Synthetic Hormones and Clot Formation

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Abstract: Combined oral contraceptives (COCs), colloquially referred to as "the pill," have been regarded as a medical breakthrough, as they have improved the lives of countless women, from simplifying family planning to the treatment of acne, endometriosis, polycystic ovarian syndrome, and dysmenorrhea. Unfortunately, COC usage has been associated with an increased occurrence of venous thrombosis and therefore a systemic hypercoagulable state in susceptible females. Here we discuss the health risks of COC usage and use viscoelastic and morphological techniques to investigate the effect of different COC constituents on clot formation, particularly fibrin network packaging and whole blood viscoelasticity. Viscoelastic properties of whole blood showed gender-specific changes while morphological alterations were person-specific, regardless of gender. Using scanning electron microscopy and thromboelastography provides great insight regarding fibrin packaging and the development of a hypercoagulable state in high-risk individuals. We proposed a three-step approach where (1) an individual's coagulation profile baseline is determined, after which (2) the "ideal" combination of constituents is prescribed, and (3) the coagulation profile of the individual is monitored to assess possible risk of thrombosis. Only in following such an individualized patient-oriented approach will we be able to avoid the many health issues due to COC usage in susceptible females.

Key words: combined oral contraceptives, hypercoagulability, venous thrombosismorphology, viscoelasticity

Introduction

Combined oral contraceptives (COCs), colloquially referred to as "the pill," have been regarded as a medical breakthrough since their debut in May of 1960. Now, more than 50 years later, COC popularity has grown worldwide to such an extent that 13% of females of reproductive age use a synthetic hormone contraceptive, whereas in developed countries such as the United States, Europe, and the United Kingdom these numbers are almost double (Department of Economic and Social Affairs, 2013; Daniels et al., 2014). In this paper, we discuss these COCs and how their usage may affect the health of female user. Although the importance of COCs and their place in family planning and other medical uses is by no means opposed, we highlight the importance of prescribing COCs with care and we show the impact they may have as a trigger for a hypercoagulable state in susceptible users. We propose an individualized patient-oriented approach, where a one-plan-for-all should not be followed, but the correct COC prescribed based on a baseline coagulation test and also in high-risk individuals on a simple laboratory investigation where the different COC constituents should be analyzed by looking at their activity on blood from the individual. The next paragraphs will first give a background regarding COCs.

All COCs contain estrogen in combination with a specific individualized progestin, as per patent. The progestin can be administered in two ways: either at the same dose daily or at varying doses that mimic the phases of progesterone production throughout the normal menstrual cycle; the first is referred to as monophasic while the latter is referred to as either biphasic or triphasic progestins (Petitti, 2003). Supplementary Table 1 indicates some of the synthetic estrogens and progestins commonly used in COCs.

Supplementary Table 1

Enovid® was the first COC introduced to the public in 1960; the first case of venous thrombosis (VT) associated with COC use was reported shortly after Jordan (1961). Subsequently, the focus over the past half a century has been to establish the relationship between COC use and the occurrence of VT (van Hylckama Vlieg & Middeldorp, 2011). Thus far researchers have established that

- *COC use is unquestionably associated with an increased risk of VT*: in 1990s, several studies approximated that COC use is associated with a two to fourfold increased risk of VT (Thorogood et al., 1992; Vandenbroucke et al., 1994; Farmer et al., 1997). More recently, a meta-analysis corroborated the initial findings that COC use significantly increases the risk of VT, but added that the generation of COC, the type of outcome, as well as the presence of a genetic mutation indeed play a role in

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the strength of this association, with odds ratio's ranging from 3 to 5 (Manzoli et al., 2012).

- *By lowering the dose of ethinylestradiol (EE) the associated risk of VT is reduced* (Lidegaard et al., 2002): a higher risk of VT is associated with COCs that contain 30 μg of EE than those containing 20 μg of EE (Lidegaard et al., 2009; van Hylckama Vlieg et al., 2009).
- *COC containing third or fourth-generation progestins are associated with double the risk of VT compared with COCs containing second-generation progestins* (Jick & Hernandez, 2011; Lidegaard et al., 2011; Parkin et al., 2011).
- *Drosperinone (DRSP)-containing COCs increase the risk of deep vein thrombosis and pulmonary embolism*: however, it is not associated with increased risk of transient ischemic attack or cerebrovascular attack compared with COCs containing second and third-generation progestins (Gronich et al., 2011).
- *The ten most commonly used COCs increase the risk of first VT*: levenorgestrel (LNG) in combination with 20 μg EE poses the lowest risk, whereas DRSP combined with 30 μg EE, cyproterone acetate combined with 35 μg EE, and LNG combined with 50 μg EE poses the greatest risk of VT (Stegeman, 2013).
- *COC use after a thrombotic event is associated with a threefold increased risk of recurrent VT*: however, depending on the EE dose, the specific progestin involved, and whether the COC is monophasic or multiphasic (Stegeman, 2013).

