Estradiol valerate in COC has more favorable inflammatory profile than synthetic ethinyl estradiol - a randomized trial

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SHORT TITLE: Estradiol valerate vs synthetic estrogen in COC: inflammation

KEY WORDS: estradiol valerate, ethinyl estradiol, combined contraceptives, hs-CRP, pentraxin 3, COC

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DISCLOSURE STATEMENT: OH serves occasionally on advisory boards for Bayer AG, Gedeon-Richter, Sandoz and Vifor Pharma; and has lectured at educational events organized by these companies. TTP has received honorariums related to lecturing and advisory boards from Merck, Gedeon-Richter, Duodecim, Ajaton Terveys, Roche, Ferring, MSD, Exeltis and Astra Zeneca. TTP also contributed to the clinical trial (ESTETRA, HRA-Pharma, ClinicalTrials.gov Identifier: NCT02817828). Other authors have nothing to disclose.

Abbreviations: BMI, body mass index; COCs, combined oral contraceptives; DNG, dienogest; E2, estradiol; EE, ethinyl estradiol; ER, estrogen receptor; EV, estradiol valerate; GPER, G-protein coupled estrogen receptor; HDL, high-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; NF-ΚB, nuclear factor kappa B; OGTT, oral glucose tolerance test; PTX-3, pentraxin 3; WHR, waist-to-hip ratio

ABSTRACT

CONTEXT: Combined oral contraceptives (COCs) alter inflammatory status and lipid metabolism. Whether different estrogens have different effects is poorly known.

OBJECTIVE: We compared the effects of COCs containing ethinyl estradiol (EE) or estradiol valerate (EV) and dienogest (DNG) with those containing DNG only on inflammation and lipid metabolism.

DESIGN: Randomized, controlled, open-label clinical trial.

SETTING: Two-center study in Helsinki and Oulu University Hospitals.

PARTICIPANTS: Fifty-nine healthy, young, non-smoking women with regular menstrual cycles. Age, BMI and waist-to-hip ratio were comparable in all study groups at the beginning. Fifty-six women completed the study (EV+DNG, n=20; EE+DNG, n=19; DNG only, n=17).

INTERVENTIONS: Nine-week continuous use of COCs containing either EV+DNG or EE+DNG, or DNG only as control.

MAIN OUTCOME MEASURES: Parameters of chronic inflammation (high-sensitivity C-reactive protein, hs-CRP and pentraxin 3, PTX-3) and lipid profile (HDL, LDL, triglycerides and total cholesterol).

RESULTS: Serum hs-CRP increased after 9-week use of EE+DNG (mean change±SD 1.10±2.11 mg/L) compared with EV+DNG (−0.06±0.97 mg/L, p=0.001) or DNG only (0.13±0.68 mg/L, p=0.021). Also, PTX-3 increased in the EE+DNG group compared with EV+DNG and DNG-only groups (p= 0.017 and p=0.003). In the EE+DNG group, HDL and triglycerides increased compared with other groups (HDL: EE+DNG 0.20±0.24 mmol/L vs. EV+DNG 0.02±0.20 mmol/L[p=0.002] vs. DNG 0.02±0.18 mmol/L[p=0.002]; triglycerides: EE+DNG 0.45±0.21 mmol/L vs. EV+DNG 0.18±0.36 mmol/L[p=0.003] vs. DNG 0.06±0.18 mmol/L[p<0.001]).

CONCLUSIONS: EV+DNG and DNG only had a neutral effect on inflammation and lipids, while EE+DNG increased both hs-CRP and PTX-3 levels as well as triglycerides and HDL.

TRIAL REGISTRATION: ClinicalTrials.gov NCT02352090

PRÉCIS

A contraceptive containing estradiol valerate induced less inflammation than ethinyl estradiol containing preparation during 9 weeks' continuous use.

INTRODUCTION

Combined oral contraceptives (COCs) are widely used for contraception and as a treatment for several medical conditions. The marketed preparations include various combinations of estrogen and progestin. Over time, several new progestins have been developed to avoid side effects such as androgen action. Traditionally, most COCs have included ethinyl estradiol (EE), the most common dose being 20–30 µg. EE is a synthetic estrogen, which has an effect on liver protein synthesis 500–600 times greater than that of the natural estrogen, estradiol (E2) (1). In efforts to replace EE with E2 in COCs, the poor endometrial bleeding control of various combinations of E2 and progestins has limited its use. In recent years, new COC combinations containing E2 have been developed, and the combination of estradiol valerate (EV) with dienogest (DNG) has resulted in an acceptable bleeding profile (2).

