CHAPTER 8

Discussion

Worldwide, hormonal contraceptives have had such an unmistakably positive impact on female healthcare throughout the last six decades that their omission is unthinkable. Elevation of the risks of venous thromboembolism and arterial disease has so far been their most feared side effect. The incidence of cardiovascular disease among young healthy women is very low, and the risk of steroid-induced cardiovascular disease in women without any other risk factors is far lower than the risk of VTE during pregnancy [1-3]. However, the possibility of being struck by thrombo-embolic events which could even result in death, in a productive and healthy phase of their lives has been troubling users of these contraceptives as well as health care professionals involved. In consequence, many women are hesitant to use hormonal contraceptives, despite having valid indications.

Possible causes were studied extensively, preparations with more favourable risk profiles were developed by lowering the oestrogen content of the pills and by developing less androgenic progestins to prevent adverse effects that androgenic progestins exerted on, for example lipid metabolism [4]. Unfortunately, preparations containing the less-androgenic progestins, also referred to as the third-generation pills, seemed to be associated with a higher risk of VTE. In 1990's the attrition of the susceptible subject or 'the starter effect' was described as women were found to have significantly higher VTE risk within the first year of COC use [5;6]. Later, it became apparent that this effect applies to carriers of hereditary thrombophilia as well as non-carriers, and was not restricted to the third-generation preparations [7]. Meanwhile, the pharmaceutical companies kept developing new oral contraceptives and routes of administration for contraceptive steroids in search for the ideal preparation that would provide excellent contraception, cycle control and alleviation of cycle-related problems, without additional risk of VTE. In addition biomarkers were sought that would reflect the risk involved with every hormonal contraceptive, so the risks could be estimated during development of each preparation, and taken into account by the authorities in the process of drug approval. Also exposing thousands of women to additional risks of unknown extent in large post-marketing studies to provide risk estimates for each novel preparation would become redundant.

VENOUS THROMBO-EMBOLISM

Among many biomarkers that have been studied in the context of steroid-induced VTE are sex-hormone binding globulin (SHBG) and extrinsic APC resistance (according to Rosing). The steroid-induced increases in SHBG have been shown to correlate with the risk of VTE as calculated in epidemiologic studies [8]. However, how SHBG is linked to the risk is unclear. Extrinsic APC resistance is a functional test that measures resistance to activated protein C. And although an increased value does suggest a tendency towards hypercoagulability, it cannot be considered indicator of future disease, except in presence of factor V Leiden mutation. Hence, the only way to get an idea of what cardiovascular risks a novel preparation could involve, has been comparing the pattern of changes it exerts on variables that could be relevant to the risk, to that of a CHC with a known cardiovascular risk profile. This methodology has been recommended by EMEA [9] and was used in Chapters 2 and 3 of this thesis. We compared the effects of a vaginal ring combining ethinyl estradiol (EE) and the non-androgenic Nestorone®, with a combined oral contraceptive (COC) containing EE and levonorgestrel on SHBG, haemostasis variables and angiotensinogen. We included angiotensinogen as a measure of the impact of route of delivery of EE on the hepatic protein synthesis. The effect of the studied CHCs was similar on angiotensinogen levels, however SHBG, factor VII total and the extrinsic APC resistance were elevated more, and protein S was reduced to a greater extent in users of the vaginal ring compared to those who used the COC. The differential effects on SHBG, factor VII total and the extrinsic APC resistance and protein S reflect the difference in androgenicity of progestins employed. Furthermore, EE did not affect the hepatic protein synthesis less vigorously when given vaginally as both treatments affected angiotensinogen (Chapter 3) and fibrinogen equally. The synthesis of both proteins is known to be modified by EE, but not by androgenicity of progestins [10-12]. The effects of the vaginal ring delivering Nestorone® and EE on haemostasis parameters and SHBG resemble those exerted by third-generation COCs. This means that the risk of VTE for the users of these preparations might be comparable as well. According to Lidegaard and colleagues who reported on a large national cohort study conducted in Denmark, the odds ratio for VTE for users of COCs containing 30-40 µg EE combined with desogestrel, gestodene and drosperinone were 6.6, 6.2 and 6.4 compared to non-users [13]. The same study reported an OR of 2.9 for users of COCs containing the same dose of EE combined with levonorgestrel.

