Ann Appl Sport Sci 8(2): e835, 2020.
http://www.aassjournal.com; e-ISSN: 2322–4479; p-ISSN: 2476–4981. 10.29252/aassjournal.835

ORIGINAL ARTICLE

Metabolic Profile in Active Female Students Users and Non-Users Combined Oral Contraceptives

[1]Marzena Malara*, [1]Anna Kęska, [1]Joanna Tkaczyk, [1]Grażyna Lutosławska

[1]Józef Piłsudski University of Physical Education, Warsaw, Poland.

Submitted 24 December 2019; Accepted in final form 15 March 2020.

ABSTRACT

Background. Metabolic changes induced by combined oral contraceptives (COC) use is regularly active in females. **Objectives.** Analyzing blood cortisol and other biochemical variables in active (COC) users and non-users. **Methods.** A total of 123 active female students (25 COC users and 98 non-users) volunteered to participate in the study. The COC users declared 2-3 years of using different types of combined the fourth generation COC pills. In all participants' body composition, physical activity and dietary habits were determined. Circulating cortisol and insulin were determined using standard radioimmunoassay techniques. Plasma glucose and lipoproteins were determined using colorimetrical methods. To evaluate the health risk ratio of total cholesterol to HDL-cholesterol (Castelli index I) and LDL-cholesterol to HDL-cholesterol (Castelli index II) were calculated. **Results.** There were no between-groups differences in body composition and dietary habits. The COC users had markedly elevated plasma cortisol ($p < 0.001$), triacylglycerols (TG) ($p < 0.001$), total cholesterol (TC) ($p < 0.002$) and HDL-C ($p < 0.040$) with no effect on glucose, LDL-cholesterol (LDL-C) and TC-to HDL-C ratio. In contrast, the LDL-C to HDL-C ratio in COC users was lower vs. non-users ($p < 0.002$). COC users were characterized by a higher frequency of elevated TC ($p < 0.001$) and HDL-C ($p < 0.040$). However, in all participants Castelli index I and II were within the accepted limits suggesting a low risk of cardiovascular disease. **Conclusion.** In COC users regular physical activity does not prevent changes in circulating biochemical variables, however, these changes did not affect cardiovascular risk which is low and similar to that noted in active non-users.

KEY WORDS: ***Combined Contraceptives, Physical Activity, Lipoproteins***

INTRODUCTION

Assuming a wide spectrum of cortisol action in many human tissues its elevated level brings about marked adverse changes in pancreas function, insulin sensitivity, but also in the liver triacylglycerol (TG) synthesis and very-low-density lipoprotein (VLDL) secretion into circulation (1). However, elevated cortisol is also observed in healthy individuals of both sexes under stress conditions and in women under hormone replacement therapy (HRT) and using oral contraceptives (OC) (2, 3). In consequence, OC use but especially combined OC (COC) increases the risk of metabolic disturbances including cardiovascular disease (4, 5).

However, it should be stressed that data concerning OC use and metabolic status in regularly active females are rare and focused mainly on cortisol and testosterone response to training and it consequences for sport competition (6, 7) There is only one study which showed a positive effect of regular moderate physical activity (150 min/week) on insulin sensitivity in progestin-only OC (8). However, it should be stressed that regular physical activity per se has

*. Corresponding Author:
Marzena Malara, PhD
E-mail: marzena.malara@awf.edu.pl

the potential to increase circulating cortisol (9), Thus, to eliminate the possible effect of physical activity on circulating cortisol our study was undertaken and focused on the comparison of body composition, dietary habits, biochemical variables (cortisol, insulin, glucose, lipoproteins and calculated indices of cardiovascular risk) in active females - OC users with elevated blood cortisol and active non-users with normal cortisol levels.

Innovation. Elevated blood cortisol and its effect on blood biochemical variables in combined oral contraceptive (COC) users have been thoroughly studied. However, there is no data concerning metabolic changes induced by COC use in regularly active females.

