Effect of sweet flavor carrier on T3/rT3 ratio, plasma and liver total cholesterol level in rats

Wpływ rodzaju nośnika smaku słodkiego na proporcję T3/rT3, stężenie cholesterolu całkowitego i jego poziom w wątrobie u szczurów

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Wprowadzenie. Wpływ substancji intensywnie słodzących na zdrowie budzi wiele kontrowersji wśród specjalistów zdrowia publicznego, a ich wpływ na metabolizm hormonów tarczycy – głównego regulatora przemian metabolicznych, w tym metabolizmu lipidów, nie był dotąd badany.

Cel. Określenie wpływu rodzaju nośnika smaku słodkiego na proporcję T3/rT3, stężenie cholesterolu całkowitego w osoczu oraz jego zawartość w wątrobie u szczurów na czczo i po spożyciu posiłku.

Materiały i metody. Doświadczenie wykonano na 56 ośmiotygodniowych samcach szczurów rasy Spraque-Dawley (masa początkowa 325±19 g), które podzielono na 4 grupy. Przez 3 tygodnie zwierzęta otrzymywały izoenergetyczne diety, z których jedna (NS) nie zawierała nośnika smaku słodkiego, a 3 charakteryzowały się identycznym natężeniem smaku słodkiego równym 10% zawartości sacharozy w diecie. Nośnikami smaku słodkiego były: sacharoza (SC) oraz substancje intensywnie słodzące: aspartam (AS) i sukraloza (SU).

Wyniki. Rodzaj nośnika smaku słodkiego miał istotny wpływ na proporcję T3/rT3, stężenie cholesterolu całkowitego i jego zawartość w wątrobie u szczurów. Wartość T3/rT3 na czczo była wyższa w grupie SC niż w NS, AS i SU, w których proporcja T3/rT3 nie różniła się istotnie. Po posiłku T3/rT3 w grupach AS i SU był istotnie niższy niż w dwóch pozostałych grupach. Najwyższe osoczowe stężenie oraz zawartość cholesterolu całkowitego w wątrobie na czczo wykazano w SU i choć jedynie w tej grupie poziom cholesterolu w wątrobie uległ obniżeniu po posiłku, nadal pozostał wyższy niż w NS i SC.

Wnioski. Wydajność obwodowego metabolizmu hormonów tarczycy wzrasta pod wpływem sacharozy, natomiast wpływ diet z aspartamem i sukralozą na metabolizm hormonów tarczycy nie różni się istotnie od efektu wywieranego przez dietę niesłodką. Spożywanie diety z aspartamem i sukralozą sprzyja gromadzeniu cholesterolu w wątrobie.

Słowa kluczowe: *hormony tarczycy, całkowite stężenie cholesterolu, sacharoza, sztuczne substancje słodzące*

Introduction. The impact of artificial sweeteners on health is still a matter of controversy among healthcare professionals, and their effect on the thyroid activity – a major regulator of metabolism, including lipid metabolism – has not been previously studied.

Aim. To determine the effect of sweet type flavor carrier on the T3/rT3 ratio, plasma and hepatic total cholesterol levels in rats at fasting and postprandial state.

Material & Method. The experiment was performed on 56 male 8-week old Sprague-Dawley rats (initial body weight 325±19 g) divided into 4 groups. During a 3-week period, rats received isoenergetic diets, one of which was non-sweet (NS) and three with the same sweet flavor intensity corresponding to 10% of sucrose. The sweet flavor carriers were as follows: sucrose (SC) and high-intensity sweeteners: aspartame (AS) and sucralose (SU).

Results. The obtained results indicate that the type of the sweet flavor carrier affects the T3/rT3 ratio, concentration of total cholesterol and its level in the rat liver. The T3/rT3 at fast in SC was higher than in NS, AS, and SU, while there were no significant differences between them. After the meal, T3/rT3 ratio in AS and SU were lower than in other groups. The highest plasma concentration and hepatic content of total cholesterol were noted in SU, and despite the fact that only in this group the hepatic cholesterol level decreased postprandially, it still remained higher than in NS and SC.

Conclusion. Sucrose stimulates the peripheral thyroid hormone activation, while the impact of diet with the high-intensity sweeteners and a non-sweet diet on thyroid hormone metabolism did not differ significantly. The chronic use of diet with aspartame and sucralose might promote hepatic cholesterol accumulation.