Even though the health benefits of COCs are clear, studies have shown that the use of COCs may have some adverse short- and long-term metabolic effects. According to previous studies, the use of COC increases the circulating levels of high-density lipoprotein cholesterol (HDL) and triglycerides (3-5) as well as inflammatory markers including high-sensitivity C-reactive protein (hs-CRP) and pentraxin 3 (PTX-3), the latter being known risk factors for cardiovascular diseases (3-7). In fact, a recent study showed that the use of COC was associated with a small but significantly increased risk of cardiovascular diseases and myocardial infarction (8). That study, however, did not include preparations containing bioidentical estrogens. Therefore, it is possible that COCs containing E2 or EV instead of EE may have more beneficial effects on metabolic profile (9, 10). Neutrality of COC in inflammation and lipid metabolism would be beneficial especially for the women in high metabolic risk. Still, as the overall impact of COC depends on the natures of both the estrogen and

progestin components, a strict comparison of metabolic effects between EE and E2 or EV would require the comparison of preparations containing the same progestin. To our knowledge, no previous study has compared combinations of different estrogens with DNG in terms of lipid or inflammatory profiles.

The present study is part of a randomized, controlled clinical trial comparing 9 weeks' continuous use of COCs containing EE+DNG and EV+DNG along with DNG-only preparation. Primary outcome of the trial was changes in glucose metabolism and that entity will be published on its own (11). The aim of the present study was to compare the effects of EE vs. EV combined with DNG on inflammatory markers and lipid metabolism in healthy young women.

MATERIALS AND METHODS

This randomized, controlled, open-label, two-center clinical trial was conducted at the Helsinki and Oulu University Hospitals, Finland, between April 2015 and January 2018. Detailed study protocol has been described in our previous study (11). The independent Ethics Committee of Helsinki University Central Hospital and The Finnish Medicines Agency (FIMEA) approved the study. The Regional Ethics committee of the Northern Ostrobothnia Hospital District was informed of the approval. The study was registered with the Clinical Trials database (identifier code NCT02352090; https://clinicaltrials.gov/) and EU Clinical trials register (EudraCT Number 2014-001243-20; https://www.clinicaltrialsregister.eu). All the subjects signed a written informed consent document. This study was investigator initiated and no commercial sponsorship was received.

The power analysis for the trial was based on glucose metabolism, which was the primary outcome measure of the study. The analysis was calculated using the

decrease in the Matsuda index in response to EE-containing combined contraceptives used in our previous study (5). According to the power analysis, 48 subjects would have been needed to reach the power of 0.8, when the α error was set to a significance level of 0.05.

Subjects

Altogether 77 women volunteered for the study, and after assessment for eligibility 59 healthy Caucasian women were randomized (Fig. 1). All study subjects had regular menstrual cycles and had not used hormonal medication for at least 2 months before entering the study. Exclusion criteria were age >35 years, body mass index (BMI) ≥25 kg/m^2, blood pressure ≥140/90 mmHg, abnormal findings in 2-h oral glucose tolerance test (OGTT) or gynecological ultrasound examination, breastfeeding (minimum wash-out period 3 months prior study), smoking, alcohol or drug abuse, and any contraindication regarding the use of COCs.

Randomization and the study protocol are described in Fig. 1. The randomization list was produced in a 1:1:1 ratio and blocks of six with a web-based randomizer (www.sealedenvelope.com). Research nurses allocated the women to treatment groups according to the randomization list; 48 women were enrolled at Helsinki and 29 at Oulu. The study subjects used one of three hormonal preparations continuously for 9 weeks: EV+DNG 2 mg/2–3 mg (Qlaira®, Bayer AG, Germany), EE+DNG 0.03 mg/2 mg (Valette®, Bayer AG, Germany) or DNG 2 mg (Visanne®, Jenapharm, Bayer AG, Germany). As EV+DNG contraceptive is available only as four-phasic regimen, the amounts of dienogest differed slightly between preparations. Differences were minimized by altering the original packages to match hormonal contents as well as possible, by removing placebo pills and the pills containing only estrogen. Women were evaluated 3 times during the study: at baseline and at 5th and 9th weeks of the

