**Intrinsic Coagulation Activation and the Risk of Arterial Thrombosis in Young Women**

**Results From the Risk of Arterial Thrombosis in Relation to Oral Contraceptives (RATIO) Case-Control Study**

B. Siegerink, MSc; J.W.P. Govers-Riemslag, PhD; F.R. Rosendaal, MD, PhD; H. ten Cate, MD, PhD; A. Algra, MD, PhD

**Background**—Classically, intrinsic coagulation proteins are thought to have a minor role in hemostasis. Recently, these proteins, especially FXII, were implicated as possible key players in the pathogenesis of arterial thrombosis. This study aims to determine the risks of arterial thrombosis conferred by increased activation of intrinsic coagulation proteins in young women and the effect of oral contraceptive use on this association.

**Methods and Results**—The Risk of Arterial Thrombosis In relation to Oral contraceptives (RATIO) study is a population-based case-control study including young women (age 18 to 50 years) with myocardial infarction (n=205) and ischemic stroke (n=175) and 638 healthy controls. Intrinsic coagulation protein activation was determined by measuring activated protein-inhibitor complexes. This complex is with C1 esterase inhibitor (FXIIa-C1-INH, FXIa-C1-INH, Kallikrein-C1-INH) or antitrypsin inhibitor (FXIa-AT-INH). Odds ratios (ORs) and corresponding confidence intervals (95% CIs) were calculated with logistic regression. High levels of protein activation (>90th percentile of controls) showed an increased risk of ischemic stroke: FXIIa-C1-INH (OR, 2.1; 95% CI, 1.3 to 3.5), FXIa-C1-INH (OR, 2.8; 95% CI, 1.6 to 4.7), FXIa-AT-INH (OR, 2.3; 95% CI, 1.4 to 4.0), and Kallikrein-C1 (OR, 4.3; 95% CI, 2.6 to 7.2). If anything, myocardial infarction risk was only increased by Kallikrein-C1-INH (OR, 1.5; 95% CI, 0.9 to 2.5). Oral contraceptive use further increased the risks.

**Conclusions**—High levels of activated proteins of the intrinsic coagulation system are associated with arterial thrombosis, whereas the strength of these associations differs for myocardial infarction and ischemic stroke. This contradicts similar analyses among men in the Northwick Park Heart Study. Together with the finding that oral contraceptive use further increases the risks, the question of whether the role of intrinsic coagulation proteins in the pathogenesis of arterial thrombosis is sex-specific is raised. *(Circulation. 2010;122:1854-1861.)*

**Key Words:** thrombosis, arterial ▬ coagulation, intrinsic ▬ activation ▬ women

Arterial thrombosis occurs when an artery is occluded by a thrombus. Classical risk factors are diabetes mellitus, hypercholesterolemia, hypertension, smoking, obesity, and oral contraceptive use. The most common manifestations are peripheral arterial disease, myocardial infarction (MI), and ischemic stroke (IS). Arterial thrombosis is common among the elderly and the most common cause of death in high-income countries.¹

**Clinical Perspective on p 1861**

A thrombus is formed on activation of the coagulation system. Historically, this system has been characterized by 2 separate activating pathways: the extrinsic and intrinsic. The intrinsic coagulation pathway consists of the serine proteases coagulation factor XII (FXII), coagulation factor XI (FXI), and prekallikrein (PK). It further includes 1 nonenzymatic protein, high-molecular-weight kininogen.²

FXII has the capacity of autoactivation on binding on negatively charged surfaces.³ After activation, activated FXII (FXIIa) activates both FXI into activated factor XI (FXIa) and PK into its active form kallikrein (KAL). High-molecular-weight kininogen acts as a cofactor in both steps, during which the potent vasodilator bradykinin is released. FXIa further activates coagulation factor IX which forms, together with cofactor VIIIa, Ca²⁺ ions and phospholipids, the tenase complex thereby activating the common pathway of the

Received February 17, 2010; accepted August 13, 2010.

From the Department of Clinical Epidemiology (B.S., F.R.R., A.A.), Einthoven Laboratory for Vascular Medicine (B.S., F.R.R.), and Department of Thrombosis and Hemostasis (F.R.R.), Leiden University Medical Center, Leiden; Laboratory for Clinical Thrombosis and Hemostasis, Department of Internal Medicine, and Cardiovascular Research Institute Maastricht (J.W.P.G.-R., H.t.C.), Maastricht University Medical Center, Maastricht; and Julius Center for Health Sciences and Primary Care (A.A.) and Department of Neurology (A.A.), University Medical Center Utrecht, Utrecht, the Netherlands. Correspondence to Dr Ale Algra, Professor of Clinical Epidemiology, Department of Clinical Epidemiology, LUMC, PO Box 9600, 2300 RC Leiden, the Netherlands. E-mail a.algra@umcutrecht.nl
© 2010 American Heart Association, Inc.

