Cardiac repolarization during hypoglycaemia and hypoxaemia in healthy males: impact of renin–angiotensin system activity

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Aims Activity in the renin–angiotensin system (RAS) may influence the susceptibility to cardiac arrhythmia. To study the effect of basal RAS activity on cardiac repolarization during myocardial stress induced by hypoglycaemia or hypoxaemia in healthy humans.

Methods and results Ten subjects with high RAS activity and 10 subjects with low RAS activity were studied on three different occasions: (i) hypoglycaemia (nadir P-glucose 2.7 ± 0.5 mmol/L), (ii) hypoxaemia (nadir pO2 5.8 ± 0.5 kPa), and (iii) normoglycaemic normoxia (control day). QT parameters were registered by Holter monitoring. Hypoglycaemia and hypoxaemia induced QTc prolongation (P < 0.001, both stimuli). The QT/RR slope and the VR increased as a function of hypoglycaemia, but were unaffected by hypoxaemia. Low RAS activity was associated with a steeper QT/RR slope in the recovery phase after both stimuli: hypoglycaemia: P = 0.04; hypoxia: P = 0.03. RAS activity had no impact on QTc [P = 0.48 (hypoglycaemia) and P = 0.40 (hypoxaemia)] or any of the other outcome variables.

Conclusion Basal RAS activity has significant impact on QT dynamics, but not the corrected QT interval, during recovery from hypoglycaemia and hypoxaemia. The impact, however, is modest and more subtle than initially expected. The clinical relevance is unclear.

KEYWORDS
Renin–angiotensin system; Cardiac repolarization; QT; Hypoglycaemia; Hypoxaemia; Substrate deficiency

Introduction
Renin–angiotensin system (RAS) activity seems to be an important factor in the pathogenesis of cardiac arrhythmia.1 Low RAS activity, either genetically determined or pharmacologically induced, may be associated with a reduced risk of ventricular arrhythmia.2 Thus, the D (deletion) allele of the angiotensin converting enzyme (ACE) I/D polymorphism (which confers high ACE activity)3 is related to an increased occurrence of reperfusion-induced ventricular arrhythmia after myocardial infarction1 and to QTc prolongation in end-stage renal disease.4 Furthermore, a meta-analysis shows that ACE inhibitor treatment after myocardial infarction reduces the risk of sudden cardiac death.2 This effect may partly be ascribed to a positive impact of ACE inhibitors on cardiac repolarization, since ACE inhibitor treatment reduces QTc in hypertensive patients5 and QT dispersion in patients with myocardial infarction.6 The QT interval and its dynamics are surrogate measures of repolarization known to carry independent prognostic information on risk of sudden cardiac death7–9 and development of ventricular tachyarrhythmia.10,11

RAS activity also influences physical capacity in endurance training12 and vulnerability to hypoglycaemia,13 a common and feared adverse effect to insulin therapy, which has been suggested to play a role in the pathogenesis of the 'dead-in-bed' syndrome observed among young patients with type 1 diabetes. Patients with high spontaneous RAS activity have an increased risk of severe hypoglycaemia13–16 suggesting a more pronounced general susceptibility to glucose deficiency. Hence, the RAS seems to be involved in cellular energy expenditure, and spontaneous RAS activity
may therefore constitute a risk marker of cardiac arrhythmia during substrate depletion.
The primary aim of this study was to investigate, if basal RAS activity has impact on the QT interval and its dynamics during hypoglycaemia and hypoxaemia, thereby being a potential risk marker of ventricular arrhythmia in situations characterized by lack of glucose or oxygen. The secondary aim was to determine the effect of hypoglycaemia and hypoxaemia on QT dynamics.