COCs are not only used for contraceptive purposes. Although pregnancy prevention is the leading motivation for COC use (accounting for 86%), almost 60% of COC users rely on this method for its additional non-contraceptive health benefits. The latest statistics indicate that 1.5 million women (14% of all COC users) rely on "the pill" for exclusively non-contraceptive purposes (Jones, 2011). Some of the main non-contraceptive benefits of COCs include the treatment of acne vulgaris (Huber & Walch, 2006; Koltun et al., 2008, 2011; Harper, 2009; Arowojolu et al., 2012; Kim et al., 2015), endometriosis (Mabrouk et al., 2012; Morotti et al., 2014; Khan, 2015; Tafi et al., 2015; Zorbas et al., 2015), polycystic ovarian syndrome (PCOS) (Mathur et al., 2008; Bird et al., 2013; Georgescu, 2015), as well as menstrual cramps and dysmenorrhea (Proctor et al., 2001; Al-Jefout & Nawaiseh, 2016; Graziottin, 2015; Witjes et al., 2015).

The considerable predicament we face with regard to COC and VT risk can be attributed to the vast number of women using COCs (Stegeman et al., 2013), not only for contraceptive purposes but also for its various non-contraceptive benefits. As such a vast number of females between the age of 15 and 44 years are using a drug therapy that is known to increase the risk of VT, a condition associated with increased disability and mortality, it is essential to determine the effect of COCs on blood clot formation. With the considerable evidence suggesting a real problem regarding a general hypercoagulable state during COC usage, that was eluded to in the previous paragraphs, we now address the following research question in this paper:

- What is the effect of different COC constituents on the viscoelastic properties of whole blood (WB)?
- What is the effect of different COC constituents on the viscoelastic properties of fibrin clots?
- What morphological changes to the fibrin network are associated with each COC constituent?
- How do changes to viscoelasticity and morphology as mentioned above relate to increased VT risk associated with COC use and how can it be prevented?

Materials and Methods

Viscoelastic and morphological techniques were employed to investigate the effect of different COC constituents on clot formation. The conversion of soluble fibrinogen to insoluble fibrin is fundamental to clot formation and ultimately wound healing as it acts as a plug to seal an injury site. For this reason, we mainly focused on the effect of different COC constituents on fibrin network formation, although WB viscoelasticity was also investigated.

Sample Collection

Blood was collected from six healthy male and six healthy female participants. All participants were between the age of 18 and 30 years. Ethical clearance was obtained (University of Pretoria Ethics Committee, ethics number 154/2014) before blood collection. All participants were nonsmokers and had no history of thrombotic disease or used any chronic medication known to interfere with coagulation. No aspirin or aspirin analogues were used by any of the individuals before sampling.

Citrate tubes containing 0.5 mL of 3.8% sodium citrate were used to draw blood for both viscoelastic and morphological investigations. WB contained in the citrate tubes was used for viscoelastic analysis (explained below in detail) before the remaining WB was centrifuged for 10 min at 1,250 × g to separate the plasma from the blood cells. The supernatant plasma was then transferred to Eppendorf tubes and subsequently centrifuged for a second time at 1,250 × g for 5 min to obtain platelet poor plasma (PPP). The PPP samples were then frozen at −80°C for no less than 48 h. Before viscoelastic and morphological procedures the samples were removed from the freezer to thaw and reach room temperature.

Sample Preparation

The fresh WB as well as thawed PPP samples were incubated for 15 min at 37°C with different constituents of COCs before viscoelastic and morphological analysis. The concentrations were precisely that of the specific estrogen or progestin as found in COCs. The COC constituents and concentrations are described in Table 1.

Table 1. Combined Oral Contraceptive (COC) Constituents Incubated.

COC constituent	Abbreviation	Concentration in COC
Ethinylestradiol	EE	0.02 and 0.05 mg[a]
Levenorgestrel	LNG	0.05 and 0.125 mg[a]
Drosperinone	DRSP	3 mg
Medroxyprogesterone acetate[b]	MPA	400 mg
Norgestrel	NG	0.5 mg

[a]EE and LNG are used at different concentrations. For this study the lowest and highest concentrations were used.
[b]MPA is an injectable progestin. Although it not a COC, it is one of the most commonly used progestin that is injected directly into the blood. Therefore it was deemed essential to include it in this study.

Viscoelastic Analysis of WB and PPP

For both fresh WB (procedure performed right after blood collection) as well as the thawed PPP (procedures performed at least 48 h after collection and freezing) the same procedure was followed: 320 μL of either fresh WB or thawed PPP was incubated for 15 min at 37°C with 20 μL each of the different COC constituents at concentrations that in final concentration with the WB or PPP was similar to that found in COC. The WB/PPP-constituent mixtures were then individually placed in a thromboelastography (TEG) cup and 20 μL $CaCl_2$ was added to reverse the effect of the sodium citrate. The samples were loaded in a Thromboelastograph 5000 Hemostasis Analyzer System (Haemonetics) for viscoelastic analysis.

GraphPad Prism 5 was employed to perform a Wilcoxon's signed-rank test for all statistical analysis, with a p-value of ≤ 0.05 considered as significant.

Morphological Analysis of Fibrin Networks Prepared from PPP

For morphological analysis of fibrin network PPP was incubated with different COC constituents in the same manner as described for the viscoelastic procedures. Standard scanning electron microscopy (SEM) preparation was followed after the incubation step.

For each of the PPP-constituent mixtures (see Table 1 for concentrations) 10 μL of the sample were placed on an individual round glass coverslip and mixed with 5 μL thrombin. Thrombin, provided by the South African National Blood Service, was prepared in biological buffer containing 0.2% serum albumin with a final concentration of 20 U/mL before addition to the samples.