According to European Medicines Agency's guideline on clinical investigation of steroid contraceptives in women which came into effect in 2005, new hormonal contraceptives should be investigated for their effect on prothrombin fragment 1+2, APC resistance (ETP-based and APTT-based), factors II, VII, VIII, D-dimer, antithrombin, proteins C and S and SHBG to assess their potential for affecting the risk of venous thromboembolism [9]. It leaves, however, undecided which fractions of protein S (total or free), FVII (total or activated) and antithrombin (antigen or activity) are to be studied. In absence of a consensus on this issue, the guideline however does not advise to study all fractions which leaves studies measuring different fraction incomparable. Furthermore, the guideline recommends to compare new preparations to an oral contraceptive containing 30 µg EE and 150 µg LNG or DSG. However comparing absolute data or even changes from baseline between studies is hardly possible due to utilization of different assays in different studies. For example, there are three different assays for measuring factor VII total: factor VII antigen, the clotting assay (activity) and the chromogenic total FVII assay. For a COC containing 30 µg EE and 150 µg LNG, the change from baseline of FVII total measured using the mentioned assays have been reported 7.7% [14], -14.7% [15] and 9% [16], respectively. The study that found 7.7% change from baseline for FVII antigen, reported -8.6% CFB for FVII clotting assay for the same treatment [14].

Probably the most significant disabling factor is that none of the parameters investigated in this context, either within or outside the EMEA guideline, has been shown to relate causally to the elevated risk of VTE associated with the use of combined hormonal contraceptives, in individuals who are not genetically predisposed through mutations causing a hypercaoagulable state. In part, this might be due to a combination of changes in the haemostasis being responsible for the change in risk rather than alteration in one parameter. Hence EMEA

guideline is not more than a protocol for screening new preparations for abnormal effects, not knowing which degree of abnormality of which parameters actually matters. In Chapters 5 and 6, discriminant analysis (DA) is introduced for the purpose of pattern recognition between CHCs containing EE combined with androgenic and less-androgenic progestins, assuming the latter affects the VTE risk to a greater extent. This method seems useful in differentiating between treatments. However, the results depend upon parameters introduced to the analysis. Consequently, parameters that best differentiate between treatments are not necessarily the ones responsible for the main effect on the risk of VTE or arterial disease. So far, no study on CHCs has obtained data on all parameters that could possibly affect the risk of cardiovascular disease, and studies described in this thesis are no exception in that aspect. Thus, effects on SHBG which proved to be the most discriminative factor in our set of data might not per se have a causal relationship with modification of VTE risk in users of CHCs, although the two seem to be strongly associated. SHBG is simply very sensitive to the net oestrogenicity of hormonal contraceptives and differentiates between them accordingly. So far, no biological mechanism has been proposed that might include SHBG in the haemostasis cascade. The question that remains pertinent is whether oestrogenicity is a proxy for risk. If it is, it would leave SHBG as a potential marker, but raise the question which haemostasis variables are oestrogen-sensitive and show a large change possibly entering a risk range. DA also pointed out FVII, protein S and plasminogen as highly discriminating parameters. As these do participate in the process of coagulation, they might be relevant in the mechanism of steroid-induced risk modification and therefore deserve to be studied more extensively in this context.