*Circulation* is available at http://circ.ahajournals.org

DOI: 10.1161/CIRCULATIONAHA.110.943738

1854
coagulation cascade. A subform of FXIa is also known to activate coagulation factor VII, thus stimulating the common pathway by priming the extrinsic pathway. The intrinsic coagulation proteins are also involved in other biological processes, such as fibrinolysis (activation of plasminogen by FXIIa, FXIa, and KAL), vasoconstriction, and blood pressure control (KAL and bradykinin). On activation, the serine proteases of the intrinsic coagulation pathway are quickly bound by an inhibitor such as the C1-esterase inhibitor, thus forming an inhibitor complex that lacks the serine protease function. An excess of these inhibitors is readily available in plasma, thus the availability of activated intrinsic coagulation proteins forms the limiting step in the formation of these inhibitor complexes. This makes these inhibitor complexes a good measure of the level of activated proteins.

FXI deficiency causes mild bleeding in patients, whereas FXII, KAL, and high-molecular-weight kinogen deficiencies do not. These observations led to the traditional view that the intrinsic coagulation proteins do not play a major role in hemostasis and thus are not likely to be a risk factor for thrombosis. However, recent evidence, both laboratory and clinical, indicate that these proteins might play a role in thrombus formation. Murine studies with FXII and FXI knockout mice show that initiation of clot forming is not FXII-dependent, but that propagation of clot formation is. An excess of these inhibitors is readily available in plasma, thus the availability of activated intrinsic coagulation proteins forms the limiting step in the formation of these inhibitor complexes. This makes these inhibitor complexes a good measure of the level of activated proteins.

FXI deficiency causes mild bleeding in patients, whereas FXII, KAL, and high-molecular-weight kinogen deficiencies do not. These observations led to the traditional view that the intrinsic coagulation proteins do not play a major role in hemostasis and thus are not likely to be a risk factor for thrombosis. However, recent evidence, both laboratory and clinical, indicate that these proteins might play a role in thrombus formation. Murine studies with FXII and FXI knockout mice show that initiation of clot forming is not FXII-dependent, but that propagation of clot formation is.

Assays
We determined inhibitor complexes of the serine proteases of the intrinsic coagulation system as a measure of enzyme activation in citrated plasma. The inhibitor could either be a C1-esterase inhibitor for FXIIa, FXIa of kallikrein (FXIa-C1-INH, FXIa-C1-INH, KAL-C1-INH), or antitrypsin inhibitor (FXIa-AT-INH). These complexes were measured by an ELISA, as described earlier. In short, for the FXIa-C1-INH ELISA we used monoclonal antibody (mAb) KOK 12, which is specific for complexed C1-inhibitor as antigen, and mAb F3, which recognizes FXII as well as α-FXIIa and β-FXIIa subsequently as conjugate. The KAL-C1-INH assay uses the same antigen, but uses mAb K15, which is directed against prekallikrein and kallikrein as conjugate. The FXIa inhibitor assays both use the XI-5 mAb as antigen, which recognizes both the native and activated FXI form of FXI. The XI-5 mAb binds to native, complexed, and inactive C1-inhibitor, was used as conjugate for the FXIa-C1-INH assay; mAb AT-15, which is directed against complexed AT, was used as conjugate in the FXIa-AT-INH assay. All conjugates were biotinylated with EZLink N-hydroxysuccinimide ester-biotin according to instructions from the manufacturer (Pierce, Rockford, IL). Absorbance was read at 450 nm on an EL 808 Ultra microplate reader (BioTek Instruments Inc., Winoski, Vt).

Results were expressed as a proportion of fully activated normal pooled plasma; activation was performed by adding an equal volume of 0.2 mg mL⁻¹ of dextran sulfate (Mr 500 000; Sigma Chemical Co., St Louis, Mo) in the FXIa-C1-INH and KAL-C1-INH assay. Normal pooled plasma for the FXIa inhibitor assays was fully activated by adding kaolin (final concentration 5 mg mL⁻¹). Activation was stopped by adding 3 volumes of phosphate-buffered saline containing 0.1 mg mL⁻¹ of soybean trypsin inhibitor (Sigma Chemical Co.) and 0.05% (w/v) polybrene (Sigma Chemical Co.). Kaolin was removed by centrifuging the reaction mixture for 5 minutes at 13 000×g. No signal was detected in FXII-, FXI- or kallikrein-deficient plasmas as a control for the specific ELISA.