After thoroughly combining the PPP-constituent mixture and thrombin, the samples were placed on a dampened filter paper within an airtight container to create a humid environment for a 10 min incubation period at 37°C. After the incubation step, the samples were washed for 20 min in buffer solution (0.075 M sodium potassium phosphate buffer solution, pH = 7.4) with a plate shaker to remove any blood proteins that could possibly be trapped within the fibrin network.

Following the wash step samples underwent primary fixation with formaldehyde (4%) for 30 min, rinsed three times with buffer solution for 5 min each. Secondary fixation with osmium tetraoxide for 15 min was followed before the samples were rinsed again as explained above. Samples were then dehydrated with a series of ethanol concentrations of 30, 50, 70, 90%, and 100% ethanol for three times for 5 min each. Finally, the samples were submerged in hexamethyldisilazane for 30 min and air-dried in a flow hood. Samples were then mounted on an aluminum platform and coated with carbon. The coated samples were examined with a Zeiss high-resolution SEM (Zeiss) at 1 kV.

Results

Viscoelasticity

TEG was used to investigate the viscosity and elastic properties of blood clots. Supplementary Table 2 indicates the seven parameters measured with TEG.

Supplementary Table 2

Fresh WB and thawed PPP samples were analyzed for viscoelastic properties without and with the addition of different COC constituents (see Supplementary Tables 3 and 4). Wilcoxon's signed-rank test showed that thawed PPP samples did not change any of the TEG parameters, only the fresh WB samples showed changes to five of the seven TEG parameters with no changes observed with regards to the overall stability (maximum amplitude) or strength (total thrombus generation: TTG) of the clot. Table 2 shows the parameters that were influenced for WB clots.

Supplementary Tables 3 and 4

Table 2. Viscoelastic Parameters Influenced by the Combined Oral Contraceptive Constituents.

WB	Control	EE1	EE2	DRSP	LNG1	LNG2	NG	MPA
R			↓	↓▼	↓	↓	↓▼	↓
K				↓	↓	↓	↓	↓
α						↑		
MRTG						↑		
TMRTG				↓	↓	↓	↓	↓

Arrows (↓ and ↑) indicate the effect on male samples. Triangles (▼) indicate the effect on female samples (both significantly decreasing the values). Shaded areas indicate the decrease in sample values for male and female samples pooled together.
WB, whole blood; EE, ethinylestradiol; DRSP, drosperinone; LNG, levenorgestrel; NG, norgestrel; MPA, medroxyprogesterone acetate; MRTG, maximum rate of thrombus generation; TMRTG, time to maximum rate of thrombus generation.

The reaction time (R), kinetics (K), angle (α) along with the maximum rate of thrombus generation (MRTG) and time to MRTG (TMRTG) were influenced by EE at its highest concentration of 0.05 mg and the progestins tested. EE at its lowest concentration of 0.02 mg did not influence any of the viscoelastic parameters.

Arrows indicate viscoelastic changes in male plasma. Triangles indicate the decrease in reaction time value for females. The shaded areas indicate the combined effect when gender differences were excluded. As only two progestins decrease one viscoelastic parameter, namely the reaction time, but the combined effect where gender differences were not taken into consideration follows the same trend as that of males, we can assume that the male differences were the major contributors to the combined effect seen in Table 2. We can therefore deduce that changes to viscoelasticity were gender-specific alterations.

Decreased reaction time, relating to quicker clot formation, was seen for DRSP and norgestrel (NG) for females, whereas all constituents except EE at the lowest concentration of 0.02 mg decreased the time of clot formation in males denoting that these hormones all caused the WB clot to form quicker compared with the control. The kinetics of WB clot formation was also decreased for all the progestins but not for EE. This was only true for males. The progestins also decreased the TMRTG, which is closely associated with the kinetics of the clot, similar to that of the kinetics with only the male WB influenced. LNG at the highest concentration of 0.125 mg not only decreased the reaction time, kinetics, and TMRTG, but also increased the thrombin burst and MRTG.

Morphology

The various effects of the different COC constituents of fibrin network morphology is shown in Figures 1 and 2. Figure 1 is representative of alterations seen in male plasma (three individual males shown) and Figure 2 is representative of alterations seen in female plasma (three individual females shown).

The different COC constituents investigated had unique effects on fibrin network morphology of each participant (Figs. 1, 2 represent six different individuals, Fig. 1 shows three males while Fig. 2 shows three females). Some of the alterations (to varying degrees) observed include

- The formation of dense matted deposits (DMDs) as indicated with an asterisk.
- Decreased fibrin fiber diameter (increased incidence) as indicated with thin white arrows.
- Coiled fibers as indicated with a thick white arrow.

However, the most fascinating observation was that in certain cases the fibrin network morphology was not altered in any way, i.e. the fibrin network had the same evenly dispersed taught arrangement characteristic of a typical fibrin network from a healthy individual (marked with a star in the corner of the micrographs). Contrary to the viscoelastic findings, we can assume from these micrographs that COC constituents tested bring about person-specific or individualized changes not dependent on gender.