Other investigators have also been trying to find associations combinations of parameters and the risk of VTE. Raps et al measured free TFPI, free protein S and SHBG levels and APC resistance in users of different hormonal contraceptives in two different studies and attempted to relate them to epidemiologic data on VTE among users of the concerning contraceptives [17;18]. They claimed that free plasma TFPI and free protein S levels are negatively, and the SHBG concentration and APC resistance ratio positively associated with the risk of VTE. However, this association was not true for all hormonal preparations they investigated. For EE 35 µg / cyproterone acetate (CPA) 2 mg, the mean free TFPI was 2.51 ng/ml with a relative risk (RR) of 6.8 compared to non-users, while 2.91 ng/ml for EE 30 µg / DSG 150 µg OC with a RR of 7.3. A comparable discrepancy was seen for the mean free protein S levels in relation to their relative risk of VTE for the same COCs. Furthermore, the mean free protein S for EE 30 µg / LNG 150 µg was 0.32 IU/dl versus 0.28 IU/dl, for non-users and LNG -IUD users. The relative risk associated with the COC was 3.6 versus 1.0 and 0.3 for non-users and the users of LNG-IUD, respectively [15]. Obviously, one can argue that the effects on the mentioned markers as well as the change in risk of VTE are associated with the net oestrogenicity of the preparations used. Still, it does not point out the effects on markers as determinants of VTE risk. The authors also proposed the achieved levels of SHBG as a surrogate marker for steroid-induced VTE, as Odlind et al did for the change in SHBG levels in 1995 [8]. More specifically, appointing SHBG a surrogate marker in absence of any evidence of its involvement in the pathophysiology of VTE is at least doubtful. SHBG is synthetized in the liver and its synthesis is elevated by EE. The effect of EE on SHBG synthesis is opposed by androgenic progestins. Since it is not unthinkable that future progestins might alter haemostasis and thus the risk of VTE through specific characteristics other than opposing the oestrogenic effect on hepatic protein synthesis, measuring just SHBG to estimate the risk will not suffice.

The main reason for the lack of knowledge about the precise path of causality in case of steroid-induced VTE, is that the relevant clinical endpoints (e.g. deep venous thrombosis and pulmonary embolism) have never prospectively been investigated concurrently with haemostatic variables. Only in such a setting meaningful correlations can be made between the clinical endpoint and haemostatic changes in (later on) affected individuals. Such a study should include a large number of women, as the incidence of VTE among young women even when using CHCs is very low. Furthermore, the higher incidence of VTE (1:1000) among those who will be using contraceptive steroids for the first time in their lives, also known as the starter effect, should be taken into account. Conducting such a study will be a highly challenging undertaking, both financially and organisationally, but in the long run will probably be more efficient and effective than strategies used to this date.

ARTERIAL DISEASE

Use of CHCs alters lipid metabolism depending on the androgenicity of the progestin employed and has been shown to mildly affect carbohydrate metabolism towards insulin resistance as well [19-24]. It also results in increased levels of fibrinogen, D-dimer and C-reactive protein (CRP), and can lead to clinically insignificant elevation of arterial blood pressure [25]. In Chapter 3, we showed the effects of the contraceptive vaginal ring delivering EE and the non-androgenic Nestorone® versus the COC containing EE and the androgenic levonorgestrel on arterial blood pressure and lipid metabolism. In this study, the diastolic blood pressure in COC users was elevated just enough to reach significance. However, the measurements were all within the normal range for young females. With regard to the lipid metabolism, the vaginal ring elevated the high-density lipoprotein (HDL) and reduced the low-density lipoprotein (LDL) levels whereas COC achieved quite the opposite. Triglyceride levels were more elevated in users of the ring than the COC. This is in line with earlier findings. Less-androgenic progestins have been reported to increase triglyceride levels significantly when combined with EE [26;27], and even more extensively than COCs containing levonorgestrel [19;22;28]. It is well-established that individuals with endogenous hypertriglyceridemia are at high risk of cardiovascular events due to enhanced atherosclerosis. Still, in case of hormonal contraceptive use the underlying metabolic derangement might be more relevant to the risk than the mere elevation of levels [29]. Enhanced synthesis of triglycerides in COC users has been reported previously, but the rate of triglyceride catabolism was unchanged or increased [30]. This is in contrast to decreased triglyceride catabolism in endogenous hypertriglyceridemia [31]. Consequently, steroid-induced elevation in triglycerides might not be a risk factor for future arterial disease. This is in line with HDL being the only lipid included in the Reynold risk score developed for healthy, non-diabetic women and based on age, sex, systolic blood pressure, total cholesterol, HDL, high-sensitivity CRP (hsCRP), smoking and

parental history of myocardial infarction [32]. This formula fits better than the Framingham risk score in predicting the risk of cardiovascular disease in the following 10 years. The Framingham risk score includes diabetes mellitus instead of parental history of myocardial infarction and does not include hsCRP [33].

