

Sex Differences in Cardiovascular Drug Response

Eline Margaretha Rodenburg

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Sex Differences in Cardiovascular Drug Response

Geslachtsverschillen in respons op cardiovasculaire geneesmiddelen

Proefschrift

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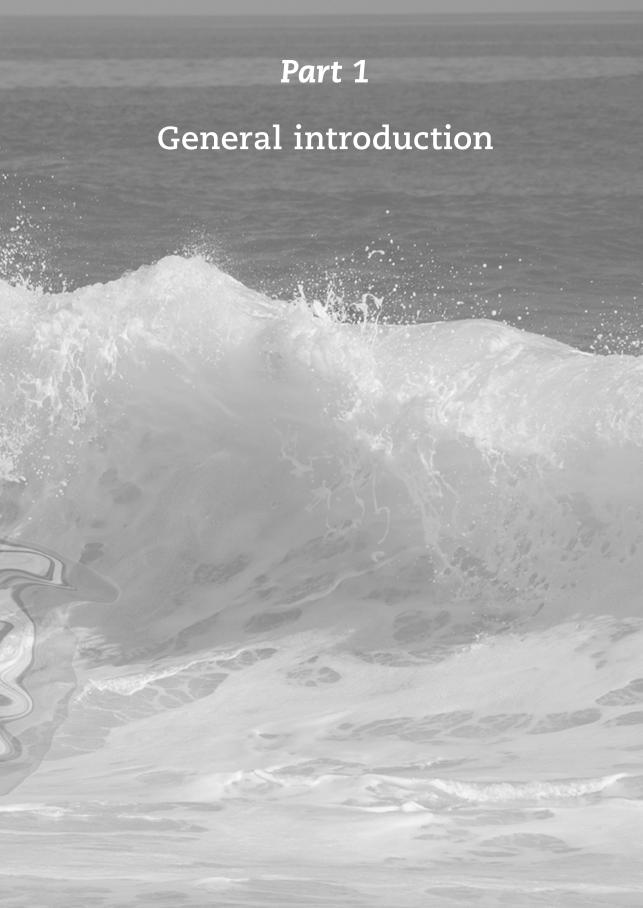
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Sex and gender differences

In the early sixties, a prominent professor in Clinical Pharmacology at the University College in London, D.R. Laurence, stated: "There are no clinically important sex differences in drug action, except, of course, to sex steroid hormones, but the subject is poorly documented. Women are said to be more liable to become excited by morphine than are men; in this respect they resemble cats." ¹ It was thought that study results in men could easily be extrapolated to women, and women were excluded from clinical studies for simplicity and protection from harmful drug effects to them and their fetuses. ² Fortunately, the following decades, this insight changed and sex differences in clinical pharmacology have gained more attention. Unfortunately, however, still not enough, as will be demonstrated in this thesis.

Sex and *gender* both refer to the differentiation in men and women. Sex refers to the biological differences; *gender* to the behavioral and psychological perception of sexual identity.³ Besides the well-known sex differences in the reproductive system and physical and psychological features,⁴ men and women differ from more perspectives, such as physiology, anatomy and pathophysiology and disease treatment. This variety also leads to differences in drug response.

Women seem to have a 1.5 to 1.7-fold higher risk of adverse drug reactions than men. 5-7 Women have lower body mass and a higher body fat percentage than men, which contributes to variation in drug response. Regarding genotype, one would expect no major differences, since the majority of genes that encode drug-metabolizing enzymes (DMEs) are not located on sex chromosomes. However, due to the complex interaction between sex steroid hormones, genetics, epigenetics, physiology and anatomy, the so-called 'sex-factor' is important in drug metabolism. Therefore, pharmacological therapy should be monitored accurately and evaluated regularly, taking into account potential sex differences in response and adverse reactions.

Genes, metabolism and transport, and sex steroid hormones

Drug metabolism is roughly divided into two phases. The cytochrome P450 (CYP) enzyme family is the primary system for phase I metabolism in the liver and intestines. Besides drug metabolism, CYP enzymes are involved in the biosynthesis and metabolism of several endogenous substances such as sex steroid hormones. The expression and activity of CYP enzymes and drug transporters is, on the other hand, partly regulated by sex steroid hormones. Expression and activity of these CYP enzymes varies during pregnancy and between the sexes.⁸⁻¹⁰

Variation in DME and transporter function affects drug accumulation and elimination, and therefore drug response. This is partly caused by genetic variation. Various single nucleotide polymorphisms (SNPs) have been identified in DMEs and transporters, which significantly changed drug response. ¹¹⁻¹³

Gene expression is influenced by nuclear receptors,¹⁴ which are for a large part under hormonal control.^{15, 16} Fluctuating hormonal levels, due to different hormonal secretion patterns, can influence the amount of functional protein that is formed. For drug metabolism, this may lead to variation in DME and transporter function and the response to a drug.^{17, 18}

Cardiovascular drug response

Cardiovascular diseases are the most important cause of mortality in both men and women. Therefore, in studying these diseases or their treatment, accurate information about both sex-related differences in response and adverse reactions is needed. A recent study showed the still lacking attention for sex-related differences in clinical trials on cardiovascular disease or drug response.¹⁹ Special attention has been given to this topic, to put sex differences in cardiovascular disease on the map.^{20, 21}

Many sex-related differences in response to cardiovascular drugs can be explained by differences in physiology and pathophysiology. For example, women have smaller organ size, ^{22,23} and cardiac output and glomerular filtration rate are lower in women than in men. ^{24,25} The renin-angiotensin-system (RAS), which is involved in the development of hypertension and is an important target of many drugs, is partly under hormonal control, also resulting in sex differences in drug response. ^{26,27} Another example pertains to cardiac repolarization disturbances. QT-length, visualized by electrocardiography (ECG), is influenced by sex steroid hormones. ²⁸ The risk of long-QT syndrome or torsade de pointes due to certain drugs is higher in women. ²⁹ In women, the pathophysiology of cardiovascular disease differs from men in the perspective of vasculature involved, endothelial function, inflammation process and plaque development. ³⁰

Sex differences have been observed in the response to cardiovascular drugs. ^{31, 32} Although there are no sex-based treatment strategies for cardiovascular diseases, pharmacological treatment is different between men and women. For example, in antihypertensive treatment, women more often used a diuretic, while men more often used a beta-blocker, angiotensin-converting enzyme (ACE) inhibitor, or calcium antagonist. ³³

Aim and outline of this thesis

In this thesis, we studied sex differences in cardiovascular drug response from an epidemiological viewpoint. The objective was to gain more insight into sex differences in drug metabolism and adverse drug reactions in the cardiovascular system. This should contribute to a better understanding of sex differences in drug response and lead to a higher awareness of the need for continuous drug surveillance.

First, in the second part of this thesis, the results are presented from three studies about differences in adverse drug reactions. In these studies, hospital admissions due to ADRs were assessed on a national basis. In the first chapter (chapter 2.1), sex differences were assessed for all ADRs. Chapter 2.2 focuses on cardiovascular drugs specifically, and chapter 2.3 focuses on ADRs in children.

In the third part, we studied sex differences in the cardiovascular system from a genetic perspective. This part focuses on the different effects of genetics on myocardial infarction in men and women. First, we studied CYP2C8 and CYP2C9, which are involved in both the metabolism of drugs and in metabolic processes of endogenous substances in the endothelium (chapter 3.1). In the second study, we assessed androgen-metabolizing enzymes and their cardiac effects (chapter 3.2). Besides the epidemiological part, in which the genetic associations were studied, this study also included a part on heart tissue samples, in which mRNA expression of these enzymes was studied. Coffee intake has been described to protect against myocardial infarction. We studied one of the major enzymes in the metabolism of coffee (CYP1A2) for sex differences and the influence of age and smoking. This study is presented in chapter 3.3.

One of the main sex differences in ADR-related hospital admissions from the studies in Part 2 was diuretic-induced hyponatremia. We focused more in detail on this specific drug-ADR combination in Part 4. The first study (chapter 4.1) describes the risks of thiazide-induced hyponatremia in the general population. Second, drug-drug interactions between, for instance, RAS-inhibitors or non-steroidal anti-inflammatory drugs (NSAIDs) with thiazides and the risk of hyponatremia were studied (chapter 4.2). The third study of this part is a study on genetic variation in the transporters involved in thiazide secretion. We studied whether genetic variation in genes encoding the organic anion transporters (OAT) 1 and 3, are associated with thiazide-induced hyponatremia. The last chapter of this part (chapter 4.4) assesses the role of sex in thiazide-induced hypokalemia.

Recently, recommendations were made to improve safety during use of thiazides and other potassium-losing diuretics by performing extra laboratory measurements. Within a general practitioner (GP) database, we studied the quantity of control measurements and the occurrence of hyponatremia and hypokalemia. In chapter 5, the results of this study are presented.

Finally, in Part 6, the major findings from this thesis are discussed in a greater perspective, including the strengths, limitations and future perspectives. A summary of this thesis is presented in Part 7.

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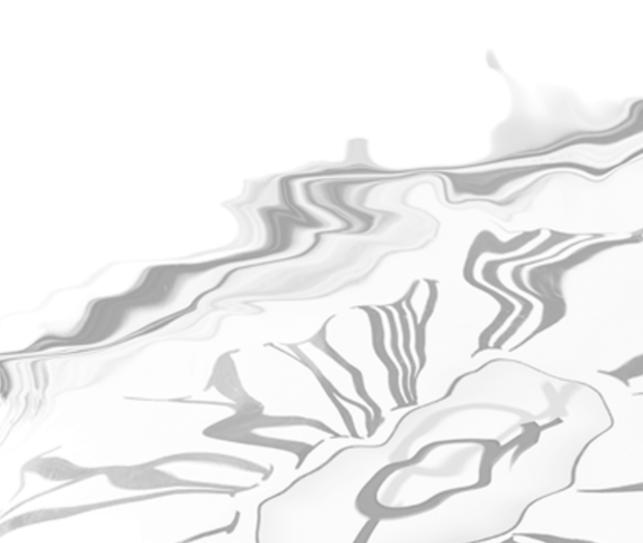




Chapter 2.1

Sex-related differences in hospital admissions attributed to adverse drug reactions in the Netherlands

Eline M. Rodenburg Bruno H.Ch. Stricker Loes E. Visser



Abstract

Background

Adverse drug reactions (ADRs) are a major burden in health care, regularly leading to hospital admission, morbidity or death. Women tend to have a higher risk of adverse drug reactions with a 1.5 to 1.7-fold greater risk than men. Our primary aim was to study differences in ADR-related hospitalizations between the sexes.

Methods

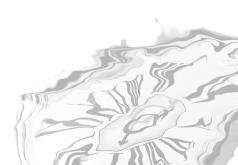
We conducted a nationwide study of all ADR-related hospitalizations in the period between 2000 and 2005 in the Netherlands, which were selected from all 9,287,162 hospital admissions in this period. ADR-drug group combinations with at least 50 admissions in one of the sexes were selected. Relative risks and confidence intervals were calculated with respect to total number of admissions and total number of prescriptions with men as reference.

Results

In total, 0.41% of the 4,236,368 admissions in men (95% CI 0.40, 0.42%) and 0.47% of the 5,050,794 admissions in women (95% CI 0.46, 0.48%) were attributed to an ADR by medical specialists (57% of all ADR-related admissions were in women). Differences between the sexes in risk of ADR-related hospitalization were found for antineoplastic and immunosuppressive drugs, antirheumatics, anticoagulants and salicylates, cardiovascular and neurological drugs, steroids, and antibiotics. In certain drug categories, risks of hospitalization changed after taking into account the total number of drug prescriptions.

Conclusion

In all different drug classes, significant differences exist between the sexes in ADR-related hospital admissions. Cardiovascular drugs account for the most pronounced differences between men and women. More research is needed to explain the clear sex differences in ADR-related hospital admissions.