MATERIAL AND METHODS

Participants. A total of 123 university students (25 OC-users and 98 non-users) active female students of participated in the study. All subjects were volunteers who responded to the advertisement in student dormitories and information provided by word–of-mouth. Both oral contraceptive users and non-users were encouraged to participate in the study. All participants lived in a big city (over 1 million inhabitants), were healthy non-smokers, and declared not taking any vitamin, mineral supplements and not taking any medication on a regular basis. Additionally, the condition for participation was engagement in regular physical activity for at least for 3 months. Additionally the OC users declared regular menstruation, and 2-3 years of using different types of the fourth generation of combined pills containing ethinlyestradiol (0.02-0.03 mg), but different progestin's (3 mg of drospirenone, 0.15 mg of desogestrel, and 0. 15 mg of levonogestrel). All participants provided written consent for participation in the study. The research protocol was accepted by the ethics committee of the Józef Piłsudski University of Physical Education in Warsaw.

Anthropometry. Body weight was measured to the nearest 0.1 kg and body height to the nearest 0.5 cm using standard medical equipment in subjects wearing light indoor clothing without shoes, jackets and sweaters. Waist circumference (WC) was measured in the midway section between the lower edge of the ribs and the iliac crest with an accuracy of 0.1 cm using non-stretchable tape. Body fat was evaluated from four skinfold measurements (biceps, triceps, suprailiac and subscapular) using Harpenden caliper (British Indicators, Burges HILL, UK). Each measurement was performed twice and was repeated for the third time in the case of discrepancies. The percentage of body fat was calculated according to Durnin and Womersley (10). Fat mass and lean body mass were also calculated. All measurements were performed by a well-trained technician.

Physical Activity. The subjects' physical activity was briefly determined by an experienced interviewer using the Seven-Day Physical Activity Recall Questionnaire (SDPAR) (11). Data concerning duration, frequency and intensity of various activities (leisure, occupational, active transportation, and sport participation) within a week were collected. Light, moderate, hard and very hard physical activity was defined as metabolic equivalent (MET) value of 1.5, 4, 6 and 10, respectively (11).

Energy and Macronutrient Daily Intake. Participants' daily energy intake was assessed using 24-h food records provided by previously instructed participants. Records contained all foods and drinks consumed during 4 days (2 weekdays and weekend). All information was carefully validated by trained technicians who established the size of meals using the Album of Photographs of Food Products and Meals (12). Subsequently, the household measures of intake were converted into grams and diet composition was analyzed using the computer program Dieta 5.0 purchased in the National Food and Nutrition Institute in Warsaw (Poland).

Biochemical Analysis. All participants were asked to refrain from physical activity for at least 24 h and to eat the last meal at 19:00 a.m. before blood sampling. Blood was withdrawn from the antecubital vein into tubes containing anticoagulant and centrifuged 15 min/4000rpm at 4° C to obtain plasma. Plasma was stored at -70° C until analysis. Plasma cortisol was determined using standard radioimmunoassay and BioSource commercial kits (Belgium). Inter- and intra assay coefficients of variation for hormones did not exceed 7%. The accepted normal level of circulating cortisol was 133.0 – 538.2 nmol/L. Plasma glucose was assayed using the GOD-PAP method. Plasma total cholesterol (TC), HDL-cholesterol (HDL-C) and triacylglycerols (TG) were assayed using colorimetric methods and commercial kits (Randox Laboratories, UK). Inter-and intra assay coefficients of variation for

the above variables did not exceed 5%. Circulating LDL-cholesterol (LDL-C) was calculated according to the Friedewald formula (13). All analyses were performed in duplicate and in the case of discrepancies were repeated for the third time. According to the information from the laboratory normal range of plasma cortisol was 133.0 – 538.2 nmol/L. Normal level of glucose was established according to the International Diabetic Association as ≤ 5.6 mmol/L (14).The following levels of lipoproteins were established as indicating low health risk - (TG < 1.7 mmol/L, TC ≤ 5.2 mmol/L, HDL-C > 1.3, LDL-C < 3.4 mmol/L (15).

Castelli index I (TC-to HDL-C ratio) and Castelli index II (LDL-C to HDL-C ratio) were also calculated as indicators of cardiovascular risk (16).The following values of both indices were accepted as normal ≤ 4.5 for index I and < 3.0 for index II (17).