For now, there is not enough evidence to support that steroid-induced changes in lipid metabolism impose the same risk as elevated LDL and triglycerides or low HDL levels do among the general population or in specific metabolic diseases. It has been shown that the steroid-induced increase in LDL levels is accompanied by a qualitative change in LDL particles. Manning and colleagues found these to be smaller and less atherogenic in cynomolgus monkeys exposed to EE and LNG [34]. There is also evidence from animal and in vitro studies that LDL uptake is enhanced with treatment by EE/DSG due to increased expression and activity of LDL receptors [35]. This is in line with the latest meta-analysis on risk of myocardial infarction and stroke in users of different CHCs[36]. According to this analysis, the pooled odds ratio (OR) for myocardial infarction is 2.9, 2.1 and 1.8 for users of first-, second- and third-generation COCs, respectively. Only the ORs of the first and third-generation preparations were significantly different. Odds ratios for the risk of ischemic stroke were 2.6, 1.9 and 1.9 for the first-, second- and third-generation COCs. The increase in risk of ischemic stroke was significantly higher for users of the first-generation than the second- and the third-generation COCs. It is not clear whether the oestrogen dose or the type of progestin is responsible for the reported difference. However, this meta-analysis shows that the remarkable differences in lipid profile associated with different androgenicity of the progestins did not translate into different risk patterns.

Other factors that might be relevant to the modification of risk of arterial disease in CHC users are plasma concentrations of fibrinogen, D-dimer and C-reactive protein. These generally are known to be elevated in users of CHCs, independent of the associated progestin. For CRP, there are however exceptions to this rule. Two studies found significantly greater increments of CRP for EE combined with less-androgenic progestins. Doring and colleagues compared effects of COCs containing the less-androgenic desogestrel and gestodene with other COCs within MONICA Ausburg survey [37]. They found more pronounced increases in CRP and fibrinogen in women treated with the COCs containing the less-androgenic progestins. The second study is described in Chapter 4 of this thesis where significantly greater elevation of CRP was seen in users of the vaginal ring delivering Nestorone® and EE compared to the COC containing LNG and EE. In this study, treatments affected fibrinogen and D-dimer to the same extent. Notably, The highest CRP values belonged to the users with the lowest baseline CRP. Considering we did not find any evidence of inflammation of the vaginal wall or the cervix in subjects involved, the difference in CRP could only be explained by a steroid-induced response, either inflammatory or non-inflammatory, to either EE or Nestorone®. Since both treatments contained EE, and the hepatic impact of EE for the treatments was equal as seen in their effect on angiotensinogen (Chapter 3), again, two explanations are possible. The differential effects on CRP are either due to characteristics of Nestorone® or to differences in users responsiveness to EE or Nestorone®. We noticed that COC users in the lowest tertile of CRP show the same pattern of change, thereby the latter possibility appears more plausible.