Key words: *thyroid hormones, total cholesterol level, sucrose, artificial sweeteners*

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Introduction

Thyroid hormones (TH) are regarded as key metabolic regulators in most tissues [1]. They have profound effects on energy and lipid homeostasis [2] including cholesterol synthesis, uptake, metabolism and accumulation in the liver [3]. Therefore, any disturbances in thyroid axis activity have an adverse effect on the serum lipid profile that might predispose

to cardiovascular disease development [4] – the leading cause of death among the Polish population [5].

Tetraiodothyronine (T4) is the main hormone secreted by thyroid gland after the pituitary thyrotropin (TSH) stimulation [6]. Peripheral T4 5'-deiodination leads to metabolically active triiodothyronine (T3) and 5-deiodination to inactive reverse triiodothyronine (rT3). Deiodination is catalyzed by Se-dependent iodothyronine deiodinases: 5' deiodination by deiodinases type 1 and type 2, 5' deiodination by deiodinases type 1 and 3 [7]. Thus, plasma T3/rT3 ratio is assumed to reflect the intensity of 5' and 5 deiodination, that is hormone activation to inactivation ratio [8].

It is well established that both quality and diet composition affect the TH synthesis and peripheral metabolism [9-11]. The amount and type of carbohydrates consumed are important modulators of circulating TH levels [12-15]. Sucrose-rich diets were shown to stimulate T4 to T3 conversion [13] and to elevate plasma T3 [13, 15], while carbohydrate-restricted feed diminished T3 concentration [14]. Diet-related alterations in thyroid hormone plasma profile could affect the plasma cholesterol concentration and its accumulation in the liver.

Artificial sweeteners have gained worldwide attention as dietary tools in the obesity and comorbidities management [16]. Sucralose and aspartame are the most popular sweeteners, respectively 600 and 200 times sweeter than sucrose, providing none or very few calories [17]. However, there are doubts whether the caloric sweetener use is truly beneficial. The evidence of association between the artificial sweeteners consumption and health effects are still a matter of controversy [18, 19]. It is hypothesized that a gap between sweetness and the expected energy dose might promote hormonal and metabolic disturbances [18].

Despite the worldwide growing prevalence of artificial sweeteners use their impact on thyroid activity has not been investigated.

Aim

To determine the effect of sweet type flavor carrier on the T3/rT3 ratio, plasma and hepatic total cholesterol levels in rats at fasting and postprandial state.

Material and method

Experimental procedure

The experiment was conducted on 56 8-week old male Sprague-Dawley rats (initial body weight 325±19 g) randomly divided into 4 groups (n=14 for each) fed semisynthetic (based on AIN-93M) isocaloric diets (3.76±0.5 kcal/g) *ad libitum* for 3 weeks: three with the same sweet flavor intensity responded to 10 g of sucrose (with sucrose – SC, AS – aspartame, SU – sucralose) and one non-sweet diet (NS). The diet composition is presented in Table I.

Table I. Composition of experimental diets (g/100 g)
Tabela I. Skład diet (g/100 g)

Ingrediences /Składniki	NS	SC	AS	SU
Wheat starch /Skrobia pszenna	62.94	52.94	62.89	62.93
Sucrose /Sacharoza	0	10	0	0
Artificial sweeteners /Sztuczne substancje intensywnie słodzące	0	0	0.05	0.0167
Casein /Kazeina	20.0	20.0	20.0	20.0
Soybean oil /Olej sojowy	7.0	7.0	7.0	7.0
Potato starch /Skrobia ziemniaczana	5.0	5.0	5.0	5.0
Mineral mixture /Mieszanka mineralna	3.5	3.5	3.5	3.5
Vitamin mixture /Mieszanka witaminowa	1.0	1.0	1.0	1.0

Food intake was monitored daily and body weight – 3 times/week as it was previously shown [7]. After three weeks the animals were euthanized: after a 16-hour fast and 180 min after a meal (7 rats from each group/time point).

Biochemical analysis

Plasma T3 and rT3 were measured by radioimmunoassays (RIA) according to the manufacturer's instructions (ZenTech SA, Belgium). The plasma total cholesterol concentration and hepatic cholesterol level were assessed by the enzymatic-colorimetric methods using commercial kits (PTH Hydrex, Poland). The content of protein in liver homogenates was determined by the Bradford method (1976).

Statistical Analysis

The results are expressed as mean±SEM. The data were analyzed by two-way ANOVA test followed by post-hoc Fisher's LSD tests. All statistical analyses were performed using the Statistica v10 software package (StatSoft, USA). The statistical significance was defined as $p=0.05$.