Statistics. All data were analyzed for normality using the Shapiro-Wilk test. The Mann-Whitney test was used for comparison of data of COC-users and non-users. Significance of differences was set at $p < 0.05$. Moreover, in both COC-users and non-users the frequency of biochemical variables exceeding the accepted limits was established. All analyses were performed using Statistica for Windows, ver. 12.

RESULTS

The COC-users and non-users did not differ in their physical activity, anthropometric characteristics, daily energy and macronutrient intake (Table 1). Circulating cortisol in COC-users was higher by 114% vs. non-users ($p < 0.001$) (Table 2). There were no between group differences in circulating glucose, insulin, and LDL-C. In contrast, in COC users blood TG, TC and HDL-C were significantly higher in comparison with non-users (by 42.8 %, $p < 0.001$, by 9.5%, $p < 0.02$ and by 17.6%, $p < 0.004$) for TG, TC and HDL-C, respectively. There were no differences between groups in the TC-to-HDL-C ratio (Castelli index I). On the contrary, in COC-users the LDL-C/HDL-C ratio (Castelli index II) was significantly lower in comparison with non-users (by 23.1%, $p < 0.002$).

Table 1. Anthropometric characteristics and dietary habits of active female students using and Non-using combined oral contraceptives

	COC – users, n = 25	COC - non-users, n = 98
Physical activity, MET - min/week	3488.5 ± 1429.7	3773.3.7 ± 1607.7
Age, years	20.9 ± 2.2	21.2 ± 1.7
Weight, kg	60.5 ± 7.9	62.0 ± 9.1
Height, cm	167.2 ± 6.5	168.3 ± 6.3
WC, cm	70.3 ± 5.8	70.9 ± 6.8
Body fat, %	25.4 ± 6.0	24.8 ± 5.9
Body fat, kg	15.4 ± 5.6	15.4 ± 5.8
LBM, kg*	45.1 ± 3.2	46.6 ± 5.1
Energy intake, kcal/day	2009 ± 579	1890 ± 515
Protein, %	15.4 ± 3.2	15.2 ± 3.6
Fat, %	32.7 ± 6.1	33.2 ± 7.9
Carbohydrates, %	51.9 ± 7.0	51.6 ± 7.6ET –

Data in table are presented as Mean ± SD.
Abbreviation: MET: metabolic equivalent; COC: users – students using combined oral contraceptives; COC: non-users – students not using combined oral contraceptives; WC: waist circumference;* LBM: lean body mass
Comparison made using the Mann-Whitney test.

Table 2. Biochemical Variables in Active Female Students Using and Non-using Oral Combined Oral Contraceptives

	COC – users, n = 25	COC - non-users, n = 98
Cortisol, nmol/L [a]	775.6 ± 328.4 a	361.8 ± 80.1
Glucose, mmol/L	4.5 ± 0.3	4.6 ± 0.3
TG, mmol/L	1.0 ± 0.5 a	0.7 ± 0.3
TC, mmol/L	4.6 ± 1.0 b	4.2 ± 0.7
HDL-C, mmol/L	2.0 ± 0.9 c	1.7 ± 0.3
LDL-C, mmol/L	2.1 ± 0.8	2.2 ± 0.5
TC/HDL-C	2.3 ± 0.6	2.5 ± 0.5
LDL-C/HDL-C	1.0 ± 0.5 d	1.3 ± 0.4

Data in table are presented as Mean ± SD.
Mean (standard deviation) for all variables; comparison made using Mann-Whitney test;
Abbreviations: TG: triacylglycerols; TC: total cholesterol; HDL-C: high-density cholesterol, LDL: low-density cholesterol; TC/HDL-C: Castelli index I; LDL-C/HDL-C: Castelli index II;
Ap < 0.001; bp < 0.020; cp < 0.004; dp < 0.002; significantly different vs. COC - non-users

Table 3. Analysis of the Frequency of Abnormal Concentrations of Biochemical Parameters and Calculated Indices of Health Risk in Active COC-user and Non-users

	COC – users, n = 25	COC - non-users, n = 98
Cortisol	100 (25) *, a	none
Glucose, mmol/L	none	1.0 (1)
TG, mmol/L	4.0 (1)	1.0 (1)
TC, mmol/L	25.0 (6) [a]	3.1 (3)
HDL-C, mmol/L	12.0 (3) [b]	3.1 (3)
LDL-C, mmol/L	4.0 (1)	2.0 (2)
TC/HDL-C	none	none
LDL-C/HDL-C	none	none