Study Design
The Risk of Arterial Thrombosis In relation to Oral contraceptives (RATIO) study is a multicenter population-based case-control study. The study consists of 3 substudies, including patients with confirmed MI, IS, or peripheral arterial disease. One control group was matched to all 3 case groups. The study was initiated to evaluate the risk of arterial thrombosis due to the changing composition of oral contraceptive pills (1990–1995). Blood and DNA were collected during the second phase of the study (1998–2002). Informed consent was obtained from all participants, and the study was approved by the medical ethics committees of the participating hospitals. For the current study we report data from the MI and IS substudies.

Participants
Patient selection has been described in detail previously. In short, all women 18 to 50 years of age who presented with a first event of MI or IS to 1 of the 16 participating hospitals in the Netherlands between 1990 and 1995 were eligible and approached for study participation. Diagnosis was made on the basis of clinical symptoms and confirmed by appropriate tests. MI was diagnosed by the presence of clinical symptoms, elevated cardiac enzyme levels, and electrocardiographic changes. Clinical symptoms of IS were confirmed by either computed tomography or magnetic resonance imaging. Women were approached to participate as a control subject by random digit dialing and were matched according to age, area of residence, and year of event. A standardized questionnaire on patient characteristics and possible cardiovascular risk factors such as (familial) medical history, use of oral contraceptives, and smoking habits was filled in by both cases and controls. Some of these questions were targeted to the year before diagnosis (cases) or the matched index year (controls). All participants were re-approached to donate blood or buccal swab for DNA analyses during the second phase of the study. Blood samples, together with a questionnaire on patient characteristics, were collected after a median of 69 months (range, 38 to 117 months) for MI and 95 months (range, 23 to 146 months) for IS cases, thereby ensuring blood was drawn after the acute phase.
Table 1. Characteristics of the RATIO Participants

<table>
<thead>
<tr>
<th></th>
<th>Myocardial Infarction (n=203)</th>
<th>Ischemic Stroke (n=175)</th>
<th>Control (n=638)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age, y</td>
<td>43</td>
<td>39</td>
<td>39</td>
</tr>
<tr>
<td>White, n (%)</td>
<td>195 (95)</td>
<td>167 (97)</td>
<td>602 (94)</td>
</tr>
<tr>
<td>History of,* n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>53 (26)</td>
<td>50 (29)</td>
<td>40 (6)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>10 (5)</td>
<td>7 (4)</td>
<td>10 (2)</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>21 (10)</td>
<td>14 (8)</td>
<td>19 (3)</td>
</tr>
<tr>
<td>Oral contraceptives use*</td>
<td>81 (40)</td>
<td>92 (53)</td>
<td>213 (33)</td>
</tr>
<tr>
<td>Smoking*</td>
<td>169 (82)</td>
<td>101 (58)</td>
<td>270 (42)</td>
</tr>
</tbody>
</table>

FXIIa-C1-INH, median (Q1–Q3)† 0.23 (0.13–0.33) 0.28 (0.15–0.45) 0.24 (0.12–0.33)
FXIa-C1-INH, median (Q1–Q3)† 0.23 (0.14–0.60) 0.37 (0.18–0.76) 0.23 (0.15–0.52)
FXIa-AT-INH, median (Q1–Q3)† 0.24 (0.20–0.29) 0.29 (0.22–0.42) 0.25 (0.21–0.31)
KAL-C1-INH, median (Q1–Q3)† 0.05 (0.03–0.13) 0.11 (0.03–0.27) 0.04 (0.03–0.12)

Q1–Q3 indicates range of first and third quartile.
In the year before event/index year.
†Levels are expressed as a proportion of fully activated normal pooled plasma.

Statistical Analysis

With the 90th percentile of the controls as a predefined cutpoint, we applied a logistic regression model to obtain odds ratios (ORs) as measures of the risk of arterial thrombosis associated with high levels of activation of the intrinsic coagulation factors. All ORs were adjusted for the frequency matching factors of area of residence, year of event, and age. Further adjustments were made for the potential confounders (diabetes mellitus, hypertension and hypercholesterolemia, and smoking) in a fully adjusted model. To assess the intrinsic coagulation protein system as a whole, a dummy variable was created, which counted the number of proteins with high activation levels. Interaction of all intrinsic coagulation proteins with oral contraceptive use in the year before the event was assessed by comparing the risk in those with either or both exposures with those with no exposures.