The other results of this study were published [20] and presented for publication (Pałkowska, et al., submitted).

Results

Plasma T3/rT3 ratio

The T3/rT3 ratio was affected by diet ($p<0.001$). At fast SC revealed the highest value ($p<0.001$), while there were no significant differences between other

groups. The meal intake in any group had a significant effect on the T3/rT3 ratio. Post-meal values in NS and SC were higher than in AS ($p<0.05$ and $p<0.001$ respectively) and SU ($p<0.001$ for both) (Fig. 1).

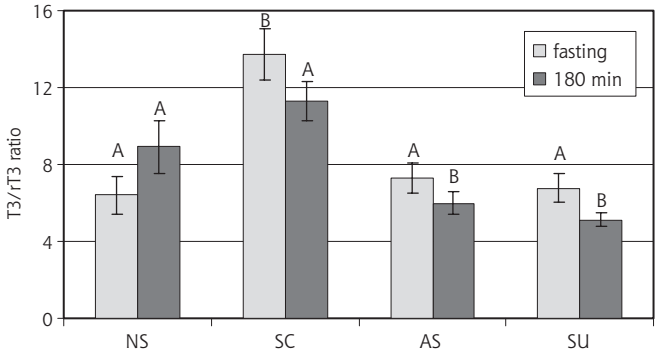

Fig. 1. Plasma T3/rT3 ratio at fast and 180 min after a meal (mean±SEM); A, B – letters indicate significant differences between dietary groups ($p<0.05$); NS – non-sweet diet, SC – diet with sucrose, AS – diet with aspartame, SU – diet with sucralose

Ryc. 1. Proporcja stężeń T3/rT3 w osoczu na czczo i po 180 min. po posiłku (średnia±SEM); A, B – różnice między grupami żywieniowymi ($p<0,05$); NS – dieta bez nośnika smaku słodkiego, SC – dieta z dodatkiem sacharozy, AS – dieta z dodatkiem aspartamu; SU – dieta z dodatkiem sukralozy

Plasma total cholesterol concentration

Total cholesterol concentration was influenced by diet type ($p<0.001$) and at fasting the highest value was noted in SU ($p<0.001$). There were no differences between NS, SC and AS. Food intake did not affect the plasma cholesterol level and its value was comparable among groups (Fig. 2).

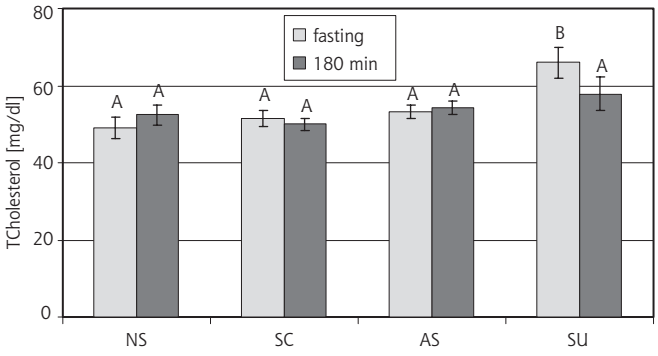

Fig. 2. Plasma total cholesterol concentration at fast and 180 min after a meal (mean±SEM); A, B – letters indicate significant differences between dietary groups ($p<0.05$); NS – non-sweet diet, SC – diet with sucrose, AS – diet with aspartame, SU – diet with sucralose

Ryc. 2. Całkowite stężenie cholesterolu w osoczu na czczo i po 180 min. po posiłku (średnia±SEM); A, B – różnice między grupami żywieniowymi ($p<0,05$); NS – dieta bez nośnika smaku słodkiego, SC – dieta z dodatkiem sacharozy, AS – dieta z dodatkiem aspartamu; SU – dieta z dodatkiem sukralozy

Liver total cholesterol level

The hepatic cholesterol content was affected by the diet type ($p<0.0001$). At baseline, the highest hepatic cholesterol level was found in SU ($p<0.05$) and only in this group postprandial changes were observed. Although it decreased, it still remained higher than in NS and SC (for both $p<0.01$), but not AS. (Fig. 3).

Moreover, only in SC the T3/rT3 ratio was negatively related to the plasma total cholesterol concentration ($r=-0.51$, $p<0.05$).

