The influence of intragastric administration of habanero fruits (*Capsicum chinense* Jacq.) on total thyroxin levels in rats

Wpływ dożołądkowego podawania owoców papryki habanero (*Capsicum chinense* Jacq.) na poziomy tyroksyny całkowitej u szczurów.

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Key words

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Słowa kluczowe

kapsaicyna, tarczycza, szczur, habanero

Abstract

Obesity is the problem in both humans and animals, induced by long-lasting positive energetic balance. Pepper fruits such as chilli habanero (*Capsicum chinense* Jacq.) are popularly used as a hot spice and ingredient of dietary supplements. Capsaicin directly influences adipocytes *in vitro* and blocks the storage of fat tissue. Thyroid hormones are involved in obesity metabolism as an important stimulator of the mitochondrial respiratory chain. **The aim.** The aim of the study was to assess total thyroxin concentration in rat serum after intragastric administration of ground dry habanero pepper fruit. **Materials and methods.** : the substance was administered to rats in the following daily doses: a high dose – 0.08g dm habanero fruit/kg b.w. (E1 group) and a low dose – 0.025g dm habanero fruit/kg b.w. (E2 group) divided into 2 equal parts and administered every 12 hours. Control animals (C group) received approximately 0.5 ml of pure peanut oil. Each group of animals consisted of 12 individuals. Total T4 concentration in serum was measured with ELISA (GenWay Biotech Inc., San Diego). The body weight of each animal was measured with analytical balance. **Results.** There was a statistically significant decrease in the total T4 concentration in day 8 in group E1. There was a negative correlation between the result obtained in day 8 of the experiment and the concentration of the habanero doses.

Streszczenie

Otyłość, zarówno u ludzi jak i u zwierząt, stanowi problem spowodowany przewlekłym dodatnim bilansem energetycznym. Owoce papryki, w tym habanero (*Capsicum chinense* Jacq.), są powszechnie używane jako ostra przyprawa i składnik suplementów diety. Zawarta w nich kapsaicyna bezpośrednio wpływa na adipocyty *in vitro* i wstrzymuje odkładanie tkanki tłuszczowej. Hormony tarczycy biorą zaś udział w metabolizmie otyłości jako istotny stimulator mitochondrialnego łańcucha oddechowego. **Celem pracy** była ocena stężenia tyroksyny całkowitej w surowicy szczurów po dożołądkowym podawaniu suszonych owoców papryki habanero. **Materiał i metody.** Szczurom podawano następujące dawki dzienne: dawka wysoka – 0,08g suchej masy owoców habanero / kg masy ciała (grupa E1) i dawka niska – 0,025 g suchej masy owoców habanero / kg masy ciała (grupa E2) zawieszane w oleju arachidowym, podzielone na 2 równe części podawane co 12 godzin. Zwierzęta kontrolne otrzymywały około 0,5 ml czystego oleju arachidowego. Każda grupa zwierząt składała się z 12 osobników. Całkowite stężenie T4 w surowicy zmierzono za pomocą ELISA (Genway Biotech Inc., San Diego). Masę ciała zwierząt mierzono za pomocą wagi laboratoryjnej. **Wyniki.** Stwierdzono istotne statystycznie obniżenie całkowitego stężenia T4 w 8 dniu w grupie E1 oraz ujemną korelację pomiędzy wynikiem uzyskanym w 8 dniu

Findings. The obtained results provide some support for the new function of capsaicinoids in weight reduction treatment based on modulating the function of the thyroid gland. They also suggest that we should be cautious in interpreting the results of routine examinations in diagnosing thyroid diseases in patients consuming large amounts of capsaicinoids.

