artery FMD represents a largely nitric oxide-mediated, endothelium-dependent dilation and is attenuated by preceding forearm IR. FMD is a well-validated model to study IR-injury in humans. Forearm ischemia was induced by inflating a blood pressure cuff around the upper arm level. FMD analysis was performed offline by investigators blinded to the treatment arm.

Results: Baseline FMD did not differ between metformin pretreatment and no pretreatment (6.9% [3.6%] and 6.1% [3.5%], respectively; P = 0.27). FMD was significantly lower after forearm IR in both treatment arms (4.4% [3.3%] and 4.3% [2.8%], respectively; P < 0.01 in both groups). A 2-way repeated measures ANOVA revealed that metformine treatment did not prevent the decrease in FMD by IR (P = 0.50).

Conclusion: In this study, we investigated for the first time whether treatment with metformin limits IR-injury in humans in vivo. In contrast with previous studies in animal models of myocardial infarction, we did not observe any protective effect of metformin on endothelial IR-injury, measured with brachial artery FMD, in healthy middle-aged volunteers. Our study does not exclude that metformin has protective effects in patients with cardiovascular disease or patients with DM. As such, additional studies, including studies in these patient groups, are needed to explore the discrepancy between the previous preclinical findings and our current results.

Disclosure of Interest: None declared.

PP078—COMPARISON OF A NEW ELISA-BASED WITH THE FLOW CYTOMETRIC ASSAY FORVASODILATOR-ASSOCIATED STIMULATED PHOSPHOPROTEIN (VASP) PHOSPHORYLATION TO ASSESS P2Y12-INHIBITION AFTER TICAGRELOR INTAKE

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Introduction: Ticagrelor is a P2Y12 receptor antagonist, with superior effects but also ensuing enhanced bleeding risk compared with clopidogrel. Determination of platelet inhibition may be useful to confirm efficient platelet inhibition on an individual patient level and to identify patients at risk for bleeding, particularly in a preoperative setting. The vasodilator-associated stimulated phosphoprotein (VASP) phosphorylation assay specifically measures platelet P2Y12 inhibition but has so far required special flow cytometric equipment and individual sample processing. A new ELISA-based VASP assay has been developed that allows batch analysis after initial platelet activation. Due to the reversible binding of ticagrelor, it is unclear if the ELISA and flow cytometric assays provide comparable results.

Patients (or Materials) and Methods: We hypothesized that the conventional and new methods may be comparable when the reversible P2Y12 inhibitor ticagrelor is used. We pair-wise compared the platelet reactivity index (PRI) between assays in a prospective clinical trial. Healthy volunteers received a single 180-mg loading dose of ticagrelor.

Results: PRI-values of the 2 methods correlated well (r = 0.97, P < 0.001). Ticagrelor rapidly decreased PRI values on average after 50 minutes, but nadir levels 2 to 6 hours after ticagrelor intake were 15% higher when PRI% was measured with the flow cytometric method. Bland-Altman analysis showed that the flow cytometric assay measured markedly higher PRI levels than the new ELISA-based technique (mean difference, 13%).

Conclusion: The new ELISA-based VASP assay offers an alternative to the currently used flow cytometric method, but measures lower PRI levels, particularly when PRI falls below 20% after ticagrelor intake.

Disclosure of Interest: None declared.

PP080—THE ROLE OF THE KCNJ5 POTASSIUM CHANNEL VARIANTS IN ALDOSTERONE RELEASE

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Introduction: Primary aldosteronism (PA) is the most prevalent form of endocrine hypertension, due to autonomous aldosterone production. The 2 main causes of PA are aldosterone-producing adenoma (APA) and bilateral adrenal hyperplasia (BAH). Aldosterone, synthesized and secreted by the zona glomerulosa (ZG) of the adrenal gland, is physiologically regulated by angiotensin II (AngII), plasma potassium concentration [K+], and ACTH. However, the molecular mechanisms that lead to the aldosterone hyperproduction are not completely understood. Recent studies have show the presence of somatic mutations of the inwardly rectifying potassium channel (KCNJ5) gene in APA, coding for the K+ channel KCNJ5. These mutations lie near or within the selectivity filter of the Kir3.4 channel, changing the normal Na+/K+ permeability of the channel. Mutation-scanning studies conducted on an Australian cohort with PA has identified germline single nucleotide polymorphisms (SNP) in the KCNJ5 gene (Q282E, E246K, and G247R) that may be relevant to the pathophysiology of PA in these subjects. However, the functionality of these SNPs is unknown, so we have tested this directly.