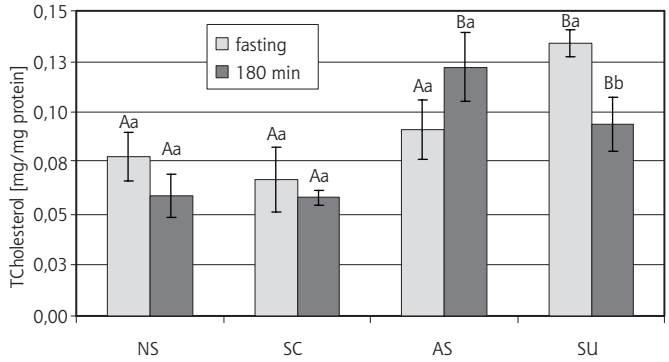

Fig. 3. Liver total cholesterol content level at fast and 180 min after a meal (mean±SEM); A, B – letters indicate significant differences between dietary groups ($p<0.05$); a, b – letters indicate significant differences between time points ($p<0.05$); NS – non-sweet diet, SC – diet with sucrose, AS – diet with aspartame, SU – diet with sucralose

Ryc. 3. Zawartość cholesterolu w wątrobie na czczo i po 180 min. po posiłku (średnia±SEM); A, B – różnice między grupami żywieniowymi ($p<0,05$); a, b – różnice między monitorowanymi punktami czasowymi w obrębie grupy ($p<0,05$); NS – dieta bez nośnika smaku słodkiego, SC – dieta z dodatkiem sacharozy, AS – dieta z dodatkiem aspartamu; SU – dieta z dodatkiem sukralozy

Discussion

The flexibility of thyroid axis activity in response to modification in the diet quantity and quality play the key role in metabolic adaptation to ingested food [1]. Normal plasma TH levels are important for regulation of crucial steps of cholesterol synthesis, metabolism and its hepatic accumulation [21]. Although carbohydrates have been shown to affect the circulating TH level [12, 15], the influence of high-intense sweeteners as sucrose substitutes on the thyroid profile and thus the total cholesterol level remained as yet unknown.

Therefore, in the current study, we focused on the effect of diets with added sucrose, aspartame, and sucralose on the T3/rT3 ratio as a marker of extrathyroidal TH metabolism to explore it potential association with the plasma total cholesterol level and hepatic cholesterol content in rats.

We noted that all monitored parameters were affected by the diet type. The diet containing sucrose was shown to potentiate peripheral T4 to T3 conversion, while the non-sweet diet and those with artificial sweeteners exerted comparable effects. This allowed us to suspect that the effect was solely dependent on the sucrose presence. This observation confirmed the previously established stimulating effect of sucrose on peripheral T4 to T3 metabolism [13].

The fasting total cholesterol concentration in SU was significantly higher than in other groups, although the average daily sucralose intake did not exceed the acceptable daily intake (ADI, 95% of its value). These results differ from the data provided by Shastry et al. [22] which showed that both sucralose and aspartame at ADI dose did not cause changes in the rat plasma total cholesterol concentration as compared to the controls receiving water. There were also no significant differences in the fasting total and HDL-cholesterol concentration between the noninsulin-dependent diabetes mellitus individuals for 6 weeks receiving the diet with sucrose (9% of daily energy intake) and those on a diet with an equivalent amount of aspartame [23]. In another study, a 3-month diet with added aspartame (36 mg/day) was not able to affect the LDL-, HDL-cholesterol and triglyceride levels in prediabetic patients [24]. Furthermore, the fasting cholesterol concentrations of healthy non-obese subjects after the 10-week exposure to a high-sucrose diet (23% of total energy intake) did not differ in comparison with the group on a diet of the sweetness matched with the artificial sweeteners mixture (54% aspartame, 22% acesulfame-K, 23% cyclamate and 1% saccharin – each below ADI) [25].

Significantly higher values of total, LDL- and HDL-cholesterol were observed in groups in which sucralose and aspartame were administered in a double dose of ADI [22]. Similar observations were made by Saad et al. [26] who reported significantly higher levels of total and LDL-cholesterol levels in both diabetic and non-diabetic rats treated with sucralose at a dose two times higher than ADI.