Introduction

Drug action and biological reaction is a continuous topic of interest, as pharmacotherapy is the most frequently employed medical intervention, and the continuous development of new drugs and removal of old products from the market are representative of a dynamic discipline. Both beneficial and adverse drug reactions are important considerations for defining optimal treatment strategies. The World Health Organisation (WHO) defined an adverse drug reaction (ADR) as "a response to a drug that is noxious and unintended and occurs at doses normally used in man for the prophylaxis, diagnosis or therapy of disease, or for modification of physiological function".¹

Sex is an important determinant of drug use and drug response. Women tend to have a higher risk of adverse drug reactions with a 1.5 to 1.7-fold higher risk as compared with men.²⁻⁴ More data on drug response in women are needed. Although the authorities emphasized the importance of including more women in clinical trials as early as 1986, women are still underrepresented in clinical research nowadays.⁵⁻⁷ The policies and guidelines, set up by the National Institutes of Health (NIH), Food and Drug Administration (FDA), and the European Medicines Agency (EMA), have unfortunately not resolved this inequality.⁸⁻¹⁰

A clear overview of sex differences in pharmacology is complicated by the large variety of drugs, indications for use, and pharmacokinetic and pharmacodynamic differences between the sexes. Pharmacokinetics, pharmacodynamics and the number and amount/dose of drugs used all contribute to the risk of occurrence of adverse reactions.¹¹⁻¹⁴

Sex differences in drug use can be explained by differences in incidence of disease (e.g. rheumatoid diseases) or by drug response itself. The effect of a drug on the body depends on the combination of pharmacokinetic factors. Women have a different volume of distribution and clearance than men, which could result in differences in effective drug concentrations. A sex difference in pharmacodynamics, the response of the body to the drug, is for example the occurrence of drug-induced torsade de pointes, which is much more frequent in women than in men. 16, 17

ADRs are a major healthcare issue, regularly leading to hospital admission, morbidity or death. ¹⁸⁻²³ In a population-based study in Sweden, fatal ADRs were the seventh cause of death. ²⁰ In hospital patients, ADRs were even ranked from the fourth to sixth cause of death. ²⁴ Data on ADRs leading to hospital admissions vary among smaller and larger studies (0.2-41.3%). ²³ Generally, the incidence of hospital admission caused by ADRs is between 3 and 6% of all hospital admissions. ^{18, 19, 23-25}

In the Netherlands, three major studies focussed on different aspects of ADR-related hospitalizations in the Dutch population. Van der Hooft et al. have studied ADR-related hospitalizations in the Netherlands in 2001. The proportion of females with ADR-related hospitalizations varied between the different age categories, increasing with increasing age from 50.5% in the age group 65-79 years to 66.6% in the highest age group (80 years and older). The proportion of ADR-related hospitalizations increased with age from 0.8% in patients aged <18 years to 3.2% in patients aged \geq 80 years. Another population-based study in the Netherlands showed a prevalence of ADR-related admissions of 5.35% after standardizing to the Dutch population. A third study in 21 Dutch hospitals showed that important patient-related risk factors for admission with an adverse drug effect (ADE) due to medication use or medication error, were impaired cognition, presence of 4 or more diseases, dependent living situation, impaired renal function and nonadherence to the medical regimen. The latter two studies did not focus on sex differences in specific adverse events and drug groups.

While female sex has been identified as a risk factor for ADRs, sex-related differences in hospital admissions attributed to ADRs have not been studied as a primary outcome in large populations. We studied the differences between the sexes in hospital admission attributed to ADRs in a nationwide study over a 6-year period, taking into account the different ADRs, drug groups involved, and differences in drug prescriptions.

Methods

Data sources

Data on hospital admissions and drug use were obtained from separate sources. Data on hospital admissions were obtained from a nationwide registry of hospital discharges (LMR). This registry contains patient characteristics, demographics, dates of admission and discharge, main diagnoses at discharge (coded), secondary diagnoses (coded), medical specialisms (coded) and special codes indicating drug-related hospitalizations (E-codes), based on the ICD-9-CM coding system. Characteristics of all hospital admissions are registered by medical doctors on the basis of hospital discharge letters and coded by professional code clerks. For every admission, one discharge/main diagnosis (mandatory), and up to nine secondary diagnoses (optional) are registered. The coding is independent of hospital or specialist. All diagnoses are submitted in the same format, mostly

electronically. All patients with an admission to a Dutch hospital in the period between 2000 and 2005 were included in the study.

Data on drug use were retrieved from 'Stichting Farmaceutische Kengetallen' (SFK), where information on drug prescriptions is collected from 1,805 pharmacies in the Netherlands (of the 1,960 pharmacies in total). Data from this database were selected on ATC-4 level per year within the study period. Per ATC code, the cumulative number of prescriptions was calculated.

Adverse drug reactions

An ADR-related hospitalization was defined as a hospitalization with an E-code as secondary diagnosis, indicating an ADR as the reason for hospitalization (E-code referring to main diagnosis). ADRs occurring during hospital admission were not included in the analysis. The E-code indicates the drug group involved in the ADR. E-codes referring to intended overdoses, errors in administration and therapeutic failure were not included in the analysis. Unique combinations of main diagnoses and E-codes were selected, resulting in assessment of ADRs per drug group.

Data analysis

We assessed the number of ADR-related hospital admissions and expressed this as the proportion of all admissions in the Netherlands between 2000 and 2005. We calculated relative risks and 95% confidence intervals (95% CIs) for hospitalizations due to an ADR with respect to all hospitalizations for women compared with men. We adjusted for the possible confounding effect of age using logistic regression analyses. Given the size of the study population, odds ratios (OR) are a good proxy for relative risks (RR). The analyses were performed for all possible ADR-drug group combinations separately. To make a more valid comparison, we only included the ADR-drug group combinations with at least 50 admissions in the study period in at least one of the sexes. Adverse drug reactions pointing out the same reaction, but described in different terms, were clustered (e.g. congestion and constipation). Furthermore, ADRs within the drug group annotated with the terms 'other drugs' and 'unspecified drugs' were excluded from further analyses.

Separate calculations were performed for all ADR-drug group combinations to measure the RRs for ADR-related hospitalizations in relation to the total number of prescriptions per drug group. For every ADR-drug group combination the number of hospitalizations per sex was divided by the total number of

prescriptions within the study period for the involved drug group. Prescription data were combined with the data on hospitalizations based on drug(s) covered by the E-code. Codes were combined as specific as possible. Calculations were performed using SPSS software (version 15.0; SPSS Inc., Chicago, Illinois, USA) and Microsoft Office Excel 2003.

Results

In the period between 2000 and 2005, 9,287,162 hospital admissions were registered in the Netherlands; 4,236,368 in men (46%) and 5,050,794 in women (54%). Of these hospital admissions, 41,260 admissions had an E-code referring to the main diagnosis, indicating that the admission was attributed to an adverse drug reaction. For men, ADR-related admissions in this period accounted for 17,561 admissions (0.41% of all admissions in men and 43% of all ADR-related admissions); for women 23,699 admissions occurred (0.47% of all admissions in women and 57% of all ADR-related admissions). Figure 1 shows the total number of ADR-related admissions per sex. The total number of prescriptions was nearly two times higher in women than in men with an increasing number over the years in both sexes. In women, more than 455 million prescriptions were recorded in the period of 2000 to 2005 as compared with nearly 286 million prescriptions

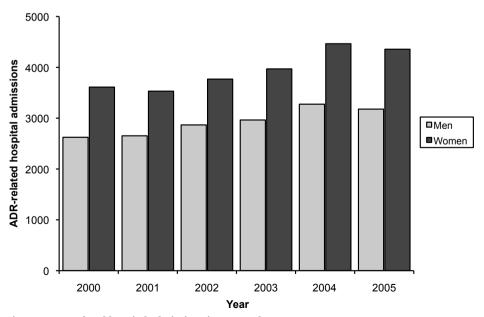


Figure 1. ADR-related hospital admissions in men and women per year.

in men. With these prescriptions, women were prescribed nearly 20,989 million defined daily doses (DDDs) and men were prescribed 13,580 million DDDs (see Table 1 for an overview). Figure 2 shows the difference in hospital admissions between the sexes during the study period, taking into account the total number of prescriptions.

Causes of admission varied widely. In total, 4,750 unique combinations of diagnosis and ADR-associated drug groups were identified in the database. Eighty of these combinations led to at least fifty hospital admissions per combination within the study period in either one of the sexes. Eighteen of the selected com-

Table 1. ADR-related hospital admissions and prescriptions in men and women between 2000 and 2005

Year	Year ADR-related admission		Total Pre	escriptions	Total Define	Total Defined Daily Doses		
	Men	Women	Men	Women	Men	Women		
2000	2,624	3,611	43,348,659	70,877,424	1,940,059,960	3,169,985,513		
2001	2,653	3,531	45,283,605	73,431,636	2,075,935,477	3,334,278,357		
2002	2,867	3,768	46,687,293	75,185,906	2,167,674,617	3,422,053,655		
2003	2,964	3,969	48,717,748	77,967,105	2,333,542,622	3,627,575,647		
2004	3,275	4,464	50,004,412	78,045,769	2,459,947,493	3,639,307,300		
2005	3,178	4,356	51,813,349	79,949,354	2,603,010,013	3,795,615,958		
Total	17,561	23,699	285,855,066	455,457,194	13,580,170,182	20,988,816,430		

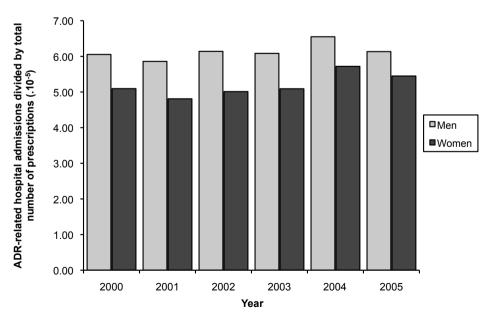


Figure 2. ADR-related hospital admissions divided by total prescriptions in men and women per year.

binations could be combined with another selected, similar drug-ADR combination. Six combinations were excluded from further analyses because of lack of additional information.

Seven large drug classes could be distinguished as drug groups leading to hospitalization: antineoplastic and immunosuppressive drugs, antirheumatics, anticoagulants and salicylates, drugs acting on the nervous system, drugs acting on the cardiovascular system, steroids and antibiotics. Tables 2-4 show the number of ADR-related hospitalizations and relative risks in women compared with men due to antineoplastic and immunosuppressive drugs, antirheumatics, and anticoagulants and salicylates, respectively. Tables 5-8 show the number of ADR-related hospitalizations due to drugs acting on the nervous system, drugs acting on the cardiovascular system, steroids, and antibiotics.

Of the seven drug groups in total, three drug groups were most prominently associated with adverse drug reactions, i.e. antineoplastic and immunosuppressive drugs, anticoagulants and salicylates, and drugs acting on the cardiovascular system. Frequently occurring adverse drug reactions in the group with antineoplastic and immunosuppressive drugs included agranulocytosis, fever, and nausea/vomiting. In the drug group with anticoagulants and salicylates, frequent reactions included gastro-intestinal bleeding, epistaxis, intracranial bleeding and other haemorrhages. For drugs acting on the cardiovascular system, poisoning by cardiotonic glycosides, collaps due to coronary vasodilators, and hypovolemia and electrolyte disorders due to diuretics accounted for the majority of adverse reactions.

Sex differences

Antineoplastic and immunosuppressive drugs

Per ADR-drug group combination, as shown in Table 2-8, relative risks (RRs) were calculated. The tables show the RRs of occurrence of the specific ADR in women compared with men, with and without adjustment for age. ATC codes are given to show the drug groups used to present drug use. Due to antineoplastic and immunosuppressive drugs, women were more frequently hospitalized with agranulocytosis, fever, and symptoms such as nausea and vomiting (Table 2); men were more frequently admitted due to pneumonia. Relative to total admissions, hospital admission because of fever attributed to antineoplastic and immunosuppressive drugs was higher in women than in men, but after adjustment for drug prescriptions the results showed the opposite. Only the RR for hospitalization due to nausea/vomiting remained significantly higher for women after taking into account the total number of prescriptions.

Table 2. Antineoplastic and immunosuppressive drugs (ATC-code 'L')

Adverse reaction	Women (N)*	Men (N)*	RR (95% CI) ¹	RR (95% CI) ²	RR (95% CI) ³
Agranulocytosis	839	548	1.28 (1.15, 1.43)	1.31 (1.18, 1.46)	0.95 (0.85, 1.05)
Anemia	510	419	1.02 (0.90, 1.16)	1.05 (0.92, 1.19)	0.75 (0.65, 0.86)
Fever	1,182	903	1.10 (1.01, 1.20)	1.13 (1.03, 1.23)	0.81 (0.74, 0.88)
Malaise or fatigue	227	159	1.20 (0.98, 1.47)	1.24 (1.01, 1.52)	0.88 (0.72, 1.08)
Nausea/vomiting	542	248	1.83 (1.57, 2.13)	1.87 (1.61, 2.18)	1.35 (1.16, 1.57)
Non-infectious/toxic Gastro-enteritis	257	228	0.95 (0.79, 1.13)	0.98 (0.82, 1.17)	0.69 (0.58, 0.83)
Unwanted drug effect	377	299	1.06 (0.91, 1.23)	1.09 (0.93, 1.27)	0.78 (0.67, 0.91)
Pneumonia	43	67	0.54 (0.37, 0.79)	0.56 (0.38, 0.82)	0.40 (0.27, 0.58)
Poisoning by cytostatics	78	74	0.88 (0.64, 1.21)	0.90 (0.66, 1.24)	0.65 (0.47, 0.89)

^{*} Number of admissions in the period 2000-2005;

Antirheumatics

Gastro-intestinal bleeding was the major ADR-related cause of admissions due to antirheumatic drug use. Ulcers were significantly more frequent in men. Regarding all hospital admissions attributed to this drug group, women were more frequently hospitalized with an ADR. However, after taking into account the total number of prescriptions in this drug group, the risk of ADR-related hospitalizations attributed to antirheumatic use was higher in men (Table 3).