study. Baseline assessments were performed during the first 1–5 days of the menstrual cycle, and the use of study preparations was begun the following day after confirmation of normal baseline OGTT. Women were advised to use a barrier contraception method for a week in cases when the COC was started later than cycle day 2 and during the whole study period in all women randomized to the DNG-only group. After randomization there were two drop-outs in the DNG group after the first appointment, due to general malaise and mood changes, and one drop-out in the EE+DNG group after the second appointment, due to minor non-specific side effects (Fig. 1).

Measurements

Fasting blood samples were collected at baseline and at 5th and 9th weeks of the study to analyze hs-CRP, PTX-3, total cholesterol, low-density lipoprotein cholesterol (LDL), HDL and triglycerides. There were technical difficulties in blood sampling for two subjects during the week 5 visit, leading to missing data for lipid measurements (see Table 1). Samples for PTX-3 measurement at week 5 were collected only in Oulu. Weight and blood pressure were measured at every appointment; waist and hip circumferences were measured at baseline and at the 9-week appointment. Assays for inflammatory markers were not performed for any drop-out cases.

Assays

Analyses of serum hs-CRP were performed at Helsinki University Hospital using the immunoturbidimetric method (Abbott Architect c8000 & reagent Abbott CRP Vario, Abbott, USA), whereas plasma PTX-3 analyses were performed at Oulu University Hospital with ELISA (Human Pentraxin 3/TSG-14 Quantikine ELISA Kit, R&D Systems, USA). Serum measurements for lipids were performed directly after sampling using accredited enzymatic and photometric methods at Helsinki (Abbott

Architect c16000/c8000, Abbott, USA) and Oulu (Advia Chemistry XPT, Siemens, Germany).

Statistics

The Statistical package for the Social Sciences (SPSS) software version 24 was used for statistical analyses. All measurements were analyzed using the hierarchical linear mixed model in which treatment and time were fixed effects, and treatment*time interaction was included in the model to examine whether mean change over time was different between treatments. Compound symmetry covariance structure was used for repeated measures, and the normal distribution assumption was checked using residuals. Missing values were assumed to be completely at random. Logarithmically transformed hs-CRP and PTX-3 were used in statistical analyses due to skewed distribution. Measured hs-CRP values >10 mg/L were presumed to indicate acute infection, and the subjects (n=3) having hs-CRP >10 mg/L (at any time point) were excluded from the hs-CRP and PTX-3 analyses. For the lipid analysis, all subjects except for two drop-out cases were included.

RESULTS

Values for clinical and metabolic characteristics are presented in Table 1. At baseline, the mean age, BMI, waist circumference, waist-to-hip ratio (WHR) and blood pressure were comparable in all study groups. Waist circumference showed a slight decrease during the treatments in all study groups but remained, in general, fairly stable. Systolic blood pressure decreased in EV+DNG and EE+DNG groups during the first 5 weeks but reverted to the baseline level at the 9-week study visit. No change in BMI was observed in any of the study groups throughout the study period.

Markers for systemic low-grade inflammation

The changes in metabolic measurements within study groups are shown in Table 1. In the EE+DNG group, the serum level of hs-CRP increased significantly and remained higher compared with the other study groups throughout the intervention. The difference in hs-CRP at 5 and 9 weeks was significant in the EE+DNG group compared with both EV+DNG and DNG groups, whereas no difference emerged between the EV+DNG and DNG-only groups (see Fig. 2). Pentraxin levels acted similarly to hs-CRP: there was a significant increase within the EE+DNG group, which was also significant compared with the other two groups, in which the levels of PTX-3 remained stable.

Serum lipids

HDL increased significantly at the 5th week of treatment in the EV+DNG and EE+DNG groups and remained elevated at the 9th week visit in the EE+DNG group (Table 1). The increase in HDL was significantly greater in the EE+DNG group compared with both EV+DNG and DNG groups (Fig. 2). Triglycerides increased in both EE+DNG and EV+DNG groups, but the difference was not statistically significant between the EV+DNG and DNG groups. However, the increase in triglycerides was significantly higher in the EE+DNG group compared with the other study groups. Total cholesterol and LDL remained stable during the study in all treatment groups.