Results

The first phase of the RATIO study included 248 cases with MI, 203 cases with IS, and 925 controls. During the recruitment of the second phase of, 30 cases with MI, 63 cases with IS, and 158 controls either could not be traced or refused to participate. With 50 additional IS cases, 218 cases with MI, 190 with IS, and 767 controls were included in the second phase of the study. Blood was successfully collected from 203 MI cases, 175 IS cases, and 638 controls.

Table 1 displays the baseline characteristics of the study participants. As expected, cases had more cardiovascular risk factors such as smoking, hypercholesterolemia, diabetes mellitus, and hypertension than controls. The median levels of activated inhibitor complexes of the intrinsic coagulation proteins are displayed in Table 1. The Figure shows the distribution of the 4 inhibitor complexes for cases and controls separately.

The odds ratios of arterial thrombosis and high levels of activated intrinsic coagulation protein levels, as well as the corresponding cutoffs, are displayed in Table 2. By our definition, 10% of controls have high levels of inhibitor complexes. No association between MI and FXIIa-C1-INH was found (10% of cases exposed; OR, 0.82; 95% confidence interval [CI], 0.46 to 1.47). Also, FXIa-C1-INH (9%; OR, 0.96; 95% CI, 0.54 to 1.71) and FXIa-AT-INH (9%; OR, 0.94; 95% CI, 0.53 to 1.68) were not associated with MI. KAL-C1-INH slightly increased the risk of MI (15%; OR, 1.50; 95% CI, 0.91 to 2.47). In IS patients, high levels of FXIIa-C1-INH were more frequent than in controls (23% exposed; OR, 2.10; 95% CI, 1.27 to 3.48). Also, high levels of FXIa-C1-INH (21%; OR, 2.77; 95% CI, 1.63 to 4.73) and FXIa-AT-INH (20%; OR, 2.33; 95% CI, 1.37 to 3.96) were more frequent. KAL-C1-INH conferred a 4-fold increase in ischemic stroke (28%; OR, 4.34; 95% CI, 2.62 to 7.18). Further adjustments for hypertension, diabetes mellitus, hypercholesterolemia, and smoking did not change the overall pattern, as is displayed in Table 2 ("fully adjusted" ORs).

The use of an oral contraceptive in the year before the event roughly doubled the risk of both MI (OR, 2.3; 95% CI, 1.6 to 3.4) and IS (OR, 2.8; 95% CI, 1.8 to 4.2). The risk of ischemic stroke conferred by a combination of high levels of intrinsic coagulation protein activation (ie, >p90) and prior oral contraceptive use was higher than could be expected by the separate effects, as can be seen in Table 3. The risk of MI was not further increased by the combination of high levels of intrinsic coagulation protein activation and oral contraceptives, except for KAL-C1-INH. The combination of high levels of KAL-C1-INH and oral contraceptives use when compared with women who had neither risk factor increased the risk of MI 6-fold (OR, 6.1; 95% CI, 2.5 to 15).

Table 4 shows the ORs per number of proteins with high levels of activation. For each additional high level of activated protein inhibitor complex, the risk of IS is increased 2-fold (OR, 2.11; 95% CI, 1.31 to 3.38), whereas the risk for
MI was not affected (OR, 1.14; 95% CI, 0.75 to 1.73). When compared with those without a high level of intrinsic coagulation protein activation, people with ≥2 high levels had a 4-fold increase in risk for IS (OR, 4.39; 95% CI, 2.44 to 7.90), but no such association was found between multiple high levels and MI (OR, 1.30; 95% CI, 0.66 to 2.57).

In total, 19 MI and 21 IS patients were on anticoagulants. The results of the analyses when restricted to those patients who did not take oral anticoagulant therapy at the time of blood drawing did not change. Therefore, oral anticoagulant therapy does not change our results. The associations of FXIa-C1-INH, FXIa-AT-INH, and KAL-C1-INH persist in IS patients whose blood was sampled years (7) after the event (data not shown). The association of FXIIa-C1-INH appeared to attenuate with increasing time between event and blood drawing. However, absence of association cannot be established because of the broad CIs.