Methods

Population

A total of 303 healthy young men participated in a screening procedure involving a venous blood sample and a health screening questionnaire. Blood samples were analysed for ACE activity, angiotensinogen concentration and the AT2 (1675A/G) polymorphism—the three RAS parameters associated with risk of hypoglycaemia. Based on a scoring system, we selected 20 subjects (26 ± 4 years) differing in terms of high or low RAS activity. The high RAS activity group was characterized by ACE activity and angiotensinogen concentration in the highest quartile and the AT2 receptor genotype AA corresponding to low expression [maximum stimulation via the angiotensin II receptor subtype 1 (AT1 receptor)]. The low RAS activity group was characterized by ACE activity and angiotensinogen concentration in the lowest quartile and the AT2 receptor genotype GG corresponding to high expression (minimum stimulation via the AT1 receptor).17

Design

The study was a single-blinded, randomized, counter-balanced, cross-over study. Each subject was studied on three different experimental occasions: at hypoglycaemia, at hypoxia and at a normoglycaemic, normoxaemic control day, separated by at least 3 weeks. The study complies with the declaration of Helsinki. All participants gave written consent to participate and the study was approved by the Regional Ethics Committee.

Experimental set-up

Subjects were studied after an overnight fast upon arrival to the laboratory at 8.00 a.m. A three channel digital Holter Monitor (DelMar Aria recorder, Delmar Avionics, Irvine, CA, USA) was mounted. Two cannulae for blood sampling were placed in the ante-cubital vein and in the radial artery of the non-dominant arm. Baseline blood samples were drawn at time 15 and 45 min. Hereafter hypoglycaemia, hypoxaemia, or euglycaemia/normoxia (control) was induced. The stimulus period lasted 60 min. Blood samples were obtained at 100 (only plasma glucose), 115, 130 (only plasma glucose), and time 145 min. Samples at the recovery phase were drawn at 215 min. A flow diagram showing the study timeline is provided in Figure 1.

Hypoglycaemia

Hypoglycaemia was induced by subcutaneously administered fast-acting human insulin (Actrapid; Novo Nordisk, Bagsvaerd, Denmark; 0.2 U/kg administered twice—a dosage scheme which resulted in stable hypoglycaemia within the target range [plasma glucose 2.5–2.9 mmol/L] in pilot studies) with the aim of standardizing the dose of insulin. Euglycaemia was restored by ingestion of a small meal consisting of 400 mL of apple juice and a slice of brown bread (55 gm).

Hypoxaemia

Hypoxaemia was induced by spontaneous breathing of a gas mixture containing 12% oxygen administered via a soft facial mask. This oxygen content is well tolerated by healthy subjects, and mild dyspnœa was the only side effect experienced by the participants. During the study, oxygen saturation and plasma glucose were measured regularly using a pulse oximeter (N-180, Nellcor; Tyco Healthcare, Pleasanton, CA, USA) and bed-side plasma glucose measurements (HemoCue, HemoCue AB, Angelholm, Sweden), respectively. To keep participants from knowing the kind of stimulus on a particular day, atmospheric air was delivered on a facial mask during hypoglycaemia and placebo injections were given during hypoxaemia. On the control day, the subjects were subjected to two placebo injections without active insulin and atmospheric air via the mask as well.

QT parameters

QT intervals were calculated from Holter recordings obtained with modified chest leads V2, V3, and V5 for three 30-min periods each study day: baseline (15–45 min), hypoglycaemia/hypoxaemia/placebo (control) (115–145 min), and recovery (215–245 min). Semiautomatic QT analysis was performed in the lead with the best defined T-wave using a modified Laguna algorithm to identify the end of the T-wave.18 Mean values of all accepted RR intervals and QT parameters were calculated for the three periods and the QT interval was corrected for differences in heart rate (HR) according to Bazett’s (QTc=QT/RR1/2) and Fredericia’s formulas (QTc=QT/RR1.5).

The adaptation of the QT interval to changes in the heart rate was assessed as the slope and the intercept of the linear regression line obtained by analysing pairs of QT interval length and the immediately preceding RR interval. SDQT and SDNN were defined as the standard deviations of all accepted QT intervals and RR intervals, respectively. The variability ratio (VR) was defined as SDQT/SDNN and reflects the variation in QT not explained by heart rate variability.8

