Dietary Sugar and Salt Represent Real Risk Factors for Cataract Development

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Key Words
Sugar, dietary - Salt, dietary - Cataract risk - Protein leakage - Lens histopathology - Porcine model

Abstract
Dietary sugar and salt represent etiological risk factors of human cataract. To verify etiological data on the basis of histological findings, 9 pigs with a body weight of 40 kg, 3 months of age, in groups of 3 were continuously fed with 5% of refined dietary sugar (sucrose - C_{12}H_{22}O_{11}), 0.5% of salt (NaCl) and a sugar-salt mixture (2.5 + 0.25% accordingly) in their crude (unboiled) meal food during 3 months, which resulted in minor cataractous changes in the lens. In the second experiment, 10 weight- and age-matched animals were fed a chronic sugar and intermittent salt diet during 6 months; the other 10 animals served as controls. During the second experiment, crystallin leakage into the aqueous humor of the lens was detected, and a marked swelling of the lens fibers and fiber tips was noticed, indicating that excessive amounts of dietary sugar and salt are risk factors for the development of cataract in normal (nondiabetic) animals.

Introduction
Etiological risk factors for the development of human cataract include age, diabetes, shortsightedness, irradiation, heavy smoking and alcohol consumption, and, according to more recent data, excessive consumption of salt and sugar, too [1-3]. These substances are of great importance because they represent common components of human food, which are often consumed simultaneously and in surplus amounts (e.g. sweet and salty meals and snacks, concurrent with other risks like smoking and alcohol). On the basis of etiological data, it can be supposed that salt and sugar influence human cataract development through hyperosmolarity. To the best of our knowledge, there are no data on normal experimental animals fed simultaneously with excessive amounts of sugar and salt. Salt addition has been described in hypertensive salt-sensitive rats given 0.9% NaCl drinking water to produce cataract [4]. Sugar cataract developed by addition of 25-50% galactose to the food of experimental animals [5-7]. Human senile and diabetic cataracts are studied best, and an association between dietary sugar and diabetic cataract formation has been largely proved [5]. However, the role of dietary sugar (sucrose) in nondiabetic cataract development has been overlooked because of the seemingly harmless impact of sugar on nondiabetics, only etiological data
on the effect of salt and sugar on human lenses are obtainable.

The aim of the present study was to demonstrate that dietary sugar and salt can initiate cataractous changes in the porcine lens, with protein leakage and irregular lens fiber swelling being characteristics of cataract development [6, 8, 9]. In our study, the amounts of sucrose and salt and the mode of their administration (chronic or intermittent) were chosen a priori, keeping approximate consumption habits of sugar and salt in humans in mind.

### Materials and Methods

**Animals and Diet**

The experiments were initiated on age- and weight-matched pigs of the Big White strain (body weight: 40 kg, age: 3 months). The animals were fed once a day (in the morning) throughout the experiment with the crude unboiled cereal whole-grain meal diet and water ad libitum. The diet consisted of barley (37.5%) and wheat (50%) to which 12.5% of the following concentrate (recipe of Provimi, Germany) was added. The concentrate consisted of 41.3% protein, 4.9% fat, 2.7% fibers, 25.8% ash, 6.3% calcium, 3.5% phosphor and 6.0% amino acids (lysine, methionine, cysteine and tryptophan).

**Sugar and Salt Addition**

Refined dietary salt (NaCl) and refined sugar (sucrose – C_{12}H_{22}O_{11}) were added to the food of the animals in two experiments. In the first experiment, three groups of 3 animals each were fed continuously with either 5% sugar, 0.5% salt or a mixture of 2.5% sugar and 0.25% salt in their food until 6 months of age; thereafter, the lenses were studied histologically. In the second experiment 10 animals were given 5% sugar continuously and intermittently 0.5% salt (a 1-week 0.5% salt diet alternating with a 2-week salt-free diet; fig. 1). As the weight of the animals increased during the experiment, the salt and sugar supplements were also increased in order to keep their concentration in food at a level assumed to be effective for producing cataractous changes but not yet noxious for the animals (table 1) [10]. Ten control animals were kept in similar farm conditions without sugar and salt supplementation. Eye tissues, aqueous and vitreous humors and blood serum were collected from fasted experimental and control animals on the 2nd and 17th days and at the end of the 1st to the 6th months after the beginning of the experiment, after the sacrifice of animals.

Glucose levels in the aqueous and vitreous humors and in the blood serum were determined by an enzymatic amperometric method (EBIO Compact, Eppendorf, Hamburg, Germany).

**Histology**

Eyes were enucleated, and lenses were fixed in Carnoy's solution and embedded in paraffin, followed by an examination of the hematoxylin-eosin-stained sagittal sections of the central part of the lens using a light microscope.

