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Gender specific differences of the ethanol and nicotine toxicity verified by the use of mathematical models

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ABSTRACT

Beside the pandemic of obesity, the binge drinking becomes a huge problem. The toxicity of consuming alcohol and smoking in the late adolescent population was examined using mathematical models. The body was divided into compartments for two different models; (i) the ethanol model (4 compartments: central compartment, muscle and fat compartment, liver compartment and gastrointestinal compartment) and (ii) the nicotine model (2 compartments: liver compartment and central compartment). Different alcohol contents simulated consumptions of 90 mL of spirits; 900 mL of beer or 600 mL of wine. Nicotine metabolism simulation was performed for three different initial doses of nicotine (light, medium and strong cigarettes). Significant differences are observed regarding the gender; where the maximum ethanol concentration is reached at 0.5 h (males: 27 mmol/dm3 and females: 33 mmol/dm3) in the gastrointestinal compartment while complete nicotine degradation in the liver takes approximately 10 h and in the central compartment 15 h, respectively. The skewness and kurtosis of the toxin concentrations showed their relation with the symmetry of the toxin retention in the body. Results show preferable positively skewed distribution which implies a shorter retention time in the organism while higher kurtosis implies higher toxin concentration.

Introduction

Eating and drinking is the source of energy and nutrients, but the intake should be in accordance to the recommendations for age and gender (Gatley, 2016). But beside the pandemic increase of obesity, alarming becomes the increase of binge drinking and cigarette smoking in the adolescent population. This is an additional fact making them an important target for public health efforts. This generation is an online communication generation which was robustly positively associated with alcohol use (Larm et al., 2017). From the population of young adults, 37.9% reported binge drinking at least once in the past 30 days, in 2013, and 37.0% of them reported smoking tobacco (Gubner and Delucchi, 2016). According to the National Institute on Alcohol Abuse and Alcoholism (NIH, 2017), binge drinking means drinking so much within about 2 hours that blood alcohol concentration (BAC) levels reach 0.08 g/dL, the legal limit of intoxication. Studies report that tobacco and alcohol use is high in early adulthood implying middle adolescence; aged 15-17, and late adolescence; aged 18-20 (Giovino et al., 2012). During the college years, the separation from parents is often seen as liberation "from the shackles" and time when they tackle new responsibilities turning to smoking and excessive alcohol consumption. According to the results of the study of Jackson et al. (2014), adolescent drinking poses significant public health concerns. Results published in the National Epidemiologic Survey on Alcohol and Related Conditions (Barry and Petry, 2009) indicate that a greater level of harm can be conducted by young people from their drinking then in comparison to older drinkers. Alcohol is a high-energy donor that releases 7.1 kcal per gram. It is first metabolized in the liver and directly related with liver

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diseases, and indirectly with brain damage, ulcers, pancreatitis and other diseases as well as increased risk of different types of cancers (Kayani and Parry, 2010). This association has strongly suggested that alcohol intake can result in DNA damage. Ethanol is classified into Group 1 (human carcinogen) by International Agency for Research on Cancer (Baan et al., 2007). Nevertheless, there has been limited evidence that ethanol can be directly carcinogenic and acetaldehyde has been regarded, as the metabolite of ethanol, possibly responsible for ethanol induced toxicity (Kayani and Parry, 2010, Baan et al., 2007). In humans, more than 90% of ingested alcohol is eliminated via metabolic degradation mainly in the liver. Ethanol is firstly metabolized into acetaldehyde through enzymatic and non-enzymatic pathways (Baan et al., 2007, Johnston et al., 2013). Much concern has been expressed about this harmful behaviour because nearly 50% of college students in the U.S. report engaging in heavy drinking in the past year (SAMHSA, 2014). On average, 87 percent of students had tried alcohol at least once in their lifetime, 79 percent have been drinking alcohol in the last 12 months, and 57 % of students have reported that they drank in the past 30 days. The trend in Europe was observed in the study ESPAD (SAMHSA, 2014, Hibell et al., 2012). Drinking alcohol 'in the last 30 days' is present in about 75 % of students in the Czech Republic and Denmark, but only 17 % in Iceland and 32 % in Albania. Among the countries there is no clear geographical regularity, although the study of Hibell et al. (2012) showed the lower prevalence of drinking in the Nordic and Balkan countries. Cigarettes are also one of the opiates that young people experiment with in the middle and late adolescence. Smoking is one of the most prevalent addictions worldwide, affecting millions (Hibell et al., 2012, DiFranza et al., 2004). Alcohol consumption and tobacco use are known to be strongly related behaviours (Giovino et al., 2012; USDHHS, 2012) and the association between these two substances has been found to become stronger with the heavier use of either substance. Recent studies have found that smoking urges increase rapidly following heavy drinking, even among light smokers (Gubner et al., 2016). In the study of Cerjak, Haas and Kovačić (2010) the adolescents were very familiar with the beer brands, what is a results of the frequent use of such drinks. Research of the ESPAD study has shown that on average 54% of students had tried cigarettes at least once, and 28% said they had smoked in the last 30 days (Hibell et al., 2012). Countries with high flaring smoking are Bulgaria, Croatia, Czech Republic, France, Latvia, Monaco and Slovakia (40%), while the lowest prevalence in Albania, Iceland, Montenegro and Norway (about 12%). In Croatia, according to the study, 41% of children said they smoked in the past month ,which places us in the third place and well above the European average. Nichter et al. (2010) refer to results of the national study in the U.S. where approximately 30% of college students report having smoked in the past 30 days, and 40% report having smoked in the past year (Nichter et al., 2010, Johnston et al, 2010). Numerous studies have shown that adolescents are more sensitive to the positive rewarding and reinforcing effects of nicotine (Sawyer et al., 2017) „helping“ to develop cigarette addiction/dependence (DiFranza et al., 2004).