Laboratory analyses

All biochemical analyses were performed blinded to the other results. Angiotensinogen was determined as the maximal quantity of angiotensin I generated during incubation of plasma in the presence of excess recombinant human renin as described previously.19 Serum ACE activity was determined by a commercial kinetics-based assay (Sigma Diagnostics, St Louis, MO, USA). DNA was extracted from peripheral blood leukocytes using a standard salting-out method. The ACE I/D variant and the AT2 receptor 1675G/A polymorphism were determined by polymerase chain reaction. Blood for measurements of adrenaline was sampled into chilled tubes containing 100 μL EDTA glutathione and heparin, centrifuged, drawn off by pipette and frozen (−80 C) within half an hour. Analyses were done using HPLC with fluorimetric detection.20 Glucose, potassium, and blood gases were analysed immediately by the local laboratory [plasma glucose concentrations were measured enzymatically (COBAS INTEGRA, Roche, Basel, Switzerland), pO2 by amperometry, pH by potentiometry (both ABL, Radiometer, Broenshoej, Denmark) and plasma potassium using a standard ion-selective electrode].

Figure 1 Timeline. The stimulus period lasted 60 min. asterisks indicate sampling of complete blood sample package (including plasma glucose), dagger indicates measurement of plasma glucose only.
Hypoglycaemia

The hypoglycaemic stimulus lasted 60 min and was similar in both groups with mean pO2 of 5.9 ± 0.6 kPa and nadir pO2 of 5.8 ± 0.5 kPa. A small but significant increase in pH from 7.40 to 7.42 was observed (\( P < 0.05 \)) during the hypoglycaemic period.

QTC (Figure 3) and QTf prolonged, the RR interval, SDQT, and SDNN decreased, whereas VR was unaltered (Tables 1 and 3). Plasma adrenaline increased slightly but significantly from 33 to 53 pmol/L (aggregate means of both groups), \( P < 0.05 \) (Figure 2), whereas plasma potassium was unchanged. Plasma adrenaline was positively correlated to the QTc during hypoxia (\( R^2 = 0.38, P < 0.005 \)), whereas the correlation with QTf did not reach statistical significance. The hypoglycaemic stimulus was not correlated to the QT prolongation.

RAS activity had no significant impact on any outcome variables during hypoglycaemia, although the QT/RR slope tended to be steeper in the low RAS group (\( P = 0.08 \)).

During recovery, QTC and QTf returned to baseline (Tables 1 and 3), whereas the effect of hypoglycaemia on the RR interval, SDQT, and SDNN extended into this phase. Plasma adrenaline declined to baseline levels (Figure 2).

Low RAS activity was significantly associated with a steeper QT/RR slope when compared with the high RAS group. No other outcome variables were influenced by RAS group.

Discussion

Hypoglycaemia/hypoxaemia and QT parameters

In the present study, hypoglycaemia induced QTc/QTf prolongation, which is in accordance with previous studies.\(^{21}\)

The effect of hypoglycaemia on QT dynamics is unclear. In the present study, we found a significantly increased slope in the recovery phase of hypoglycaemia when compared with baseline. The variability ratio, VR, also increased in response to hypoglycaemia. These changes in QT dynamics may reflect alterations in the QT heart rate dependency, which are potentially harmful, as increases in slope and VR predicts sudden cardiac death in patients with heart failure and myocardial infarction.\(^{8,9,22}\) The impact of hypoglycaemia on QT dynamics may therefore be of relevance for the increased mortality rates seen among young patients with type 1 diabetes,\(^{23}\) who regularly experience hypoglycaemic episodes.\(^{24}\) The ‘dead-in-bed syndrome’ describes unexplained over-night death in a young patient with type 1 diabetes without late complications,\(^{25}\) and is suggested to involve hypoglycaemia-induced ventricular arrhythmias.\(^{26}\) The altered QT dynamics observed during hypoglycaemia tentatively supports this hypothesis. The slope is altered in patients with diabetes in general\(^{27}\)—but this phenomenon seems to be related to autonomic neuropathy.\(^{28}\)

The QT prolongation in response to hypoglycaemia is believed to be induced primarily by raised adrenaline levels, but also by insulin- and adrenaline-induced hypokalaemia. Adrenaline affects L-type Ca\(^ {2+} \) channels,\(^ {29} \) but do also increase Tpeak–Tend,\(^ {30} \) which provides an index of transmural dispersion of repolarization (TDR).\(^ {31} \) The idea is that the peak of the T-wave reflects the time of largest transmural dispersion, i.e. when the epicardium is fully