Indicated on the micrographs (Figs. 1, 2) are also changes to specific viscoelastic parameters, i.e. increased values for MRTG and TTG along with decreased values for TMRTG which are specifically related to hypercoagulable fibrin clot formation. It should be noted that these increases for MRTG and TTG, and also the decrease for TMRTG, were not statistically significant, but show small alterations when compared with the individual's baseline/control values. No specific trend could be distinguished; however, the following should be noted:

- It appears as if changes were more pronounced for females (49%) than for males (30%).
- For males overall, there was no specific correlation between morphological alterations and viscoelastic changes (see Table 3 for percentage increased MRTG and TTG as well as percentage decreased for TMRTG) with 57% of micrographs showing changes to the mentioned parameters.
- Male 3 with EE2 showed no morphological or viscoelastic alterations, although when LNG2 was added no morphological alterations were visible but TTG was increased, and NG showed morphological changes with no associated viscoelastic alterations.
- Females also showed no specific correlation between morphological alterations and viscoelastic changes (see Table 3 for percentage increase of MRTG and TTG as well as percentage decrease for TMRTG), although they were more pronounced than the males with 81% of the micrographs showing changes to the specific parameters.
- Female 3 with DRSP showed no morphological alterations, although MRTG and TTG was increased and when LNG2 was added no morphological changes were visualized although TTG was increased.

This non-specific SEM-TEG correlation again points to person-specific or individualized changes when the person's own TEG values serve as the baseline.

Discussion

Main Findings

Viscoelasticity revealed gender-specific changes to WB clot formation. This could possibly be explained by known gender-specific changes in erythrocyte concentration. Due to menstruation females of reproductive age have lower red blood cell counts compared with age-matched males (Rushton et al., 2001). Blood viscosity has a positive correlation to erythrocyte concentration (Filatova et al., 2015) therefore male blood is more viscous than that of females. The only difference between WB and PPP is the presence of erythrocytes and platelets. As all blood coagulation factors that are present in WB remain within PPP after centrifugation we speculate that the gender-specific changes we

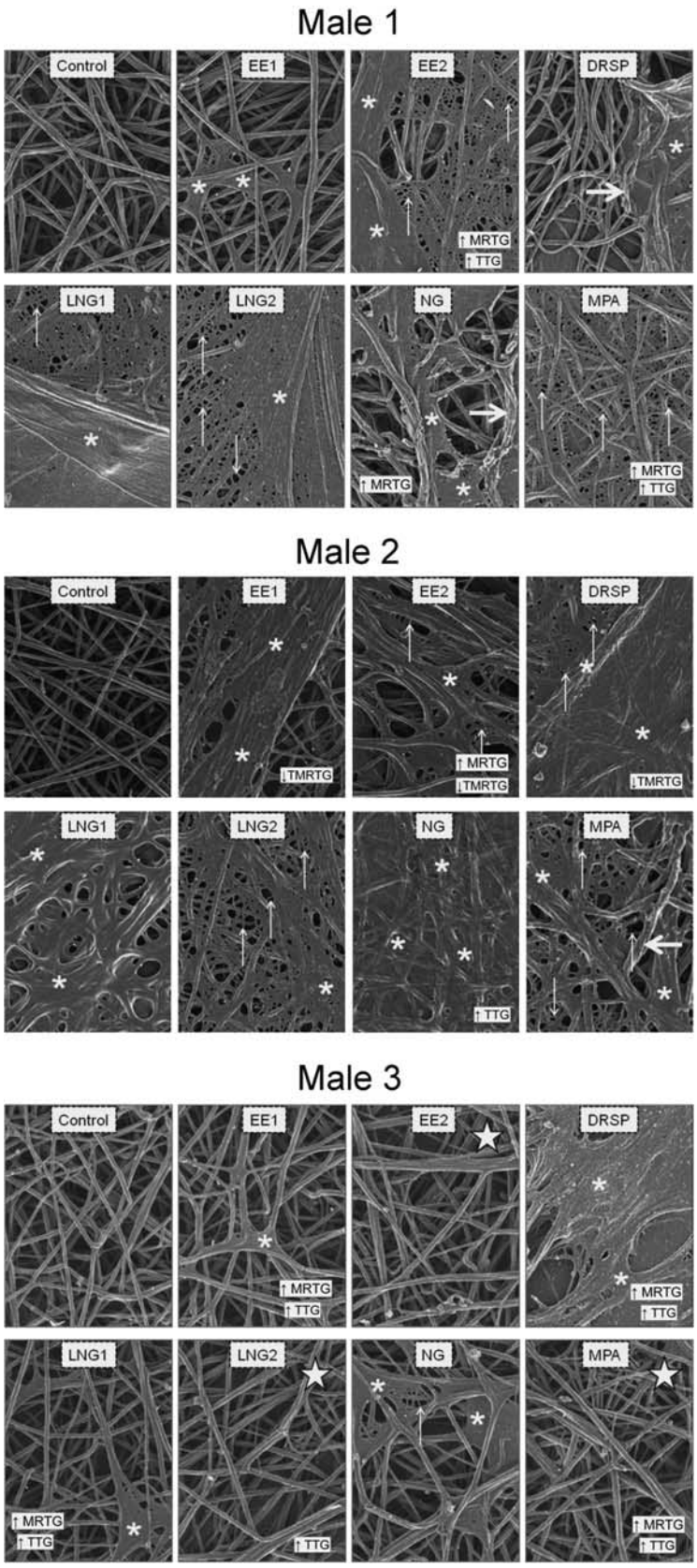

Figure 1. Alterations to fibrin networks observed with the addition of different combined oral contraceptive constituents to male plasma. Asterisk indicates dense matted deposit formation; thin white arrow indicates fibrin fibers with decreased diameter; thick white arrow indicates coiled fibers. EE, ethinylestradiol; DRSP, drosperinone; MRTG, maximum rate of thrombus generation; LNG, levenorgestrel; NG, norgestrel; MPA, medroxyprogesterone acetate.