The aim of this study was to investigate the toxicity of the alcohol consumption and smoking of cigarettes in the late adolescent population. Gender similarities or differences in the toxic effect are investigated by the use of mathematical models. Human body can be observed as Nature's designed reactor, in which biological, mechanical, electrical and chemical activities are involved (Specht, 2004, Cederbaum, 2012). By use of these models, where the model represents an imitation of the system or process in the real world, the effects on the organism by the intake of different doses of ethanol and/or nicotine may be simulated. In view of these things, here we sought to characterize the toxic effects of adolescent smoke pre-exposure (nicotine) as well as the exposure to alcohol (ethanol) by use of these models.

Materials and methods

The energy and macronutrient content of drinks was calculated from the defined binge drinking (blood alcohol concentration levels reach 0.08 g/dl) what typically occurs after 4 drinks with women and 5 drinks with men (NIH, 2017). In this work we used the value of 3 drinks to be under the binge drinking servings. The Food Composition data base: DTU Food from the Finland National Food Institute, release 2. was used for the calculation.

The body was divided into compartments, according two models (i) ethanol model and (ii) nicotine model. Ethanol model was observed controlling the ethanol intake through following four compartments: (i) gastrointestinal, (ii) liver, (iii) central and (iv) muscle and fat compartment; while the nicotine model is presented through two compartments; metabolizing compartment and central compartment, because smoking and drinking are influenced by different factors (Cederbaum, 2012, Rodríguez-Cano et al., 2016, Milicic and Leatherdale, 2017). For both models are the volumes of the organs recalculated from average body mass (BM), of late adolescents (aged from 18 – 21), for both genders (Bosanac et al., 2016).

Models were simulated for three different initial doses of both ethanol and nicotine in two groups of college students (male and female). Ethanol initial doses were calculated using the available information about ethanol

amount in three different beverages (wine, beer and distilled beverage) with assumption that three beverages were consumed (90 mL of brandies; 900 mL of beer or 600 mL of wine). Same simulations were performed for the nicotine model; the initial doses of nicotine were calculated using available information on amount of nicotine per cigarette (three types of cigarettes with different content of nicotine were used: content of nicotine ranged from 0.012 to 0.034 mg/kg).
To evaluate the normalized third moment of the concentration distributions in different compartments we used the measure of the asymmetry (μ3), the skewness, as well as the fourth moment, the kurtosis (μ4) as the measure of the heaviness of the tail of the distribution, compared to the normal distribution.

Mathematical model of the ethanol metabolism

Used pharmacokinetic model describes ethanol time change. Model divides human organism into five compartments that exchange material: (i) stomach, compartment as (ii) gastrointestinal, (iii) liver, (iv) central and (v) muscle and fat compartment (Jana et al., 2013, Umulis et al, 2005). In this work the last four compartments (ii – v) were used. All compartments, except for the liver, are modelled as stirred tank reactor; liver is modelled as a tubular flow reactor. Time change of ethanol is given in the form of ordinary differential equations developed based on the flow of the blood between the compartments. The flow rate of the ethanol metabolism is based on alcohol dehydrogenase reaction, while acetaldehyde metabolism is based on aldehyde dehydrogenase enzymatic pathway. Model included equations using 15 parameters (both kinetics and physiological) (Umulis et al., 2005). Parameters of the model and its values are given Table 1.

Mathematical model of the nicotine metabolism

This model describes nicotine and cotinine time changes in the body, based on the metabolic parameters determinated partly in vivo partly in silico (Yamazaki et al., 2010). The model consists of an adsorption compartment, a metabolizing compartment and a central compartment. Parameter relationships in the form of the ordinary differential equations, for both nicotine and cotinine, were developed for each of the compartment. In the nicotine model simulation 21 parameters were included (Yamazaki et al., 2010). Parameters of the model and their values are given in Table 2.

Global sensitivity analysis

Application of the sensitivity analysis determines the "sensitivity" of included parameters and their weight (importance) in the simulation process. The importance of the individual parameters of the mathematical model of the ethanol and both nicotine metabolism was performed numerically by Fourier Amplitude Sensitivity Test (FAST) method (Saltelli et al., 2008). FAST method is based on transformation of the multidimensional parameter space to a one dimensional space of a single parameter, s, by the nonlinear transformations of the normalized parameter x_i (Eq.1):

$$x_i = \frac{1}{2} + \frac{1}{\pi} \cdot \arcsin(\sin(\pi \cdot \omega_i \cdot s + \varphi_i) \qquad (1)$$

Table 1. Parameters of the ethanol metabolism model

No.	Parameter	Description	Value
1	$v_{max,ADH}$	maximum reaction rate for reaction catalyzed by alcohol dehydrogenase	2.2 mmol (min kg_{liver})$^{-1}$
2	$v_{rev,ADH}$	maximum reaction rate for reverse reaction catalyzed by alcohol dehydrogenase	32.6 mmol (min kg_{liver})$^{-1}$
3	$K_{m,ADH}$	Michaelis-Menten constant for ethanol in reaction catalyzed by alcohol dehydrogenase	0.4 mmol/L
4	$K_{rev,ADH}$	Michaelis-Menten constant for acetaldehyde in reaction catalyzed by alcohol dehydrogenase	1 mmol/L
5	$v_{max,ALDH}$	maximum reaction rate for reaction catalyzed by acetaldehyde dehydrogenase	2.7 mmol (min kg_{liver})$^{-1}$
6	$K_{m,ALDH}$	Michaelis-Menten constant for acetaldehyde in reaction catalyzed by acetaldehyde dehydrogenase	1.2 μmol/L
7	k_S	stomach-emptying rate	~0.4 min^{-1}
8	$c_{ETOH,S0}$	initial ethanol dose	variable
9	V_S	water volume of gastrointestinal tract	2.07 dm^3
10	v_L	liver flow rate	1.350 cm^3 min^{-1}
11	V_M	water volume of muscle and fat compartment	25.76 dm^3
12	v_M	blood flow rate to the muscle and fat compartment	1.5 cm^3 min^{-1}
13	V_C	water volume of central compartment	11.56 dm^3
14	V_L	water volume of liver compartment	1.08 dm^3
15	ΔV_L	water volume of liver sub-compartment	0.108 dm^3