DISCUSSION

We observed that the preparation containing EE promoted systemic inflammation and altered lipid metabolism compared with EV-containing preparation or DNG only. The increase in systemic inflammation was evidenced by increased hs-CRP and PTX-3 levels. During the 9-week use of EE+DNG, HDL and triglycerides, but not LDL,

increased within the study group and also compared with other groups. Triglycerides increased in the EV+DNG group, but the change was not significant compared with the DNG-only group. In the DNG-only group, the 9-week treatment did not result in significant changes in serum lipids. The study suggests that COC containing EV has a more beneficial inflammatory profile compared to a preparation containing EE.

The present results show that the use of COC containing EE promotes low-grade inflammation in women, as evidenced by increased levels of circulating hs-CRP and PTX-3. This is in line with earlier studies that have reported an increase in hs-CRP during the use of COC containing EE (3, 4, 6, 7). We have also previously demonstrated that regardless of the route of administration (oral, transdermal, vaginal), EE-containing combined contraceptives increase the serum concentrations of hs-CRP and PTX-3 (5). The complex role of estrogens in inflammatory pathways has been reviewed earlier and the effect seems to differ according to estradiol levels (12). Moreover, EE has a multifold effect on liver protein synthesis compared with natural estrogens (1), although the effects have been mainly focused on hormone-binding globulins, not on inflammatory markers.

CRP is produced mainly in the liver in response to IL-6 (13, 14) and is commonly recognized as a marker of inflammation but it also has an active role in promoting atherosclerosis through different mechanisms (14-17). CRP is also able to activate the complement system through C3 and promote leucocyte adhesion and migration (14, 16). Importantly, clinical data show that hs-CRP concentrations higher than 3.0 mg/L indicate an increased risk for cardiovascular events (18). Therefore, the mean increase of 1.1 mg/L in hs-CRP seen in the EE group in the present study suggest clinical significance. PTX-3, on the other hand, is an acute-phase protein produced by many different tissues, such as endothelial cells, mononuclear phagocytes and

adipocytes, but not hepatocytes (19). It mediates innate immunity by different mechanisms, for example through opsonization and complement activation/inhibition (19). As there was also a significant increase in PTX-3 levels during EE use, EE seems to promote low-grade inflammation beyond liver targeted effects. The mechanism by which EV induces less inflammatory effects than EE warrants further studies using both *in vivo* and *in vitro* setups.

Besides inflammatory changes, we also observed significant changes in circulating triglycerides and HDL concentrations, in line with the findings of previous studies (3-5, 20). Interestingly, EV had a significantly milder effect on these parameters compared to EE. The mean increase of triglycerides in the EE+DNG group was 0.45 mmol/L, compared to 0.18 mmol/L in the EV+DNG group, a result that might have clinical significance. If the changes would prevail also during longer exposure, it may have an atherosclerotic effect over the long term. However, the observed increase in HDL induced by EE may compensate for this risk.

The study has several strengths but also limitations that need to be addressed. The findings provide clues on the metabolic and hormonal alterations and the mechanisms of metabolic actions of these commonly used preparations in young healthy women. We were able to control progestin-related effects with progestin-only preparation and reduce selection bias by randomization and low drop-out rate. Still larger studies are needed to investigate if these effects remain in long-term use and whether the effects are similar in women with higher metabolic risk (obese or premenopausal women, women with polycystic ovary syndrome etc.). In spite of several metabolic alterations a possibility of type II error is still possible, as power calculation for the study was based on changes in glucose metabolism in our previous study (5). As there is not monophasic contraceptive with EV+DNG on the market, the

amounts of DNG differs slightly between preparations. Moreover, as the packaging and contraceptive efficiency of the preparations were different, the setup had to be non-blinded to enable proper counselling considering the lack of contraceptive indication for DNG-only preparation in Finland. In any case, this is the first study comparing the effects of contraceptives containing EE or EV with the same progestin component and progestin effect alone.