For abbreviations – see Table 2; * – in brackets - number of subjects.
[a] p < 0.001; [b] p < 0.040 – significantly different vs. COC - Non-users

Table 3 presents the analysis of the frequency of abnormal concentrations of biochemical parameters and calculated indices of health risk in active COC-user and non-users. When circulating biochemical variables in both groups were evaluated with respect to their healthy levels it became clear that COC users are at the risk of unhealthy TC observed in 25.0% of subjects. In contrast, in both groups the frequency of disturbed glucose, TG, and LDL-C did not differ significantly. In contrast, in all participants Castelli index I and II were within the accepted limits.

DISCUSSION

From our study it could be tentatively postulated that physical activity does not prevent changes in circulating lipoproteins observed in response to COC-use. It is worth noting that similar changes in circulating TG, TC and HDL-C were observed in different groups of COC-users whose physical activity was not evaluated (18, 19).These effects are probably due to the detrimental action of elevated cortisol on liver metabolism in COC-users. An early study indicated that elevated cortisol markedly stimulates the activity of 3-hydroxy-3-methyl-glutaryl CoA reductase (HMG-CoA reductase, EC 1.1.1.88), a key regulatory enzyme for TC synthesis (20). Moreover, increased circulating cortisol promotes hepatic TG synthesis and promotes lipolysis in omental fat resulting in increased portal vein free fatty acid delivery to the liver which results in elevated blood TG (21).

On the other hand, in active females COC use significantly increased circulating HDL-C due to ethinylestradiol action which mimics natural 17-ß-estradiol effects on lipoproteins (22).This effect has to be recognized as positive and was also noticed by others in females whose physical activity was not evaluated (23). Additionally, Tikkanen et al. (24) demonstrated that OC use affects mostly the HDL-C2 fraction responsible for protection against atherosclerosis. On the contrary, there are data indicating decreased paraoxonase 1 (EC 3.1.8.1) activity and elevated oxidative stress in OC-users vs. non-users which in turn adversely affects protective HDL-C effects on cardiovascular risk (25-27). Thus, at present it is difficult to determine if elevation in circulating HDL-C in COC-users really means improved protection against cardiovascular disease.

The observed lack of an effect of COC use on circulating LDL-C in our participants is in contrast to other studies (28). However, there are also data indicating no effect of COC on blood LDL-C in participants using levonogestrel-drospirenone pills which were also used by our participants (29). It should be stressed that progestins, the second compound of COC used by our participants, also affect metabolic processes. Shoupe (30) demonstrated that desogestrel decreases glucose tolerance. Moreover, Wynn and Niththananthan (31) found that levonogestrel markedly depressed HDL-C2 and decreased the HDL-C2 to LDL-C ratio and in consequence increased health risk. On the contrary, Barkfeld et al. (32) did not find any effect of progestin-only pills on lipid metabolism. In our study both COC users and non-users did not differ with respect to circulating glucose, however, this does not exclude disturbances in insulin action (33).

However, more precise information concerning COC health effects in active females was provided by analysis of frequency distribution of unhealthy biochemical variables. The only variables which markedly differentiate COC users and non-users are higher incidence of elevated TC and low HDL-C in the former than in the latter. Taking into account similar dietary habits and weekly physical activity in both groups these differences were due to COC use. It should be pointed out that circulating levels of TC, TG and LDL-C are commonly used in medical practice in cardiovascular risk assessment (34). However, there are numerous data suggesting that

ratios of lipoproteins provide more accurate information about health risk (35). Thus, as normal values of both Castelli indexes were observed, COC-users were at low risk of cardiovascular events.

Moreover, taking into account new data suggesting decreased skeletal muscle mass in COC-users (36) our data and no changes in LBM probably suggest that physical activity prevent adverse changes in body composition. However, this issue needs further studies.

CONCLUSION

Our study indicated that in active females COC use affects circulating lipoprotein with pronounced adverse effects on circulating TC but minor changes in other biochemical variables. However, the above-mentioned changes had minor effect on cardiovascular risk.

