Flavonoids are a class of natural polyphenolics (Figure 1A) that are ubiquitous in plants, vegetables, fruits, and beverages of plant origin, such as tea and wine.1 Numerous prospective epidemiological studies have found beneficial effects of flavonoid consumption on overall cardiovascular mortality.2–7 and flavonoid consumption repeatedly has been suggested to play a major role in the “French paradox.”8 Flavonoids apparently reduce the incidence of fatal complications associated with acute myocardial infarction and the postinfarction period.7 Flavonoids apparently reduce the incidence of fatal complications associated with acute myocardial infarction and the postinfarction period.7

Conventionally, these beneficial effects have been attributed primarily to nonspecific antioxidant and antithrombotic properties of flavonoids6; however, major fatal complications of acute and chronic myocardial infarctions are malignant ventricular arrhythmias.9 Therefore, we examined the effects of dietary flavonoids on cardiac electrophysiology in vitro and in vivo.

Methods

Subjects

Ten volunteers (5 women and 5 men) participated in the ECG study. Subject characteristics were age, 25.2±4.6 years (mean±SD) (range, 20 to 37 years); weight, 70.5±10.4 kg (range, 59 to 92 kg); and body mass index, 23.4±3.4 kg/m² (range, 18.6 to 29.7 kg/m²). The subjects were all healthy, with no history of disease of the cardiovascular system or the gastrointestinal tract. None of the subjects were taking any medications. All participants provided informed consent. The Ethics Committee of the University of Heidelberg approved the study design.

Study Design

The participants drank 1 L of freshly squeezed pink grapefruit juice within half an hour in the morning. Continuous 12-channel Holter ECG recording was started 30 minutes beforehand and continued for 12 hours afterward. To examine diurnal variation of QTc duration, a second Holter ECG recording was performed in all participants on a day during which they refrained from drinking citrus juice and tea.

Holter ECG Recording and Analysis

ECGs were recorded with continuous 12-channel Holter ECG recording and evaluated with Mortara Research Tool Software (Mortara Instrument). According to common practice, short periods with electric artifacts were excluded from analysis by use of H-Scribe Software (Mortara). Leads V1 and V6 were selected for automatic analysis because T-wave signals were optimal in those leads. Measurement of RR and QT values was performed automa-
ically, without any manual correction. QTc values were generated automatically by use of Bazett’s formula. QTc values of all heartbeats for a period of 5 minutes were averaged to obtain a mean value. Six of those were averaged to determine mean QTc values for periods of 30 minutes. Synchronized data from all ECGs were fitted with a polynomial regression curve by use of Origin 4.0 software (Microcal Inc) to obtain a graphical fit (Figure 2).

Molecular Biology and Electrophysiology
The HERG cDNA (GenBank accession No. hs04270) was a gift from Dr M.T. Keating (Salt Lake City, Utah). Heterologous expression in *Xenopus* oocytes was performed as described previously.10 The double-electrode voltage-clamp configuration was used as described previously.10 This investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication No. 85-23, revised 1996).

Solutions and Drug Administration
All substances were purchased from Sigma. Flavonoids were dissolved in DMSO to prepare stock solutions (100 mmol/L) and stored at −20°C.

Data Analysis
Dose-effect curves were fitted with the Hill equation as described previously.10 Statistical data are expressed as mean±SEM, with n representing the number of experiments performed. Statistical significance was evaluated by use of the unpaired Student t test (electrophysiological experiments and comparison of ECGs after ingestion of grapefruit juice with control ECGs) and the paired Student t test (comparison of ECGs before and after ingestion of grapefruit juice).