CONCLUSION

The present study demonstrates that COC containing EV seems to trigger less metabolic effects compared with preparations containing EE, as evidenced by the unchanged inflammation profile and neutral effect on triglyceride levels in the EV-DNG and DNG-only groups. Conclusions concerning the possible long-term effects of these preparations and the effects in metabolically compromised female populations cannot be drawn from this study, and larger, long-term follow-up studies are required.

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AUTHORS' ROLES

The study was designed by JST and OH in collaboration with TTP and AH. MHK, AH, KL, OH, JST and TTP contributed to the data collection. MHK conducted the statistical analysis and wrote the first draft of the manuscript; all authors contributed to revision and approved the final version of the manuscript. EKK, RKA and JKH shared in discussion and figure drawing. TTP, JST and OH supervised the project.

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CONFLICTS OF INTEREST

This study was investigator initiated by JST and OH, and no commercial sponsorship was received. TTP has received honorariums related to lecturing and advisory boards from Merck, Gedeon-Richter, Duodecim, Ajaton Terveys, Roche, Ferring, MSD, Exeltis and Astra Zeneca. TTP also contributed to the clinical trial (ESTETRA, HRA-Pharma, ClinicalTrials.gov Identifier: NCT02817828). OH serves occasionally on advisory boards for Bayer, Gedeon Richter, HRA-Pharma and Vifor Pharma and has lectured at educational events for Bayer, Gedeon Richter and Sandoz. The other authors confirm having no conflict of interest.

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FIGURE LEGENDS:

Figure 1. (a) Flow chart of the study. Women were randomized to oral EV+DNG, EE+DNG or DNG-only treatments for 9 weeks.
*Drop-out due to minor non-specific side-effects.
** One drop-out due to general malaise; one drop-out due to mood changes.
(b) Hormonal contents of the preparations used in the study. Treatments were used for three consecutive cycles, i.e. 3×21 days.

Figure 2. Changes in blood measurements during trial. Data are represented as mean+SD. The SPSS hierarchical linear mixed model was used for statistical analysis.
*Change within the group: $p<0.05$.

Table 1. Clinical characteristics and biochemical measurements of the study subjects during the 9-week trial.