Table 2. Parameters of the nicotine metabolism model

No.	Parameter	Description	Value
1	$logP_N$	octanol-water partition coefficient for nicotine	0.930
2	$CL_{h,int,N}$	hepatic intrinsic clearance for nicotine	5.44 $dm^3\ h^{-1}$
3	$K_{p,h,N}$	Liver-plasma concentration ratio for nicotine	0.797
4	$CL_{r,N}$	renal clearance for nicotine	0.0994 $dm^3\ h^{-1}$
5	$f_{u,p,N}$	plasma unbound fraction for nicotine	0.688
6	$R_{b,N}$	ratio of blood to plasma concentration for nicotine	1
7	$V_{l,N}$	volume of systematic circulation for nicotine	0.746 dm^3
8	$V_{h,N}$	hepatic volume for nicotine	0.0085 dm^3
9	$Q_{h,N}$	hepatic blood flow rate of systemic circulation to the tissue compartment for nicotine	0.853 $dm^3\ h^{-1}$
10	$k_{a,N}$	absorption rate constant for nicotine	0.746 dm^3
11	F_aF_g	Fraction absorbed x intestinal available	1
12	Dose	dose of nicotine	variable
13	$logP_C$	octanol-water partition coefficient for cotinine	0.040
14	$CL_{h,int,C}$	hepatic intrinsic clearance for cotinine	0.208 $dm^3\ h^{-1}$
15	$K_{p,h,C}$	Liver-plasma concentration ratio for cotinine	0.680
16	$CL_{r,C}$	renal clearance for cotinine	0.00421 $dm^3\ h^{-1}$
17	$f_{u,p,C}$	plasma unbound fraction for cotinine	0.743
18	$R_{b,C}$	ratio of blood to plasma concentration for cotinine	1
19	$V_{l,C}$	volume of systematic circulation for cotinine	0.451 dm^3
20	$V_{h,C}$	hepatic volume for cotinine	0.0085 dm^3
21	$Q_{h,C}$	hepatic blood flow rate of systemic circulation to the tissue compartment for cotinine	0.853 $dm^3\ h^{-1}$

Where s is sampling parameter in the range of s ∈[-1, 1], ω_i are selected frequencies and φ_i are randomly selected phase angles. The φ_i is element of the range [-π, π], while frequencies are integers and form an incommensurate set. A sequence of the scan parameter s generates a large set of random and uncorrelated sequences of the input variables.

Response of the output variable are expanded into series of Fourier series by which the overall variance D (Eq.2) of the output function is decomposed into summands of squares of Fourier coefficients, Aω and Bω (Eq.3-4).

$$D_T = 2 \cdot \sum_{\omega=1}^{\infty} (A_\omega^2 + B_\omega^2) \quad (2)$$

$$A_\omega = \frac{1}{2 \cdot \pi} \cdot \int_{-\pi}^{\pi} y(s) \cdot \cos(\omega \cdot s)\, ds \quad (3)$$

$$B_\omega = \frac{1}{2 \cdot \pi} \cdot \int_{-\pi}^{\pi} y(s) \cdot \sin(\omega \cdot s)\, ds \quad (4)$$

Individual parameter contributions in the total dispersion are calculated by the corresponding harmonics (Eq.5):

$$D_T = 2 \cdot \sum_{\omega=k\cdot\omega_i}^{\infty} (A_\omega^2 + B_\omega^2) \quad (5)$$

The relative sensitivity coefficient Si, measure of the global influence of the parameter x_i on the output, and it is given as the ratio of the partial dispersion Di and the total dispersion D_T (Eq.6):

$$S_i = {}^{D_i}/_{D_T} \quad (6)$$

Results

Caloric and macronutrient content of beer, spirits and wine in the amounts that present volumes consumed in 2 hours and defined as binge drinking are presented in Table 3 (900 ml of beer, 600 ml of wine and 90 ml of spirits). Beer energy content is not just from alcohols but also from macronutrients as carbohydrates and a small part from proteins, what is a result of the production process. In some spirits carbohydrates are a source of energy as well, but those are sugars added during the production.

The adolescents of both genders were the target group. The degradation of toxins in the body was simulated for both genders in different organs (central, muscles and fat; liver and/or gastrointestinal compartments). Gender specific similarities and/or differences were verified and the first reason is different compartments volume for female and male adolescents calculated on the basis of average body mass (females, 61.6 kg; males, 72.8 kg) of adolescents aged 18 to 21.

Significant differences are confirmed regarding the gender were the maximum ethanol concentration is reached at 0.5 h and is about 27 mmol/dm^3 for males and 33 mmol/dm^3 for females in the gastrointestinal compartment. The profile of acetaldehyde in different body compartments was also observed, for different inlet ethanol concentrations of both male and female adolescents.