**Electrophoresis**

The aqueous and vitreous humors were kept at -20°C until studied electrophoretically. Freezing and thawing makes the vitreous more liquid in comparison with the native one. The thawed aqueous and vitreous were centrifuged at 14,000 rpm for 20 min, thereafter the supernatants were diluted for electrophoresis in an SDS-PAGE sample buffer, boiled for 5 min and electrophoresed in a gradient gel (8-25%) at a maximum of 200 V and 30 mA for 4 h in Hoefffer’s unit, using a discontinuous system with tricine in the cathode buffer [11].

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**Table 1.** Approximate body mass and food consumed per animal [10] and a priori chosen chronic sugar and intermittent salt diet administered in the course of the experiment

<table>
<thead>
<tr>
<th>Duration in months</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass, kg</td>
<td>40</td>
<td>55</td>
<td>70</td>
<td>85</td>
<td>100</td>
<td>115</td>
<td>130</td>
</tr>
<tr>
<td>Food, kg/animal per day</td>
<td>2.0</td>
<td>3.0</td>
<td>3.5</td>
<td>4.0</td>
<td>4.0</td>
<td>5.0</td>
<td>6.0</td>
</tr>
<tr>
<td>Sucrose, kg/animal per day</td>
<td>0.100</td>
<td>0.166</td>
<td>0.200</td>
<td>0.250</td>
<td>0.300</td>
<td>0.400</td>
<td>0.500</td>
</tr>
<tr>
<td>Sucrose, %</td>
<td>5.0</td>
<td>5.6</td>
<td>5.6</td>
<td>6.3</td>
<td>7.5</td>
<td>8.0</td>
<td>8.3</td>
</tr>
<tr>
<td>Sucrose, g/kg body weight per day</td>
<td>2.5</td>
<td>3.0</td>
<td>2.9</td>
<td>2.9</td>
<td>3.0</td>
<td>3.5</td>
<td>3.8</td>
</tr>
<tr>
<td>Salt, kg/animal per day</td>
<td>0.010</td>
<td>0.016</td>
<td>0.020</td>
<td>0.025</td>
<td>0.030</td>
<td>0.040</td>
<td>0.050</td>
</tr>
<tr>
<td>Salt, %</td>
<td>0.50</td>
<td>0.56</td>
<td>0.56</td>
<td>0.63</td>
<td>0.75</td>
<td>0.80</td>
<td>0.83</td>
</tr>
<tr>
<td>Salt, g/kg body weight per day</td>
<td>0.25</td>
<td>0.29</td>
<td>0.29</td>
<td>0.29</td>
<td>0.30</td>
<td>0.35</td>
<td>0.38</td>
</tr>
</tbody>
</table>

**Fig. 1.** Time scale of sucrose and salt supplementation in the second experiment. The numbers indicate the duration of the experiment in months. □ = Weeks when sucrose was added; □ = weeks when both sucrose and salt were added.
Fig. 2. The results of gel electrophoresis of the aqueous and vitreous humors (lines 1–6) of the eyes studied in parallel with crystallins (line 7) and molecular weight markers (in kD; line 8). 1 = Aqueous after 2-day sugar and salt diet; 1a = corresponding aqueous of the control eye; 2 = vitreous after 2-day diet; 2a = the corresponding vitreous of the control eye; 3 = aqueous after 2-month diet; 3a = corresponding aqueous of the control eye; 4 = vitreous after 2-month diet; 4a = corresponding vitreous of the control eye; 5/6 = aqueous/vitreous humors after a 6-month diet; 5a/6a = corresponding aqueous/vitreous humors of the control eyes.

Results and Discussion

The microscopic examination of the lenses of the animals that were kept under chronic sugar, salt and sugar-salt regimens revealed only slightly fiber swelling and formation of tiny vacuoles in the cortical region of the lens by the end of the experiment. More pronounced histological changes in the lenses were produced when a chronic sugar and intermittent salt diet was applied. This mode of administration might approximately model human sugar-salt consumption habits, as most individuals eat sweet and salty meals alternately or both concomitantly. In the following, the second experiment with continuous sugar and intermittent salt administration will be described and discussed.

The first event indicating cataract development preceding histological changes was crystallin leakage from the lens into the aqueous 2 days after the start of the experiment (fig. 2). The thin lens capsule of adolescent animals and the holes formed in the lens fiber cell membranes [12] facilitate both glucose and salt influx, and crystallin leakage from the lens. The later cessation of crystallin leakage might be associated with the thickening of the lens capsule and with the development of a layer of tightly packed anterior and posterior subcapsular lens fibers in adolescent and adult animals during lens growth (fig. 3c), which can be resistant to oxidative and osmotic damage and also protect the lens cortical fibers from oxidative and osmotic damage caused by surplus sugar and salt [6] as well as prevent protein leakage. Cessation of protein leakage from the lens may also result from decreased enzyme activity (e.g., aldolase reductase) in the lens as the animals grow up [13, 14]. In the vitreous, crystallins were not detected because of the assumed high density in the anterior vitreous of the animals. The electrophoretic similarity of the protein patterns of the aqueous and vitreous humors was due to the similar composition of the aqueous and the liquid vitreous, as vitreous gel collagen and hyaluronic acid were not present in the electrophoretic samples. The absence of protein leakage in control animals throughout the experiment confirms that the leakage must have been initiated by the diet.