This was cross-sectional and single-center study with small number of recruited COC-users. However, the latter was the consequence of low use of contraception, but especially modern contraception in Eastern Europe vs. Western countries (37).

APPLICABLE REMARKS

The authors suggest that physical activity may be recommended for COC-users as a simple way to prevent adverse health effects.

FINANCIAL DISCLOSURE

Authors declare no conflict of interests.

ACKNOWLEDGEMENTS

We express our thanks to all students participating in the study.

FUNDING/SUPPORTS

This study was partially supported by the Grant DS-230 from the University of Physical Education in Warsaw.

REFERENCES

1. Rafacho A, Ortsäter H, Nadal A, Quesada I. Glucocorticoid treatment and endocrine pancreas function: implications for glucose homeostasis, insulin resistance and diabetes. *J Endocrinol*. 2014;**223**(3):R49-R62. **doi:** 10.1530/joe-14-0373
2. Edwards KM, Mills PJ. Effects of estrogen versus estrogen and progesterone on cortisol and interleukin-6. *Maturitas*. 2008;**61**(4):330-333. **doi:** 10.1016/j.maturitas.2008.09.024
3. Hertel J, König J, Homuth G, Van der Auwera S, Wittfeld K, Pietzner M, et al. Evidence for Stress-like Alterations in the HPA-Axis in Women Taking Oral Contraceptives. *Sci Rep*. 2017;**7**(1). **doi:** 10.1038/s41598-017-13927-7
4. Asare GA, Santa S, Ngala RA, Asiedu B, Afriyie D, Amoah AG. Effect of hormonal contraceptives on lipid profile and the risk indices for cardiovascular disease in a Ghanaian community. *Int J Womens Health*. 2014;**6**:597-603. **doi:** 10.2147/IJWH.S59852 **pmid:** 24940082
5. Samson ME, Adams SA, Merchant AT, Maxwell WD, Zhang J, Bennett CL, et al. Cardiovascular disease incidence among females in South Carolina by type of oral contraceptives, 2000-2013: a retrospective cohort study. *Arch Gynecol Obstet*. 2016;**294**(5):991-997. **doi:** 10.1007/s00404-016-4143-5 **pmid:** 27402505
6. Roli L, De Vincentis S, Rocchi MBL, Trenti T, De Santis MC, Savino G. Testosterone, cortisol, hGH, and IGF-1 levels in an Italian female elite volleyball team. *Health Sci Rep*. 2018;**1**(4):e32. **doi:** 10.1002/hsr2.32 **pmid:** 30623067
7. Crewther BT, Obminski Z, Cook CJ. Serum cortisol as a moderator of the relationship between serum testosterone and Olympic weightlifting performance in real and simulated competitions. *Biol Sport*. 2018;**35**(3):215-221. **doi:** 10.5114/biolsport.2018.74632 **pmid:** 30449938
8. Melhado-Kimura V, Batista GA, de Souza AL, Silva Dos Santos PN, Alegre SM, Pavin EJ, et al. Hyperinsulinemic-euglycemic clamp over the first year of use of depot-medroxyprogesterone acetate as a contraceptive. *Contraception*. 2018. **doi:** 10.1016/j.contraception.2018.04.003 **pmid:** 29665358
9. Alghadir AH, Gabr SA, Aly FA. The effects of four weeks aerobic training on saliva cortisol and testosterone in young healthy persons. *J Phys Ther Sci*. 2015;**27**(7):2029-2033. **doi:** 10.1589/jpts.27.2029 **pmid:** 26311920
10. Durnin JV, Womersley J. Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. *Br J Nutr*. 1974;**32**(1):77-97. **doi:** 10.1079/bjn19740060 **pmid:** 4843734