Table 3. Energy and nutrient contents in alcoholic beverages

	Energy (kJ)	Protein (g)	Fat (g)	SFA	MFA	PFA	CHO (g)	TAS (g)	Alcohol (g)
Beer (600 mL)									
Beer, lager, 2.6 %.	990	1.8	0	0	0	0	24.3	0	18.9
Beer, household, low alcohol	1701	2.7	0	0	0	0	77.4	0	11.7
Beer, lager, 4.4 %	1422	2.7	0	0	0	0	24.3	0	33.3
Beer, export, 5.6 %	1755	3.6	0	0	0	0	28.8	0	41.4
Beer, strong, 7.6 %	2331	3.6	0	0	0	0	41.4	0	54
Liqueur/spirit (90 mL)									
Liqueur, brown cocoa	1307	0	0	0	0	0	47.7	0	17.1
Liqueur, Cherry Brandy	888	0	0	0	0	0	25.6	25.6	15.7
Liqueur, Crème de Menthe	1424	0	0.3	0	0	0.2	37.4	37.4	26.8
Liqueur, Curacao	1139	0	0	0	0	0	24.2	24.2	25.1
Liqueur, coffee	1294	0.1	0.3	0.1	0	0.1	42.1	42.1	19.5
Liqueur, coffee with cream	1246	2.5	14.1	7.3	3.4	0.5	18.8	18.0	12.4
Liqueur, average values	1010	0	0	0	0	0	26.1	26.1	19.5
Spirits, 70 % proof	867	0	0	0	0	0	0.5	0	29.6
Vodka	872	0	0	0	0	0	0	0	30.1
Brandy, gin, rum, 40 %	870	0	0	0	0	0	0	0	29.7
Cognac, 38 %	859	0	0	0	0	0	0	0	28.8
Whisky	923	0	0	0	0	0	0	0	31.5
Wine (600 mL)									
Wine, white, medium	1980	0.6	0	0	0	0	14.4	0	59.4
Wine, white, sparkling, champagne	1896	1.8	0	0	0	0	8.4	0	59.4
Wine, white, sweet	2400	1.2	0	0	0	0	35.4	0	61.2
Wine, white, dry	1686	0.6	0	0	0	0	1.2	0	57
Wine, rosé	1782	1.2	0	0	0	0	8.4	0	55.8
Wine, red	1824	1.2	0	0	0	0	9	0	57

SFA: saturated fatty acids; MFA: monounsaturated fatty acids; PFA: polyunsaturated fatty acids; CHO: carbohydrates; TAS: total added sugar

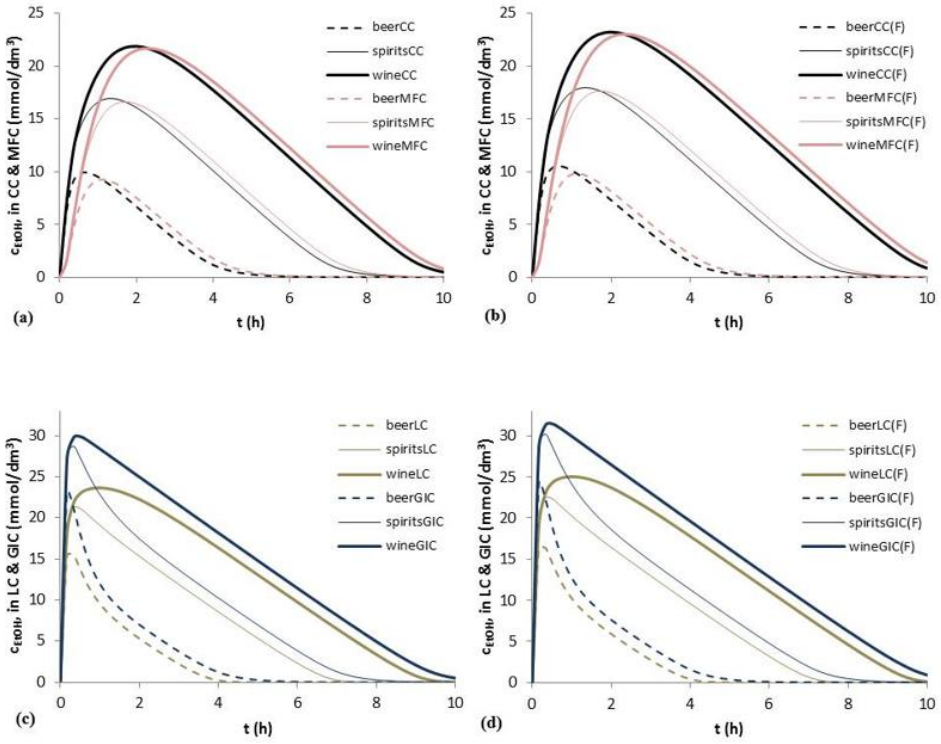

Fig. 1 Concentration profiles of the ethanol model (EtOH) for beer, spirits and wine in the: (i) central compartment (CC) and muscle and fat compartment (MFC), for male (a) and female (b) adolescents; (ii) liver compartment (LC) and gastrointestinal compartment (GIC) for male (c) and female (d) adolescents