Patients (or Materials) and Methods: The electrophysiology of these mutants was studied by applying a 2-electrode voltage clamp technique to Xenopus oocytes expressing the mutant KCNJ5 channels. A human H295R adrenocortical cell line was used as the experimental model to study the effects of the mutations on aldosterone release. Cells were transiently transfected with the WT KCNJ5 or mutant forms, and the aldosterone release was evaluated using a radioimmunoassay, both under normal conditions and after depolarization with high extracellular K+ and Ang II.

Results: The Q282E and E246K KCNJ5 showed change in the selectivity of the channel, with Na+/K+ currents being observed in both of them. However, the G247R KCNJ5 behaved like the WT KCNJ5 channel. Differences were also observed in the levels of aldosterone release in subjects with PA.

Conclusion: The findings from this work suggest that SNPs and rare variants outside the selectivity filter of KCNJ5 are functionally important and may be have a role in the autonomous aldosterone release in subjects with PA.

Disclosure of Interest: None declared.

PP081—EVALUATION OF HYPOGLYCEMIC ACTIVITY OF A NOVEL LONG ACTING INSULIN ANALOGUE

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Introduction: Hypoglycemic activity is one of the most important features of insulin derivatives and can be examined both in vitro and in vivo. The aim of the study was to evaluate activity of a new long-acting insulin analogue using 3 different test systems.
Patients (or Materials) and Methods: New insulin analogue AKR of a potential long-acting feature was evaluated in the Institute of Biotechnology and Antibiotics. Bioactivity of AKR was compared with that of USP human insulin standard in bioassays on cells and rabbits, while the pharmacodynamic activity on rats was referred to control group received 0.9% NaCl. The 3T3-L1 cells differentiated into adipocytes were used for the performance of insulin dependent glucose uptake experiment. Dose-response relationship of log insulin concentration and Deoxy-D-glucose uptake of AKR was determined. The biological activity on animals was assessed on 24 albino rabbits according to US Pharmacopoeia. The glucose concentrations were measured with spectrophotometer in 2 time points (1.0 and 2.5 hours) after administration of 1 U and 2 U of insulin. Potency of AKR was calculated with 95% CI. Pharmacodynamic effect was based on glucose concentration measurement in rats with hyperglycemia induced by streptozotocin. The overall glycomic profile up to 36 hours was evaluated after subcutaneous single dosing at range 2.5 to 10.0 U/kg b.w. In the 28-day multiple dose study, AKR was administered twice a day at dose 5.0 U/kg b.w.

Results: Percentage of maximum Deoxy-D-glucose uptake in 3T3-L1 cells was similar for AKR and human insulin, except the highest concentration in which the hook effect was observed. The absolute potency of a new analogue determined in rabbits was 23.94 U/mg, and the potency ratio of AKR versus the insulin standard was 0.90. The relative potency was 38.35 U/mL with confidence limit of 32.99–44.42 U/mL. The experiment on rats confirmed with a statistical significance (P < 0.05) hypoglycemic activity of AKR in comparison of control group both after single and multiple doses. Characteristics for AKR profile was rather fast beginning of action (0.5-1.0 hour) with the maximal effect at 6 hours postdose and quite prolonged return to initial values. The glucose levels were stable during 4-week administration.

Conclusion: Bioactivity of the novel insulin analogue AKR, connected with glucose metabolism was confirmed in both in vitro and in vivo conditions. AKR is a candidate for a hypoglycemic drug product in diabetes care.

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Disclosure of Interest: None declared.