Statistics

Test for normality was done using Kolmogorov–Smirnov’s model. To reduce the amount of statistical analyses performed, and thereby minimizing the risk of mass significance, mean values for the baseline period and the stimulus period were calculated and included in the statistical models. An unpaired T-test was used to compare baseline values in the two groups. To evaluate both the impact of the RAS group and the intervention (hypoglycaemia or hypoxaemia vs. placebo) on the outcome variables, a mixed linear model (ANCOVA) was applied. Hypoglycaemia and hypoxaemia data were analysed separately. ‘RAS group’ and ‘intervention’ were defined as fixed factors and ‘number of participants’ as a random factor. To correct for potential minor group differences in baseline values (and to eliminate the risk of outcome variables increases/decreases regressing towards the mean), the baseline value of the particular variable being investigated was included in the model as a covariate. The mixed model was applied on data obtained in the stimulus period and the recovery period, respectively. Correlations were evaluated using Pearson’s correlation coefficient. A level of significance < 5% (\( P < 0.05 \); two-sided) was considered significant. Statistical analyses were performed using SPSS (version 13.0). Values are given as mean ± 1 SEM unless otherwise mentioned.

Results

The distribution of the ACE I/D genotypes were as follows: Low RAS activity, 4/6/0 (II/ID/DD); high RAS activity, 0/4/6 (II/ID/DD).

No parameters differed between RAS groups at baseline (Table 1 and Figure 2).

Hypoglycaemia

The hypoglycaemic stimulus was similar in both RAS groups with mean plasma glucose in 60 min lasting stimulus period of 2.8 ± 0.3 mmol/L and nadir plasma glucose of 2.7 ± 0.5 mmol/L.

Hypoglycaemia induced QTC (Figure 3) and QTf prolongation [18 ± 2 ms (aggregate mean of both groups)], whereas the dynamic QT parameters reacted ambiguously (Tables 1 and 2). The VR increased significantly. Plasma adrenaline increased by 1500%, \( P < 0.001 \) (Figure 2) and plasma potassium decreased by 20% (nadir: 3.4 mmol/L), \( P < 0.05 \). Neither plasma glucose, nor plasma adrenaline, nor plasma potassium concentrations were directly correlated to the QT prolongation.

RAS group had no impact on any of the outcome variables in the hypoglycaemic period.

During recovery, QTC and QTf were still prolonged and VR was still increased (Tables 1 and 2). The RR interval decreased and the QT/RR slope increased significantly (Figure 4). Plasma adrenaline (Figure 2) and plasma potassium tended to normalize in the recovery phase, but did not reach baseline levels.

Low RAS activity was significantly associated with a steeper QT/RR slope when compared with the high RAS group, whereas no other outcome variables were influenced by RAS group.

Hypoxaemia

The hypoxaemic stimulus lasted 60 min and was similar in both groups with mean pO2 of 5.9 ± 0.6 kPa and nadir pO2 of 5.8 ± 0.5 kPa. A small but significant increase in pH from 7.40 to 7.42 was observed (\( P < 0.05 \)) during the hypoxaemic period.

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repolarized and the endocardium still is depolarized. Hence, Tpeak–Tend is a window of vulnerability, where a transmural arrhythmogenic substrate exists. Increases in TDR seem to facilitate propagation of early afterdepolarizations, which again provide the substrate for the development of Torsade de Pointes. Hence, analysing Tpeak–Tend would have been another way of quantifying risk of arrhythmia in the present study. However, transmural dispersion is not the solely gradient in the ventricles. Action potentials are shorter in the right ventricle than in the left ventricle.