So far, no evidence is at hand for a steroid-induced systemic inflammation response being responsible for elevated CRP in CHC users. In a study conducted by van Rooijen en colleagues, the elevation of CRP in COC users was not accompanied by a response in other indicators of inflammation such as IL-6 and tumour necrosis factor (TNF) alpha [38]. In another study lower soluble IL-6 receptor (sIL-6R) concentrations were found during pill intake compared to the pill-free week in women who had been using COCs for at least 3 months [39]. IL-6 exerts its pro-inflammatory effects through binding to sIL-6R. In the same study IL-6 levels were not different during and on pause from pill intake. Interestingly, production of sIL-6R by neutrophils can be enhanced to 3-fold when CRP is added in concentrations seen in acute phase response (50 µg/ml) [40]. Under the same conditions CRP has been shown to increase shedding of the membrane-bound IL-6 receptors as well. This way an initial elevation of CRP through non-inflammatory processes like induction of hepatic protein synthesis, could theoretically initiate an inflammatory chain reaction. However, such high CRP levels have never been reported for CHC use. Eventually, the elevated CRP in CHC users, and its larger increase in some users of the vaginal ring in the study described in Chapter 4 of this thesis can at this moment not be attributed to an induced inflammatory state, but rather a direct effect on the hepatic protein synthesis.

Effects on fibrinogen and D-dimer have been addressed in many studies on CHC use. Fibrinogen is dose-dependently increased by EE and is not affected by the progestin type [41-43]. D-dimer also has been shown to be elevated in CHC users. However, this effect seems to be depending on neither EE dose nor progestin type employed [41-43]. Despite some earlier reports on their involvement in the risk of arterial disease, fibrinogen and D-dimer are not included in the Reynolds risk score developed to predict the risk of future cardiovascular events in healthy women [32].

EXTENDED REGIMEN

So far, studies investigating the effects of COCs on lipid and glucose metabolism and on haemostasis have not found any evidence of a modified risk profile of arterial disease or venous thromboembolism in users of extended regimens compared to those who take COCs according to the classic 21-days-on, 7-days-off sequence [44-48].

As mentioned earlier in this chapter, ethinyl estradiol is known to elevate fibrinogen levels in COC users in a dose-dependent manner [41-43]. Progestins have no modulating effect on this process. In our study presented in Chapter 7, effects of a continuous regimen of oral EE 20 µg and LNG 90 µg were compared to those of the cyclically used EE 20 µg combined with LNG 100 µg. Fibrinogen levels of users of the continuous COC did not rise between cycles 7 and 13, and were comparable to those measured in users of the cyclically taken COC. Therefore, accumulation of EE after continuous use of 1 year was ruled out. As different doses of LNG were in this study associated with different regimens, no conclusion could be drawn on its possible accumulation in association with extended regimen.

Since skipping pill-free days have gained popularity, the 'mismatch theory' has been re-introduced into discussion on risk of thrombo-embolic events . According to this theory, the most relevant factor in steroid-induced VTE risk is not merely the extent of alterations in different haemostasis variables, but the asynchronous course of change in pro- and anti-thrombotic effects while starting and pausing COC intake each cycle. As different variables peak and drop at different rates, the net thrombotic effect could at some points be greater than the net anti-thrombotic effect and so, haemostasis could be off balance [49].

However of speculative nature, one could suggest that extended regimen could improve the risk profile associated with the cyclically used COCs by skipping the pill-free days, by maintaining a new pro- / anti-thrombotic balance inflicted by the COC. This effect however would only be observed in epidemiological studies and will take large numbers of users to stand out.

The effects of continuous use of 20 µg EE and 90 µg of LNG during one year did not have a significantly different effect on carbohydrate or bone metabolism than the traditional 21-days-on 7-days-off regimen of 20 µg EE and 100 µg LNG, while total cholesterol and LDL were affected unfavourably by the continuously used COC. As argued in Chapter 7, the 11% increase in LDL found for the continuous COC falls within the range that earlier studies have reported for the cyclically used COC that was used as comparator in our same study, and will therefore be unlikely to have differential long-term consequences for the risk of arterial disease. Interestingly, smokers seem to show a different pattern of change in LDL than non-smokers, namely a decrease versus an increase in LDL, respectively. This differential effect is probably the cause of the variability in effects (3.2 to 16.9% increase) reported in the literature [50-54]. The larger the number of smokers in a study, the smaller the increase in LDL levels due to use of the low-dose oral EE/LNG.