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Introduction

One of the common beliefs concerning the causes of obesity in both humans and animals is that is induced by long-lasting positive energetic balance [1,2]. One of the ways to counteract obesity is therefore to fasten metabolism, which is, in practice, much easier than introducing radical changes in diet or eating habits [3]. The stimulation of energy expenditure aimed at reducing body mass can be performed using substances naturally present in food, which include capsaicinoids such as capsaicin [4,5]. They can be found in pepper fruits such as chilli habanero (*Capsicum chinense* Jacq.), which are popularly used as a hot spice and which constitute an ingredient of dietary supplements [5,6]. Europeans and North Americans consume on average 0.77mg of capsaicin per person (maximum of 2.64 mg daily). However, in countries with culturally higher consumption of hot spices (Thailand, Mexico, India), the daily intake of capsaicinoids is 0.5-4mg/kg bw, which calculates to 25-200mg/person/day, assuming that the average body weight is 50kg [7]. The recommended limit of capsaicin in ready made meals is 5mg/kg [8].

Rodents on a diet containing a 0,014% addition of capsaicin were not shown to increase the intake of calories. However, they were reported to have reduced visceral fat by 30% and to have increased bowel blood flow, while blood flow in subcutaneous fat was found to have decreased. Capsaicin directly influences adipocytes in vitro and delays storing fat tissue [9]. It not only stimulates the metabolism of lipids, but also the secretion of catecholamines, which results in an increase in thermogenesis and use-up of energy for temperature regulation [10-13]. Apart from catecholamines, thyroid hormones are an important stimulator of the mitochondrial respiratory chain. In hypothyreosis they cause the weakening of the respiratory chain and an increase in body mass de-

doświadczenia a dawką papryki habanero. **Wnioski.** Uzyskane wyniki sugerują nową rolę kapsaicynoidów w leczeniu otyłości, polegającą na modulacji funkcji gruczołu tarczowego. Sugerują również, że powinniśmy zachować ostrożność w interpretacji wyników rutynowych badań w diagnostyce chorób tarczycy u pacjentów spożywających duże ilości kapsaicynoidów.

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spite no changes in appetite [14,15]. The functional changes in the hypothalamic-pituitary-thyroid axis caused by diet ingredients containing capsaicinoids can be of interest in both the profilaxis and treatment of obesity.

The aim of the study was to assess total thyroxin (total T4) concentration in rat serum after intragastric administration of ground dry habanero pepper fruit.

Methods

Animals. Experiments were conducted with due approval of the procedures by the Local Animal Ethics Committee with regard to the care and use of animals. The study was conducted on 36 male albino Wistar rats, originating from breeding laboratory animals. They were maintained in an air-conditioned room at a relative humidity of 45-47% and a temperature of 22-23°C, with a light cycle of at 12h light/12h darkness. They were fed commercial feed for laboratory animals (LSM, Agropol Motycz, Poland) and were given tap water to drink ad libitum. The acclimation period was 16 days before the experiment. The animals were divided into 2 experimental groups (E1 and E2) and control group (C). Each consisted of 12 individuals.

Test Substance. The test substance was ground dry habanero fruit (dm) with a specific HPLC capsaicinoid (capsaicin and dihydrocapsaicin) content of 7.64mg/g dm. It was suspended in peanut oil and administered in the following daily doses: a high dose – 0.08g dm habanero fruit/kg bw (E1 group) and a low dose – 0.025g dm habanero fruit/kg bw (E2 group). After immobilizing the animal by gripping the nape of the neck, the suspension was applied using an atraumatic special plastic feeding tube for rodents (Instech Laboratories, Inc., Plymouth Meeting, PA USA). These daily doses were divided into 2 equal parts and administered every 12

hours. The concentrations of the suspensions were prepared so that each animal received approximately 0.5ml. Control animals (C group) received approximately 0.5 ml of pure peanut oil using the same method of administration.

Blood Sampling for Testing. On the 8th and 29th day of the experiment, after a 12h fast, six rats from each of the three groups (E1, E2, C) were anesthetized using ketamine (Vetaketam; Vet-Agro, Poland) in a dose of 80 mg/kg bw intramuscularly. Under general anesthesia, blood was drawn by puncturing the right ventricle using a 0.8mm needle. Blood samples were put into tubes in order to obtain serum. Serum was obtained by centrifuging at 4°C for 30 minutes at 4000rpm.

Hormone analysis. Total T4 concentration in serum was measured with ELISA (GenWay Biotech Inc., San Diego). Prior to the measurement, the serum was kept at -70°C.

Body weight measurement. On the 8th and 29th day of the experiment before anesthesia the body weight of each animal was measured with analytical balance.