**Discussion**

Our study shows that high levels of activated proteins of the intrinsic coagulation system are associated with IS, and are not or are to a lesser extent associated with MI, in young women. In general, high levels increased the risk of ischemic stroke 2.5-fold, whereas the risk of MI was hardly affected. The increased risk for IS was further increased by oral contraceptive use. When the intrinsic coagulation proteins were assessed as a whole, high levels were more frequent in IS when compared with myocardial infarction.

The rate-limiting step in the formation of the inhibitor complexes is the availability of activated coagulation proteins, because the inhibitors are present in excess in plasma. The cause of the elevated complexes is not clear. It could either be a relative increase in which the absolute levels of zymogens remain stable with an increased activation rate, or it could be an absolute increase of zymogens with a stable activation rate.

Our study has some limitations; because of the case-control study design, blood was collected after the event in the case groups. This might have led to reverse causation, a process in which a consequence of an event is mistaken for the cause of the event. Because blood sampling in the RATIO study is several months after the event, we can safely rule out the
possibility that our findings directly reflect the transient effects of the acute phase, which lasts days to weeks. However, slowly subsiding effects that are induced by IS could explain some of the relation between FXIIa-C1-INH and IS. Nontransient effects (or chronic effect) can still be the cause of reverse causation in our study and can only be ruled out in a prospective study.

Furthermore, the necessity of a blood sample after the event implicates that our results are only valid for nonfatal arterial thrombosis. Although our study can be considered to be of reasonable size, or even relative large because of the rare nature of cardiovascular disease in young women, the RATIO study lacks power to detect small effects. Lack of precision, which is reflected in the wide CIs, sometimes hampers definite interpretation of our results. This is most clear when the intrinsic coagulation system is assessed as a whole; only few patients and controls are positive on 3 or all 4 assays, making it difficult to interpret the ORs for these strata. However, the pattern is clear: the risk increase is essentially similar after reanalysis with the 90th percentile as cutoff (analyses done in collaboration with the NPHS II Heart Study). This observation suggests that the pathogenic role of the intrinsic coagulation proteins is not similar for all manifestations of arterial thrombosis.

The results indicate that the risks conferred by high levels of intrinsic coagulation proteins were further increased by oral contraceptive use. This raises the question of whether women who want to start with oral contraceptives should be screened. The highest risk, after adjustment for potential confounders, was found in women who both had high levels of activated prekallikrein (>p90 of controls) and used oral contraceptives: a 23-fold increase in risk compared with women with neither risk factor (fully adjusted model). Even with this relative risk, a total of 15686 women have to be screened for their kallikrein activation levels before the start of oral contraceptives. If the 10% of these women with the highest kallikrein activation levels do not start to use oral contraceptives, 1 IS case will be prevented per year. Although a formal cost-benefit analyses could show a different number, these calculations show that screening is clearly not desirable, mainly because of the low incidence of IS in this population. This is even more clear when one considers the beneficial effects of oral contraceptive use in this population, such as a reduction of pregnancy-associated morbidity and mortality and ovarian and endometrial cancer.

A nested case-control study in the second Northwick Park Heart Study (NPHS II) also assessed the relationship between the inhibitor complexes of intrinsic coagulation proteins and the risk of coronary heart disease (CHD, n=231) and stroke (n=56, of which 12 were hemorrhagic). CHD was defined as definite MI (fatal and nonfatal), possible MI (fatal), angina pectoris, or coronary angiographic findings requiring intervention and ECG changes at 5 years of follow-up. Strokes were diagnosed and categorized on the basis of clinical presentation, computed tomography, lumbar puncture, and autopsy findings.

CHD risk was decreased in the second tertile of FXIIa-C1-INH; if anything, the third tertile also showed a decrease in risk, but to a lesser extent, thus resulting in a U-shaped relation. Other inhibitor complexes (FXI-C1-INH, FXI-AT-INH, KAL-C1-INH) did not seem to alter CHD risk. A similar U-shaped pattern was observed for the relation between KAL-C1-INH complexes and stroke. These results are essentially similar after reanalysis with the 90th percentile as a cutoff (analyses done in collaboration with the NPHS II He...
investigators; results not shown here). There are several possible explanations for the discrepant findings in the RATIO and the NPHS II analyses. First, the differences might be due to chance. Second, the NPHS II is a nested case-control study, so blood draw was before the event. Although reverse causation in the RATIO study is not a likely explanation for our results, because of the timing of blood draw and the different effects for MI and IS, it cannot be ruled out. Third, the case definitions in the RATIO study were more stringent when compared with those in the NPHS. In the NPHS, ischemic and hemorrhagic strokes were combined, whereas the RATIO study included only relatively young women. This difference is of particular interest because FXII transcription is estrogen sensitive, because of an estrogen-responsive element in the promoter region of the F12 gene. Furthermore, a recent murine study suggests that estrogens influence the transcription of more coagulation proteins. The mechanism(s) by which these proteins are involved in the pathogenesis of IS in young women still have to be established. Perhaps such an increased activity of these proteins results in an imbalance of the several systems in which the intrinsic coagulation proteins are involved (eg, coagulation, fibrinolysis, and inflammation). Ultimately, this imbalance then leads to a hypercoagulable state, which