In the presented experimental schedule, the meal did not affect the plasma total cholesterol level in any group. We were not able to find data regarding the aspartame and sucralose influence on the postprandial cholesterol level. However, according to Vega-Lopez et al. [27] the type of ingested carbohydrate (white bread with 50 g of available carbohydrate vs 50 g of glucose) had no significant effect on the postprandial total, LDL- and HDL-cholesterol concentrations over the 5-hour time period. Langsted, et al. [28] however reported that after common food consumption the total, LDL-, HDL-cholesterol, and albumin levels were reduced down to 3 to 5 hours as compared with the fasting levels. A negative correlation between the T3/rT3 ratio and plasma cholesterol level in our study is in accordance with the inverse association of plasma T3 level with the total, HDL- and LDL-cholesterol concentrations reported by others [3, 29]. This strong relation in the SC group confirms the relationship between efficient TH peripheral activation and the total cholesterol concentration.

Hepatic cholesterol content in SU at fast and postprandially in both groups on a diet with intense-sweetener was higher as compared with those receiving non-sweet and sucrose-added diets. Although Abhilash et al. [30] reported that the long-term aspartame consumption resulted in leukocyte infiltration and altered antioxidant status which may cause liver damage manifested by impaired plasma lipid profile, the aspartame dose (1000 mg/kg b.wt.) applied in this study was much higher than in our examinations. According to Kahnamoei and Ranjbar [31], who based their conclusion on the liver enzymes activity, there was no adverse effect of sucralose treatment (15 mg/kg b.wt./day, for one month) on liver function in rats. However, as it was reported earlier, sucralose administration alters the rat serum lipid profile which could be related to changes in the liver cholesterol metabolism [26]. As toxicological issues regarding mechanisms by which chronic exposure to high-intense sweeteners might interfere with metabolic parameters in the liver are yet uninvestigated, we could not explain the hepatic cholesterol increase in the group fed aspartame- and sucralose-added diets. It might be only assumed that lower hepatic cholesterol level in SC than AS and SU could be the effect of an increased conversion of cholesterol into bile acid, as the key enzyme in this process-7-hydroxylase (CYP7A1) is under the TH control [32].

Conclusion

Sucrose stimulates the peripheral TH metabolism, while the impact of diet with artificial sweeteners and without a sweet flavor carrier on the thyroid hormone metabolism did not differ significantly. Sucrose stimulates, while the non-sweet diet and the diet with added aspartame and sucralose diminish the peripheral thyroid hormone activation. The use of artificial sweeteners might promote hepatic cholesterol accumulation. This study shows that artificial sweeteners are metabolically active and advocates caution that their overuse may exacerbate rather than prevent metabolic disorders.