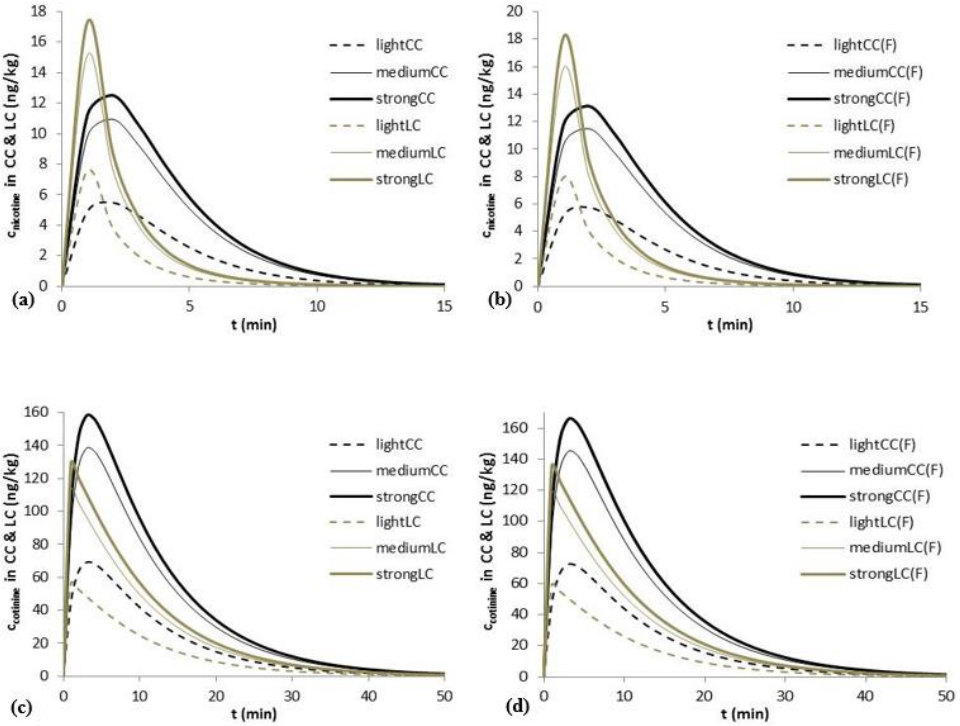

Fig. 2 Concentration profiles of nicotine models for male (a) and female (b) adolescents and cotinine model for male (c) and female adolescents (d) in two observed compartments; (i) central compartment (CC) and (ii) liver compartment (LC) based on the content of nicotine and cotinine in light, medium and strong cigarettes

For the highest ethanol inlet concentration for male gender after 10 h approximately 10% of acetaldehyde stays non-metabolized, while in the case of female adolescents this percentage is around 30.

The nicotine simulation model was performed for three different initial doses of nicotine (cigarettes with different amount of nicotine) expressed on average body mass of both genders; for male adolescents 0.029 mg/kg, 0.025 mg/kg and 0.012 mg/kg and for female adolescents 0.034 mg/kg, 0.029 mg/kg, and 0.015 mg/kg.

Concentration profiles for nicotine and cotinine in central compartment and liver compartment for both male and female adolescents showed no difference regarding the gender and the maximum of the nicotine concentration in liver compartment is reached after approximately 0.9 h and in central compartment after approximately 1.6 h, for all three initial nicotine concentrations. The complete nicotine degradation in the liver takes approximately 10 h and in central compartment, approximately 15 h. Concentration profiles of cotinine obtained by model simulation show that it takes approximately 40 h to complete cotinine elimination from central body compartment of both genders after smoking only three cigarettes. Long cotinine half-life in body organs indicates the possible toxic effect of that compound. Due to the complex analytical procedure necessary for determination of nicotine and cotinine concentrations in human blood or saliva, mathematical model confirmed their usefulness by getting better insight into distribution and degradation of mentioned compounds.

To examine the relevance of the model parameters, the general sensitivity analysis was conducted.

Obtained results allow the detail analysis of the model parameters and their importance in the observed models. They can also be used in possible model parameters reduction.

Skewness and kurtosis are parameters used to present the deviation of the concentration distribution from a symmetric or normal distribution. If they are equal to 0, the distribution is symmetric.

The absolute value of skewness (|μ3|), in the range from 0 to 0.25 shows no significant deviation from a symmetric distribution. Ethanol degradation showed no deviation from a symmetric distribution for wine while spirits and beer show deviations from the symmetry. Wine (μ3(GIC)=0.06; μ3(CC)=-0.20, for male adolescents and μ3(GIC)=0.03; μ3(CC)=-0.24), for female adolescents); spirits (μ3(MFC)=0.24; μ3(GIC)=-0.71, for male adolescents and μ3(GIC)=0.66; μ3(LC)=0.50 for female adolescents) and beer (μ3(MFC)=0.99; μ3(GIC)=1.9, for male

adolescents and μ3(GIC)=1.85; μ3(MFC)=0.95 for female adolescents). For nicotine and cotinine are all skewness values over 1, what is an indication of positively skewed distribution.
This is verification that for those two alcoholic drinks, the toxins will be kept longer in the body i.e. will be secreted more slowly. The kurtosis, on the other hand, with its deviation from the value 0 presents higher concentrations of the toxins in observed compartments. Very high values indicate high concentrations of the toxin, what is particularly evident for nicotine and cotinine (μ4>2 in the liver compartment).