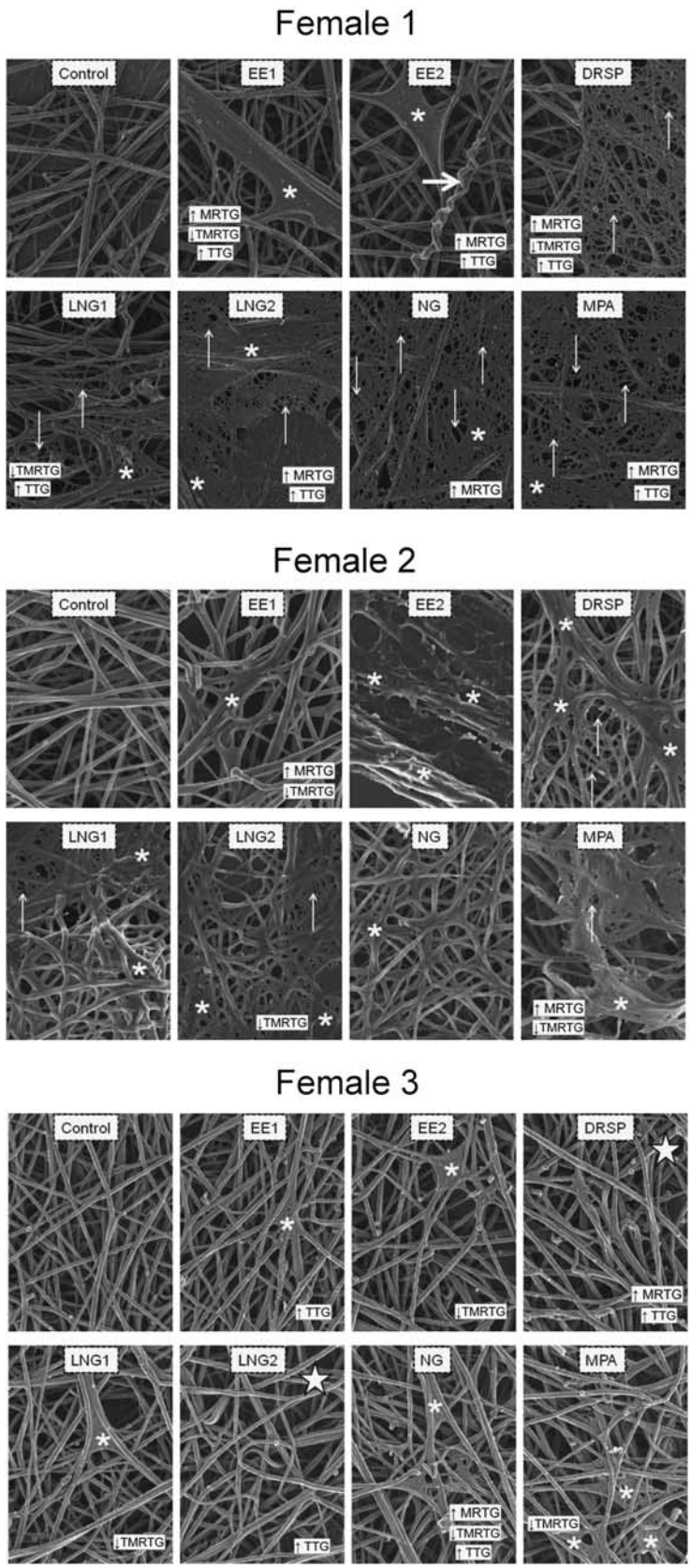

Figure 2. Alterations to fibrin networks observed with the addition of different combined oral contraceptive constituents to female plasma. Asterisk indicates dense matted deposit formation; thin white arrow indicates fibrin fibers with decreased diameter; thick white arrow indicates coiled fibers. EE, ethinylestradiol; DRSP, drosperinone; MRTG, maximum rate of thrombus generation; TMRTG, time to maximum rate of thrombus generation; LNG, levenorgestrel; NG, norgestrel; MPA, medroxyprogesterone acetate.

Table 3. Changes to Specific Viscoelastic Parameters (%) Correlating to Morphological Alterations for Males and Females.

	Males (%)	Females (%)
Increased MRTG	38	52
Decreased TMRTG	14	48
Increased TTG	38	48

MRTG, maximum rate of thrombus generation; TMRTG, time to maximum rate of thrombus generation.

observed in this study could be attributed to erythrocyte and/or platelet influence. Future studies are crucial to identify the specific cause of the gender-specific viscoelastic changes.

The addition of EE at 0.05 mg and all progestins tested resulted in WB clots that formed at a quicker rate and with greater amplification. LNG at 0.125 mg additionally increased the rate of fibrin build up and maximum rate of clot growth. LNG at 0.125 mg therefore follows a trend that is indicative of hypercoagulability; however, it did not influence the clot strength and stability in the same manner.

The viscoelastic properties of fibrin clots with the addition of the constituents remained same as that of control samples. However, morphological alterations were seen with SEM. As the changes to fibrin network morphology were person-specific (unlike the gender-specific trend seen with the WB viscoelastic analysis) it is possible that these changes are negated when values are grouped together with viscoelastic methods. It is only when the person's own TEG values are used as baseline that subtle changes to the mentioned parameters are revealed. SEM is a very sensitive and precise technique that provides great detail. Therefore these results can also indicate that these constituents bring about such subtle changes to fibrin network formation that it can only be visualized with SEM and not as readily be detected with viscoelastic methods that are dependent on previous determined reference ranges.