ROUTE OF DELIVERY

Non-oral routes of administration of CHCs were developed to reduce hepatic exposure to steroidal contraceptives and consequently the risk of VTE associated with them. In Chapter 3, we reported on similar effects of the vaginal ring delivering 15 µg EE and 150 µg Nestorone® a day, and the COC containing 30 µg EE and 150 µg LNG per tablet on plasma angiotensinogen. Furthermore, the vaginal ring exerted more pronounced biochemical effects on variables sensitive to oestrogenic/androgenic ratio of CHCs (SHBG, protein S, APC resistance and triglycerides) compared to the COC, as shown in Chapters 2 and 3 of this thesis. Hence, we can conclude that the oestrogenic impact of EE is not tempered by vaginal administration.

The number of comparative studies on the effects of contraceptive hormones delivered through different routes is limited. These studies often also differ in the type of oestrogen and progestins they have compared as well as haemostasis parameters they have included. This degree of variability is a great limitation for interpreting data on how haemostasis is affected by the administration route. In 2007, Hemelaar and colleagues published an extensive review of the effects of different routes of delivery of hormone replacement therapy (HRT) [55]. They reported less substantial unfavourable alterations in biomarkers of cardiovascular risk due to non-oral versus oral treatment. However, the non-oral oestrogen given in all studies included in this review was oestradiol (E_2), and it was compared to oral E_2 and conjugated equine oestrogens. Since oral EE induces larger changes in haemostasis parameters than oral E_2 [56], effects of different delivery routes for contraceptive steroids should be stratified by type of oestrogen as well as progestin.

With regard to the non-oral versus oral delivery of EE, it is noticed that both affect almost all variables of interest to the risk of steroid-induced VTE in the same direction [15;16;57-59]. In general, among variables that have been studied in the ten reports on non-oral EE, FVII total [15;16], protein S [16;59], protein C [15], ext APCr [16;57;59], global APTT-based APCr [16;58] and SHBG [16;22;58;-62] were subjected to more substantial changes by transvaginal rather than by oral EE. These differential effects are however considered to be due to the difference in androgenicity of the associated progestins rather than the route of delivery. The reasoning is based on two factors. First, the differences were found only in biomarkers known to be modified depending on androgenecity of progestins. Second, a study conducted by Johnson and colleagues who compared a transdermal patch containing EE and norelgestromin and a COC containing EE and norgestimate [58], observed similar effects for the most included haemostasis parameters. As norgestimate is converted to norelgestromin in the body [63], the androgenicity of the studied preparations is not different.

In contrast to others, some markers have been found to be affected by oestrogen dose rather than by the oestrogenicity / androgenicity ratio of a CHC, for example fibrinogen and t-PA [41]. An exception is the vaginal ring delivering EE and etonogestrel (ENG) which caused smaller decrease in tissue plasminogen activator (t-PA) compared to the EE/LNG OC [15]. Considering the effects of these treatments on fibrinogen were similar and that according to studies described in Chapters 2 and 3 of this thesis 15 µg of EE administered by a vaginal ring was found equipotent to 30 µg given orally, it is difficult to clarify the difference in effects on t-PA.

In Chapter 2 of this thesis, we described a more pronounced elevation of plasminogen levels in users of the vaginal ring delivering EE and NES compared to EE/LNG COC. So far, there have been no other reports on differential effects on plasminogen due to differences in delivery route or androgenicity. Specific properties of this vaginal ring remain the alternative for this pro-fibrinolytic effect.

The reviewed reports contain evidence that in case of CHCs containing EE, keeping the liver from exposure to peaking high concentrations in the portal system, is not effective in reducing steroidal impact on haemostasis variables, CRP and SHBG. Apart from this, Devineni and colleagues found significantly higher SHBG levels in users of EE/norelgestromin transdermal patch (Ortho Evra) than in users of the EE/norgestimate (precursor of norelgestromin oral contraceptive in a cross-over design [64], suggesting an even higher impact of the patch on the liver compared to the COC. This suggestion would be in line with the finding of an adjusted relative risk of 7.9 versus 3.6 for venous thromboembolism in users of the contraceptive patch and the COCs containing norgestimate compared to non-users, respectively [65], and at the same time contradicts the earlier finding of similar risk of non-fatal VTE involved with use of the same preparations, reported by Jick and colleagues [66].