<table>
<thead>
<tr>
<th>Table 4. Risk of Arterial Thrombosis With Increasing Numbers of High Levels of Activated Intrinsic Coagulation Proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Myocardial Infarction</strong></td>
</tr>
<tr>
<td>No. of High Levels</td>
</tr>
<tr>
<td>-------------------</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ischemic Stroke</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (%)</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
</tbody>
</table>

Odds ratios are obtained from logistic regression and are adjusted for the stratification factors age, area of residence, and year of event. Odds ratios depicted as "fully adjusted" are additionally adjusted for diabetes mellitus, hypertension and hypercholesterolemia, and smoking. ref indicates reference.
could further increase the risk of ischemic stroke. According to this hypothesis, some event, such as a plaque rupture or changes in blood flow, acts as a trigger of the start of coagulation. The extent of the following thrombus formation is dependent on the tendency to clot: Patients suffering from a hypercoagulable state are more likely to form an occlusive thrombus with clinical effects than those without such a predisposition. This mechanism predicts that a dose-dependent effect should be observed: the greater the imbalance, the larger the tendency to coagulate, the greater the risk of ischemic stroke. Not all forms of arterial thrombosis need to be subject to this hypothesized mechanism. Possibly, exposure of the highly thrombogenic surface after rupture of a coronary plaque inevitably will start clot formation, whether the patient is more prone to coagulation or not. Although the formation of a thrombus in itself is involved in the pathogenesis of both MI and IS, we hypothesize that the tendency to start a clot is an important risk factor for IS, but not for MI.

Conclusions

Results from the RATIO study showed that increased levels of activated intrinsic coagulation proteins are associated with IS, but not MI, in young women. These risks are further increased by oral contraceptive use. These results differ from an earlier study in middle-aged men (NPHS II). This raises 2 questions: Why are the effects of intrinsic coagulation proteins different for MI and IS in young patients, and are these effects sex-specific?

Answers to these questions could come from both basic and epidemiological research. Basic research is needed to further unravel the function of activated intrinsic coagulation proteins and perhaps identify different mechanisms for different forms of arterial thrombosis. Epidemiological research could also provide more insight in the potentially different mechanisms for MI and IS; for example, by specifying the associations per stroke subtype. Furthermore, epidemiological research could provide more insight on the possible sex-specific effects by including both male and female patients. A prospective study design could also diminish the risk of reverse causation, but the low incidence of arterial thrombosis in the young would make such a study very inefficient.

Acknowledgments

The authors wish to thank the participants and the earlier contributors to the RATIO study. We are indebted to H. Ghafouri, who assisted with the measurements. A special thanks to Dr J.A. Cooper from the NPHS for providing the additional analyses.

Sources of Funding

This study was supported by grants from the Netherlands Heart Foundation (grant 2001.069) and the Leducq Foundation, Paris, France, for the development of Transatlantic Networks of Excellence in Cardiovascular Research (grant 04 CVD 02).


