the period of December-May: ME 1,150 kcal/kg (%ME of protein 52.0, fat 36.0, carbohydrate 12.0), 11 May till weaning: ME-1,250kcal/kg (%ME of protein, 45.0 fat 43.0, carbohydrate 12.0), 16 July till 15 September: 1,550kcal/kg (%ME of protein 36.0, fat 52.0, carbohydrate 12.0), 16 September till slaughter: 1,620 kcal/kg (%ME of protein 32.0, fat 52.0, carbohydrate 14.0). The feeding dose was established individually, depending on the animal condition - in the quantity of 180 g/24 h per one female. Between April 15 and May 8 each female had a feed limited to 130 g/24 h. After May 8 the dose was increased to the earlier value. The feed of both groups was supplemented with a vitamin-mineral premix in the ratios covering full demand for these elements, a preservative – sodium pyrosulphite and Rendox anti-oxidant.
The blood was collected twice: after weaning (collection I) and at the end of the production cycle (collection II).

The activity of selected enzymes, which are oxidative state markers, was determined in serum. The activity of superoxide dismutase (EC-SOD) was determined according to adrenaline method of Misra (1), Glutathione peroxidase (GPx), glutathione reductase (GR), and total antioxidant status (TAS) were established by means of diagnostic tests by Randox, using a spectrophotometer. Additionally, the content of soluble protein, as a conversion factor for the enumerated oxidative state enzymes, was measured.

Anatomic and histopathological examinations were conducted after the production cycle. Samples of the liver, kidneys, small intestine, and ovaries were preserved in 10% neutral formalin and stained with haematoxylin-eosin (HE) and Sudan IV.

The obtained results were statistically analysed with Student’s t-test (Microsoft Excel NT). The significance of differences depending on analysed factors and the significance of interaction were established by means of two-factor variance analysis for a double non-orthogonal cross classification.

**Results**

The activity of SOD ranged from 0.234 to 0.258 U/mgb in control group K, and from 0.266 to 0.342 U/mgb in the experimental group E. Similarly, peroxidase and reductase reached lower activity in the animals whose situation was reverse in reference to collection II. In this experimental group (82.17 mg/mL) as compared to of this parameter were found in minks from the first collections in the control group. No statistical differences were found between the groups in the activity of particular enzymes. TAS, in both I and II collections, showed statistically higher values in animals from score 3. In the case of soluble protein, statistically significant differences were revealed between the first and second collections from the analysed groups of animals. In collection I, statistically higher mean levels of this parameter were found in minks from the experimental group (82.17 mg/mL) as compared to individuals from the control group (74.33 mg/mL). The situation was reverse in reference to collection II. In this case, lower levels were observed in the animals whose weight was lower than in control group. The obtained results are included in Table 1.

**In the liver samples from group K in comparison with group E (Fig. 1), the trabecular structure of the lobule was effaced and regressive changes were more prominent. Numerous clusters of erythrocytes were present in sinusoidal spaces, as well as in the lumen of the central vein. Moreover, smaller and bigger vacuoles occurred in numerous hepatocytes of the intermediary and circumferential zones of the lobule. Sudan IV staining showed an increased intensity of the reaction to neutral fats in the liver of group K animals in comparison with group E. It was expressed the best in the intermediary and circumferential zones of the lobules. HE staining revealed normal kidney structure in group E animals (Fig. 2). On the other hand, kidneys of group K animals showed degenerative changes in the epithelial cells of the tubules of the excretory part, especially in the proximal tubules. These changes were accompanied by numerous small and singular bigger vacuoles, which caused that the cell nucleus was pushed to the circumference of the cell, or it even disappeared (Fig.3).

Degenerated epithelial cells occurred along the whole excretory part of the tubule, and they concerned 10%-20% of all renal tubules. Sudan IV staining revealed the presence of orange Sudan-absorbing bodies and indicated a lipid character of vacuoles in the cells. No visible pathological changes were observed in other organs examined.

**Discussion**

A response to the oxidative stress manifests itself with the definite antioxidation state that can be presented as the TAS. It is likely to be a perfect marker of this state, as there are numerous interactions recorded between the antioxidants that are easily overlooked, when being individually determined (1). Glutathione belongs to the antioxidants operating on the basis of the non-enzymatic mechanism. A glutathione thiole group readily reacts with free radicals, fastest with hydroxyl radicals and a little slower with organic radicals occurring at the aqueous phase (1). Several other investigators have also reported that menopause is associated with increase in lipid peroxidation and CAT, and a decrease in SOD and GPx (9).