Statistical analysis. The mean and standard deviation (SD) were calculated. Statistical analysis was conducted by the Mann-Whitney U test at p-values of p≤0.05 (Statistica 10.0 software). For each parameter, statistically significant differences were calculated between control and experimental groups in day 8 and 29.

Results

The results of the assessment of total T4 concentration are presented in Table 1. In day 8 of the experiment a statistically significant decrease in the total T4 concentration was observed in the animals in group E1. A smaller decrease in the concentration of total T4 was also reported in group E2, but it was not statistically significant. There was a negative correlation between the result obtained in day 8 of the experiment and the concentration of the habanero doses. In day 29 of the experiment a rise in thyroxin levels proportionate to the administered doses was reported in both groups compared to the control group, but the observed differences were not statistically significant.

Table 2 presents the results of body weight measurements. In day 8 of the experiment a statistically significant decrease in body weight was observed in group E1 compared to group C. A similar, but less significant decrease in body weight

Table 1. The results of the assessment of total T4 level (mmol/L) *Statistically significant differences compared to the C group at p≤0.05

Day	Group			
		C	E1	E2
8	x	89.23	69.49*	86.66
	SD	2.65	10.54	3.52
29	x	72.08	80.66	77.22
	SD	14	8.09	6.09

Table 2. The results of body weight measurements *Statistically significant differences compared to group C at p≤0.05

Day	Group			
		C	E1	E2
8	x	286.67	251.33*	276.67
	SD	5.16	7.23	10.59
29	x	338.00	323.67	331.00
	SD	24.85	20.98	70.99

was also noted in group E2. The results obtained in groups E1 and E2 were reversely proportional to the administered amount of the test substance. In day 29 there no statistically significant differences in body weight were present between the experimental groups and the control group, but the differences were reversely proportional to the habanero dose, similarly to day 8 of the experiment.

Discussion

The problem of obesity in both humans and in animals has grown to an epidemic around the world [1,16,17]. Naturally, the need to find new methods of treatment has appeared. Among popular methods, fastening metabolism combined with reducing the intake of food has been found to be most effective. Dietary supplements with capsaicinoids have been increasingly used to this purpose [5]. A well described case of a man who suffered from arterial hypertensive crisis, acute heart failure and increased levels of thyroid stimulating

hormone (TSH) after consuming excessive quantities of peppers and chilli peppers should trigger a more careful examination of the impact which foods containing capsaicinoids exert on the functioning of the thyroid gland [18]. The available publications do not concentrate on such relationships, and capsaicinoids are only discussed with respect to treating cancer [6,19–21]. The excitation of the peripheral nervous system caused by capsaicinoids may lead to an increase in thyrotropin (TRH) secretion as a result of the activation of α -adrenergic receptors in the paraventricular nucleus (PVN). This, in turn, leads to an increase in the synthesis and secretion of TSH responsible for stimulating the release of total T4 by the thyroid gland [22]. It is difficult to explain the dose-dependent lowering of the total T4 level in the examined animals in day 8 of the experiment. It can be connected with smaller levels of thyroglobulin in the thyroid gland as a result of earlier increased stimulation of the gland. It is probable because total T4 levels in the experimental groups lowered inversely proportionately to the habanero pepper dose used. The obtained results show that in the following days the hormone reserves in the thyroid gland could have levelled out, which would

ensure the levels of thyroxine secretion observed in day 29 of the experiment. Despite the fact that the results obtained at that time in groups E1 and E2 do not differ statistically significantly from the results in group C, it can be speculated that habanero peppers administered intragastrically can stimulate total T4 release proportionate to the dose used. Body weight analysis, and particularly the negative correlation between body weight and T4 levels observed in day 29 of the experiment, corroborates these findings.

Findings

The obtained results provide some support for the new function of capsaicinoids in weight reduction treatment conducted with dietary supplements, which is based on modulating the function of the thyroid gland. They also suggest that we should be cautious in interpreting the results of routine examinations in diagnosing thyroid diseases in patients consuming large amounts of capsaicinoids. The results need a further confirmation based on longitudinal studies on larger samples of the population.

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