Table 3. Antirheumatics (ATC-code 'M01')

Adverse reaction	Women (N)*	Men (N)*	RR (95% CI) ¹	RR (95% CI) ²	RR (95% CI) ³
Anaphylactic shock	136	88	1.30 (0.99,1.70)	1.32 (1.01,1.73)	0.97 (0.74,1.27)
Gastro-intestinal bleeding	190	133	1.20 (0.96,1.50)	1.24 (0.99,1.55)	0.89 (0.72,1.12)
Poisoning by antirheumatics	73	47	1.30 (0.90,1.87)	1.29 (0.90,1.87)	0.97 (0.67,1.40)
Unwanted drug effect	117	81	1.21 (0.91,1.61)	1.22 (0.92,1.63)	0.90 (0.68,1.20)
Duodenal/ventricular ulcer	59	69	0.90 (0.76,1.06)	0.92 (0.78,1.09)	0.67 (0.57,0.79)

^{*} Number of admissions in the period 2000-2005;

Anticoagulants and salicylates

The risk of hospitalizations for bleeding in any specific form due to anticoagulant use or use of salicylates was significantly higher in men (except for non specified haemorrhage and, after taking into account the total number of prescriptions, gastro-intestinal bleeding) (Table 4). Hospitalizations for haematuria and hae-

¹ Relative risk of ADR-related hospitalizations with respect to total number of hospital admissions;

² Relative risk of ADR-related hospitalizations with respect to total number of hospital admissions, age adjusted;

³ Relative risk of ADR-related hospitalizations with respect to total number of prescriptions.

¹ Relative risk of ADR-related hospitalizations with respect to total number of hospital admissions;

 $^{^2}$ Relative risk of ADR-related hospitalizations with respect to total number of hospital admissions, age adjusted;

³ Relative risk of ADR-related hospitalizations with respect to total number of prescriptions.

Table 4. Anticoagulants and salicylates (ATC-code 'B01A')

Adverse reaction	Women (N)*	Men (N)*	RR (95% CI) ¹	RR (95% CI) ²	RR (95% CI) ³
Anemia	222	138	1.35 (1.09, 1.67)	1.29 (1.04, 1.59)	1.75 (1.42, 2.17)
Gastro-intestinal bleeding	1,067	1,064	0.84 (0.77, 0.92)	0.82 (0.75, 0.89)	1.09 (1.00, 1.19)
Epistaxis	285	337	0.70 (0.60, 0.83)	0.71 (0.60, 0.83)	0.92 (0.79, 1.08)
Haemoptysis	59	115	0.43 (0.31, 0.59)	0.44 (0.32, 0.60)	0.56 (0.41, 0.76)
Haematuria	74	223	0.28 (0.22, 0.36)	0.28 (0.21, 0.36)	0.36 (0.28, 0.47)
Intracranial bleeding	370	598	0.52 (0.46, 0.59)	0.51 (0.45, 0.58)	0.67 (0.59, 0.77)
Haemorrhage non specified	930	692	1.13 (1.02, 1.25)	1.13 (1.02, 1.24)	1.46 (1.33, 1.61)
Duodenal/ventricular ulcer	351	479	0.61 (0.54, 0.71)	0.61 (0.53, 0.70)	0.80 (0.70, 0.92)

^{*} Number of admissions in the period 2000-2005;

moptysis were much more frequent in men than in women with a RR of 0.28 (95% CI 0.22, 0.36) and 0.43 (95% CI 0.31, 0.59), respectively. These differences remained after adjusting for age and taking into account the total number of prescriptions (RR 0.36; 95% CI 0.28, 0.47 and RR 0.56; 95% CI 0.41, 0.76), respectively).

Drugs acting on the nervous system

Considering all hospitalizations related to use of drugs acting on the nervous system, admissions due to ADRs were in general higher in women (Table 5). Poisoning and constipation were the most frequent ADRs related to use of drugs acting on the nervous system. Relatively more women were hospitalized due to poisoning than men, but after taking into account the differences in drug prescriptions, this relative risk of admission disappeared. Risk to be hospitalized for constipation was highest in men (RR 0.59; 95% CI 0.48, 0.71). Nausea and vomiting causing hospital admission due to (other) opiates and related narcotics was more profound in women both with respect to admissions (RR 2.81; 95% CI 1.64, 4.83) and with respect to prescriptions (RR 1.90; 95% CI 1.10, 3.26).

Drugs acting on the cardiovascular system

Drugs acting on the cardiovascular system cover several different drugs causal to various ADRs (Table 6). Within this category, the risks of ADR-related admissions were most pronounced, as compared with other drug classes. Diuretics appeared to be the main causing drugs for hospital admissions. Differences between the sexes are remarkable. Women had a RR of 5.33 (95% CI 4.32, 6.58) for hospitalization due to hypo-osmolarity or hyponatremia and a RR of 3.42 (95% CI 2.41, 4.83) for hospitalization due to hypokalemia as compared with men.

¹ Relative risk of ADR-related hospitalizations with respect to total number of hospital admissions;

² Relative risk of ADR-related hospitalizations with respect to total number of hospital admissions, age adjusted:

³ Relative risk of ADR-related hospitalizations with respect to total number of prescriptions.

Table 5. Drugs acting on the nervous system

Drug type	Women (N)*	Men (N)*	RR (95% CI) ¹	RR (95% CI) ²	RR (95% CI) ³		
Antidepressants (N06A)							
Poisoning (unintended)	60	22	2.29 (1.41, 3.73)	2.27 (1.39, 3.69)	1.29 (0.79, 2.11)		
Aromatic analgesics (N02B)							
Poisoning (unintended)	72	32	1.89 (1.25, 2.86)	1.87 (1.24, 2.84)	1.05 (0.69, 1.59)		
Opiates and related narcotics	(N02A)						
Constipation	208	200	0.87 (0.72, 1.06)	0.90 (0.74, 1.10)	0.59 (0.48, 0.71)		
Nausea / vomiting	57	17	2.81 (1.64, 4.83)	2.91 (1.69, 5.00)	1.90 (1.10, 3.26)		
Poisoning (unintended)	89	42	1.78 (1.23, 2.57)	1.84 (1.28, 2.66)	1.20 (0.83, 1.73)		
Anticonvulsants (N03A)							
Poisoning (unintended)	69	67	0.86 (0.61, 1.20)	0.87 (0.63, 1.22)	0.88 (0.63, 1.23)		
Benzodiazepine-based tranq	uillizers (N05B)						
Poisoning (unintended)	163	91	1.50 (1.16, 1.94)	1.51 (1.17, 1.95)	0.89 (0.69, 1.16)		
Psychotropics (N06B)							
Poisoning (unintended)	186	89	1.75 (1.36, 2.25)	1.82 (1.41, 2.34)	6.38 (4.96, 8.22)		
Unwanted drug effect	58	19	2.56 (1.53, 4.29)	2.66 (1.58, 4.46)	9.32 (5.55, 15.65)		

ATC codes are presented between brackets;

These higher risks for women remained after adjustment for the total number of prescriptions of these drugs (RR 3.33; 95% CI 2.70, 4.10 and RR 2.13; 95% CI 1.51-3.01, respectively). Cardiotonic glycosides were also more frequent as a cause for hospital admissions in women, with a RR of 2.07 (95% CI 1.59, 2.70) for unwanted drug effect and 2.42 (95% CI 1.93, 3.03) for poisoning. Syncope or collapse due to coronary vasodilators and hypovolemia due to diuretics occurred more frequently in men (RR 0.68; 95% CI 0.54, 0.86 and RR 0.79; 95% CI 0.67, 0.93, respectively, after adjustment for number of prescriptions).

Steroids

Sex differences in ADR-related hospital admissions due to steroids were as expected (Table 7). A few causal ADRs were not assessable, because use of these hormones is sex dependent and therefore none or seldom hospital admissions occurred in men (anterior pituitary hormones, ovarian hormones). Adrenal cortical steroids and insulins and antidiabetic agents were equally frequently associated with ADRs in both sexes. Admission for osteoporosis due to adrenal cortical steroids was more frequent in women (RR 2.50; 95% CI 1.82, 3.43, after adjustment for total number of drug prescriptions), whereas diabetes due to ad-

^{*} Number of admissions in the period 2000-2005;

¹ Relative risk of ADR-related hospitalizations with respect to total number of hospital admissions;

² Relative risk of ADR-related hospitalizations with respect to total number of hospital admissions, age adjusted;

³ Relative risk of ADR-related hospitalizations with respect to total number of prescriptions.

Table 6. Drugs acting on the cardiovascular system

Drug type	Women (N)*	Men (N)*	RR (95% CI) ¹	RR (95% CI) ²	RR (95% CI) ³
Cardiac rhythm regulator (C01B)					
Heart dysrhythmia	49	52	0.79 (0.54, 1.17)	0.76 (0.52, 1.13)	1.10 (0.74, 1.62)
Cardiotonic glycosides (C01A)					
Unwanted drug effect	190	77	2.07 (1.59, 2.70)	1.94 (1.49, 2.53)	1.66 (1.27, 2.16)
Poisoning	291	101	2.42 (1.93, 3.03)	2.30 (1.84, 2.89)	1.93 (1.54, 2.43)
Coronary vasodilators (C01D)					
Syncope/collaps	128	170	0.63 (0.50, 0.79)	0.62 (0.50, 0.79)	0.68 (0.54, 0.86)
Diuretics (C03A/C)					
Disorder kidney / ureter	57	48	1.00 (0.68, 1.47)	0.95 (0.64, 1.39)	0.62 (0.42, 0.91)
Hypoosmolarity/ hyponatriemia	642	101	5.33 (4.32, 6.58)	5.02 (4.06, 6.19)	3.33 (2.70, 4.10)
Hypokalemia	163	40	3.42 (2.41, 4.83)	3.53 (2.50, 4.99)	2.13 (1.51, 3.01)
Hypovolemia	348	231	1.26 (1.07, 1.49)	1.15 (0.97, 1.36)	0.79 (0.67, 0.93)
Renal failure	57	34	1.41 (0.92, 2.15)	1.38 (0.90, 2.11)	0.88 (0.57, 1.34)
Sympatholytics (C04A)					
Heart dysrhythmia	118	82	1.21 (0.91, 1.61)	1.20 (0.90, 1.59)	1.04 (0.79, 1.38)
Other antihypertensive agents (CO2	2A/C)				
Angioneurotic edema	83	66	1.05 (0.76, 1.45)	1.09 (0.79, 1.51)	0.89 (0.64, 1.23)

ATC codes are presented between brackets;

Table 7. Steroids

Drug type	Women (N)*	Men (N)*	RR (95% CI) ¹	RR (95% CI) ²	RR (95% CI) ³
Adrenal cortical steroids (H02A)					
Diabetes mellitus	143	136	0.88 (0.70, 1.12)	0.91 (0.72, 1.15)	0.78 (0.62, 0.99)
Osteoporosis	165	49	2.82 (2.05, 3.88)	2.93 (2.13, 4.03)	2.50 (1.82, 3.43)
Anterior pituitary hormones (H01A/	'B)				
Ovarian hyperfunction	216	0	N.a.	N.a.	N.a.
Ovarian disorder (non- inflammatory)	64	0	N.a.	N.a.	N.a.
Unwanted drug effect	66	0	N.a.	N.a.	N.a.
Insulins and antidiabetic agents (A	10)				
Hypoglycemia	1,126	946	1.00 (0.92, 1.09)	1.04 (0.95, 1.13)	1.00 (0.92, 1.09)
Hypoglycemic coma	108	120	0.75 (0.58, 0.97)	0.78 (0.60, 1.02)	0.76 (0.59, 0.98)
Ovarian hormones (G03 (exG03B))					
Pulmonary embolism or lung infarction	63	1	52.84 (7.33, 380.95)	52.30 (7.25, 377.10)	0.30 (0.04, 2.16)

ATC codes are presented between brackets; N.a.=not applicable;

^{*} Number of admissions in the period 2000-2005;

¹ Relative risk of ADR-related hospitalizations with respect to total number of hospital admissions;

² Relative risk of ADR-related hospitalizations with respect to total number of hospital admissions, age adjusted;

³ Relative risk of ADR-related hospitalizations with respect to total number of prescriptions.

^{*} Number of admissions in the period 2000-2005;

¹ Relative risk of ADR-related hospitalizations with respect to total number of hospital admissions;

² Relative risk of ADR-related hospitalizations with respect to total number of hospital admissions, age adjusted;

 $^{^{\}scriptscriptstyle 3}$ Relative risk of ADR-related hospitalizations with respect to total number of prescriptions.