Table 1  RR intervals and QT parameters, hypoglycaemia, and hypoxaemia

<table>
<thead>
<tr>
<th></th>
<th>Hypoglycaemia</th>
<th>High RAS</th>
<th>P-value</th>
<th>Hypoglycaemia</th>
<th>High RAS</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RR–baseline</td>
<td>903 (55)</td>
<td>842 (51)</td>
<td>0.09</td>
<td>697 (49)</td>
<td>628 (51)</td>
<td>0.03</td>
</tr>
<tr>
<td>RR–stimulus</td>
<td>894 (50)</td>
<td>847 (45)</td>
<td>0.06</td>
<td>692 (47)</td>
<td>594 (51)</td>
<td>0.001</td>
</tr>
<tr>
<td>RR–recovery</td>
<td>920 (51)</td>
<td>875 (48)</td>
<td>0.01</td>
<td>701 (45)</td>
<td>637 (47)</td>
<td>0.001</td>
</tr>
<tr>
<td>QTf–baseline</td>
<td>132 (11)</td>
<td>124 (10)</td>
<td>0.03</td>
<td>120 (10)</td>
<td>110 (10)</td>
<td>0.03</td>
</tr>
<tr>
<td>QTf–stimulus</td>
<td>129 (12)</td>
<td>123 (11)</td>
<td>0.05</td>
<td>116 (10)</td>
<td>108 (10)</td>
<td>0.05</td>
</tr>
<tr>
<td>QTf–recovery</td>
<td>134 (12)</td>
<td>126 (11)</td>
<td>0.03</td>
<td>121 (10)</td>
<td>113 (10)</td>
<td>0.03</td>
</tr>
<tr>
<td>SDQT–baseline</td>
<td>7.30 (2.11)</td>
<td>8.32 (3.46)</td>
<td>0.38</td>
<td>8.19 (2.11)</td>
<td>8.56 (2.41)</td>
<td>0.70</td>
</tr>
<tr>
<td>SDQT–stimulus</td>
<td>10.16 (2.20)</td>
<td>10.69 (2.42)</td>
<td>0.39</td>
<td>8.10 (2.31)</td>
<td>7.09 (1.38)</td>
<td>0.70</td>
</tr>
<tr>
<td>SDQT–recovery</td>
<td>9.01 (2.42)</td>
<td>8.81 (2.83)</td>
<td>0.39</td>
<td>7.40 (1.61)</td>
<td>6.67 (2.18)</td>
<td>0.70</td>
</tr>
<tr>
<td>SDNN–baseline</td>
<td>107.03 (26.6)</td>
<td>112.37 (44.06)</td>
<td>0.75</td>
<td>114.66 (39.92)</td>
<td>110.56 (53.68)</td>
<td>0.85</td>
</tr>
<tr>
<td>SDNN–stimulus</td>
<td>109.92 (36.3)</td>
<td>115.66 (31.49)</td>
<td>0.75</td>
<td>93.98 (42.02)</td>
<td>93.43 (31.75)</td>
<td>0.85</td>
</tr>
<tr>
<td>SDNN–recovery</td>
<td>95.99 (89.3)</td>
<td>103.28 (31.81)</td>
<td>0.75</td>
<td>103.13 (39.36)</td>
<td>96.28 (43.83)</td>
<td>0.85</td>
</tr>
<tr>
<td>VR–baseline</td>
<td>0.069 (0.014)</td>
<td>0.080 (0.03)</td>
<td>0.32</td>
<td>0.076 (0.018)</td>
<td>0.089 (0.047)</td>
<td>0.43</td>
</tr>
<tr>
<td>VR–stimulus</td>
<td>0.097 (0.024)</td>
<td>0.099 (0.037)</td>
<td>0.32</td>
<td>0.093 (0.03)</td>
<td>0.081 (0.025)</td>
<td>0.43</td>
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<tr>
<td>VR–recovery</td>
<td>0.095 (0.022)</td>
<td>0.088 (0.021)</td>
<td>0.32</td>
<td>0.076 (0.017)</td>
<td>0.074 (0.022)</td>
<td>0.43</td>
</tr>
</tbody>
</table>

Means ± 1 SD. P-values represent RAS group comparison at baseline. Units all ms unless otherwise mentioned. RR, interval between two succeeding R-waves; QTc = RR/QT1/2; QTf = RR/QT1/3; Slope, slope of linear regression line (QT on RR); Intercept, intercept of linear regression line (QT on RR); SDNN, standard deviation of all normal RR intervals; SDQT, standard deviation of all QT intervals; VR, variability ratio (SDQT/SDNN).