PP082—PRESCRIPTION OF RECOMMENDED DRUGS FOR ACUTE MYOCARDIAL INFARCTION IN ESTONIA IN 2001 VS 2007 AND IN 2007 VS 2011

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Introduction: Current acute myocardial infarction (AMI) guidelines recommend the use of platelet aggregation inhibitors, beta-blockers, angiotensin-converting enzyme inhibitors (ACEI), or angiotensin II receptor blockers (ARB) and statins for in-hospital and long-term treatment. The aim of the study was to evaluate the changes in the prescription of these drugs in Estonian hospitals in 2001 versus 2007 and in 2007 versus 2011.

Patients (or Materials) and Methods: We performed a retrospective cross-sectional study including random samples of hospitalized AMI cases. Chi-square test and logistic regression were used to study the changes in the prescription of the drugs.

Results: Final analysis included 423 cases in 2001, 687 cases in 2007, and 740 cases in 2011. The prescription rates of most drugs recommended for in-hospital and for out-patient use improved (Table).

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Aspirin</td>
<td>83.7%</td>
<td>90.1%</td>
<td>0.021</td>
<td>88.5%</td>
<td>0.333</td>
</tr>
<tr>
<td>P2Y12 inhibitors</td>
<td>8.8%</td>
<td>10.1%</td>
<td>&lt;0.001</td>
<td>42.3%</td>
<td>0.004</td>
</tr>
<tr>
<td>Beta-blockers</td>
<td>77.8%</td>
<td>80.1%</td>
<td>0.363</td>
<td>76.2%</td>
<td>0.080</td>
</tr>
<tr>
<td>ACEI/ARB</td>
<td>53.7%</td>
<td>68.3%</td>
<td>&lt;0.001</td>
<td>63.5%</td>
<td>0.039</td>
</tr>
<tr>
<td>Statins</td>
<td>16.1%</td>
<td>14.8%</td>
<td>&lt;0.001</td>
<td>28.0%</td>
<td>0.001</td>
</tr>
<tr>
<td>Outpatient use</td>
<td>82.6%</td>
<td>88.5%</td>
<td>0.013</td>
<td>92.0%</td>
<td>0.069</td>
</tr>
<tr>
<td>P2Y12 inhibitors</td>
<td>9.9%</td>
<td>11.1%</td>
<td>&lt;0.001</td>
<td>52.9%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Beta-blockers</td>
<td>70.1%</td>
<td>80.4%</td>
<td>0.001</td>
<td>82.9%</td>
<td>0.307</td>
</tr>
<tr>
<td>ACEI/ARB</td>
<td>52.6%</td>
<td>73.7%</td>
<td>&lt;0.001</td>
<td>75.9%</td>
<td>0.434</td>
</tr>
<tr>
<td>Statins</td>
<td>23.5%</td>
<td>57.6%</td>
<td>&lt;0.001</td>
<td>72.3%</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Conclusion: Prescription of recommended drugs for AMI patients increased continuously during last years in Estonia. Continuous education programs and future studies should focus to differences of drug utilization in tertiary and nontertiary hospitals, also to different patient subgroups (ie, elderly).

Disclosure of Interest: None declared.

PP083—DABIGATRAN—DEMONSTRATES THE NEED FOR COMPREHENSIVE APPROACHES TO OPTIMISE THE USE OF NEW DRUGS

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Introduction: There are potential conflicts between authorities and companies to fund new premium-priced drugs, especially where there are safety and/ or budget concerns. Dabigatran exemplifies these issues due to variable drug concentrations, no known antidote, dependence on renal elimination, and considerably more expensive than warfarin. The latter is a concern with the growing prevalence of atrial fibrillation (AF). There are also issues with potentially re-designing anticoagulant services. As a result, there is a need to review authority activities regarding dabigatran and use the findings to develop new models to better manage the entry of new drugs in the future.

Patients (or Materials) and Methods: Descriptive review and appraisal of educational and other activities regarding dabigatran pre- to postlaunch in a systematic manner among > 30 European countries and regions. The findings were used to develop a new model.