### Table 1

Mean values of the blood oxidation state parameters of minks (n=40)

<table>
<thead>
<tr>
<th>Group</th>
<th>Collection</th>
<th>Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Superoxide dismutase (U/mgb)</td>
<td>0.238 ± 0.1542</td>
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<tr>
<td></td>
<td></td>
<td>Glutathione peroxidase (U/mgb)</td>
<td>0.253 ± 0.0909</td>
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<tr>
<td></td>
<td></td>
<td>Glutathione reductase (U/mgb)</td>
<td>0.0015 ± 0.0005</td>
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<tr>
<td></td>
<td></td>
<td>Total antioxidant status (µmol/mgb)</td>
<td>0.0045 ± 0.0002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Protein (mg/mL)</td>
<td>74.33A ± 13.68</td>
</tr>
<tr>
<td>Control</td>
<td>I</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental</td>
<td>I</td>
<td></td>
<td>0.342 ± 0.1239</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td></td>
<td>0.266 ± 0.0526</td>
</tr>
</tbody>
</table>

Means marked with capital letters differ statistically at P≤0.01; means marked with small letters differ statistically at P≤0.05.
Fig. 1. Liver of the mink from the group E. Fine Sudan-stained droplets in hepatocyte cytoplasm. Sudan IV+ haematoxylin, 120x.

Fig. 2. Fine Sudan-stained droplets in the renal tubule epithelium of minks from the group E. Sudan IV + haematoxylin, 240x.

Fig. 3. Intense reaction for Sudan-positive particles in the renal tubule epithelium of minks from the group K. Sudan IV + haematoxylin, 240x.
GPx is an adaptive enzyme. Its activity increases in response to the oxidation stress. It catalyses the hydrogen peroxide reduction and organic peroxides by the reduced glutathione (1, 14). Other studies have reported an association between increased oxidative stress and conditions such as dyslipidaemia, arthritis, hypertension, and diabetes, which coexist with obesity (9, 13).

Obesity is an epidemic problem impacting global health. The most frequently used measure of obesity is body mass index (BMI), which is calculated using the formula weight/ height² (kg/m²) (2). Obesity per se may induce systemic oxidative stress, which is, in turn the underlying cause of selective increase in ROS, dysregulation of adipocytokines, and development of metabolic syndrome (6). The link between obesity per se and oxidative stress has been suggested in some studies and a good correlation between BMI and oxidative stress has been reported, indicating obesity as an independent risk factor for plasma lipid peroxidation and depletion of erythrocyte cytoprotective enzymes in humans (6, 9).

In the light of the obtained results, it should be stated that higher activities of selected enzymes were obtained in the animal group with better body condition. It should be emphasised that both the activity of enzymes and TAS in group E animals decreased in collection II as compared to collection I. This may be explained by the fact that collection I was performed directly after finishing restrictive feeding, whereas collection II – at the end of the production cycle - which was the main subject of our other studies (1).

The results of histopathological examination demonstrated that the observed lesions were parallel to changes in enzyme activity. These changes concerning group K of animals confirm the studies by other authors (7, 12). According to Tove et al. (13) they might appear as a result of a marked increase in autophagy, amino acid catabolism, and gluconeogenesis due to the increased demands for energy in the lactating female.

Negative influence of high-energy nutrition on anatomical and histopathological changes was observed in polar foxes. The histopathological examinations revealed hyperaemia in the liver, kidneys, and ovaries, with increased content of blood in the capillary and central vessels of obese foxes (8).

Genetic conditions of minks, a diet poor in unsaturated fatty acids n-3, the size of litter, a tendency to increased fat deposition, increased age, and lack of movement, are the major factors which can contribute to the worse condition of minks during perinatal period (11). The results of current studies concerning the function of adipose tissue showed its importance as a para-, auto- and endocrine secreting tissue. It has been demonstrated in humans that obesity has a major impact on fertility, obstetric complications, and offspring health. Obesity affects the ovulation and may increase miscarriage rates. Offspring of obese mothers are more likely to have macrosomia and are at risk for developing obesity and metabolic syndrome later in life (2, 10).

Free radicals cause fast glycation of the long-living proteins, and oxidation of saccharides triggers, for example, increased oxidation of haemoglobin. Frequent interference of free radicals from high-energy feed in the cell organelles damages the structure of lipids, proteins, and DNA. This can trigger the disease state (12, 13). An inter-relationship seems to exist between the processes of lipid peroxidation and oxidation of saccharides and glycosylated proteins.

References