Figure 2  Adrenaline profiles during hypoglycaemia and hypoxaemia. Plasma adrenaline increased 15-fold as a response to hypoglycaemia. The increase seen during hypoxaemia was significant, but discrete. ———, High RAS, hypoglycaemia; ————, Low RAS, hypoglycaemia; ————, Low RAS, hypoxaemia.

Figure 3  QTc during hypoglycaemia and hypoxaemia. QTc prolonged independently of basal RAS activity both during hypoglycaemia and hypoxaemia. ———, High RAS, hypoglycaemia; ————, Low RAS, hypoglycaemia; ————, Low RAS, hypoxaemia.
Table 2: Holter parameters, statistical analysis, hypoglycaemia

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Parameter (fixed)</th>
<th>Estimated effect (b)</th>
<th>95% CI</th>
<th>P-value</th>
<th>Estimated effect (b)</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RR (ms)</td>
<td>Low RAS, hypoglycaemia</td>
<td>0.52 (0.52)</td>
<td>98.3–124.1</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>QT (ms)</td>
<td>Low RAS, hypoglycaemia</td>
<td>0.48 (0.48)</td>
<td>90.3–123.1</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>QTc (ms)</td>
<td>Low RAS, hypoglycaemia</td>
<td>0.48 (0.48)</td>
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<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>QTf (ms)</td>
<td>Low RAS, hypoglycaemia</td>
<td>0.52 (0.52)</td>
<td>98.3–124.1</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Slope</td>
<td>Low RAS, hypoglycaemia</td>
<td>0.6 (0.6)</td>
<td>34.4–102.1</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Intercept (ms)</td>
<td>Low RAS, hypoglycaemia</td>
<td>9.2 (9.2)</td>
<td>34.4–102.1</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td></td>
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<tr>
<td>SDQT (ms)</td>
<td>Low RAS, hypoglycaemia</td>
<td>2.5 (2.5)</td>
<td>10.6–34.7</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>SDNN (ms)</td>
<td>Low RAS, hypoglycaemia</td>
<td>9.1 (9.1)</td>
<td>10.6–34.7</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>VR</td>
<td>Low RAS, hypoglycaemia</td>
<td>0.01 (0.01)</td>
<td>0.001–0.021</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td></td>
</tr>
</tbody>
</table>

Values indicate the estimated effects of (i) belonging to the low RAS activity group (high RAS group serves as a reference point) and (ii) inducing hypoglycaemia (placebo serves as a reference point). Values are in absolute numbers. Bracketed values indicate 1 SEM. RR, interval between two succeeding R waves; QTc, QT corrected for heart rate; QTf, QT corrected for heart rate; Slope, slope of linear regression line (QT on RR); Intercept, intercept of linear regression line, nor the fall in plasma potassium, was correlated to QTc/QTf prolongation. The unexpected lack of correlation between potassium concentrations during hypokalaemia and QT prolongation probably has several explanations. First of all, the hypokalaemic stimulus was mild with a nadir plasma potassium concentration just below the reference range (3.5–4.5 mmol/L). Second of all, the participants were all healthy young men, who are known to have a large repolarization reserve when compared with women and patients with organic heart disease.

Hypokalaemia inhibits the human ether-a-go-go-related gene (HERG) K\(^+\) channel directly, and thereby reduces the rapid delayed rectifier potassium current (I\(\text{Kr}\)). I\(\text{Kr}\) is an important repolarizing current, and reductions in I\(\text{Kr}\) can lead to QT prolongation. In correspondence with this, hypokalaemia is a known risk factor for arrhythmogenesis in congenital and acquired long QT syndromes. Hypokalaemia itself may also have the capacity to change the QT interval, just as hypoglycaemia also may affect repolarization directly by blocking the HERG K\(^+\) channel. In the present study, neither the highly significant rise in plasma adrenaline, nor the fall in plasma potassium, was correlated to QTc/QTf prolongation. The unexpected lack of correlation between potassium concentrations during hypokalaemia and QT prolongation probably has several explanations. First of all, the hypokalaemic stimulus was mild with a nadir plasma potassium concentration just below the reference range (3.5–4.5 mmol/L). Second of all, the participants were all healthy young men, who are known to have a large repolarization reserve when compared with women and patients with organic heart disease.