The presence of DMDs decreased fibrin diameter and coiled fibers are all indicative of altered fibrin formation. DMDs are closely associated with hypercoagulability. Fibrin fiber formation influences the rate at which a fibrin clot can be dissolved. Denser thickened masses are not lysed as readily as dispersed fibers (Bucay et al., 2015). The same holds true for fibers that deviate from the normal structure. Thinner fibers also have decreased degradation potential as the amount of fibrin substrate is directly proportional to the lysis capacity (Collet et al., 2000). Therefore the studied constituents have a hypercoagulable and/or hypofibrinolytic effect on fibrin clot formation. This confirms and explains the increased risk of VT associated with COCs. Not only is the tendency to form clots increased with these constituents, but the ability of the clots to be lysed is impaired.

Strengths and Limitations

Viscoelastic and morphological techniques, specifically TEG and SEM, provide great detail pertaining to alterations in clot formation. As we are facing a dilemma with regards to the vast amount of females using COCs (not only for family planning but also for various other non-contraceptives uses, and that from a very young age) and subsequent VT risk our findings show the importance of a new approach to prescribing COCs, which is in line with the National Institute of Health's (NIH's) individualized patient-centered precision medicine approach (Collins & Varmus, 2015).

Although the sample size and specific constituents were limited in this study, it should be noted that sample collection was done at random therefore ensuring a basic representative population of healthy individuals who did not smoke or have any inflammatory or hypercoagulable condition. This representative population again falls in line with the NIH's approach (Collins & Varmus, 2015) to individualized patient-centered precision medicine. Morphological analysis has been regarded as a time-consuming technique, but as it provides such accurate detail pertaining to individualized hematological changes as exhibited in our current work it should be regarded as a crucial part in maintaining female health.

Interpretation

Although COC use is closely related to increased risk of VT, not all females will suffer a thrombotic event. SEM revealed that in some cases the fibrin network was not influenced by specific constituents. This novel finding provides us with a possible solution to the significant COC and VT risk predicament we face (see Supplementary Fig. 1).

Supplementary Figure 1

We propose a three-step approach. Step 1: before prescribing a female any COC (for either contraceptive or non-contraceptive purposes) a blood sample should be taken to determine the individual's personal coagulation profile baseline. Step 2: by adding the different estrogen and progestin constituents available (not only the specific constituents investigated in this manuscript) it can be determined which constituents have either a hypercoagulable and/or hypofibrinolytic effect compared with the individual's baseline and can thus be avoided. The constituents that do not influence the fibrin network morphology or viscoelastic properties of the individual's baseline can then be prescribed. Step 3: once the "ideal" combination of constituents have been determined and prescribed, the individual should be monitored for a 3-month period to track any changes to the coagulation profile.

Sensitive viscoelastic and morphological techniques such as TEG and SEM, respectively, are the best methods to establish an individual's coagulation profile baseline and any changes to that profile after COC use. This method of testing

is in line with a preventative and individualized patient-centered precision medicine approach recently suggested by NIH (Collins & Varmus, 2015).

Conclusion

COCs have improved the lives of countless women from simplifying family planning to assisting in the treatment of acne, endometriosis, PCOS, and dysmenorrhea. It is, however, essential to ensure the health of all females using COCs by decreasing the VT risk associated with COC use. As the focus is on preventative and individualized patient-centered precision medicine, an "umbrella-approach" cannot be followed as each female needs to be assessed individually to determine her risk of VT. We proposed a three-step approach where (1) an individual's coagulation profile baseline is determined, after which (2) the "ideal" combination of constituents is prescribed, and (3) the coagulation profile of the individual is monitored for a 3-month period to assess any possible risk of thrombosis. Only in following such an individualized patient-oriented approach will we be able to avoid the many health issues due to COC usage in susceptible females.

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Supplementary Material

Table S1. Estrogens and progestins commonly used in COCs. Adapted from (4)

Estrogens		
Used in first COC, not commonly used		Mestranol
Most commonly used estrogen		Ethinyl estradiol (EE)
Progestins		
First generation	Estranes derived from testosterone	Norethynodrel
	Pregnanes derived from 17-OH progesterone	Medroxyprogesterone acetate (MPA)
Second generation	Gonanes derived from testosterone	Levonorgestrel (LNG)
		Norgestrel (NG)
Third generation	Gonane (Levonorgestrel) derivatives	Desogestrol
		Gestodene
Fourth generation	Non ethylated estranes:	Drospirenone (DRSP)

Table S2. Viscoelastic parameters measured.

Parameter	**Unit of measurement**	**Description**
Reaction time (R)	Minutes	Initiation time
Kinetics (K)	Minutes	Amplification
Angle (α)	Angle in degrees	Thrombin burst
Maximal amplitude (MA)	mm	Overall stability of the clot
Maximum rate of thrombus formation (MRTG)	Dyn.cm-2.s-1	The maximum velocity of clot growth
Time to maximum rate of thrombus formation (TMRTG)	Minutes	Time interval observed before maximum speed of the clot growth
Total thrombus generation (TTG)	Dyn.s-1	Clot strength