Table 8. Antibiotics

Drug type	Women (N)*	Men (N)*	RR (95% CI) ¹	RR (95% CI) ²	RR (95% CI) ³
Penicillins (J01C)					
Dermatitis	68	43	1.33 (0.91, 1.95)	1.33 (0.91, 1.95)	1.35 (0.92, 1.97)
Anaphylactic shock	59	56	0.88 (0.61, 1.27)	0.89 (0.62, 1.29)	0.90 (0.62, 1.29)
Unwanted drug effect	129	78	1.39 (1.05, 1.84)	1.40 (1.06, 1.85)	1.41 (1.06, 1.86)
Other specified antibiotics (J01G/M/l	R/X)				
Non-infectious gastro-enteritis or colitis	66	39	1.42 (0.96, 2.11)	1.46 (0.99, 2.18)	0.53 (0.36, 0.79)
Unwanted drug effect	59	58	0.85 (0.59, 1.22)	0.87 (0.60, 1.25)	0.32 (0.22, 0.46)
Sulfonamides (J01E)					
Unwanted drug effect	65	40	1.36 (0.92, 2.02)	1.38 (0.93, 2.05)	0.54 (0.36, 0.80)

ATC codes are presented between brackets;

renal cortical steroids and hypoglycemic coma due to insulins and antidiabetic agents were more frequent in men (RR 0.78; 95% CI 0.62, 0.99 and RR 0.76; 95% CI 0.59, 0.98, respectively, after adjustment).

Antibiotics and other drugs

Overall, the risk of ADR-related admissions due to antibiotics seemed to vary per type of ADR between the sexes as a part of total admissions. However, if drug prescriptions were taken into account, men were more frequently hospitalized for ADRs following antibiotic use (Table 8).

Discussion

The primary aim of our study was to give an overview of the differences in ADR-related hospitalizations of the most frequent adverse drug reactions between men and women. Both drug group and type of ADR were of interest in our study. Because the incidence of ADR-related hospitalizations is related to drug use, drug use of the total population within the study period was taken into account in the analyses. However, we should be careful when interpreting the results, since no individual data were used in this ecological design.

Overall, the risk of ADR-related hospital admissions was higher in women than in men, with respect to the total number of hospital admissions. This is in accordance with other studies focusing on ADR-related hospital admissions. ^{2-4, 18, 19, 22} However, the cumulative incidences of ADR-related admissions

^{*} Number of admissions in the period 2000-2005;

¹ Relative risk of ADR-related hospitalizations with respect to total number of hospital admissions, crude;

 $^{^2}$ Relative risk of ADR-related hospitalizations with respect to total number of hospital admissions, age adjusted;

³ Relative risk of ADR-related hospitalizations with respect to total number of prescriptions.

(0.41% in men and 0.47% of total admissions in women) from this study were lower than the incidences reported in the literature. This might be due to underrecognition and to the coding system in which notification of causes is done on a voluntary basis.

Drug use within the study period was higher among women and after adjustment for this use, ADR risk clearly changed in all different drug groups. For various drug-related admissions, risks for the sexes turned in the opposite direction. This was surprising, since female sex is usually indicated as a major risk factor for developing an ADR.

As far as we are aware, this is the first study in which ADRs are combined with prescription data on a national basis. Previous studies have taken into account drug prescriptions, but these studies focused more on drug use per patient when admitted to the hospital, instead of taking into account background use.^{2,4,19,21,22} Martin et al.³ studied the incidence of ADRs in the sexes per drug exposure time. However, this concerned prescriptions for a variety of drugs, not specified per drug group.

According to earlier studies, risk of ADRs due to antineoplastic agents was highest.^{2, 19} In our study, risk of hospitalization due to an ADR following use of antineoplastic and immunosuppressive drugs was higher in men in the majority of the most frequent reactions. This drug group is a good example of personalized drug dosing. Men receive much higher doses of drugs due to the adjustment for body surface or body weight in the majority of these drugs. A possible explanation for this sex difference is the variation in activity of the various drug metabolizing agents involved. Among others, this accounts for cytochrome P450 (CYP) 2B6 and CYP3A4.^{11, 12} Differences between the sexes in metabolizing capacity of these cytochrome enzymes, or involved transporters, could result in prolonged drug exposure.

Three major drug classes that are a burden in drug-related hospitalizations, as described in the literature, are NSAIDs, anticoagulants and cardiovascular drugs. 18, 19, 21 We found higher risks of ADR-related hospitalization due to antirheumatics in men as compared with women after adjustment for total number of prescriptions. Especially, hospitalization for gastrointestinal ulcers differed significantly. A possible explanation for this higher risk in men is that men are more exposed to other risk factors for gastrointestinal ulcers, such as alcohol use, coffee, smoking, H. pylori infection or use of other drugs (e.g. aspirin). 29, 30 Another theory can be that non-selective COX inhibitors are used more frequently by men.

Regarding use of anticoagulants and salicylates, risk of ADR-related hospitalization varied per type of reaction. Where men seemed to have a higher risk of

being hospitalized with specific haemorrhages (haematuria, haemoptysis and cerebral bleeding), women seemed more prone to be hospitalized with anemia. However, non-specified haemorrhages and gastrointestinal bleeding, which comprised the largest number of hospitalizations, resulted significantly more often in hospital admissions in women than in men. Cytochrome P450 (CYP) 2C9 plays an important role in the metabolism of anticoagulants and salicylates, as well as of certain antirheumatics. Genetic influence of the CYP2C9 enzyme on bleeding risk has been shown, but so far, no clear difference in amount or activity of this enzyme has been determined between the sexes.^{11, 12, 14, 15, 31, 32} A possible role for drug transporters must be considered.

Drugs acting on the cardiovascular system include a range of drugs with different sites of action. Men seemed to experience more hypovolemic symptoms, regarding coronary vasodilators and diuretics. Women were more at risk to be hospitalized due to adverse effects of cardiotonic glycosides and electrolyte disorders following use of diuretics. Adverse effects due to cardiotonic glycosides are well known. Because of slower renal clearance of these drugs in women, drug effects may be greater if doses have not been adjusted. The remarkable difference in risk of electrolyte disorders between the sexes has been noticed earlier³³ and could be explained by higher exposure levels due to lower clearance in women. Genetic variation in drug transporters (e.g. OATP1B1, OAT1, OAT4) might be considered,^{33, 34} but so far no major sex differences have been found.^{11, 14} Adverse reactions due to cardiovascular drugs are of major clinical relevance because of the high impact of potential consequences. Although these ADRs concern known reactions, the sex differences as shown by this study emphasize the importance of sex-based dosing or prescribing.

Antidepressants and other neurological drugs are often thought to cause more ADRs in women.^{32, 35} Despite the fact that this can partly be explained by pharmacodynamics, pharmacokinetics and drug use,^{32, 36} a recent review showed that current evidence has been derived from small studies.³⁵ In our study, interpretation of the results was impeded by the coding of the events. Poisoning was the most frequent drug reaction to neurologic drugs, but intended overdose could not be ruled out due to contradiction of the E-code and main code. In these cases, the E-code refers to drugs causing adverse effects in therapeutic use while the main code refers to poisoning by drugs, excluding adverse effects. Further studies are needed to further assess sex differences within these drug groups.

One of the strengths of our study was the availability of nationwide data on discharge diagnoses of all hospitalizations and data on drug use over a 6-year period. Data on drug use were also available for the same 6-year period, which made it possible to illustrate the use of the various drugs as a background of

ADR occurrence. Because of the ecological design of the study, it was not possible to match the data on drug use (which were not discernable on an individual basis) with the ADR-related hospitalizations. Therefore, interaction between the various drugs could not be studied. Although adjustment for age was done in the first analysis, unfortunately this was not possible in the analysis with total number of drug prescriptions. Another limitation is that the data within the drug categories did not match in an exact manner. This was due to the different coding systems used by the two databases in our study. Hospitalizations were only taken into account if the secondary diagnosis of the admission was coded as being due to an ADR. Because of the passive coding of ADRs related to the admission diagnosis at discharge, the cumulative incidence of ADR-related admissions is probably substantially underestimated. However, this underestimation is probably the same for men and women and would not influence the RRs.

Female sex is considered as a risk factor for development of ADRs to a variety of drug groups. When prescribing drugs to women, one should be aware of the differences in pharmacokinetics and pharmacodynamics compared with men. Although the overall number of ADR-related hospital admissions in our study confirmed the higher risk of women to be hospitalized due to an ADR, our study also suggests that the differences in drug use play a role in this gender difference. However, men were also at risk of ADRs but to other drug groups and the risk in men should not be overlooked.

It should be acknowledged that the above-mentioned factors are not the only ones accounting for the sex differences in drug metabolism. For instance, sex steroid hormones are likely to contribute to drug response to a great extent. First of all, sex steroid hormones have shown to influence target tissues, such as cardiac channel density and thiazide receptor density in the kidneys. Second, besides direct effects on drug metabolizing enzyme (DME) activity and drug transporters, sex steroid hormones also modulate gene expression. Sex differences in patterns of growth hormone (GH) secretion by the hypothalamus result in different expression patterns.

To obtain more insight into the difference in risk of adverse drug reactions between men and women, more research is needed to study the underlying mechanisms. Additional clinical trials and experimental research are necessary to further determine the role of sex steroid hormones and their effects on drug response.

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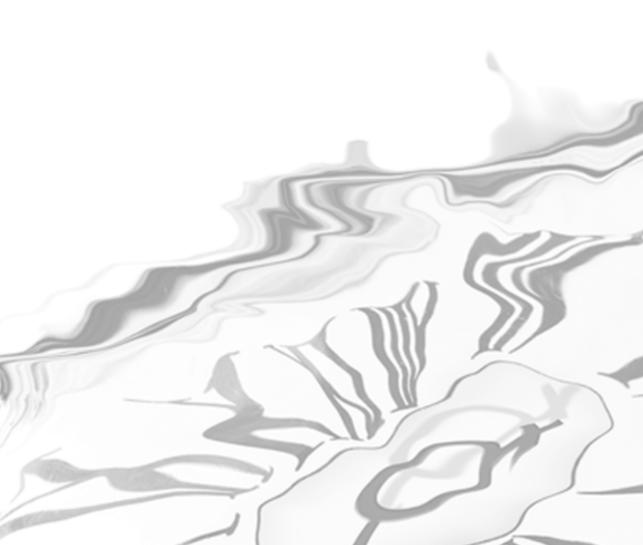
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Chapter 2.2

Sex differences in cardiovascular drug-induced adverse reactions causing hospital admissions

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Abstract

Background

Cardiovascular disease in women is often underestimated. The response to cardiovascular drugs differ between the sexes because of pharmacokinetic and pharmacodynamic differences. Adverse drug reactions (ADRs) within these drug classes may have serious consequences, leading to hospital admission. We aimed to study differences between men and women in hospital admissions for adverse drug reactions due to cardiovascular drugs.

Methods

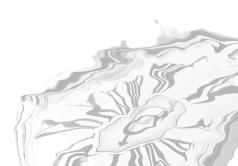
We conducted a nationwide study of all hospital admissions between 2000 and 2005 with data from the Dutch National Medical Register. Relative risks were calculated of hospital admissions due to ADRs to the different cardiovascular drug groups for women compared with men. By an ecological design, risks were adjusted for the total number of Dutch inhabitants, and total number of prescriptions.

Results

In total, 14,207 of the hospital admissions (34% of all ADR-related admissions) were attributed to cardiovascular drugs (7,690 in women (54%; 95% CI 53%, 55%)). 'Anticoagulants and salicylates' (N=8,988), 'thiazide and loop diuretics' (N=2,242) and 'cardiotonic glycosides' (N=932) were responsible for the majority of the ADR-related hospital admissions. The most pronounced sex differences were seen in users of thiazide diuretics (RR 4.02; 95% CI 3.12, 5.19), cardiotonic glycosides (RR 2.38; 95% CI 2.06, 2.74), loop diuretics (RR 2.10; 95% CI 1.91, 2.32) and coronary vasodilators (RR 0.77; 95% CI 0.65, 0.91).

Conclusion

Clear sex differences exist in ADRs requiring hospital admission for different cardiovascular drug groups. Sex differences should be taken into account in the prescription and evaluation of cardiovascular drugs.



Introduction

Cardiovascular disease is one of the most frequent causes of death in both men and women.^{1,2} Presentation of disease and disease outcome differ between the sexes.^{3,4} Differences in physiology and in regulation of cardiovascular function exist between men and women. Heart size is less, heart rate is higher and the QTc interval is longer in women than in men.⁵⁻⁸

Development of cardiovascular disease involves different biological pathways and the effects of cardiovascular risk factors differ between men and women. 9-11 For example, low values of high density lipoproteins (HDL) contribute more to the risk profile in men, whereas the risk in women is more dependent on low-density lipoproteins (LDL) and triglycerides. 12 The cardiovascular risk profile is also partly determined by sex steroid hormones; androgens and estrogens influence insulin sensitivity, atherosclerosis formation, blood pressure regulation and lipid levels. 12-16

Pharmacotherapy following myocardial infarction (MI) and the use of antihypertensives differs between men and women.¹⁷⁻¹⁹ For example, women are less likely to be treated with glycoprotein IIb/IIIa inhibitors after MI, and hypertensive women are more likely to use a diuretic than a beta-blocker, angiotensin-converting enzyme (ACE) inhibitor or calcium antagonist.^{17, 18} Regarding drug response, plasma levels of metoprolol and statins reach higher levels in women.^{20, 21} Furthermore, verapamil shows higher bioavailability in women.²² and response to ACE-inhibitors differs between the sexes.²³ However, there are no sex-based treatment guidelines for cardiovascular diseases.²⁴⁻²⁶ In 2001, a report was brought about by the Institute of Medicine (IOM), which induced debate on the importance of sex in health research.²⁷ Regulatory guidelines have been changed to enhance inclusion of women in clinical trials.^{28, 29} Differences in both presentation of disease and drug response between the sexes might require different treatment strategies to optimize efficacy and to prevent adverse drug reactions (ADRs).

