Influence of caffeine
used at various temperature ranges
on the concentrations of glucose and total serum protein
as well as body weight gain in pregnant rats

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Abstract

Caffeine (120 mg/kg) was administered intragastrically to pregnant rats daily on gestational days 8-21. An increase in serum concentration of glucose and total protein was found in animals, which were given caffeine. The protein content proved to be highly significant in the experimental group of animals. The control group showed a negative interdependence between body weight gain and glucose concentration. No correlation was found between body weight gain and total protein concentration, yet the glucose concentration significantly influenced the total protein concentration in this group of animals. Among animals which received caffeine, correlations between total protein and glucose concentrations were observed. The analysis did not show that the glucose or total protein concentration significantly influenced the body weight gain of pregnant female rats in the experimental group. The research conducted suggests the possibility of modulating effects of caffeine on adaptive processes during pregnancy.

Key words: rats, pregnancy, caffeine, metabolism.

Introduction

One of the problems of contemporary civilisation is stimulant abuse. Caffeine is one of them; it is the most widely used psychoactive substance. It is a natural alkaloid remarkably widespread in nature, yet its synthetic form is also commonly used. In nature, it is present in the leaves, seeds, and fruit of more than 60 plant species including the coffee and tea plant (18).

It is converted into over 25 metabolites, the majority of which have a biological influence similar to the original substance. Genetic predispositions influence the pace of caffeine biotransformation (17). Caffeine use depends on culture or eating habits but not on age or gender. Daily intake of caffeine on a global scale is estimated at 3–7 mg/kg b.w., which equals approximately 200 mg (18).

Annual consumption of caffeine around the world reaches about 4.5 kg per person (1). Caffeine can be consumed at various temperature ranges, which determine its bioavailability. The most popular sources of caffeine in a diet are coffee (60%–75%) and tea (15%–30%). Among children and teenagers, its main sources are chocolate and fizzy drinks containing caffeine (17). A great variety of products increase its availability for different age groups including children or pregnant women. It is underlined that caffeine intake among children and teenagers ranges from 3 up to 7 mg/kg b.w. It is worth noting that the consumption of caffeine by pregnant women in the USA is 1 mg/kg,
During pregnancy a mother's body undergoes numerous adaptive changes. They are evolutionary adaptations which create and preserve an ideal environment for the developing foetus. Progressive hypoglycaemia, which is observed among physiological changes in pregnancy, is linked to post-prandial hyperglycaemia. There is also a risk of increased insulin-resistance and consequent hyperinsulinaemia (5). Adaptive changes during pregnancy are also manifested in hepatic metabolic activity. The total protein concentration in serum is reduced mainly due to a decrease in albumin concentration by 20%-40%. To some extent this drop is caused by dilution of serum as the result of an increase in total blood volume. The concentration of fibrinogen is significantly raised and an increased concentration of ceruloplasmin, transferrin, and many specific binding proteins such as thyroxin-binding globulin (TBG) and corticosteroid-binding globulin (CBG) is also observed. The physiology of the changes is not fully explained, but it seems that it is dependent on the interaction between oestrogen, progesterone, and placental lactogen (5). Several factors have been identified which influence the homeostasis of total protein and glucose contents in serum. They include liver function, hormonal convergence, an appropriate diet, and the use of medicines or stimulants.

The fact that caffeine is the most commonly consumed psychoactive substance in the world recommended an evaluation of its influence on selected biochemical indices of serum and body weight gain in pregnancy. This study made such an evaluation on pregnant rats.

### Material and Methods

The experiments were conducted on Wistar female rats. Inseminated females were randomly divided into caffeine-exposed and control groups. The day of insemination was considered the first day of their pregnancy. In the experiment, caffeine was used (Caffeine anhydrous powder, Sigma-Aldrich Chemie GmbH, Germany) at the dose of 120 mg/kg b.w. Directly prior to administration of the substance, it was mixed with Tween 80 (Sigma), and then placed into sterile distilled water (2 mL/kg). Control animals were exposed only to Tween 80 water suspension (water-exposed group). The caffeine and water suspensions, at temperatures of 10, 25, and 45°C, were given intragastrically from the 8th to 21st day of pregnancy once a day at the volume of 2 mL/kg b.w. Each caffeine-exposed and control group was divided into three subgroups according to the temperature of the administered solution.

During the experiment the animals were observed at least three times per day. Every three days their body weight was checked. On the 21st day of pregnancy, the rats were decapitated. In blood serum the concentrations of total protein (TP) and glucose (GLU) were determined with the use of mono tests (BioMaxima, Poland). The findings were analysed statistically with the use of the Shapiro-Wilk test, Student's t-test, Mann-Whitney U test, and Spearman's rank correlation. Statistically significant differences were estimated at P ≤ 0.05.

### Results

No clinical symptoms of caffeine toxicity were observed among the examined animals. Since the temperature caused only insignificant parameter changes within each group, all caffeine-exposed and control animals were pooled into two groups (39 caffeine-exposed and 37 water-exposed animals) to increase the statistical power of the analysis. An increase in both examined biochemical parameters were found in the group exposed to caffeine (Table 1).

A significant negative correlation between body weight gain and glucose concentration was found in the control group. Moreover, the glucose concentration positively correlated with total protein content (Table 2).

In the group of caffeine-exposed animals, a positive correlation between the total protein and glucose was established. No significant correlation was found between body weight gain and both biochemical parameters (Table 2).