Discussion

Binge drinking energy and macronutrients were calculated on the basis of 3 drinks what resulted with 90 ml of /spirits; 600 ml of wine and 900 ml of beer. Results are presented in Table 3. The results are based on the data from the Food Composition data base: DTU Food from the Finland National Food Institute, release 2. For 900 ml of beer the energy content will range from 990-2331 kJ with an average intake of 1589 kJ with the majority of calories gained from alcohol (53.4%) followed by calories from carbohydrates (43.5%). Caloric intake is the highest in wine consuming, 1918 kJ with the domination of the energy from alcohol (87.8%) while in the spirits the energy intake is the lowest (1049 kJ) with the highest share of alcohol in the total energy content (63.6%). The added sugars are also important for the spirit category and they are not a result of the production process and they are not natural (30%, CHO). Based on gained values, the ethanol model was simulated. Mathematical model of alcohol (ethanol, EtOH) metabolism was used to analyse the ethanol degradation and distribution in organs of male and female adolescents. Over the years, several studies were performed to analyse the alcohol consumption differences between genders (Gubner et al., 2016). According the study of Mumenthaler et al. (1999), women have proportionally more body fat and less water than men of the same body masses. Because alcohol is dispersed in body water, women reach higher peak blood alcohol concentration than men after consuming equivalent doses of alcohol, even when doses are adjusted for body masses.
In this work difference between genders was modelled based on the tissue water volumes of specific organ compartment. The tissue for water volumes gastrointestinal tract, liver, central compartment and muscle and fat compartment,respectively, were adjusted from the 69.4 kg "standard man" to average body masses of both genders of adolescents (BM girls, 61.6 kg; boys, 72.8 kg). The initial ethanol concentrations were calculated based on available information on amount of alcohol in vine, beer and distilled beverage. Due to the difference in average body mass, the initial ethanol concentrations were for males: 0.734 g/kg, 0.588 g/kg, and 0.338 g/kg while for females: 0.926 g/kg, 0.694 g/kg and 0.399 g/kg. The concentration profiles for ethanol in organ compartments of both genders are given in Figure 1. The effects of ethanol on various tissues depend on its blood alcohol concentration over the time. Blood alcohol concentration is determinated by how quickly alcohol is absorbed, distributed, metabolized and excreted (Zakhari, 2006). Comparing concentration profiles for ethanol in organ compartments it can be noticed that there are no significant differences in profiles for both genders. It can be noticed that all curves have specific form – they are all bell shaped with fast increase and slow decrease. A number of potentially dangerous by-products are generated in liver (Kuntsche et al., 2015), regardless the gender and sexual orientation (Coulter et al., 2016). Central organ compartment (CC) includes kidneys, blood, brain, heart, spleen, bone and skin. Analysing the obtained simulation results it can be noticed that maximum of the ethanol concentrations in central organ compartment is reached after approximately 2 h for both genders and for all analysed ethanol intake concentrations (Figs. 1a and 1b). As expected, the residence time of the ethanol in the central organ compartment is longer for the female gender. In case of muscle and fat compartment (MFC), the maximum ethanol concentration is reached after approximately 2.5 h for male adolescents and for approximately 1 h in case of female gender (Figs. 1a and 1b). The concentration of alcohol differs in the drinks, so the beer has an average of 28 g in 900 ml (Table 3) while in the wine it will reach the value of 58 in 600 ml. The maximum of the ethanol concentration in gastrointestinal compartment is achieved after approximately 0.5 h and it is higher with females (Fig. 1d). It can also be observed that ethanol concentration decreases after the maximum value and reaches zero in gastrointestinal compartment after approximately 5 h for the lowest initial ethanol concentration in male body (Fig. 1c). For the higher initial ethanol concentration in male body, it takes 10 h to reach zero in gastrointestinal tract. In case of female body, at 10 h the highest ethanol concentration is still not completely metabolized. According to Bujanda (2000) alcohol facilitates development of gastroesophageal reflux disease by reducing the pressure of the lower oesophageal sphincter and oesophageal motility. Longer residence time of the alcohol in the gastrointestinal compartment has stronger negative

effect. After alcohol is swallowed, it is absorbed from the small intestine into the veins and carried to the liver where it is metabolized (Zakhari, 2006).Only 2% to 10% of absorbed ethanol is eliminated through the kidneys and lungs and the rest is metabolized primarily in the liver (Lieber, 1997). The major pathway for alcohol metabolism involves ADH and enzyme that catalyses the conversion of alcohol to acetaldehyde (Lieber, 1997). Simulation results show that it takes from 3.5-8.5 h to complete alcohol degradation in the liver (liver compartment, LC) for male adolescent (Fig. 1c) and from 5.5-10.5 h for female adolescents (Fig 1d). The obtained results indicate the toxicity of the alcohol on the liver tissue due to the long residence time. Liver is practical susceptible to alcohol related injuries because it is primarily site of alcohol metabolism. Ethanol cannot be excreted and must be metabolized, primarily by the liver (Cederbaum, 2012) and its metabolism in the liver depends on two enzymes; alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH). Metabolism of ethanol with ADH produces acetaldehyde, a highly reactive and toxic by-product that may contribute to tissue damage and, possibly, the addictive process.

Mathematical model of nicotine metabolism (Yamazaki et al., 2010) was used to analyse the nicotine consumption and distribution in organs of male and female adolescents from Croatia. Used model consists of an absorption compartment, a metabolizing compartment and central compartment. Model simulations were preformed for three different initial doses of nicotine (with assumption of three smoked cigarettes from three different trademark with different amount of nicotine) expressed on average body mass of both genders; for male adolescents 0.029 mg/kg, 0.025 mg/kg and 0.012 mg/kg and for female adolescents 0.034 mg/kg, 0.029 mg/kg, and 0.015 mg/kg. When tobacco smoke reaches the small airways and alveoli of the lungs, nicotine is rapidly absorbed (Rodríguez-Cano et al., 2016, Milicic and Leatherdale, 2017). Blood concentration of nicotine rises quickly during smoking (Benowitz, 2010). The rapid absorption of nicotine from cigarette smoke through lungs can be explained with large surface area of the alveoli and small airways. After absorption, nicotine enters the bloodstream and is being distributed to body tissues. The extensive nicotine metabolism takes place in the liver, were it is being metabolized into six metabolites from which cotinine is quantitatively the most important (Bao et al., 2005). Concentration profiles for nicotine and cotinine in central compartment and liver compartment for both male and female adolescents are given in Figure 2.