**CLINICAL PERSPECTIVE**

Arterial forms of thrombosis are leading causes of death and disability in the Western world. The Risk of Arterial Thrombosis in Relation to Oral Contraceptives (RATIO) study is a population-based case-control study that focuses on arterial thrombosis in young women (18 to 50 years of age) and has a unique opportunity to identify risk factors that are difficult to identify in older patients because they could be obscured by age-related comorbidities. The identification of these new risk factors could aid in the prevention and treatment of arterial thrombosis in all age groups. This study investigated whether proteins of the intrinsic coagulation pathways are risk factors for arterial thrombosis. Historically, the role of some of these factors was thought to be minor. However, both animal and clinical studies have implicated these proteins in pathophysiological thrombus formation. Furthermore, these proteins also play a role in other relevant biological systems, such as inflammation. Inhibitor complexes of coagulation factors XIIa, Xia, and kallikrein were determined as a measure of protein activation. It was found that these complexes were higher in ischemic stroke cases, but were not higher in myocardial infarction cases, when compared with matched controls. The risk of stroke was further increased (up to 23-fold) among users of oral contraceptives. However, because of the low incidence of arterial thrombosis in young women, screening for these factors before the start of arterial thrombosis does not seem to be warranted. Further studies should be performed to investigate the exact pathophysiological mechanism and whether these mechanisms are different for the subtypes of arterial thrombosis, especially for subtypes of ischemic stroke.
Intrinsic Coagulation Activation and the Risk of Arterial Thrombosis in Young Women: Results From the Risk of Arterial Thrombosis in Relation to Oral Contraceptives (RATIO) Case-Control Study

B. Siegerink, J.W.P. Govers-Riemslag, F.R. Rosendaal, H. ten Cate and A. Algra

_Circulation_. 2010;122:1854-1861; originally published online October 18, 2010; doi: 10.1161/CIRCULATIONAHA.110.943738

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2010 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/122/18/1854

Data Supplement (unedited) at:
http://circ.ahajournals.org/content/suppl/2013/10/17/CIRCULATIONAHA.110.943738.DC1

**Permissions:** Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

**Reprints:** Information about reprints can be found online at:
http://www.lww.com/reprints

**Subscriptions:** Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org/subscriptions/
Dysfonction vasculaire chez la femme ayant des antécédents de prééclampsie et de retard de croissance intra-utérin
Eléments d’appréciation du risque vasculaire à venir

Yoav Yinon, MD ; John C.P. Kingdom, MD ; Ayodele Odutayo, BSc ; Rahim Moineddin, PhD ; Sascha Drewlo, PhD ; Vesta Lai, RN ; David Z.I. Cherney, MD, PhD ; Michelle A. Hladunewich, MD

Contexte—Les femmes ayant présenté une maladie plaquettaire sont exposées à un risque accru de développement d’une affection vasculaire. On ignore toutefois si la préexistence d’une dysfonction endothéliale intervient dans la prédisposition aux pathologies plaquetaires et dans la survenue ultérieure d’un trouble vasculaire. Cette étude a donc pour objet d’évaluer la fonction vasculaire chez des femmes en période de postpartum et d’établir si son altération diffère selon le type d’affection plaquettaire.

Méthodes et résultats—Des patientes qui avaient respectivement des antécédents de prééclampsie de début précoce (n =15), de prééclampsie de survenue tardive (n = 9) et de retard de croissance intra-utérin sans prééclampsie (n = 9) ont été suivies pendant une durée de 6 à 24 mois après leur accouchement parallèlement à des femmes dont la grossesse s’était déroulée normalement (n = 16). La vasodilatation médiane par le flux sanguin et celle indépendante de celui-ci (induite par la trinitrine) ont été étudiées au niveau de l’artère humérale par écho-Doppler de haute résolution. La rigidité artérielle a été appréciée par analyse de l’onde de pouls (indice d’augmentation). Le bilan biologique a porté sur les taux de facteurs angiogéniques circulants (facteur de croissance de l’endothélium vasculaire, fms-like tyrosine kinase 1 soluble, facteur de croissance placentaire et endogline soluble). La vasodilatation dépendante du flux est apparue significativement plus faible chez les femmes qui avaient des antécédents de prééclampsie de début précoce et de retard de croissance intra-utérin que chez celles ayant antérieurement présenté une prééclampsie de survenue tardive et chez les témoins (respectivement, 3,2 ± 2,7% et 2,1 ± 1,2% contre 7,9 ± 3,8% et 9,1 ± 3,5% ; p <0,0001). La vasodilatation indépendante du flux a été comparable dans tous les groupes. De même, l’indice d’augmentation de la pression artérielle radiale s’est révélé significativement majoré chez les femmes ayant des antécédents de prééclampsie de début précoce et de retard de croissance intra-utérin, alors qu’il a été normal chez celles ayant présenté une prééclampsie tardive et chez les témoins (p = 0,0105). Les taux de facteurs angiogéniques circulants ont été similaires dans tous les groupes.