We conducted a nationwide study to investigate the differences between men and women in adverse drug reactions associated with cardiovascular drugs requiring hospital admission. In this study, we also took into account the numbers of drug prescriptions. The aim of our study was to gain insight into the major differences in risk of hospital admissions for men and women and to determine the major cardiovascular drug groups causing these differences.

Methods

Data sources

Data on hospital admissions were obtained from a Dutch nationwide registry of hospital discharges (LMR). From 1 January 1986, all hospitals in the Netherlands were linked to the registry. This registry contains patient characteristics, dates of admission and discharge, main diagnosis at discharge (coded), secondary diagnoses (coded), medical specialisms (coded) and special codes indicating drug-related hospitalizations (E-codes), based on the ICD-9-CM coding system.³⁰ For every admission, one discharge/main diagnosis (mandatory), and up to nine secondary diagnoses (optional) are registered. Discharge diagnoses are made by medical specialists and coded by professional code clerks on the basis of hospital discharge letters. Subsequently, the data are coded by the medical registration and registered in the local computer system before transmission to the database. Coding is performed independently of reimbursement and is independent of hospital or specialist. All diagnoses are submitted in the same format, mostly electronically. All registered admissions in the period between 2000 and 2005 were included (N=9,287,162).

Information on prescriptions of cardiovascular drugs was obtained from the database of the Dutch Foundation of Pharmaceutical Statistics (SFK). Information on drug prescriptions is collected from 1,805 pharmacies in the Netherlands (of the 1,960 pharmacies in total). Data from this database were selected on pharmacological subgroup level according to the Anatomical Therapeutic Chemical (ATC) classification system. The information from these two data sources was not linked individually, but combined in an ecological manner.

Adverse drug reactions

An ADR-related hospitalization was defined as a hospitalization with an E-code as secondary diagnosis, indicating an ADR as the main reason for hospitalization (E-codes are auxiliary to the discharge diagnosis). The E-codes indicate the drug group involved in the ADR. Cardiovascular drugs were covered by the E-codes 942.0-942.9 ('agents primarily affecting the cardiovascular system'), 934.2 and 935.3 ('anticoagulants and salicylates') and 944.0-944.5 ('water, mineral, and uric acid metabolism drugs'). These drug groups included systemic agents acting on the water-balance. ADRs occurring during hospital admission were not included in the analysis.

Data analysis

ADR-related admissions associated with cardiovascular drugs were selected from all hospital admissions within the total study period. Per drug group, the number of admissions was studied per sex.

Subsequently, relative risks (RRs) for hospital admission due to the specific cardiovascular drug groups were calculated for women compared with men. To make a more valid comparison, these calculations were performed only for the groups with more than 50 hospital admissions. The risks were calculated with respect to the mean number of inhabitants of the Netherlands, with respect to total number of admissions adjusted for age, and with respect to total number of prescriptions within the specific drug group. In the first and latter analysis, RRs were calculated using Microsoft Office Excel 2003, according to the following calculation: (number of ADR-related admissions in women / mean number of female inhabitants or total number of drug prescriptions) / (number of ADR-related admissions in men / mean number of male inhabitants or total number of drug prescriptions). Information on drug prescriptions (ATC code) was combined with the specific drug groups as specific as possible (ICD-9 code).

For the age-adjusted analyses, logistic regression analyses were used, within the total number of admissions. Given the size of the study population and the rareness of the 'disease', the odds ratios (ORs) retrieved from this analysis will approximate the relative risks (RRs).³¹ Stratification by age (age < 50 years and age \geq 50 years) was performed to study the potential influence of menopause in women.

Third, we selected the cardiovascular drug groups causing significant sex differences in the number of ADR-related hospital admissions. For these groups, the most frequent reactions were studied per sex. ADRs accounting for at least 2.5% of the admissions within each particular drug group are demonstrated in the tables.

Results

Over the 6-year period covered in the analysis, 41,260 hospital admissions were ADR-related (17,561 in men and 23,699 in women), of which 14,207 admissions were associated with cardiovascular drugs (accounting for 34% of all admissions due to an ADR). Of these admissions, 7,690 admissions occurred in women (54%; 95% CI 53%, 55%). The cardiovascular drugs mainly responsible for hospital admissions were 'anticoagulants and salicylates', accounting for more than half of

Table 1. Number of hospital admissions attributed to cardiovascular drugs

Code*	Drug group		er of hospital a	lmissions
		Total	Women (%)	Men (%)
934.2/5.3	Anticoagulants and salicylates	8,988	4,392 (49)	4,596 (51)
944.4	Loop diuretics	1,864	1,272 (68)	592 (32)
942.1	Cardiotonic glycosides	932	660 (71)	272 (29)
942.6	Antihypertensive agents (other)	716	396 (55)	320 (45)
942.4	Coronary vasodilators	572	252 (44)	320 (56)
944.3	Thiazide diuretics	378	304 (80)	74 (20)
942.0	Cardiac rhythm regulators	324	173 (53)	151 (47)
942.9	Other and unspecified agents primarily affecting the cardiovascular system	178	100 (56)	78 (44)
942.2	Antilipemic and antiarteriosclerotic drugs	74	40 (54)	33 (46)
944.1	Purine derivative diuretics	47	24 (51)	23 (49)
942.5	Vasodilators (other)	43	19 (44)	24 (56)
944.5	Electrolytic, caloric, and water-balance agents	43	28 (65)	15 (35)
944.2	Carbonic acid anhydrase inhibitors	32	20 (63)	12 (37)
	Other [†]	16	10 (62)	6 (38)

^{*} Code according to the ICD-9 classification;

all cardiovascular drug-related admissions (63%). Loop diuretics were in second place and they were responsible for almost half of the remainder of the admissions together with the thiazide diuretics (16% of the total). Cardiotonic glycosides and antihypertensive agents accounted for 7% and 5% of all cardiovascular drug-related admissions, respectively. Table 1 represents all ADR-related admissions attributed to these cardiovascular drug groups.

Clear sex differences in ADR-related hospital admissions were seen after calculating relative risks. (Table 2) With respect to the total number of hospital admissions, women were more frequently admitted with an ADR-related to loop diuretics, thiazide diuretics and cardiotonic glycosides than men. The relative risk of hospital admissions due to thiazide diuretics was highest (RR 4.02; 95% CI 3.12, 5.19). In the age-adjusted analysis and the analysis taking into account the total number of prescriptions within this drug group the increased risk in women remained (RR 2.14; 95% CI 1.66, 2.75). Risks of admission related to loop diuretics were also higher in women, remaining after adjustment for the number of prescriptions (RR 1.13; 95% CI 1.02, 1.24). Cardiotonic glycosides accounted for a risk of women to be hospitalized with an ADR which was twice as high as for men (RR 2.38; 95% CI 2.06, 2.74). Adjustment for number of prescriptions still showed a 63% higher risk in women for an ADR-related hospital admission.

[†] Other drugs include: Antivaricose drugs, including sclerosing agents (942.7), Capillary-active drugs (942.8), Mineral salts, not elsewhere classified (other) (944.6), Ganglion-blocking agents (942.3), Mercurial diuretics (944.0).

Table 2. Relative risks for women for an ADR-related hospital admission attributed to a cardiovascular drug

Code*	Drug group	RR† (95% CI) ¹	RR† (95% CI) ²	RR† (95% CI) ³
934.2/5.3	Anticoagulants and salicylates (B01A)	0.94 (0.90, 0.98)	0.80 (0.77, 0.84)	1.04 (1.00, 1.08)
944.4	Loop diuretics (C03C)	2.10 (1.91, 2.32)	1.73 (1.57, 1.91)	1.13 (1.02, 1.24)
942.1	Cardiotonic glycosides (C01A)	2.38 (2.06, 2.74)	1.92 (1.66, 2.21)	1.63 (1.41, 1.88)
942.6	Antihypertensive agents (other) (C02A/C)	1.21 (1.05, 1.40)	1.08 (0.93, 1.25)	0.87 (0.75, 1.01)
942.4	Coronary vasodilators (C01D)	0.77 (0.65, 0.91)	0.66 (0.56, 0.78)	0.71 (0.60, 0.84)
944.3	Thiazide diuretics (C03A)	4.02 (3.12, 5.19)	3.44 (2.66, 4.43)	2.14 (1.66, 2.75)
942.0	Cardiac rhythm regulators (C01B+C07A)	1.12 (0.90, 1.20)	0.98 (0.78, 1.21)	0.89 (0.71, 1.10)
942.2	Antilipemic and antiarteriosclerotic drugs (C10A)	1.19 (0.75, 1.88)	1.05 (0.67, 1.67)	1.49 (0.94, 2.37)

ATC codes are presented between brackets;

ADR-related admissions associated with coronary vasodilators were more frequent in men. The age-adjusted relative risk with respect to the total number of admissions was 0.66 (95% CI 0.56, 0.78). After taking into account the total number of prescriptions, the relative risk changed only slightly (RR 0.71; 95% CI 0.60, 0.84). Hospital admissions with an ADR due to 'anticoagulants and salicylates' were also more frequent in men (RR 0.94; 95% CI 0.90, 0.98). After taking into account the total number of drug prescriptions, the risk estimate changed (RR 1.04; 95% CI 1.00, 1.08); no significant sex difference remained. Stratification by age (age < 50 years and age \geq 50 years) showed only few admissions below 50 years of age for all drug groups. Consequently, in the age group \geq 50 years, relative risks were similar to the total group and menopausal differences could not be studied adequately (data not shown).

For the drug groups with significant sex differences, the types of adverse reactions that were reported as cause of admission are shown in Table 3. The most frequent reaction in women due to loop diuretics was hypo-osmolality/ hyponatremia (36%), followed by volume depletion (27%). In men, volume depletion accounted for 38% of the ADR-related admissions in this drug group. Hypo-osmolality/hyponatremia was also the most frequent reason for admission in thiazide diuretics. However, in women this proportion was remarkably higher: 63% as compared with 31% in men. A low blood level of potassium was the second most frequent ADR in this group, accounting for 19% and 18% of ADR-related admissions in women and men, respectively. A remarkable difference was seen in the risk of the various types of reactions due to loop diuretics and

^{*} Code according to the ICD-9 classification;

[†] Relative risks show the risk for women to be hospitalized with men as the reference;

¹ Relative risk with the number of Dutch inhabitants used as denominator;

² Relative risk within total number of hospital admissions, adjusted for age;

³ Relative risk with total number of prescriptions used as denominator.

Table 3. Most frequently occurring reactions per cardiovascular drug group

Drug group	Women	Men	RR† (95% CI)
	N (%)*	N (%)*	_
Loop diuretics			
Volume depletion	340 (26.7)	225 (38.0)	0.79 (0.67, 0.94)
Hypoosmolality/hyponatremia	451 (35.5)	78 (13.2)	3.04 (2.39, 3.86)
Hypopotassemia	106 (8.3)	27 (4.6)	2.06 (1.35, 3.15)
Kidney/ureter disorder, unsp	57 (4.5)	48 (8.1)	0.62 (0.42, 0.92)
Hyperpotassemia	33 (2.6)	42 (7.1)	0.41 (0.26, 0.65)
Renal failure	52 (4.1)	29 (4.9)	0.94 (0.60, 1.48)
Thiazide diuretics			
Hypoosmolality/hyponatrmia	191 (62.8)	23 (31.1)	4.32 (2.80, 6.66)
Hypopotassemia	57 (18.8)	13 (17.6)	2.28 (1.25, 4.17)
Volume depletion	8 (2.6)	6 (8.1)	0.69 (0.24, 2.00)
Poisoning	8 (2.6)	2 (2.7)	2.08 (0.44, 9.80)
Cardiotonic glycosides			
Poisoning	291 (44.1)	101 (37.1)	1.93 (1.54, 2.43)
Unspecified adverse effect	190 (28.8)	77 (28.3)	1.66 (1.27, 2.16)
Cardiac dysrhythmias	48 (7.3)	31 (11.4)	1.04 (0.66, 1.63)
Coronary vasodilators			
Syncope/ collaps	128 (50.8)	170 (53.1)	0.68 (0.54, 0.86)
Cardiac dysrhythmias	22 (8.7)	18 (5.6)	1.10 (0.59, 2.06)
Poisoning	10 (4.0)	10 (3.1)	0.90 (0.38, 2.17)
Thyrotoxicosis	7 (2.8)	10 (3.1)	0.63 (0.24, 1.66)

^{*}The percentages show the number of specific reactions in relation to the total number of ADR-related hospital admissions within the sexes;

thiazide diuretics; women suffered more frequently from electrolyte disorders, where men suffered more from volume depletion.

Within the cardiotonic glycosides, poisoning, unspecified adverse effects, and cardiac dysrhythmias accounted for the highest frequency of ADR-related admissions in both men and women. Of the admissions related to coronary vasodilators, more than half was due to syncope/collapse (51% in women versus 53% in men).