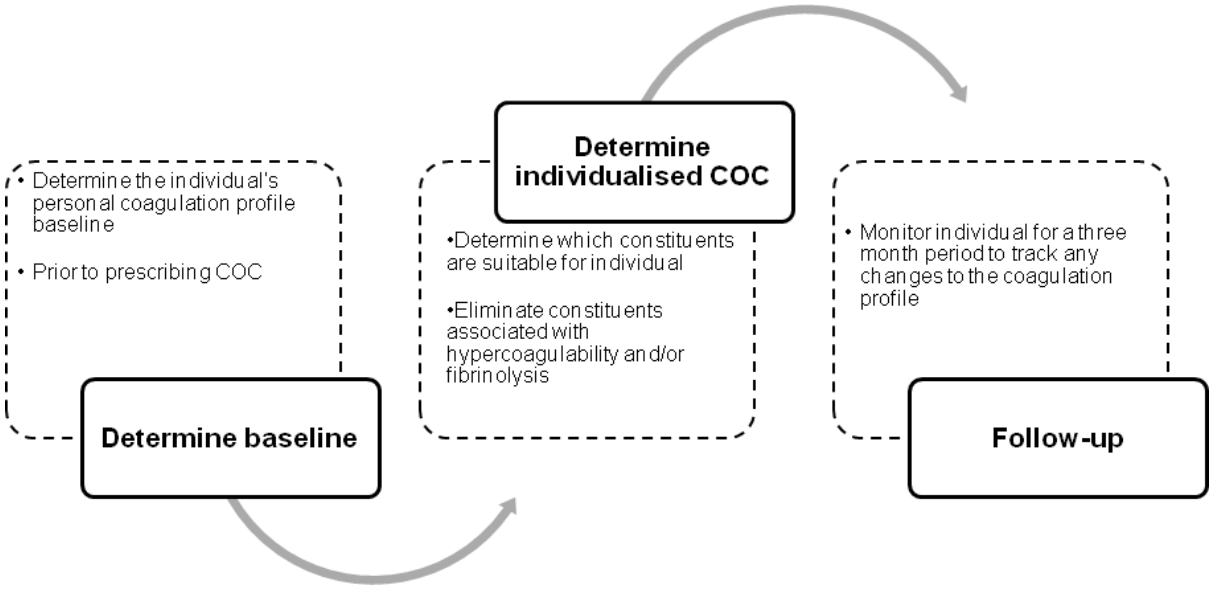
Table S3. TEG values for fresh WB samples without and with added constituents.

			C	EE1	EE2	DR	LE1	LE2	MX	NO
R	M	Median	9.20	8.80	7.15	8.55	8.40	9.30	8.20	10.10
		Stdev	2.75	3.64	4.26	1.60	4.28	6.93	3.24	5.42
	F	Median	9.00	6.70	9.45	6.80	5.15	7.70	8.20	8.20
		Stdev	1.76	1.96	2.75	2.55	2.47	1.69	2.09	1.66
	C	Median	9.20	7.60	9.15	7.50	6.90	8.25	8.20	8.80
		Stdev	2.21	2.82	3.51	2.05	3.88	5.12	2.62	4.03
K	M	Median	4.00	3.35	2.20	3.95	3.15	4.45	3.30	2.60
		Stdev	4.36	6.46	2.50	2.60	2.11	2.01	2.72	3.76
	F	Median	5.45	3.75	2.80	5.10	3.35	3.60	3.35	4.10
		Stdev	2.68	2.81	2.84	6.44	2.74	1.35	1.54	2.55
	C	Median	4.75	3.45	2.80	4.25	3.15	4.05	3.30	3.20
		Stdev	3.47	4.78	2.55	4.87	2.34	1.63	2.12	2.98
α	M	Median	60.05	59.60	63.50	62.85	59.20	54.15	58.10	63.90
		Stdev	7.79	5.97	17.10	9.00	7.36	13.49	9.85	11.88
	F	Median	64.40	62.95	64.45	66.00	60.70	57.90	60.15	62.45
		Stdev	7.28	9.76	10.18	11.85	9.44	10.24	11.65	9.44
	C	Median	60.65	61.80	64.30	63.55	59.20	57.90	58.10	63.70
		Stdev	7.32	7.76	13.81	10.14	8.38	11.47	10.33	10.42
MA	M	Median	29.00	25.90	31.85	25.15	25.50	24.85	30.10	35.80
		Stdev	7.94	6.29	13.42	8.38	3.75	2.52	13.17	4.61
	F	Median	26.60	28.50	29.35	27.10	26.45	30.55	27.45	24.25
		Stdev	4.93	6.91	3.01	10.15	8.52	4.44	6.59	11.72
	C	Median	26.60	26.20	29.95	25.15	26.45	27.00	28.90	34.70
		Stdev	6.43	6.24	9.28	8.91	6.47	3.92	10.18	9.71
MRTG	M	Median	4.01	4.12	5.53	4.50	4.35	3.33	5.66	6.43
		Stdev	3.86	2.05	2.94	1.78	1.55	2.48	5.33	2.32
	F	Median	3.89	4.14	5.41	4.03	3.72	3.63	4.38	3.60
		Stdev	1.66	4.44	2.42	4.23	2.64	0.36	1.77	5.76
	C	Median	3.89	4.14	5.41	4.50	4.13	3.52	4.59	5.12
		Stdev	2.88	3.47	2.60	3.12	2.10	1.72	3.98	4.34
TMRTG	M	Median	11.50	11.09	9.50	10.67	10.63	11.84	10.35	13.58
		Stdev	2.96	3.09	6.53	2.66	4.96	7.72	3.75	9.18
	F	Median	10.21	9.08	11.05	7.96	7.80	10.96	9.79	10.13
		Stdev	2.05	2.45	3.30	3.18	2.46	2.04	3.79	1.89
	C	Median	10.96	10.25	10.88	9.55	9.00	10.96	10.25	10.67
		Stdev	2.44	2.74	4.95	2.93	4.37	5.64	3.61	6.83
TTG	M	Median	209.96	175.23	233.02	168.58	171.67	165.77	214.03	280.15
		Stdev	79.36	70.60	114.25	93.34	34.36	23.17	204.27	63.05
	F	Median	181.73	180.47	210.41	200.22	180.87	220.74	194.30	148.27
		Stdev	44.60	68.81	28.95	108.67	114.84	45.87	86.00	144.87
	C	Median	181.73	175.23	216.25	168.58	180.78	185.23	204.33	265.77
		Stdev	63.15	66.58	79.59	97.20	83.97	40.01	153.58	117.63