Conclusions—Seules les femmes ayant des antécédents de prééclampsie de survenue précoce et de retard de croissance intra-utérin sans prééclampsie sont sujettes à une altération de leur fonction vasculaire, ce qui pourrait expliquer leur présédposition aux pathologies plaquetaires et leur propension supérieure à développer secondairement une affection vasculaire. (Traduit de l’anglais : Vascular Dysfunction in Women With a History of Preeclampsia and Intrauterine Growth Restriction; Insights Into Future Vascular Risk. Circulation. 2010;122:1846–1853.)

Mots clés : endothélium ■ prééclampsie ■ vasodilatation ■ femmes ■ maladies vasculaires ■ pathologies plaquetaires ■ femme en postpartum

Activation de la voie intrinsèque de la coagulation et risque de thrombose artérielle chez la femme jeune

Résultats de l’étude cas-témoins Risk of Arterial Thrombosis in Relation to Oral Contraceptives (RATIO)

B. Siegerink, MSc ; J.W.P. Govers-Riemslag, PhD ; F.R. Rosendaal, MD, PhD ; H. ten Cate, MD, PhD ; A. Algra, MD, PhD

Contexte—L’opinion qui prévaut est que les protéines de la voie intrinsèque de la coagulation jouent un rôle mineur dans l’hémostase. De récentes données suggèrent toutefois que ces protéines, et plus particulièrement le facteur XII, seraient des acteurs clés dans la pathogenèse des thromboses artérielles. La présente étude a donc été entreprise pour évaluer les risques...
de thrombose artérielle associés à l’augmentation de l’activation de ces protéines chez la femme jeune et pour déterminer l’influence exercée par la contraception orale sur cette association.

**Méthodes et résultats**—L’étude RATIO (Risk of Arterial Thrombosis in Relation to Oral Contraceptives [Risque de thrombose artérielle associé à la prise de contraceptifs oraux]) est une étude de population cas-témoin menée chez des femmes jeunes (âge compris entre 18 et 50 ans) ayant été victimes d’un infarctus du myocarde (n = 205) ou d’un accident vasculaire cérébral ischémique (n = 175) ainsi que chez 638 témoins sains. L’activation des facteurs de la voie intrinsèque de la coagulation a été évaluée en dosant les taux de complexes protéine-inhibiteur activés. Ces complexes sont respectivement formés avec l’inhibiteur de la C1 estérase (FXIIa-C1-INH, FXIa-C1-INH, kallicrène-C1-INH) ou l’inhibiteur de l’antitrypsine (FXIa-AT-INH). Les odds ratios (OR) et leurs intervalles de confiance (IC) à 95 % ont été calculés à partir d’une analyse par régression logistique. Il est ainsi apparu qu’un degré élevé d’activation de chacune de ces protéines (au-delà du 90ème percentile de la valeur mesurée chez les témoins) avait contribué à augmenter le risque d’accident vasculaire cérébral ischémique : FXIIa-C1-INH (OR : 2,1 ; IC à 95 % : 1,3 à 3,5), FXIa-C1-INH (OR : 2,8 ; IC à 95 % : 1,6 à 4,7), FXIa-AT-INH (OR : 2,3 ; IC à 95 % : 1,4 à 4,0) et kallicrène-C1 (OR : 4,3 ; IC à 95 % : 2,6 à 7,2). En revanche, seul le complexe kallicrène-C1-INH a été associé à une augmentation du risque d’infarctus du myocarde (OR : 1,5 ; IC à 95 % : 0,9 à 2,5). La prise de contraceptifs oraux a eu pour effet de majorer les risques.

**Conclusions**—Des taux élevés de protéines activées de la voie intrinsèque de la coagulation favorisent les thromboses artérielles, bien que la puissance de cette association diffère pour l’infarctus du myocarde et pour l’accident vasculaire cérébral ischémique. Ces observations tranchent avec les résultats d’analyses similaires effectuées chez l’homme dans le cadre de la Northwick Park Heart Study. Compte tenu du fait que les risques apparaissent majorés par la contraception orale, la question se pose de savoir si le rôle joué par les facteurs de la voie intrinsèque de la coagulation dans la pathogénèse des thromboses artérielles serait propre au sexe féminin. (Traduit de l’anglais. Intrinsic Coagulation Activation and the Risk of Arterial Thrombosis in Young Women; Results From the Risk of Arterial Thrombosis in Relation to Oral Contraceptives (RATIO) Case-Control Study. *Circulation*: 2010;122:1854–1861.)

**Mots clés** : thrombose artérielle ■ coagulation, voie intrinsèque ■ activation ■ femme