[†] Relative risks (RRs) show the risk for women to be hospitalized with the particular ADR with men as the reference. RRs are calculated with the total number of prescriptions per sex as a denominator; Adverse reactions of 'antihypertensive agents (other)' were not further analyzed in this table, because the unspecific additional value.

Discussion

Cardiovascular drugs were responsible for a major part of ADRs requiring hospital admission, as described previously in the literature. These drugs were also the main ones responsible for potentially preventable medication-related hospital admissions, involved in all five major reasons for preventable admissions. In our study, women were more frequently admitted to the hospital with an ADR-related to cardiovascular drugs than men in general, but risks differed according to the drug group. Previous studies already showed different effects within the sexes for different cardiovascular drugs.

Our results show that within the class of cardiovascular drugs, 'anticoagulants and salicylates' accounted for over half of all ADR-related hospital admissions, mainly hemorrhages. Other studies also showed a relatively high contribution of these drugs. ³²⁻³⁴ One should be careful about interpreting results based on indication, because analgesic use of salicylates could not be distinguished from use as a platelet inhibitor. However, prescribed salicylates are mainly indicated for platelet inhibition, so analgesic use probably concerns a low percentage of use of the total number of prescriptions.

Of the other hospital admissions related to cardiovascular drugs, thiazide diuretics and loop diuretics together accounted for almost half (and 16% of the total) of these admissions. Diuretics were responsible for the major difference between the sexes in risk of an ADR-related admission. ADRs due to these drugs are predominantly type A reactions, which are mainly augmented pharmacological effects, and are well documented.^{39, 40} The reason, however, why women would suffer more frequently from a specific ADR, is less clear. It is often thought that diuretics are more frequently prescribed in women.¹⁸ A reason for this might be that increased blood pressure in postmenopausal women is partly due to an increase in salt-sensitivity.⁴¹ Therefore, diuretics are thought to be most effective in the treatment of hypertension. Our study shows that even after adjustment for drug use, this sex difference remains.

In a rat model, a lower clearance of loop diuretics was shown in females with an increased diuretic, natriuretic and kaliuretic response.^{42, 43} In other animal studies, differences were seen between males and females in expression of the organic anion transporters (OAT) and the thiazide-sensitive NaCl cotransporter (NCC) in the kidney, which play a role in this mechanism.^{44, 45} Regulation and expression of these transporters is influenced by the sex steroid hormones testosterone and estrogen.⁴⁶⁻⁴⁸ It is therefore very likely that hormonal differences also result in different treatment effects of diuretics. In our study, differences were also seen in type of adverse reactions; volume depletion was more pro-

nounced in men and women suffer more from electrolyte disorders. This supports the idea that diuretic-related ADRs in general are more water-related than salt-related and have different consequences in men and women.

Cardiotonic glycosides and coronary vasodilators, which also contribute significantly to the total number of ADR-related admissions, are two of the other drug groups showing sex differences in risk of admission. Cardiotonic glycosides are responsible for a two times higher risk in women than men to be admitted with an ADR. In a previous trial in heart failure patients, digoxin treatment resulted in a higher mortality in women, but not in men.⁴⁹ Digoxin, which is probably the most well-known and most frequently used drug within this drug group, is transported by P-glycoprotein (P-gp). Genetic variation within the gene encoding this transporter (MDR1), was shown to affect the kinetics of digoxin, resulting in a change in digoxin levels.⁵⁰ Since expression of this drug-efflux transporter is highest in men, drug transport is faster in men than in women.⁵¹ In women, this leads to a reduction in drug elimination and prolonged half-life. Sex steroid hormones modify P-gp activity, with an inhibitory effect of progestins.⁵² This also suggests differences between pre-and postmenopausal women. Unfortunately, we were not able to study this with our data.

Coronary vasodilators were also responsible for a large number of hospital admissions. Risk of admissions, related to this drug group, was higher in men. The most commonly used drugs within this drug group are the nitrates, with nitroglycerine (glyceryl trinitrate) probably being the most well-known example. The metabolism of glyceryl trinitrate is complex. Two major mechanisms have been distinguished in the biotransformation of this drug. One of the mechanisms leads to direct nitric oxide (NO) production; the second mechanism leads to production of inorganic nitrite ions (NO²), both directly affecting vasodilation and blood flow.⁵³ For these two major mechanisms, at least five metabolizing pathways have been hypothesized: two non-enzymatic and three enzymatic pathways.^{54,55} Due to the complex metabolism of these coronary vasodilators, it is hard to explain the sex difference found in this study. Possible explanations are the difference in vessel size and differences in the endothelial mechanisms involved in vasodilation.^{56,57} It is unknown if sex influences vascular tolerance, which is a known phenomenon of these drugs.

One of the strengths of our study is the availability of nationwide data on discharge diagnoses of all hospital admissions over a 6-year period. Data on drug use were also available for the same 6-year period, and served as a denominator. Because of the ecological design of the study, it was not possible to match the data on drug use (which were not discernable on an individual basis) with the ADR-related hospital admissions. Therefore, interaction between the vari-

ous drugs could not be studied. Another limitation is that the data within the drug categories did not match completely. This is due to the different coding systems used by the two databases in our study. Furthermore, it might have occurred that an ADR presented itself during admission and was not the direct reason for admission. However, in our opinion, the frequency with which ADRs occurring during hospitalization are coded as main diagnosis is most likely low. Moreover, as this misclassification will be non-differential, it will most likely not bias our comparison between men and women. Owing to the passive coding of ADR-relatedness of the admission diagnosis at discharge, the cumulative incidence of ADR-related admissions is probably substantially underestimated. In general, we expect that underrecognition of serious ADRs (i.e. requiring hospital admission) will be non-differential, since symptoms are serious and will lead to admission anyhow.

In conclusion, clear sex differences exist in ADRs requiring hospital admission for some of the cardiovascular drug groups. In addition to the pharmacokinetic and pharmacodynamic factors of the specific drugs, the number and types of simultaneously used drugs are important in identifying high-risk patients. Furthermore, comorbidity, such as depression, and severity of the underlying disease are of importance in the determination of ADRs. Depression is associated with a higher risk of drug intolerance and adverse reactions. ^{58, 59} As perception of symptoms may differ between men and women, due to sympathetic and parasympathetic differences, one should be aware of differences in presentation of diseases and ADRs. ⁶⁰ More research is needed to study the possible causative mechanisms and contributing factors for the specific drugs, and whether interactions play a role. Furthermore, this study underlines the necessity of studying (adverse) effects of drug therapy in both sexes. Prescription adjustments for high-risk drugs should be considered to prevent serious ADRs, requiring hospital admission.

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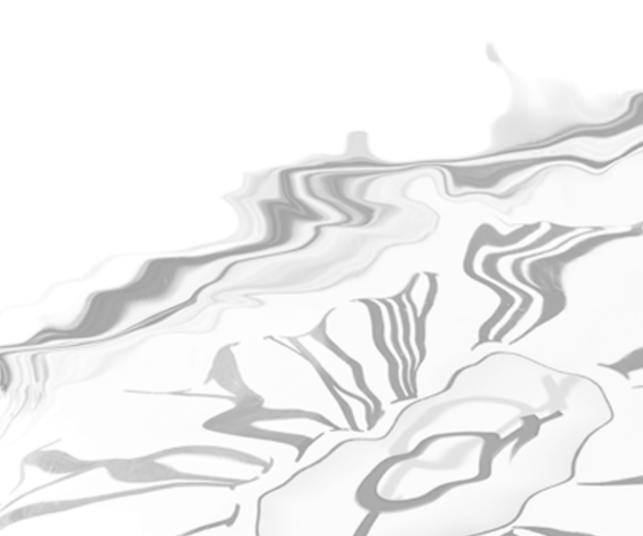
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Chapter 2.3

Adverse drug reaction-related hospital admissions in children

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Abstract

Background

Many drugs lack information on efficacy and adverse drug reactions (ADRs) in children. Several authorities have taken measures to increase this knowledge. The aim of this study was to determine the extent of ADRs causing hospital admissions in children, to reflect the importance of surveillance in this vulnerable patient group.

Methods

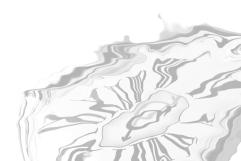
We performed a nationwide study of all hospital admissions in children and adolescents (aged 0 to 20 years) between 2000 and 2005. Hospital admissions were evaluated within predefined age categories, sex and calendar-year. The most prominent drug groups leading to hospitalization were determined. Risks of hospital admissions were calculated in relation to the total number of Dutch inhabitants and total number of drug prescriptions per age category.

Results

Of all acute admissions in children, 0.75% (N=5,570) was attributed to an adverse drug reaction. The proportion of hospitalizations was highest in the youngest children, aged 0 to <2 years (169/100,000 children). The majority of the admissions in this age category was related to drug effects through placenta and breast milk and increased over the years. Most frequently involved drugs groups in general were 'antineoplastic and immunosuppressive drugs' (N=344), 'insulins and antidiabetic agents' (N=173), 'pertussis vaccine' (N=109), and 'penicillins' (N=70), with the youngest at highest risk. There were no consistent differences between boys and girls.

Conclusion

These results emphasize the importance of extra precaution regarding drug use in young children, during pregnancy and lactation of the mother.



Introduction

Drugs in children are often prescribed outside their licensed indications (off-label use). ¹⁻⁴ Until recently, clinical trials in children were considered as unethical. For this reason, multiple drugs are not registered in this young age-group with respect to dosage, age, indication, or route of administration (unlicensed use). Off-label and unlicensed prescription rates in children range from 11-80% in general, with the highest proportion on neonatal wards. ⁵ The problem with off-label and unlicensed drug use in children is the fact that the drug was not appropriately evaluated before marketing. This may result in deficient information on the risks of the prescribed drugs which may lead to adverse drug reactions (ADRs). ⁶

In 2003, the United States (US) changed legislation to improve drug safety in children. Since July 1, 2007, the Paediatric Regulation has been implemented in the European Union (EU), focusing on registration and research (pre- and postauthorization) of drugs in children.^{7,8} Before registration of a new drug in the EU, the marketing authorization holder should compose a 'paediatric investigation plan' (PIP) including details on their efforts to study efficacy, safety, dosage adaptations and administration routes of the particular drug in children. Furthermore, they should describe how they will assess post-marketing drug safety. For products not intended for the paediatric population, e.g. due to indication, a waiver or partial waiver can be granted. For medicinal products that are already off-patent a new type of marketing authorization has been established: 'Pediatric Use Marketing Authorisation' (PUMA). This PUMA is intended for the development of new pediatric formulations for generic drugs used in children. By completing this centralized application, and submitting study results on use in the pediatric population, the pharmaceutical company can benefit from 10 years market protection. The PIPs, waivers and PUMAs are assessed by the Pediatric Committee (PDCO) of the European Medicines Agency (EMA). The Dutch legislation is even more restrictive. The act 'medical research involving human subjects' (WMO) requires negligible risks and minimal objections for medical research in children.9,10

The Pediatric Regulation and WMO are implemented to lead to lower the risk of ADRs in this vulnerable patient group. We conducted a nationwide study to investigate ADR-related hospital admissions in children and adolescents, including prescription data, age categories and sex.

Methods

Data sources

Data on hospital admissions were obtained from a Dutch nationwide registry of hospital discharges which contains patient characteristics, dates of admission and discharge, main diagnoses at discharge, secondary diagnoses and special codes indicating drug-related hospitalizations (E-codes) based on the ICD-9-CM coding system. Characteristics of hospital admissions are registered by medical doctors on the basis of hospital discharge letters and coded by professional code clerks. The coding is independent of hospital or specialist. All diagnoses are submitted in the same format, mostly electronically. For this study, all patients between 0 and 20 yrs of age with an acute, non-planned admission to a Dutch hospital in the period between 2000 and 2005 were included. Numbers of inhabitants in the Netherlands per calendar-year, sex and age were obtained from 'Statistics Netherlands'.

Information on prescriptions was obtained from the database of the Dutch Foundation of Pharmaceutical Statistics (SFK), which includes information from 1,805 pharmacies in the Netherlands (92% of total). Data from this database were accessible on pharmacological subgroup level according to the Anatomical Therapeutic Chemical (ATC) classification system.¹³ Prescription information was available per calendar-year for the predefined age categories: 0 to <2, 2 to <11 and 11 to 20 years. Per ATC group, the cumulative number of prescriptions was calculated.