As described in literature, nicotine concentration increases very fast after the intake in both analysed compartments for both genders. The maximum of the nicotine concentration in liver compartment is reached after approximately 0.9 h and in central compartment after approximately 1.6 h for all three initial nicotine concentrations in both genders. It can also be noticed that it takes approximately 10 h for complete nicotine degradation in liver and approximately 15 h for nicotine consumption in central compartment. These results can be compared with dose obtained for ethanol degradation where it also takes approximately 10 h for complete ethanol degradation. The rate of nicotine metabolism is hypothesized to be a determinant of how much a person smokes (Sweeting and Hunt, 2015). Rapid metabolizers would be expected to need more nicotine and therefore smoke more than slow metabolizers. Nicotine, a highly lipid-soluble alkaloid, is converted to cotinine in a two-step process involving cytochrome P450 and aldehyde oxidase. Nicotine is eliminated primarily by hepatic metabolism by way of C-oxidation to cotinine, the major metabolite. While nicotine has a relatively short half-life of about 2 hours, cotinine has a half-life of approximately 20 hours. Therefore, cotinine provides a more stable marker of exposure in the person since there is less variability in cotinine throughout the day than that observed for nicotine (Jain, 2014, Yuki et al., 2013). The maximum level of cotinine in both liver and central compartment is reached after approximately 4 h for both genders and all three different inlet nicotine concentrations. Concentration profiles of cotinine obtained by model simulation show that it takes approximately 40 h for complete cotinine elimination from central body compartment of both genders after smoking only three cigarettes. Long cotinine half life in body organs indicates the possible toxic effect of that compound. Due to the complex analytical procedure necessary for determination of nicotine and cotinine concentrations in human blood or saliva (Yuki et al., 2013), mathematical model are very useful for getting the better insight into distribution and degradation of mentioned compounds.

In this work, global sensitivity analyses dealing with the output uncertainties over the entire range of values of the input parameters simultaneously was applied. In case of ethanol metabolism model, the output variable was ethanol concentration in central compartment, while in the case of the nicotine metabolism model, nicotine concentration in the blood was chosen as output variable. The effects of the simultaneous perturbations of all parameters included into models on selected output variables were analysed. Obtained results are presented in Figure 3.

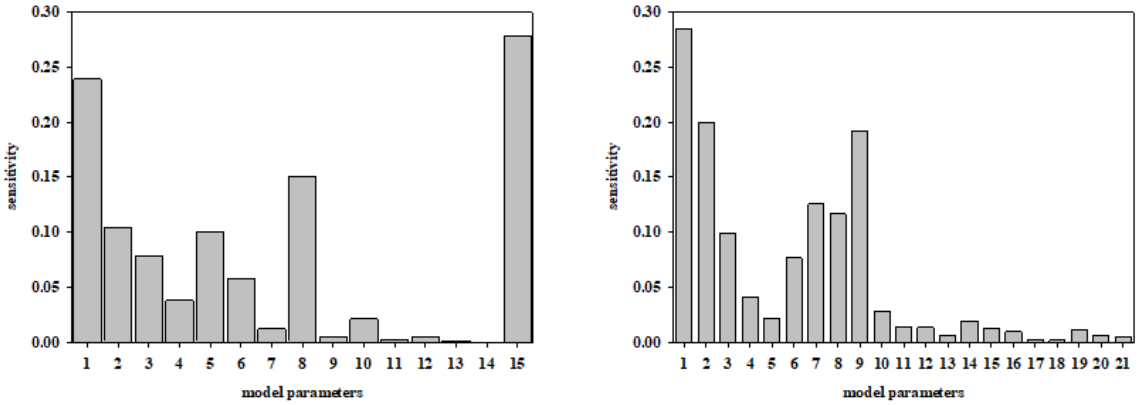

Fig. 3 Results of the FAST analysis for the (A) ethanol and nicotine (B) metabolism model

It can be seen that in the case of ethanol metabolism model the most important parameters are 15th, 1st and the 8th. Parameter listed as 15th is the volume of the liver sub-compartment. As mentioned before, ethanol metabolism model is developed describing the organs as reactors. Liver is modelled as tubular reactor composed of the series of batch reactors. Sensitivity analysis shows that the volume of the individual batch reactors describing liver is the most important for the ethanol central compartment concentration. The second most important model parameter is shown to be maximum reaction rate of the reaction catalysed by alcohol dehydrogenase; the ethanol concentration in central compartment decrees when the alcohol dehydrogenase maximum reaction rate increases. Sensitivity analysis revealed that the initial amount of the ethanol in the stomach (parameter number 8) has important effect on the ethanol concentration in central compartment. Results also revealed that parameters from 9 to 14 ((9) volume of gastrointestinal compartment, (10) perfusion rate in liver, (11) volume of muscle and fat compartment, (12) perfusion rate in muscle and fat, (13) central compartment volume and (14) liver volume) have almost no effect on the ethanol concentration in central compartment. In case of the nicotine metabolism model sensitivity analysis revealed the nicotine concentration in blood is mostly affected by the first nine model parameters (Table 2). The most important are shown to be (1) partition coefficient for the nicotine, (2) hepatic intrinsic clearance and (9) hepatic blood flow rate of systemic circulation. Partition coefficient determinates which amount of the nicotine form the cigarette smoke will be absorbed and it is highly dependent on pH. Hepatic intrinsic clearance quantifies the loss of the component during it passage through the liver. Knowing that the nicotine metabolism primarily takes place in the liver it is obvious that hepatic intrinsic clearance perturbation has significant effect on nicotine blood concentration. The third most important parameter is shown to be hepatic blood flow rate systematic circulation; by increasing this parameter the blood comes faster into the liver where the nicotine metabolism takes place. Model parameters between 3 and 8 ((3) liver-plasma concentration ratio, (4) renal clearance, (5) plasma unbound fraction, (6) ratio of blood to plasma concentration, (7) volume of systemic circulation and (8) hepatic volume) show to have effect on the nicotine blood concentration while parameters form 10 to 21 (parameters included into cotinine metabolism) show to have very small effect on nicotine blood concentration. Obtained results allow the detailled analysis of the model parameters effects and also can be used in possible model parameters reduction.