In contrast, non-oral CHCs that contain non-EE oestrogens seem either not to affect haemostasis variables at all or to cause the opposite of the effects that EE-containing preparations yield. The former is true for fibrinogen [67;68], factor X (FX) [69], plasminogen [68;69], plasminogen activator inhibitor (PAI) activity [69], antithrombin (AT) antigen [69], fibrinogen degradation products (FGDP) [67], APTT [69] and SHBG [68], and the latter for FVII total [69], FX [69], protein C activity [69], PT [69] and APTT [69]. These observations are in accordance with those made in users of hormone replacement therapy (HRT) [55]. There are however two exceptions noted in the reviewed data. PAI activity was reduced more by oral EE than by non-oral E_2 in a study conducted by the United Nations Development Programme in 2003 [69]. This could mean a greater fibrinolytic activity imposed by the oral EE relative to the non-oral E_2 treatments. Furthermore, the injectable oestradiol valerate /norethisterone decreased AT antigen similar to the oral EE/norethisterone, while oestradiol cypionate / medroxyprogesterone injectable left it unchanged [69]. Also worth mentioning is the study by Vexiau and colleagues in which the combination of E_2 and oral cyproterone acetate increased AT activity in women with hyperandrogenism irrespective of the delivery route of E_2 [69].

Whether these observations are meaningful in terms of their relevance to VTE risk is hard to say due to the small number of reports and observations per preparation. Among all effects reviewed, the decreased protein C levels due to the injectable CHCs containing E_2 is unique since it seems to be the only unfavourable effect of the non-oral CHCs containing E_2, with regard to the risk of VTE. This is in contrast to orally and transvaginally administered EE which enhances protein C levels [15;69]. Whether these differential effects modify the risk of VTE in users remains however unknown.

Although Whigham and colleagues have not mentioned any difference in treatment effects in their publication, they did report reduced FVII due to the injectable progestin-only, medroxyprogesterone acetate. The same treatment left other haemostasis variables unaffected [70]. Remarkably, the levonorgestrel-only pill even lowered SHBG in the study by Pakarinen and collegues [71]. The difference in effect of oral and intra-uterine administration of LNG on SHBG, however, could be ascribed specifically to the intra-uterine route of administration. As the action of a progestin-releasing intra-uterine device (IUD) is meant to be primarily local (proliferation inhibition of endometrium and thickening of cervical mucus), the serum levels and thus the systemic effects associated with its use are much lower than those needed for and associated with suppressing the ovulation which is the aim in case of other delivery routes [72;73].

To summarize, as shown in Chapters 2 through 4 of this thesis, non-oral delivery of EE does not reduce its biochemical impact compared to oral treatment. This is in contrast to non- EE oestrogens for which differential effects of route of delivery have been described in the literature. Furthermore, non-oral non- EE oestrogens seem to have less unfavourable impact on haemostasis parameters and SHBG than the non-oral EE, as do oral non-EE oestrogens compared to oral EE. Although these conclusions follow from an indirect comparison of results, they are in line with findings on oral and non-oral HRT [55] and that the risk of VTE is not reduced by non-oral routes of administration of EE-containing CHCs [74].

So far, no gold standard exists for estimating the risk of cardiovascular disease during the course of development of hormonal contraceptives. This is mainly due to the lack of data on the exact mechanism of steroid-induced disease. This has resulted in a lack of consensus on which biomarkers or biomarker fractions to study. Alteration of several biomarkers measured within one study is often analysed with epidemiological data on the risk of disease extracted from another in an attempt to find correlations, while epidemiological reports are often inconsistent on risk of disease of different CHCs. In this light, the only solution would be to investigate biomarkers and the relevant clinical outcome prospectively in one study. Such a study will entail a great deal of expense, but will provide with reliable information and prove more efficient in the long term.

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