Adverse drug reactions

An ADR-related hospital admission was defined as a hospital admission with an E-code between 930-950 as secondary diagnosis or a main diagnosis specifically indicating an ADR. E-codes are auxiliary to the main discharge diagnosis, indicating an ADR as the primary reason for admission. The E-code is indicative for the drug group involved in the ADR. E-codes referring to intended overdoses, errors in administration and therapeutic failure were not included in the analysis. Main diagnoses specifically indicating an ADR consisted of 15 separate codes: iatrogenic hypothyroidism (244.3), polyneuropathy due to drugs (357.6), toxic myocarditis (422.93), shock due to anesthesia (995.4), encephalitis, myelitis, and encephalomyelitis following immunization procedures (323.5), drug-induced or radiation-induced myelopathy (336.8), toxic hepatitis (573.3), contact dermatitis and other eczema due to drugs and medicines in contact with skin (692.3), der-

matitis due to substances taken internally due to drugs and medicines (693.0), drug reactions and intoxications specific to newborn (779.4), noxious influences affecting fetus or newborn via placenta or breast milk caused by narcotics (760.72), hallucinogenic agents (760.73), anti-infectives (760.74), other (760.79), and other and unspecified adverse effect of drug, medicinal and biological substance (due) to correct medicinal substance properly administered (995.2).

Data analysis

ADR-related hospitalizations were identified in total and for separate age categories. Age categories were based on the classification of the available prescription data (0 to <2, 2 to <11, 11 to 20 years). For the initial analysis, the two highest categories were subdivided in two sub-categories to show more details of the effect of age (0 to <2, 2 to <7, 7 to <11, 11 to <16 and 16 to 20 years). The absolute number of ADR-related hospitalizations was determined separately for boys and girls, per age category, and per calendar-year. Per age category, the most frequently occurring reactions were studied.

Second, we studied the specific drug groups most frequently causing ADRs, and we selected the ten drug groups related to the highest number of hospital admissions. In this analysis, we used three age categories: 0 to <2, 2 to <11 and 11 to 20 years of age. Proportions of ADR-related admissions were calculated per 100,000 inhabitants and per 1,000 prescriptions. Relative risks (RRs) with 95% confidence intervals (95% CI) were calculated for the two youngest age categories compared with the highest age category, and for girls compared with boys. In the denominator, we used the total number of prescriptions in the study period. The information on prescriptions was combined with the data on hospitalizations based on drug(s) covered by the E-code.

Results

Within the study period, 740,280 hospital admissions were registered for children and adolescents. Of these admissions, 5,570 children (0.75%) were hospitalized due to an adverse drug reaction (ADR). Fifty percent of these children were boys (Table 1). Four children died during hospital admission (aged 0, 14, 14 and 19 years).

The majority of adverse drug reactions occurred in the children aged 0 to <2 years (N=4,112; 74%) and 16 to 20 years (N=511; 9%). In the lowest age category, the proportion of ADR-related admissions was 169/100,000 children. For the

Table 1. ADR-related hospital admissions, stratified per age category, sex and calendar-year within the study period (2000-2005)

Age catego (years)	ory	Number of children N (%)	ADR-related admissions N (%)	ADR-related admissions / 100,000 children	RR (95% CI)
0 to <2	Total	2,434,000	4,112	169	15.07 (14.47, 15.68)
	Boys (%)	1,247,000 (51)	2,052 (50)	165	
	Girls (%)	1,187,000 (49)	2,060 (50)	174	
2 to <7	Total	5,990,000	393	7	0.59 (0.53, 0.65)
	Boys (%)	3,065,000 (51)	245 (62)	8	
	Girls (%)	2,925,000 (49)	148 (38)	5	
7 to <11	Total	4,777,000	202	4	0.38 (0.33, 0.43)
	Boys (%)	2,443,000 (51)	120 (59)	5	
	Girls (%)	2,334,000 (49)	82 (41)	4	
11 to <16	Total	5,905,000	352	6	0.53 (0.48, 0.59)
	Boys (%)	3,020,000 (51)	184 (52)	6	
	Girls (%)	2,885,000 (49)	168 (48)	6	
16 to 20	Total	4,557,000	511	11	Ref
	Boys (%)	2,334,000 (51)	190 (37)	8	
	Girls (%)	2,223,000 (49)	321 (63)	14	
All ages	Total	23,663,000	5,570	24	
	Boys (%)	12,109,000 (51)	2,791 (50)	23	
	Girls (%)	11,554,000 (49)	2,779 (50)	24	
Calendar-y	ear				
2000		3,870,000	673	17	
2001		3,908,000	695	18	
2002		3,941,000	797	20	
2003		3,967,000	994	25	
2004		3,988,000	1,148	29	
2005		3,989,000	1,263	32	

Abbreviations: ADR=adverse drug reaction; RR=relative risk; ref=reference category;

oldest children, the proportion was second highest: 11/100,000 children. In the middle age categories, numbers were more or less similar between boys and girls. Within the lowest and highest age categories, girls exceeded boys in number and proportion of ADR-related admissions. The proportion of ADR-related admissions almost doubled over the years from 17 to 32 per 100,000 children (relative risk (RR) 1.88; 95% CI 1.05, 3.39).

The admissions for drug effects through the placenta or breast milk accounted for the majority of the ADR-related admissions (N=3,666, 89%), all within the lowest age category (50% in boys). In 2000, the first year of the analysis, 56% of

the ADR-related admissions in children were due to drug effects through the placenta or breast milk, increasing to 72% in 2005. Of these, 7% was related to narcotics, 6% to hallucinogenic agents, and 2% was related to anti-infective agents; the majority of these ADRs was related to 'other' drugs. Within the separate age categories, 'antineoplastic and immunosuppressive drugs' and 'insulins and antidiabetic drugs' were the most frequently reported drugs (Table 2).

The five classes with the largest number of admissions due to an ADR were 'primarily systemic agents' (N=402, 7.2%), 'hormones and systemic substitutes' (N=266, 4.8%), 'other and unspecified drugs and medicinal substances' (N=145, 2.6%), 'antibiotics' (N=124, 2.2%), and 'bacterial vaccines' (N=123, 2.2%). Following in this range of admissions, were 'analgesics, antipyretics and antirheumatics', 'psychotropic agents' and 'anticonvulsants and anti-Parkinsonism drugs'.

Within these drug classes, some drug groups caused the majority of reactions. For the five largest classes of admission-causing drugs, these were 'antineoplastic

Table 2. Most frequently reported drugs causing ADR-related hospital admissions per age category

Age category	Drug related to admission	N (% within age category)
0 to <2*	Pertussis vaccine	108 (26)
	Penicillin	26 (6)
	Thyroid	25 (6)
	Antineoplastic and immunosuppressive drugs	25 (6)
2 to <7	Antineoplastic and immunosuppressive drugs	116 (33)
	Insulins and antidiabetic drugs	33 (9)
	Unspecified	25 (7)
	Anticonvulsants	20 (6)
7 to <11 [†]	Antineoplastic and immunosuppressive drugs	57 (33)
	Insulins and antidiabetic drugs	37 (22)
	Unspecified	11 (6)
	Anticonvulsants	8 (5)
11 to <16 [†]	Antineoplastic and immunosuppressive drugs	64 (23)
	Insulins and antidiabetic drugs	61 (22)
	Adrenal corticosteroids	12 (4)
	Anticonvulsants	12 (4)
16 to 20†	Antineoplastic and immunosuppressive drugs	82 (19)
	Insulins and antidiabetic drugs	39 (9)
	Antirheumatics	33 (8)
	Penicillin	17 (4)

^{*} ADR-related admissions attributed to drug effects through placenta or breast milk (N=3,666) are not included in this table. Another large group of ADRs ("drug reaction and intoxication to the newborn"; N=57) is not included because information on related drugs was missing. Percentages are calculated without these admissions;

 $^{^\}dagger$ Drug groups causing the ADR "toxic hepatitis" could not be included because of missing information on drugs related to the admission.

Table 3. Numbers and proportions for the ten drug groups most prominent in causing ADR-related hospital admissions

Drug group	Total		0 to <2 yr	yr		2 to <11 yr	yr		11 to 20 yr	yr
	z	z	N/100,000 children	N/1,000 prescriptions	z	N/100,000 children	N/1,000 prescriptions	z	N/100,000 children	N/1,000 prescriptions
Antineoplastic and	344	25	1.45	31.37	173	1.03	4.77	146	1.61	1.90
immunosuppress drugs (L)										
Boys	500	10	1.73	19.46	119	0.80	8.18	8	2.16	2.19
Girls	135	15	1.17	53.00	54	1.26	2.48	99	1.03	1.64
Insulins and antidiabetic drugs (A10)	173	3	0.73	2.26	20	0.12	1.00	100	0.65	0.44
Boys	88	2	0.73	2.63	37	0.16	1.01	49	0.67	0.42
Girls	85	Т	0.74	1.77	33	0.08	0.99	51	0.63	0.46
Pertussis vaccine*	109	108	0.46	n.a.	П	4.44	n.a.	0	0.01	n.a.
Boys	99	99	0.55	n.a.	0	5.29	n.a.	0	0.00	n.a.
Girls	43	42	0.37	n.a.	⊣	3.54	n.a.	0	0.02	n.a.
Penicillins (101C)	70	26	0.30	0.03	19	1.07	0.01	25	0.18	0.02
Boys	34	15	0.28	0.03	∞	1.20	0.01	11	0.15	0.02
Girls	36	11	0.31	0.03	11	0.93	0.01	14	0.21	0.02
Other anticonvulsants (N03A)	99	14	0.28	0.87	28	0.58	0.12	24	0.26	0.07
Boys	33	9	0.27	0.65	17	0.48	0.13	10	0.31	90.0
Girls	33	∞	0.29	1.16	11	0.67	0.11	14	0.21	0.09

Table 3. Numbers and proportions for the ten drug groups most prominent in causing ADR-related hospital admissions (Cont)

Drug group	Total		0 to <2 yr	yr		2 to <11 yr	yr		11 to 20 yr	£.
	z	z	N/100,000 children	N/1,000 prescriptions	z	N/100,000 children	N/1,000 prescriptions	z	N/100,000 children	N/1,000 prescriptions
Antirheumatics (M01+M02)	56	∞	0.24	0.84	72	0.33	0.20	43	0.05	0.01
Boys	19	33	0.16	0.55	2	0.24	0.17	14	0.04	0.01
Girls	37	2	0.32	1.15	8	0.42	0.23	29	90:0	0.02
Anti-allergic / anti-emetic drugs (A04A+R06A)	51	6	0.22	0.08	22	0.37	0.02	20	0.20	0.01
Boys	20	4	0.17	5.61	10	0.32	0.29	9	0.18	0.59
Girls	31	2	0.27	15,97	12	0.42	0.51	14	0.23	1.00
Adrenal corticosteroids (H02A/B)	34	8	0.14	0.54	∞	0.12	0.13	23	0.07	0.10
Boys	21	\vdash	0.17	0.45	2	0.08	0.10	15	0.09	0.10
Girls	13	2	0.11	0.71	co	0.17	0.18	∞	90:0	0.10
Other antibiotics (J01X)	34	6	0.14	0.77	12	0.37	0.16	13	0.11	0.10
Boys	17	2	0.14	0.45	9	0.40	0.65	9	0.11	2.05
Girls	17	4	0.15	1.19	9	0.34	0.07	7	0.11	0.03
Thyroid and derivatives (H03A/B)	27	25	0.11	4.45	2	1.03	0.06	0	0.02	N.a.
Boys	14	12	0.12	4.64	2	96.0	0.14	0	0.04	N.a.
Girls	13	13	0.11	4.28	0	1.10	N.a.	0	0.00	N.a.

ATC codes are presented between brackets;

N.a.=not applicable; absence of cases in the reference category;
* Complete prescription data of this vaccine were not available; administration of this vaccine is part of the National Vaccination Program.

and immunosuppressive drugs' (86% of 402 admissions), 'insulins and antidiabetic agents' (65% of 266 admissions), 'unspecified drug or medicinal substance' (66% of 145 admissions), 'penicillins' (57% of 124 admissions), 'pertussis vaccine, including combinations with a pertussis component' (89% of 123 admissions).

For the ten most prominent drug groups, most of the proportions of ADR-related admissions were highest in the middle age category, except for 'anti-neoplastic and immunosuppressive drugs' and 'insulins and antidiabetic drugs' (Table 3). Taking into account the total number of prescriptions of these drug groups, the youngest age group was at the highest risk to be admitted to a hospital due to an ADR compared with the oldest children (Table 4). Risks for boys and girls showed large overlap within the different drug groups. However, the risk to be hospitalized due to an ADR related to 'antineoplastic and immunosuppressive drugs' was remarkably high in girls in the youngest age category, compared with both older children and boys (RR 16.53; 95% CI 10.88, 25.11 and RR 2.72; 95% CI 1.24, 5.98, respectively).

Hypoglycemia, due to 'insulins and antidiabetic drugs', accounted for the majority of admissions (Table 5). For 'antineoplastic and immunosuppressive drugs' the most frequent ADR was neutropenia. Adverse drug reactions due to anticonvulsants were mainly poisoning or unspecified drug reactions. Of the specified reactions, penicillin most frequently caused dermatitis as a reason for hospital admission; most frequent reactions following pertussis vaccine were syncope/collapse and convulsions. Although the number of hospital admissions related to ADRs attributed to pertussis vaccine was relatively high overall, these numbers decreased over the years from 30 admissions in 2000 to 2 admissions in 2005.

Discussion

Our study showed the variety of adverse drug reaction (ADR)-related hospital admissions in children and adolescents in the Netherlands over a 6-year period. As far as we are aware, this is the first study in which ADRs are combined with prescription data on a national basis.