		A) EV+DNG					B) EE+DNG					C) DNG				
		N	Mean	±	SD	p-value*	N	Mean	±	SD	p-value*	N	Mean	±	SD	p-value*
Age. years		20	24.1	±	3.5		20	25.7	±	3.7		17	24.0	±	3.7	
hs-CRP. mg/L	week 0	18	0.62	±	0.51		18	0.95	±	0.86		17	0.65	±	0.57	
	week 5	18	0.82	±	1.28		18	**2.11**	**±**	**2.08**	**0.001**	17	1.14	±	1.40	
	week 9	18	0.56	±	0.91		18	**2.05**	**±**	**2.05**	**0.001**	17	0.79	±	0.84	
PTX-3. ng/mL	week 0	18	0.81	±	0.53		18	0.59	±	0.24		15	0.62	±	0.20	
	week 5	8	1.25	±	0.69		8	**0.94**	**±**	**0.51**	**0.041**	7	0.71	±	0.30	
	week 9	18	0.80	±	0.53		18	**0.81**	**±**	**0.44**	**0.012**	15	0.59	±	0.26	
Total Cholesterol. mmol/L	week 0	20	3.97	±	0.72		20	4.13	±	0.57		17	4.07	±	0.45	
	week 5	18	3.97	±	0.71		20	4.22	±	0.69		17	4.14	±	0.70	
	week 9	20	3.81	±	0.62		19	4.25	±	0.77		17	4.18	±	0.63	
LDL. mmol/L	week 0	20	2.19	±	0.65		20	2.15	±	0.60		17	2.39	±	0.55	
	week 5	18	2.17	±	0.80		20	2.03	±	0.55		17	2.46	±	0.66	
	week 9	20	2.05	±	0.58		19	1.98	±	0.63		17	2.41	±	0.64	
HDL. mmol/L	week 0	20	1.61	±	0.35		20	1.79	±	0.38		17	1.62	±	0.30	
	week 5	18	**1.71**	**±**	**0.27**	**0.022**	20	**1.95**	**±**	**0.40**	**0.004**	17	1.59	±	0.39	
	week 9	20	1.59	±	0.34		19	**2.00**	**±**	**0.47**	**0.001**	17	1.60	±	0.32	
Triglycerides. mmol/L	week 0	20	0.69	±	0.25		20	0.68	±	0.17		17	0.65	±	0.17	
	week 5	18	0.74	±	0.22		20	**1.08**	**±**	**0.30**	**<0.001**	17	0.78	±	0.22	
	week 9	20	**0.87**	**±**	**0.38**	**0.011**	19	**1.14**	**±**	**0.28**	**<0.001**	17	0.71	±	0.22	
Weight. kg	week 0	20	61.44	±	5.80		20	62.71	±	5.01		17	57.98	±	7.10	
	week 5	20	60.88	±	6.09		20	62.48	±	4.88		17	**57.29**	**±**	**6.97**	**0.002**
	week 9	20	61.03	±	6.19		19	63.14	±	4.38		17	**57.41**	**±**	**7.17**	**0.010**
BMI. kg/m2	week 0	20	22.45	±	1.61		20	22.99	±	1.90		17	21.87	±	1.94	
	week 5	20	22.24	±	1.70		20	22.94	±	1.91		17	21.61	±	1.89	
	week 9	20	22.29	±	1.65		19	23.06	±	1.92		17	21.92	±	2.50	
Waist. cm	week 0	20	73.55	±	5.18		20	75.78	±	4.62		17	73.76	±	4.87	
	week 9	20	**73.04**	**±**	**5.21**	**0.030**	19	**74.55**	**±**	**3.97**	**0.007**	17	**72.60**	**±**	**5.34**	**0.003**
Hip. cm	week 0	20	96.65	±	3.83		20	96.78	±	4.78		17	94.18	±	5.78	
	week 9	20	**96.07**	**±**	**4.16**	**0.028**	19	97.79	±	4.60		17	93.53	±	6.02	
WHR	week 0	20	0.76	±	0.04		20	0.78	±	0.05		17	0.78	±	0.03	
	week 9	20	0.76	±	0.04		19	0.76	±	0.05		17	0.78	±	0.03	
sRR. mmHg	week 0	20	118.60	±	7.31		20	117.00	±	9.35		17	111.94	±	9.73	
	week 5	20	**111.60**	**±**	**7.96**	**<0.001**	20	**111.10**	**±**	**8.82**	**0.013**	17	108.41	±	9.69	
	week 9	20	115.85	±	9.31		19	114.53	±	9.38		17	108.53	±	9.27	
dRR. mmHg	week 0	20	75.05	±	6.96		20	72.45	±	8.13		17	72.53	±	7.38	
	week 5	20	**71.60**	**±**	**7.94**	**0.005**	20	71.10	±	7.17		17	71.65	±	7.19	
	week 9	20	73.45	±	8.99		19	72.58	±	7.08		17	70.00	±	7.37	

Missing data are due to drop-out after second study visit, difficulties in sample collection or hs-CRP >10 mg/L (due to presumed infection). The SPSS hierarchical linear mixed model was used for statistical analysis. Units mmol/L can be converted to mg/dL by multiplying values with following conversion factors: total cholesterol, HDL and LDL by 38.67 and triglycerides by 88.57.
*Values with p <0.05 compared to the baseline are marked in bold.

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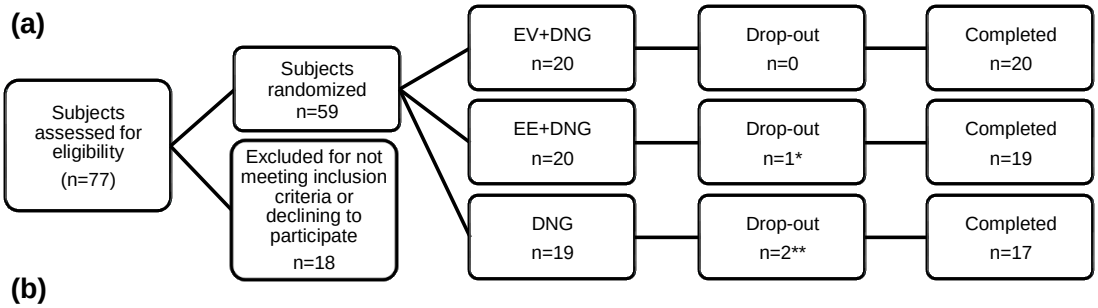
399 **Figure 1**.