Table S4. TEG values for thawed PPP samples without and with added constituents

			C	EE1	EE2	DR	LE1	LE2	MX	NO
R	M	Median	8.25	6.90	5.55	5.40	4.95	4.50	3.85	4.25
		Stdev	2.34	1.64	1.96	1.26	1.84	1.97	2.26	2.03
	F	Median	9.05	6.70	7.15	6.15	7.00	6.85	6.05	7.20
		Stdev	1.42	1.02	0.88	1.20	2.61	2.39	1.74	2.61
	C	Median	8.70	6.75	6.65	5.95	5.35	6.30	4.70	6.05
		Stdev	1.85	1.32	1.54	1.29	2.36	2.44	2.08	2.53
K	M	Median	5.10	4.90	4.35	3.55	3.40	3.15	3.45	3.30
		Stdev	2.19	1.23	0.76	0.94	0.91	0.44	1.24	0.82
	F	Median	4.30	3.15	3.70	3.35	3.40	3.45	4.25	3.65
		Stdev	2.20	1.09	2.87	0.80	1.67	1.92	1.47	1.37
	C	Median	4.60	3.65	4.20	3.45	3.40	3.30	3.65	3.55
		Stdev	2.18	1.47	2.00	0.87	1.29	1.40	1.32	1.12
α	M	Median	49.00	51.20	51.90	57.55	56.25	58.80	58.10	59.50
		Stdev	4.40	4.06	6.62	10.83	5.06	3.29	9.52	6.74
	F	Median	48.75	59.45	58.60	59.85	56.85	55.65	54.90	54.95
		Stdev	10.08	7.41	8.28	6.82	8.98	9.27	8.10	8.40
	C	Median	48.75	55.65	56.05	57.95	56.25	56.40	55.45	56.40
		Stdev	7.67	8.12	8.13	9.21	6.98	6.70	8.43	7.43
MA	M	Median	50.15	47.15	52.50	48.95	51.70	53.55	54.20	52.30
		Stdev	7.91	6.55	5.23	6.84	8.56	3.30	7.97	3.09
	F	Median	52.20	53.15	46.30	54.80	49.65	48.70	51.20	52.15
		Stdev	7.11	7.91	17.13	6.73	10.03	6.43	9.55	7.94
	C	Median	51.35	51.45	51.40	51.00	50.90	53.00	52.50	52.30
		Stdev	7.18	7.76	12.66	7.08	8.95	5.28	8.62	5.76
MRTG	M	Median	2.48	2.78	3.38	3.56	3.72	4.65	3.92	3.75
		Stdev	0.88	0.74	0.30	1.58	1.00	1.03	1.30	1.11
	F	Median	3.07	3.56	3.23	3.74	3.53	3.58	3.30	3.41
		Stdev	1.81	2.63	2.89	1.29	3.10	1.90	1.94	2.29
	C	Median	2.75	3.38	3.38	3.61	3.60	4.04	3.49	3.58
		Stdev	1.42	2.08	2.02	1.38	2.26	1.56	1.59	1.72
TMRTG	M	Median	13.34	13.05	11.25	8.96	7.54	7.59	7.29	7.33
		Stdev	4.23	2.95	1.49	2.68	2.55	2.05	3.13	3.13
	F	Median	12.79	9.21	9.67	10.25	10.38	11.33	9.79	11.17
		Stdev	2.76	1.40	1.67	1.87	3.69	4.30	2.73	3.66
	C	Median	13.29	11.38	10.29	9.63	8.29	8.09	9.00	9.96
		Stdev	3.47	2.74	1.69	2.25	3.31	3.72	2.93	3.65
TTG	M	Median	501.60	447.43	552.83	480.77	552.51	577.51	593.14	550.30
		Stdev	167.48	140.31	140.08	145.27	182.22	81.33	174.77	76.51
	F	Median	552.03	565.99	417.41	604.54	527.01	556.36	529.72	555.28
		Stdev	142.03	276.29	394.81	199.54	318.51	117.40	265.72	199.79
	C	Median	528.08	531.74	528.75	522.08	536.64	567.56	552.61	550.30
		Stdev	148.36	227.79	284.89	179.77	257.44	98.47	223.59	145.73

Figure S1


Determine baseline
• Determine the individual's personal coagulation profile baseline
• Prior to prescribing COC
Determine individualised COC
•Determine which constituents are suitable for individual
•Eliminate constituents associated with hypercoagulability and/or fibrinolysis
Follow-up
• Monitor individual for a three month period to track any changes to the coagulation profile