Piśmiennictwo / References

1. López M, Alvarez CV, Nogueiras R, Diéguez C. Energy balance regulation by thyroid hormones at the central level. Trends Mol Med 2013, 19(7): 418-427.
2. Mullur R, Liu YY, Brent GA. Thyroid hormone regulation of metabolism. Physiol Rev 2014, 94(2): 355-382.
3. Sinha RA, Singh BK, Yen PM. Thyroid hormone regulation of hepatic lipid and carbohydrate metabolism. Trends Endocrinol Metab 2014, 25(10): 538-545.
4. Rizos CV, Elisaf MS, Liberopoulos EN. Effects of Thyroid Dysfunction on Lipid Profile. Open Cardiovasc Med J 2011, 5: 76-84.
5. Poznańska A, Wojtyniak B, Seroka W. Najważniejsze przyczyny zgonów Polaków w 2030 roku. Prz Epidemiol 2011, 65(3): 483-489.
6. Bianco AC, Kim BW. Deiodinases: implications of the local control of thyroid hormone action J Clin Invest 2006, 116(10): 2571-2579.
7. St Germain DL, Galton VA, Hernandez A. Minireview: Defining the roles of the iodothyronine deiodinases: current concepts and challenges. Endocrinology 2009, 150(3): 1097-1107.
8. Ruhla S, Arafat AM, Weickert MO, et al. T3/rT3-ratio is associated with insulin resistance independent of TSH. Horm Metab Res 2011, 43(2): 130-134.
9. Flier JS, Harris M, Hollenberg AN. Leptin, nutrition, and the thyroid: the why, the wherefore, and the wiring. J Clin Invest 2000, 105(7): 859-861.
10. Kopp W. Nutrition, evolution and thyroid hormone levels – a link to iodine deficiency disorders? Med Hypotheses 2004, 62(6): 871-875.
11. Lachowicz K, Koszela-Piotrowska I, Rosołowska-Huszcz D. Thyroid hormone metabolism may depend on dietary fat. J Anim Feed Sci 2008, 17(1): 110-119.
12. Smith SM, Lukaski HC. Type of dietary carbohydrate affects thyroid hormone deiodination in iron-deficient rats. J Nutr 1992, 122(5): 1174-1181.
13. Shafrir E. Overnutrition in spiny mice (Acomys cahirinus): beta-cell expansion leading to rupture and overt diabetes on fat-rich diet and protective energy-wasting elevation in thyroid hormone on sucrose-rich diet. Diabetes Metab Res Rev 2000, 16(2): 94-105.
14. Bisschop PH, Sauerwein HP, Endert E, Romijn JA. Isocaloric carbohydrate deprivation induces protein catabolism despite a low T3-syndrome in healthy men. Clin Endocrinol (Oxf) 2001, 54(1): 75-80.
15. Garg M, Mehra P, Bansal DD. Hormonal imbalance and disturbances in carbohydrate metabolism associated with chronic feeding of high sucrose low magnesium diet in weanling male wistar rats. Mol Cell Biochem 2014, 389(1-2): 35-41.
16. Bellisle F, Drewnowski A. Intense sweeteners, energy intake and the control of body weight. Eur J Clin Nutr 2007, 61(6): 691-700.
17. Mortensen A. Sweeteners permitted in the European Union: safety aspects. Scand J Food Nutr 2006, 50(3): 104-116.
18. Pereira MA, Odegaard AO. Artificially sweetened beverages – do they influence cardiometabolic risk? Curr Atheroscler Rep 2013, 15(12): 375.
19. Tandel KR. Sugar substitutes: Health controversy over perceived benefits. J Pharmacol Pharmacother 2011, 2(4): 236-243.
20. Bigos A, Pałkowska E, Rosołowska-Huszcz D. Effect of artificial and natural sweeteners on glucose and insulin in plasma of rats. J Pre-Clin Clin Res 2012, 6(2): 93-97.
21. Malik R, Hodgson H. The relationship between the thyroid gland and the liver. Q J Med 2002, 95(9): 559-569.
22. Shastry CS, Yatheesh CK, Aswathanarayana BJ. Comparative evaluation of diabetogenic and mutagenic potential of artificial sweeteners- aspartame, acesulfame-K and sucralose. NUJHS 2012, 2(3): 80-84.
23. Colagiuri S, Miller JJ, Edwards RA. Metabolic effects of adding sucrose and aspartame to the diet of subjects with noninsulin-dependent diabetes mellitus. Am J Clin Nutr 1989, 50(3): 474-478.
24. Koyuncu BU, Balci MK. Metabolic Effects of Dissolved Aspartame in the Mouth before Meals in Prediabetic Patients; a Randomized Controlled Cross-Over Study. J Endocrinol Diabetes Obes 2014, 2(2): 1032.
25. Raben A, Møller BK, Flint A, et al. Increased postprandial glycaemia, insulinemia, and lipidemia after 10 weeks' sucrose-rich diet compared to an artificially sweetened diet: a randomised controlled trial. Food Nutr Res 2011: 55.
26. Saada HN, Mekky NH, Eldawy HA, Abdelaal AF. Biological effect of sucralose in diabetic rats. Food Nutr Sci 2013, 4(7A): 82-89.
27. Vega-López S, Ausman LM, Matthan NR, Lichtenstein AH. Postprandial lipid responses to standard carbohydrates used to determine glycaemic index values. Br J Nutr 2013, 110(10): 1782-1788.
28. Langsted A, Freiberg JJ, Nordestgaard BG. Fasting and nonfasting lipid levels: influence of normal food intake on lipids, lipoproteins, apolipoproteins, and cardiovascular risk prediction. Circulation 2008, 118(20): 2047-2056.
29. Gullberg H, Rudling M, Saltó C, et al. Requirement for thyroid hormone receptor beta in T3 regulation of cholesterol metabolism in mice. Mol Endocrinol 2002, 16(8): 1767-1777.
30. Abhilash M, Paul MV, Varghese MV, Nair RH. Effect of long term intake of aspartame on antioxidant defense status in liver. Food Chem Toxicol 2011, 49(6): 1203-1207.
31. Kahnamoei JR, Ranjbar A. Comparative study of the effect of sucralose and sugar on some serum biomarkers of rats. Ind J Fund Appl Life Sci 2014, 4(1): 10-15.
32. Lammel Lindemann JA, Angajala A, Engler DA, et al. Thyroid hormone induction of human cholesterol 7 alpha-hydroxylase (Cyp7a1) in vitro. Mol Cell Endocrinol 2014, 388(1-2): 32-40.