Results
We screened a large number of flavonoids for their inhibitory activity to HERG channels, the molecular correlate of the rapid delayed rectifier potassium current *I*<sub>Kr</sub>,11 which may provide a basis of potential antiarrhythmic effects. We selected major compounds within different structural flavonoid subgroups to cover a broad spectrum: flavanone glycosides (neohesperidin, hesperidin, naringin), flavanone aglycones (galangin, hesperetin, naringenin), flavone glycosides (rutin), flavone aglycones (flavone, chrysin, fisetin, kaempferol, morin, quercetin, myricetin, apigenin), furanocoumarin derivatives (bergapten, methoxsalen, psoralen), and

Figure 2. Transient QTc prolongation after ingestion of grapefruit juice. Time course of mean QTc prolongation measured in 10 persons after ingestion of grapefruit juice is displayed in front of a background (shaded) showing schematic plasma kinetics of naringenin after single-dose oral ingestion of grapefruit juice as described by Erlund et al.12 in arbitrary units. Second curve, synchronized to other ECG data, demonstrates mean QTc shortening in same subjects as observed under control conditions as a consequence of diurnal variation of QTc duration. Asterisks indicate a significant difference from control ECGs and from baseline recordings.
coumarin derivatives (scopoletin, umbelliferone, coumarin, 7-ethoxycoumarin) (Figure 1B).

In *Xenopus* oocytes heterologously expressing the channels, typical HERG currents were recorded with a standard protocol. From a holding potential of −80 mV, a first test pulse to +30 mV (400 ms) evoked activating currents, and a return pulse to −60 mV (400 ms) elicited large outward tail currents characteristic of HERG channels. Flavonoids were washed into the bath at a concentration of 1 mmol/L, and the test pulse protocol was repeated at a frequency of 0.07 Hz for 15 minutes. Peak tail currents of the recordings at the beginning and end of the perfusion were compared to quantify the amount of block.

Six experiments were performed with each flavonoid and compared with vehicle controls with DMSO at a concentration of 1% (vol/vol) (n = 6). Ten compounds caused a significant inhibition of HERG currents: naringenin, hesperetin, morin, flavone, kaempferol, quercetin, methoxsalen, scopoletin, umbelliferone, and 7-ethoxy coumarin (Figure 1B).

In the screening experiments, naringenin, morin, and hesperetin exhibited the highest potency of HERG channel blockade. Dose-effect relations of these compounds measured in the *Xenopus* oocyte expression system yielded IC50 values of 102.3 μmol/L for naringenin, 111.4 μmol/L for morin, and 288.8 μmol/L for hesperetin (n = 6 each) (Figure 1C). In HEK cells, the IC50 for naringenin was 36.5 μmol/L (n = 5) (Figure 1C). At a concentration of 1 μmol/L, naringenin exerted a mild inhibitory effect, reducing HERG currents by 13.8 ± 2.4% (n = 5).

The glycosides of naringenin are the major flavonoid components in pink grapefruit juice (>1000 μmol/L).12 Given our findings of HERG blockade by naringenin, we examined whether grapefruit juice can mimic the effects of class III antiarrhythmic drugs, ie, induce QT prolongation.

Ten healthy volunteers drank 1 L of freshly squeezed pink grapefruit juice in the morning. Software analysis of Holter ECG recordings revealed a transient QTc prolongation in the ensuing hours (Figure 2). Mean QTc prolongation 4 and 5 hours after ingestion of the grapefruit juice was 10.6 ± 2.8 and 12.5 ± 4.2 ms, respectively (n = 10). The difference from the initial recordings, ie, before drinking the juice, was highly significant (P = 0.004 and P = 0.024, respectively). Using Fridericia’s formula for heart rate correction instead of Bazett’s formula, peak QTc prolongation is 10.3 ± 3.0 ms (t = 5 hours, P = 0.01). QTc prolongation declined again and approached baseline values (3.0 ± 2.2 ms) 9 hours after ingestion of the juice. Other relevant surface ECG parameters were not affected (data not shown).

Diurnal variation of QTc duration has been described in healthy persons, with a decrease during daytime and an increase at night.13,14 We verified these previously published observations in the participating individuals by performing a second continuous ECG recording on a day during which they refrained from drinking citrus juice and tea (Figure 2). At the time corresponding to the observed QTc prolongation after ingestion of grapefruit juice, we found a mean QTc shortening of −8.0 ± 3.0 ms (t = 4 hours) and −5.9 ± 6.0 ms (t = 5 hours), respectively (n = 10). This was significantly different from the effects of grapefruit juice (P = 0.002 and P = 0.024, respectively).