11. Sallis JF, Buono M, Roby J, Micale F, Nelson J. Seven-day physical activity recall. *Med Sci Sports Exerc*. 1997;**29**(Suppl 6):89-103.
12. Szponar L, Wolnicka K, Rychlik E. Album of Photographs of Food Products and Dishes; National Food and Nutrition Institute Press: Warsaw, Poland, 2000. Warsaw: National Food and Nutrition Institute; 2000
13. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem*. 1972;**18**(6):499-502. **pmid:** 4337382
14. Bergmann N, Gyntelberg F, Faber J. The appraisal of chronic stress and the development of the metabolic syndrome: a systematic review of prospective cohort studies. *Endocr Connect*. 2014;**3**(2):R55-80. **doi:** 10.1530/EC-14-0031 **pmid:** 24743684
15. Jellinger PS, Handelsman Y, Rosenblit PD, Bloomgarden ZT, Fonseca VA, Garber AJ, et al. American Association of Clinical Endocrinologists and American College of Endocrinology Guidelines for Management of Dyslipidemia and Prevention of Cardiovascular Disease. *Endocr Pract*. 2017;**23**(Suppl 2):1-87. **doi:** 10.4158/EP171764.APPGL **pmid:** 28437620
16. Bhardwaj S, Bhattacharjee J, Bhatnagar M, Tyagi S. Atherogenic index of plasma, castelli risk index and atherogenic coefficient-new parameters in assessing cardiovascular risk. *Int J Pharm Biol Sci*. 2013;**3**(3):359-364. **doi:** 10.1016/j.dsx.2017.03.038 **pmid:** 28404515
17. Millan J, Pinto X, Munoz A, Zuniga M, Rubies-Prat J, Pallardo LF, et al. Lipoprotein ratios: Physiological significance and clinical usefulness in cardiovascular prevention. *Vasc Health Risk Manag*. 2009;**5**(3):757-765. **pmid:** 19774217
18. Guedes JVM, Nunes NR, Ferreira LG, Vilar TG, Pinheiro MB, Domingueti CP. Evaluation of lipid profile, high-sensitivity C-reactive protein and D-dimer in users of oral contraceptives of different types. *J Bras Patol Med Lab*. 2018;**54**(1):14-20.
19. Pettersson-Pablo P, Nilsson TK, Breimer LH, Hurtig-Wennlof A. Body fat percentage is more strongly associated with biomarkers of low-grade inflammation than traditional cardiometabolic risk factors in healthy young adults - the Lifestyle, Biomarkers, and Atherosclerosis study. *Scand J Clin Lab Invest*. 2019;**79**(3):182-187. **doi:** 10.1080/00365513.2019.1576219 **pmid:** 30767573
20. Mitropoulos KA, Balasubramaniam S. The role of glucocorticoids in the regulation of the diurnal rhythm of hepatic beta-hydroxy-beta-methylglutaryl-coenzyme A reductase and cholesterol 7 alpha-hydroxylase. *Biochem J*. 1976;**160**(1):49-55. **doi:** 10.1042/bj1600049 **pmid:** 12745
21. Dolinsky VW, Douglas DN, Lehner R, Vance DE. Regulation of the enzymes of hepatic microsomal triacylglycerol lipolysis and re-esterification by the glucocorticoid dexamethasone. *Biochem J*. 2004;**378**(Pt 3):967-974. **doi:** 10.1042/BJ20031320 **pmid:** 14662008
22. Mumford SL, Schisterman EF, Siega-Riz AM, Browne RW, Gaskins AJ, Trevisan M, et al. A longitudinal study of serum lipoproteins in relation to endogenous reproductive hormones during the menstrual cycle: findings from the BioCycle study. *J Clin Endocrinol Metab*. 2010;**95**(9):E80-85. **doi:** 10.1210/jc.2010-0109 **pmid:** 20534764
23. Fahraeus L, Wallentin L. High density lipoprotein subfractions during oral and cutaneous administration of 17 beta-estradiol to menopausal women. *J Clin Endocrinol Metab*. 1983;**56**(4):797-801. **doi:** 10.1210/jcem-56-4-797 **pmid:** 6833462
24. Tikkanen MJ, Nikkila EA, Kuusi T, Sipinen SU. High density lipoprotein-2 and hepatic lipase: reciprocal changes produced by estrogen and norgestrel. *J Clin Endocrinol Metab*. 1982;**54**(6):1113-1117. **doi:** 10.1210/jcem-54-6-1113 **pmid:** 7076794
25. De Groote D, Perrier d'Hauterive S, Pintiaux A, Balteau B, Gerday C, Claesen J, et al. Effects of oral contraception with ethinylestradiol and drospirenone on oxidative stress in women 18-35 years old. *Contraception*. 2009;**80**(2):187-193. **doi:** 10.1016/j.contraception.2009.02.015 **pmid:** 19631796
26. Kowalska K, Sciskalska M, Bizon A, Sliwinska-Mosson M, Milnerowicz H. Influence of oral contraceptives on lipid profile and paraoxonase and commonly hepatic enzymes activities. *J Clin Lab Anal*. 2018;**32**(1). **doi:** 10.1002/jcla.22194 **pmid:** 28276605
27. Behnaz F, Soltanpoor P, Teymourian H, Tadayon N, Mohseni GR, Ghasemi M. Sympatholytic and Anti-Inflammatory Effects of Ropivacaine and Bupivacaine After Infraclavicular Block in Arterio Venous Fistula Surgery. *Anesth Pain Med*. 2019;**9**(1):e85704. **doi:** 10.5812/aapm.85704 **pmid:** 30881912