### Table 1. Body weight and biochemical changes in caffeine-exposed and control animals

<table>
<thead>
<tr>
<th></th>
<th>Water</th>
<th></th>
<th></th>
<th>Caffeine</th>
<th></th>
<th></th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>M (g/kg)</td>
<td>SD (g/kg)</td>
<td>n</td>
<td>M (g/kg)</td>
<td>SD (g/kg)</td>
<td></td>
</tr>
<tr>
<td>Body weight gain (%)</td>
<td>37</td>
<td>7.42 (±2.82)</td>
<td>10.62 (±3.15)</td>
<td>39</td>
<td>11.92 (±3.01)</td>
<td>15.87 (±2.10)</td>
<td>0.1975</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>37</td>
<td>7.24 (±2.38)</td>
<td>3.54 (±1.12)</td>
<td>39</td>
<td>10.12 (±3.06)</td>
<td>3.92 (±0.86)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Total protein (g/dL)</td>
<td>37</td>
<td>16.83 (±4.14)</td>
<td>12.27 (±4.07)</td>
<td>39</td>
<td>29.2 (±9.85)</td>
<td>11.65 (±2.46)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

n - number, SD - standard deviation, M - average
Table 2. Correlation between body weight gain and biochemical changes in caffeine-exposed and control animals, presented as Spearman rank (RS)

<table>
<thead>
<tr>
<th></th>
<th>Water</th>
<th>Caffeine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight gain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>-0.3459</td>
<td>-0.1986</td>
</tr>
<tr>
<td>Total protein</td>
<td>-0.1986</td>
<td>-0.0324</td>
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<tr>
<td>Body weight changes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>0.0324</td>
<td>0.0796</td>
</tr>
<tr>
<td>Total protein</td>
<td>0.6969</td>
<td>-</td>
</tr>
</tbody>
</table>

Discussion

The obtained results show that caffeine significantly increases serum concentrations of glucose and total protein in pregnant female rats. The raised concentration of protein after caffeine administration to rats was also observed by Grucka-Mamczar et al. (11, 12). Birkner et al. (2, 3) consider that an increased level of protein after administration of caffeine is caused by intensified renal filtration and diuresis. In such circumstances, there is increased urea and creatinine excretion by the kidneys. According to Grucka-Mamczar et al. (11, 12) it is proved a higher concentration of protein in serum. The values obtained in our experiment correspond with the results achieved by other authors (3, 4, 15). A raised total protein content after caffeine administration to horses was observed by Delfiœt al. (7).

Constant intake of caffeine impairs glucose tolerance; therefore, increasing its concentration in the blood decreases tissue sensitivity to insulin. It results from an increase in adrenaline concentration and inhibition of glucose uptake by muscles dependent on adenosine receptor (13). Insulin deficiency caused by caffeine results in increased lipolysis, intensified oxidation of fatty acids, induction of gluconeogenesis, and inhibition of glycolysis, which additionally perpetuates and intensifies insulin resistance (6, 21). There are controversies around the opinion that caffeine inhibits the development of type 2 diabetes. In research by Pereira et al. (19), this process is explained by the intensification of energy expenditure by release of fatty acids and glycogen from tissues as well as their oxidation under the influence of caffeine. It is interesting that decaffeinated coffee shows more beneficial influence on glucose metabolism than coffee containing caffeine, which proves that other substances present in coffee can reinforce such influence. Caffeine inhibits phosphodiesterase activity, which breaks up cyclic adenosine monophosphate (cAMP) and results in its accumulation in cells and activation of metabolic processes (11, 12, 14). Grucka-Mamczar et al. (11, 12) confirm that caffeine is a phosphodiesterase inhibitor and after its administration an increase in cAMP and glucose in blood is observed. This inhibition results from the fact that due to an increased concentration of cAMP glucagon stimulates synthesis of glucose and inhibits glycolysis in the liver (idem). According to Fiebich et al. (9) and Kot and Daniel (14), caffeine intensifies the breakdown of glycogen and formation of active glucuronic acid and therefore degrades glycogen and lipids, providing the cells with energy. Adding caffeine results in an inhibition of glycolysis in extrahepatic cells by weakening aldolase activity (11, 12). The influence of caffeine is manifested by the inhibition of transport of glucose into tissue (12). MacKenzie et al. (16) demonstrated a 35% decrease in tissue sensitivity to insulin without affecting the concentrations of glucose, DHEA, androstenedione, or melatonin after caffeine administration. Moreover, Leblac et al. (15) found increased concentrations of glucose, non-estriified fatty acids (NEFA), insulin, adrenocorticotropic hormone (ACTH), and corticosterone in rats treated with caffeine. They observed no influence of caffeine on the concentration of glucagon or reciprocal correlation between the concentration of glucose and NEFA glucagon. The chronic effect of caffeine was also evaluated. Gilbert et al. (10) reported a dose-dependent increase in glucose content and decrease in body weight among pregnant female monkeys (Macaca fascicularis) chronically exposed to methylxanthines (caffeine and theophylline; 0.15 and 0.35 mg/mL).

In the study presented here, no influence of caffeine on the body weight gain of pregnant female rats was observed. According to Bajarowicz and Przygoda (4) caffeine accelerates metabolism, has thermogenic properties, boosts fat burning, and increases the release of stress hormone – cortisol, which has catabolic properties. Conducted research suggests some modulating effects of caffeine on adaptive processes during pregnancy. Caffeine in pregnant females does not change tissue sensitivity to insulin, yet it significantly increases serum concentration of glucose, providing convenient metabolic conditions for the development of carbohydrate intolerance. This process is probably reinforced by the hormonal system activated by caffeine. The influence of caffeine on the concentration...
of total protein can be manifested in disorders of fluid distribution between intravascular and extravascular spaces in pregnant individuals. Its influence on the buffering system seems important as it is responsible for maintaining acid-alkaline balance.

References