The mean glucose values of experimental and control animals (table 2) were not significantly different, indicating that the diet had little effect on the fasting serum glucose level, and that the animals were not diabetic. The mean aqueous and vitreous glucose values, however, differed between control and experimental groups, indicating (1) that the glucose content of the vitreous and aqueous are normally lower than that of the serum, and (2) a tendency to possible accelerated glucose utilization from the aqueous by glycation and/or accumulation in the lens depending on the diet.

It is known that adolescent and adult animals metabolize galactose to dulcitol in galactose cataracts, or glucose to sorbitol, when they are made diabetic, thus causing osmotic stress and oxidative damage to the lens [6, 13]. It
is possible that in our experiment in addition to some sodium accumulation, sorbitol accumulation may have also occurred in the lenses.

Fasting serum levels did not significantly differ, with respect to osmolarity in the vitreous and the aqueous between experimental and control animals, indicating that the osmotic effect of sugar and salt supplements could act on the lens during the daytime while approxi-

mately normal levels were recovered during the night, as the fluids were harvested from the fasting animals in the morning, after sacrifice.

Our histological results of the simultaneous administration of two cataractogenic substances were compared with literature data on multifactorial human cataract and a one-component (e.g. galactose) cataractogenic effect on the lens of other experimental animals [5, 8, 9, 13]. Histological examination of the lenses of the animals subjected to chronic sugar and intermittent salt administration revealed the presence of a layer of irregularly widened superficial equatorial and posterior cortical fibers, and vacuoles of various sizes in the lens cortex by the 17th day of the experiment. By the 30th day of the experiment, in addition to enlarged equatorial subcapsular fibers (fig. 3a), enlarged fiber tips were observed in the posterior suture region of the lens. This observation is in agreement with a previous study showing that the primary site of the osmotic attack of glucose is the posterior pole of the lens and that salt penetrates the lens preferably through anteri-
or and posterior surfaces [14] and over the entire lens surface (our own in vitro lens organ culture data). Enlarged fiber tips were discernible up to the end of the experiment (fig. 3b). Whether the enlargement of fibers and fiber tips might be reversible remains to be studied.

After 2 months, the continuous increase in the layer of tightly packed anterior and posterior subcapsular fibers described above had occurred in the lenses of the experimental and the control animals and persisted up to the end of the experiment (fig. 3c). The layer was less evident in the equatorial region of the lens, possibly also facilitating osmotic damage in this region of the lens. The thickened lens capsule during animal growth and the presence of tightly packed subcapsular fibers in the lenses supposedly together with the decline in the enzymatic activity of the lens [15, 16] may explain well-known difficulties related to cataract formation in experimental models of adult animals.

Protein leakage, presence of vacuoles, widened equatorial fibers and posterior sutural fiber tips in the lenses of the experimental animals are similar to the characteristics described in other cataract models [5, 6, 8, 9], which confirms that sugar and salt represent real risk factors for cataract development. As the chronic administration of both sugar and salt or their mixture caused only slight cataractous changes in the lenses, it can be concluded that it was the simultaneous influence of a chronic sugar and intermittent salt diet that caused the development of evident histological cataractous changes in the lenses, and vice versa, similar results could be obtained with the administration of chronic surplus salt and intermittent surplus sugar. As the changes observed in both experiments occurred in clear lenses, they represent preclinical cataractous changes.

Neither refined sugar nor salt are food components in nature, but they have become food components of our everyday diets. The addition of 5–8.3% of chronic sugar and intermittent addition of 0.5–0.83% salt to porcine meals is a non-natural but small amount in comparison with the continuous 25–50% galactose content in the food of other experimental animals [6, 9], or in comparison with human meals to which up to 20–30% sugar (even up to 99% sucrose in some kinds of sweets) and 1.1–2.3% salt are often added (these data were obtained from local factories producing preserves, bread). Still, modest amounts of salt and sugar added to porcine meals resulted in protein leakage and fiber swelling characteristic of human senile or animal experimental cataract. Therefore, the results of our experiments suggest that nondiabetics may also be prone to cataractous changes caused even by moderate amounts of surplus sugar and salt. This might particularly apply to children's lenses, where the lens capsule is thinner in comparison with the capsules of adults and a protective layer of tightly packed subcapsular lens fibers has not yet developed. Salty and sugar-rich meals should be avoided to prevent preclinical and clinical cataractous changes in the lenses.

**Conclusion**

In the present study the etiological risk factors for human cataract (surplus sucrose and salt) were for the first time confirmed histologically and electrophoretically as risk factors for cataract in nondiabetic experimental animals. Continuous moderate consumption of sugar or salt or both has a weak cataractogenic effect on the lens; simultaneous administration of chronic dietary sugar and intermittent salt may trigger cataract formation.

**Acknowledgement**

This study was supported by grants 4457 and 4345 of the Estonian Science Foundation.

Sugar, Salt and Cataract