28. Berenson AB, Rahman M, Wilkinson G. Effect of injectable and oral contraceptives on serum lipids. *Obstet Gynecol*. 2009;**114**(4):786-794. **doi:** 10.1097/AOG.0b013e3181b76bea **pmid:** 19888036
29. Giribela CR, Consolim-Colombo FM, Nisenbaum MG, Moraes TL, Giribela AH, Baracat EC, et al. Effects of a combined oral contraceptive containing 20 mcg of ethinylestradiol and 3 mg of drospirenone on the blood pressure, renin-angiotensin-aldosterone system, insulin resistance, and androgenic profile of healthy young women. *Gynecol Endocrinol*. 2015;**31**(11):912-915. **doi:** 10.3109/09513590.2015.1062860 **pmid:** 26172927
30. Shoupe D. Effects of desogestrel on carbohydrate metabolism. 1993;**168**(3):1041–1047.
31. Wynn V, Niththyananthan R. The effect of progestins in combined oral contraceptives on serum lipids with special reference to high-density lipoproteins. *Am J Obstet Gynecol*. 1982;**142**(6 Pt 2):766-771. **doi:** 10.1016/s0002-9378(16)32486-3 **pmid:** 6801982
32. Barkfeldt J, Virkkunen A, Dieben T. The effects of two progestogen-only pills containing either desogestrel (75 μg/day) or levonorgestrel (30 μg/day) on lipid metabolism. *Contraception*. 2001;**64**(5):295-299. **doi:** 10.1016/s0010-7824(01)00269-4
33. Ghasemi M, Behnaz F, Hajian H. The Effect of Dexmedetomidine Prescription on Shivering during Operation in the Spinal Anesthesia Procedures of Selective Orthopedic Surgery of the Lower Limb in Addicted Patients. *Anesth Pain Med*. 2018;**8**(2):e63230. **doi:** 10.5812/aapm.63230 **pmid:** 30009149
34. Piepoli MF, Hoes AW, Agewall S, Albus C, Brotons C, Catapano AL, et al. 2016 European Guidelines on cardiovascular disease prevention in clinical practice: The Sixth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of 10 societies and by invited experts)Developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR). *Eur Heart J*. 2016;**37**(29):2315-2381. **doi:** 10.1093/eurheartj/ehw106 **pmid:** 27222591
35. Upadhyay RK. Emerging risk biomarkers in cardiovascular diseases and disorders. *J Lipids*. 2015;**2015**:971453. **doi:** 10.1155/2015/971453 **pmid:** 25949827
36. Suuronen J, Sjoblom S, Tuppurainen M, Honkanen R, Rikkonen T, Kroger H, et al. Effects of ethinyl estradiol-containing oral contraception and other factors on body composition and muscle strength among young healthy females in Finland-A cross-sectional study. *Eur J Obstet Gynecol Reprod Biol*. 2019;**232**:75-81. **doi:** 10.1016/j.ejogrb.2018.11.015 **pmid:** 30502591
37. Fait T, Buryak D, Cirstoiu MM, Luczai E, Janczura R. Needs and preferences of women users of oral contraceptives in selected countries in Central and Eastern Europe. *Drugs Context*. 2018;**7**:212510. **doi:** 10.7573/dic.212510 **pmid:** 29445408