**Discussion**

To the best of our knowledge, we are the first to show direct effects on the ECG by dietary compounds. Furthermore, we have demonstrated for the first time that flavonoids act as specific antagonists of cardiac potassium channels.

The concentration used in the screening experiments was relatively high because we had to take into account the fact that higher drug concentrations are needed in *Xenopus* oocytes.15,16 We intended primarily to collect qualitative data, because for many flavonoids, quantitative data on bioavailability and resulting plasma concentrations are not yet available.17 Therefore, it is currently not possible to estimate the significance of these compounds. Naringenin is well characterized in terms of bioavailability, which enabled us to demonstrate the physiological relevance of our findings. Other important flavonoids (particularly hesperetin, quercetin, and kaempferol, all of which reach plasma concentrations of 0.7 to 2 μmol/L)17 also exhibited inhibitory effects on HERG channels, although with lower potency. Furthermore, it is remarkable that more than half of the flavonoids tested showed HERG activity, which suggests that there may be numerous other flavonoids with similar properties.

At a concentration of 1 μmol/L, naringenin reduced HERG currents in HEK cells by +14%. Naringenin is one of the most important flavonoids in grapefruit, with a concentration of >1000 μmol/L in juice.12 Plasma kinetics of naringenin18 are remarkably congruent with the time course of QTc prolongation observed in our study (Figure 2, background). The bioavailability of grapefruit-derived naringenin is high. According to the literature, plasma levels reach concentrations of 6.0 μmol/L, with a peak after 4 to 6 hours.18

Taking the HEK cell experiments into account, it seems likely that mild HERG inhibition already occurs at these concentrations. Because the mild QTc prolongation of 10 to 12 ms measured in the ECG study probably corresponds to a merely partial HERG blockade, the observed effect is probably attributable primarily to naringenin. The variety of grapefruit flavonoids with HERG activity may also elicit cumulative effects, however.

It has been found that QTc duration varies diurnally in healthy persons, with a maximum at night and a steady decline during daytime, followed by an increase in the evening.13,14 In our study, grapefruit juice was ingested in the morning, and transient QTc prolongation was observed in the ensuing 6 to 8 hours. With regard to the normal diurnal variation, one would have expected a decrease in QTc duration instead. Indeed, control recordings showed a shortening of the QTc interval in the corresponding period in the same persons. Therefore, our data provide strong evidence that grapefruit juice has direct effects on cardiac repolarization.

In summary, we have found numerous different flavonoid compounds in grapefruit juice that block cardiac HERG channels and may cause a prolongation of the QTc interval as a consequence. These findings provide a rational basis for potential effects of flavonoids on cardiac electrophysiology.
From a theoretical point of view, HERG channel blockade may have antiarrhythmic or proarrhythmic effects. Taking the positive epidemiological data into account, however, proarrhythmic properties of flavonoids appear unlikely.

On the basis of this “proof of principle,” we wish to encourage further research in this field, which may result in new dietary recommendations for patients at risk of cardiovascular events. Flavonoids may also prove to be a promising new class of antiarrhythmic agents, combining electrophysiological effects with antioxidant and antithrombotic properties.

Acknowledgments
This work was supported by grants from the Deutsche Forschungsgemeinschaft, Ki 663/1-1 to Dr Kiehn and Ka 1714/1-1 to Dr Karle, and from the Deutsche Stiftung für Herzforschung to Dr Thomas. Dr Owen is grateful to the Verein zur Förderung der Krebsforschung in Deutschland eV for funding. The excellent technical assistance of Klara Gueth, Ramona Bloehs, and Patrizia Kraft and the expert support of T. Hilbel and J. Laude concerning software design for the analysis of Holter ECG data are gratefully acknowledged. The authors thank their friends and colleagues for participating in this study.

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QTc Prolongation by Grapefruit Juice and Its Potential Pharmacological Basis: HERG Channel Blockade by Flavonoids

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_Circulation_. 2005;111:835-838; originally published online February 14, 2005; doi: 10.1161/01.CIR.0000155617.54749.09

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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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:
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