Hypokalaemia-induced changes in QT dynamics may also involve adrenaline and potassium. Adrenaline has been shown to affect QT heart rate dependence indirectly. For instance, Extramiana et al. showed that treatment with a beta blocking agent reduced the diurnal QT/RR slope in healthy subjects. In the present study, the slope increased after, and not during, hypokalaemia, which may be explained by a protracted effect of adrenaline.

Figure 4: Impact of hypoglycaemia on slope. QT/RR slope increased significantly during recovery from hypoglycaemia compared to baseline.
to decrease IKr currents. In the present study, the hypoxaemic stimulus was only moderate, and pH increased vaguely as a function of hypoxaemia-induced hyperventilation (respiratory alkalosis).

QT dynamics were ambiguously influenced by hypoxaemia. The variation in QT intervals (SDQ) was reduced during recovery probably as a function of an increased heart rate. The VR and slope were unaffected, which is in conflict with a previous study using 11% oxygen that showed an increased slope in response to hypoxaemia. The differing results observed for hypoglycaemia and hypoxaemia were not unexpected, and may very well reflect different cellular response mechanisms. Alternatively, the magnitude of the two quite different stimuli did not correspond to each other, and the cardiac impact was of different extent.

### Renin–angiotensin system activity and QT parameters

Basal RAS activity affected the QT/RR slope during recovery from both hypoglycaemia and hypoxaemia. Low RAS activity was significantly associated with a steeper slope when compared with high RAS activity. Since our definition of low basal RAS activity at some points is comparable with ACE inhibitor treatment, and since high RAS activity seems to be associated with a cellular vulnerability to hypoglycaemia, we expected low basal RAS activity to be related to an electrophysiological advantage when compared with high basal RAS activity. As a steep slope is associated with an increased risk of sudden death and ventricular arrhythmia in patients with myocardial infarction and congestive heart failure, low RAS activity was expected to be associated with a flatter slope than high RAS activity. In the present study, the opposite was observed. The finding could of course represent a statistical coincidence, but it does, nevertheless, lead to several considerations. Among these are, whether low basal RAS activity, in contrast to the initial hypothesis, constitutes a risk of arrhythmia during substrate depletion. Another is, whether a steeper slope, within the normal area, may actually be associated with a cellular vulnerability to hypoglycaemia that may very well reflect a protective antiarrhythmic effect compared to a flatter slope. Previous results from a small study tentatively support the last claim. Here, the authors found the slope to be significantly steeper in normal subjects compared with patients resuscitated from ventricular fibrillation.

In contrast to the QT/RR slope, QTc and QTf were not associated with basal RAS activity. The fact that the participants were healthy may have influenced the result, as the QT shortening effect of ACE inhibitors, and the association between the ACE D-allele and QT prolongation, were described in the presence of structural cardiac disease. Consistent with this Jeron et al. found that the association between the D-allele and increased QT dispersion in patients with myocardial infarction could not be retrieved in the healthy siblings of the subjects.

The results of the present study underline the complexity of the RAS. On a long-term basis, the ACE activity seems to be positively correlated to the QT interval. On a short-term basis, however, the effects of angiotensin II and ACE inhibitors may be completely different. Thus, angiotensin II reduces action potential duration by increasing the delayed rectifier potassium current, just as in vitro
studies found ACE inhibitors to increase action potential duration, thereby possibly mimicking class 3 antiarrhythmic agents. In conclusion, basal RAS activity has impact on QT dynamics in the recovery phases of both hypoglycaemia and hypoxaemia. Unexpectedly, low basal RAS activity is associated with a steeper QT/RR slope when compared with high basal RAS activity. The finding may be relevant for young patients with insulin-treated diabetes or pulmonary disease, even though the difference was small, and the clinical consequence questionable. QTc and QTf were unaffected by RAS activity.

Hypoglycaemia, but not hypoxaemia, induces increases in QT/RR slope and variability ratio—a finding of potential relevance for young patients with insulin-treated diabetes, who experience hypoglycaemia on a regular basis. The different responses for hypoglycaemia and hypoxaemia may indicate different cellular responses.

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