(a)

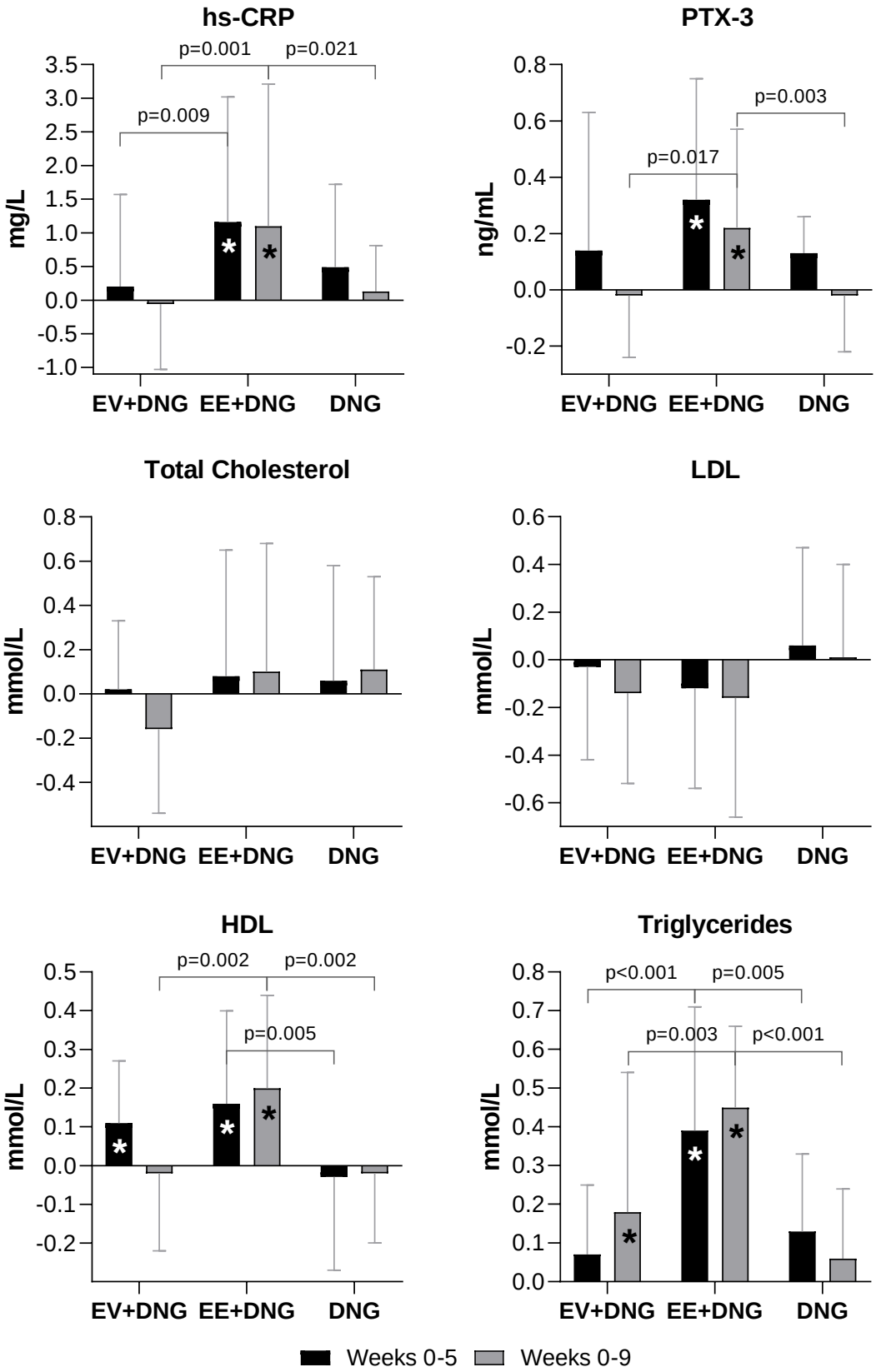


(b)

Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
EV+DNG	EV 2mg + DNG 2 mg					EV 2mg + DNG 3mg															
EE+DNG	EE 0.03 mg + DNG 2mg																				
DNG	DNG 2mg																				

400

401 **Figure 2.**



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