The bell-shaped curves can be described with the third and fourth moment describing their asymmetry and flattening, where a perfectly symmetrical curve has a skew and kurtosis of 0 (Field et al., 2012).

The skewness of the distribution of toxin concentrations could be considered as a normal distribution if the absolute value $|\mu 3|$ would be between 0 and 0.25. Absolute values of the skew moment higher than 0.25 also indicate that the curve is wider than a normal distribution curve would be, what in this study means that the retention of EtOH or nicotine and cotinine is in the body is longer (Table 1 and Fig. 1). The kurtosis, as the fourth moment, will show flattening ($\mu 4<0$) or elongation $\mu 4>0$) of the distribution of toxin concentration. In this study, flattening expresses lower concentrations while elongation means higher concentration of the observed toxins in different compartments, Based on the kurtosis values, liver compartment is dominant in detoxification of toxins as nicotine and cotinine while the distribution of toxic effect of EtOH is high in all observed compartments, what is a verification of alcohol toxicity distributed in the whole body. The toxin distribution is higher in the female population, because their volumes of observed organs are slightly smaller.

Table 4. Skewness (μ3) and kurtosis (μ4) as third and fourth moment calculated for the concentration distribution curves for ethanol models (EtOH) and nicotine model (nicotine and cotinine)

		male adolescents		female adolescents	
		μ3	μ4	μ3	μ4
EtOH					
Central compartment (CC)	beer	1.09	-1.68	1.03	-1.96
	spirits	0.25	-1.55	0.20	-1.55
	wine	-0.20	-1.41	-0.24	-1.35
Muscle and fat compartment (MFC)	beer	0.99	-1.77	0.95	-1.89
	spirits	0.24	-1.44	0.18	-1.55
	wine	-0.19	-1.40	-0.22	-1.35
Liver compartment (LC)	beer	1.78	-1.65	1.72	-1.69
	spirits	0.56	-1.51	0.50	-1.59
	wine	-0.08	-1.49	-0.13	-1.44
gastrointestinal compartment (GIC)	beer	1.90	-1.09	1.85	-0.06
	spirits	0.71	-1.00	0.66	-0.95
	wine	0.06	-0.92	0.03	-1.33
Nicotine					
Central compartment (CC)	light	1.62	-0.52	0.83	-0.53
	medium	1.78	-0.34	0.90	-0.34
	strong	1.89	-0.15	0.96	-0.16
Liver compartment (LC)	light	1.32	2.47	1.88	5.53
	medium	1.41	2.93	2.08	6.70
	strong	1.50	3.41	1.98	6.10
Cotinine					
	light	1.34	0.38	1.34	0.40
	medium	1.38	0.39	1.36	0.43
Central compartment (CC)	strong	1.39	0.46	1.38	0.46
	light	1.66	1.66	1.66	1.69
	medium	1.68	1.67	1.69	1.70
Liver compartment (LC)	strong	1.70	1.72	1.70	1.72

Conclusions

The energy intake per binge drinking presented consumption of alcoholic drinks in 2 hours, and the values showed intakes rich in energy (859 – 2400 kJ) and poor in nutritional density. But the models showed the toxic effect of drinking (EtOH model) and smoking (nicotine and cotinine model) for the adolescents. It is of global interest to develop more advanced and accurate risk assessment systems to support appropriate interpretation and communication based on human biomonitoring results. Therefore,the modelling of the toxicity of consuming alcohol and nicotine in the late adolescent population is a contribution to it. Such approach showed a human body represented as associated bioreactors and the flows in it are presented with numerous parameters. The general sensitivity analysis revealed that the initial amount of the ethanol in the stomach has important effect on the ethanol concentration in central compartment, as confirmation that if the stomach is empty, toxicity will be higher. For the female population the toxicity is even higher than for the male population. Gender is a significant factor where the maximum ethanol concentration is reached at 0.5 h and is about 27 mmol/dm^3 for males and 33 mmol/dm^3 for females in the gastrointestinal compartment.

The ethanol model simulation showed that after 10 h approximately 10% of acetaldehyde stays non-metabolized for the male population while for the female population this percentage is around 30%. Conducted sensitivity analysis in the nicotine metabolism indicated that the parameter hepatic blood flow rate systematic circulation shows that increase of this parameter will result with more rapid entry of blood into the liver where the nicotine metabolism takes place. The nicotine simulation model was performed for three different initial doses of nicotine (ranged from 0,012 – 0.0345 mg of nicotine per kilogram of average body mass). For all three initial nicotine concentrations, regardless the gender, the maximum of the nicotine concentration in liver compartment is reached after ≈ 0.9 h and in central compartment after ≈1.6 h. The complete nicotine degradation takes approximately 10 h in the liver compartment and approximately 15 h in central compartment. The skewness of the distribution of toxin retention showed high asymmetry for short toxin retention (verification that alcoholic drinks with higher ethanol content (spirits and wine), will keep the toxins longer and will be secreted more slowly while the kurtosis was higher when the toxin concentrations in the bioreactor compartments were higher. Very high kurtosis values indicate high concentrations of a toxin,

what is particularly evident for nicotine and cotinine (μ4>2 in the liver compartment).

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