In the current study, we showed that ADRs accounted for 0.75% of all pediatric hospital admissions in the period between 2000 and 2005. This is somewhat lower than expected from the literature. A review and meta-analysis from 2001 showed a range from 0.59 to 4.1% of incident ADRs leading to pediatric hospital admissions with a weighed average of the five studies of 2.09% (95% CI 1.02, 3.77). A more recent literature review showed an admission rate between 0.6 and 6% of hospitalizations due to ADRs, with a weighed average of 1.8% (95%

Table 4. Relative risks for ADR-related hospital admissions relative to the total number of prescriptions. RRs are presented with the age category (a) '11 to 20 years' and (b) 'boys' as the reference category, respectively

a.

Drug group (ATC group)	Age 0 to <2 yr	Age 2 to <11 yr	Age 11 to 20 yr
	RR (95% CI)	RR (95% CI)	RR (95% CI)
Antineoplastic and immunosuppress drugs (L)	16.53 (10.88, 25.11)	2.51 (2.02, 3.13)	1.00
Insulins and antidiabetic drugs (A10)	5.17 (1.64, 16.28)	2.29 (1.68, 3.10)	1.00
Pertussis vaccine*	N.a.	N.a.	N.a.
Penicillins (J01C)	1.37 (0.79, 2.37)	0.33 (0.18, 0.59)	1.00
Other anticonvulsants (N03A)	12.33 (6.38, 23.83)	1.67 (0.97, 2.89)	1.00
Antirheumatics (M01+M02)	58.04 (26.44, 127.44)	13.87 (7.57, 25.40)	1.00
Anti-allergic / anti-emetic drugs (A04A+R06A)	5.96 (2.72, 13.10)	1.41 (0.77, 2.59)	1.00
Adrenal corticosteroids (H02A/B)	5.38 (2.30, 12.58)	1.29 (0.59, 2.84)	1.00
Other antibiotics (J01X)	7.99 (2.40, 26.60)	1.62 (0.72, 3.62)	1.00
Thyroid and derivatives (H03A/B)	N.a.	N.a.	N.a.

ATC codes are presented between brackets;

b.

Drug group (ATC group)		Girls versus Boys	
	Age 0 to <2 yr	Age 2 to <11 yr	Age 11 to 20 yr
	RR (95% CI)	RR (95% CI)	RR (95% CI)
Antineoplastic and immunosuppressive drugs (L)	2.72 (1.24, 5.98)	0.30 (0.22, 0.42)	0.75 (0.54, 1.04)
nsulins and antidiabetic drugs (A10)	0.67 (0.06, 7.39)	0.98 (0.61, 1.57)	1.09 (0.74, 1.61)
Pertussis vaccine*	N.a.	N.a.	N.a.
Penicillins (J01C)	0.97 (0.45, 2.11)	1.42 (0.57, 3.54)	0.99 (0.45, 2.18)
Other anticonvulsants (N03A)	1.78 (0.62, 5.12)	0.84 (0.39, 1.79)	1.57 (0.70, 3.53)
Antirheumatics (M01+M02)	2.10 (0.57, 7.83)	1.36 (0.59, 3.16)	1.23 (0.47, 3.21)
Anti-allergic / anti-emetic drugs (A04A+R06A)	2.49 (0.59, 10.40)	1.86 (0.31, 11.15)	1.52 (0.80, 2.87)
Adrenal corticosteroids (H02A/B)	1.59 (0.43, 5.93)	1.77 (0.57, 5.49)	1.03 (0.35, 3.06)
Other antibiotics (J01X)	2.64 (0.24, 29.13)	0.11 (0.03, 0.44)	0.02 (0.01, 0.04)
Thyroid and derivatives (H03A/B)	0.92 (0.42, 2.01)	N.a.	N.a.

ATC codes are presented between brackets;

N.a.=not applicable; absence of cases in the reference category;

^{*} Complete prescription data of this vaccine were not available; administration of this vaccine is part of the National Vaccination Program.

N.a.=not applicable; absence of cases in the reference category;

^{*} Complete prescription data of this vaccine were not available; administration of this vaccine is part of the National Vaccination Program.

Table 5. Most frequent ADRs per drug group leading to hospitalization, accounting for minimal 50% of admissions per drug group (max 4)

Drug Group*	Adverse Drug Reaction	N
Antineoplastic and immunosuppressive drugs (N=344)	Neutropenia	100
	Fever	63
	Aplastic anemia	57
Insulins and antidiabetic drugs (N=173)	Hypoglycemia	131
Pertussis vaccine (N=109)	Syncope/collaps	32
	Unspecified	23
	Convulsion	14
Penicillins (N=70)	Unspecified	20
	Dermatitis	17
Anticonvulsants (other) (N=66)	Poisoning	16
	Unspecified	13
	Effects via placenta or breast milk	5
Antirheumatics (N=56)	Poisoning	18
	Other adverse effect	12
Anti-allergic / anti-emetic drugs (N=51)	Poisoning	17
	Other adverse effect	10
	Unspecified pyramidal disease	5
Adrenal corticosteroids (N=34)	Corticoadrenal insufficiency	5
	Diabetes with unspecfied complications	4
	Cushing's syndrome	3
	Fetus affected by drug use	3
Antibiotics (other) (N=34)	Other adverse effect	10
	Dermatitis	8
Thyroid and derivatives (N=27)	Fetus affected by drug use	24

^{*} Results are shown for the ten most prominent drug groups in causing ADR-related hospital admissions.

CI 0.4, 3.2).¹⁵ These differences in figures with our study can partly be explained by differences in study design of the studies included in the analysis, e.g. lower number of hospitals included, shorter study period and/or broader definition of the outcome. Besides, other factors can contribute to variation in the proportion of ADR-related hospital admissions like time and age, the type of drugs and sex, which we will discuss below. The passive coding of ADR-relatedness (non-mandatory E-codes) of the admission diagnosis at discharge is most probably responsible for an underestimation of the cumulative incidence of ADR-related admissions.

Time and age

An increase in the proportion of ADR-related admissions was present over time, mainly contributed by an increase in ADRs related to drug effects through the placenta and breast milk in the youngest children. Drug use during pregnancy is of high concern and therefore, pregnancy prevention programs (PPPs) have been implemented for certain teratogenic drugs, to prevent pregnancies during use. The effect of these programs is, however, questioned. The relatively high proportion of ADRs in children due to drug use of the mother instead of the child, was also seen by a study in the FDA's Adverse Event Reporting System (AERS). Although a notable proportion of these effects were due to narcotics, hallucinogenic agents and anti-infective agents, in the majority of cases the type of drug was not appointed and marked as 'other drugs' (85%). This could either indicate the involvement of different drugs or the lack of specification in the coding system. Although the administration of drugs during pregnancy cannot always be circumvented, the proportion of ADR-related admissions in children related to maternal causes is high and requires extra attention.

Besides hospital admissions related to these maternal drug effects, admission rates in the youngest children were highest compared with the other agecategories. This suggests that either the youngest children are more prone to develop ADRs requiring hospitalization, or ADRs are more frequently recognized in these children. Our results are consistent with the literature; an increased risk of ADR-related admissions in children under 12 months of age, relatively to other ages, was shown previously in a study by Kramer et al.²⁰

The relatively higher frequency and risk of ADR-related hospital admissions, relative to the number of prescriptions, in the youngest children within our study can be explained by several factors. First of all, pharmacokinetics and pharmacodynamics are age-dependent and the largest differences with adults exist in this youngest age-group.²¹ Due to these differences young children are more prone to develop ADRs. Second, young children are more likely to experience medication and dosing errors since formulations of many drugs are not suitable for young children. It is known that this leads to a higher frequency of ADRs and also more severe ADRs.²² Third, it is assumed that off-label use is related to a higher frequency of ADRs.^{23, 24} and off-label drug use is highest in the youngest children.^{1, 5} Our results emphasize that in the youngest children more effort and research is necessary to limit the off-label use and stimulate development of proper pediatric formulations and precautions.

Type of drugs

As also shown by others, the drug groups most frequently accounting for the ADR-related admissions in our study were antineoplastic and immunosuppressive drugs.²⁵ This is not surprising in view of their toxicity and the indications for which these drugs are used. Antimicrobial drugs and anticonvulsants, the fourth and fifth causing drug groups within this study, were in the top five of causative drugs in other studies as well.²⁵⁻²⁷

We found a large contribution of pertussis vaccine to the ADR-related admissions. Pertussis vaccination has been part of the Dutch National Vaccination Programme since 1952 with a vaccination coverage of approximately 96%.²⁸ Since 1996, there has been an increase in number of pertussis cases within the Netherlands, especially in children under the age of 3 months.²⁹ Effectivity of the vaccine had decreased due to adaptation of and increased toxin production by the B. pertussis strains.³⁰ In 1999, a change in the vaccination programme resulted in administration of the first four vaccines a month earlier than usual.³¹ This might have led to increased awareness in the beginning of this period, leading to increased reporting of a possible ADR as a cause for hospital admission. The number of pertussis-vaccine-related hospital admissions decreased over the study period. In 2005, a non-cellular vaccine was introduced, which was expected to be more effective and to have a lower risk of ADRs.³¹

Sex differences

Differences in ADR-related hospital admissions between boys and girls have been described in previous studies, though results are conflicting.^{20, 32, 33}

The results from our study suggest that girls are more frequently involved in ADR-related admissions in the lowest and highest age categories, whereas the proportion of ADR-related admissions in the middle age categories is comparable between the sexes. The main difference between boys and girls was seen in the 'antineoplastic and immunosuppressive drugs'. Possibly, pharmacokinetic differences at young age also play a role in this difference.^{21,34}

Strenghts and limitations

One of the strengths of our study is the availability of nationwide data on discharge diagnoses of all hospitalizations over a 6-year period. Data on drug use were also available for the same period, which illustrated the use of the various drugs as a background of ADR occurrence. Our study did not cover the years after implementation of the new European legislation, which made it impossible to study its direct effect on drug safety. Data on drug use were not discernable on an individual basis, so we were unable to match these with the ADR-related

hospitalizations. Therefore, interaction between the various drugs could not be studied. Although adjustment for age was done in the first analysis, this was not possible in the analysis with total number of drug prescriptions.

Another limitation is the different coding systems used by the two databases in our study. Because of the passive coding of ADR-relatedness of the admission diagnosis at discharge, the cumulative incidence of ADR-related admissions is probably underestimated. However, this underestimation is probably the same for all age categories and for different sexes and will not influence the relative risks.

Conclusions

Adverse drug reactions accounted for 0.75% of all pediatric hospital admissions in the period between 2000 and 2005. The risk of ADR-related hospital admissions was highest in the youngest children in comparison to the older children. Drug effects through placenta and breast milk contributed the largest part to the total number of admissions, affecting the youngest children. Differences between boys and girls in ADR-related admissions do exist, but definitive conclusions cannot be drawn from this study.

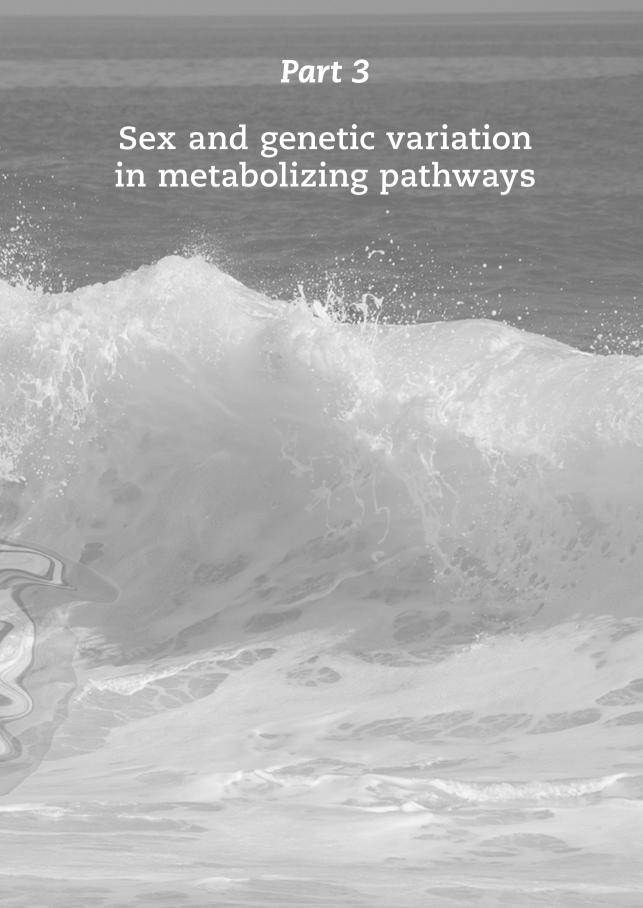
From a regulatory perspective, these results emphasize the importance of surveillance of safe drug use in children. Drug use in the youngest children and use during pregnancy and lactation might require extra precaution.

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Chapter 3.1

Genetic variance in CYP2C8 and increased risk of myocardial infarction

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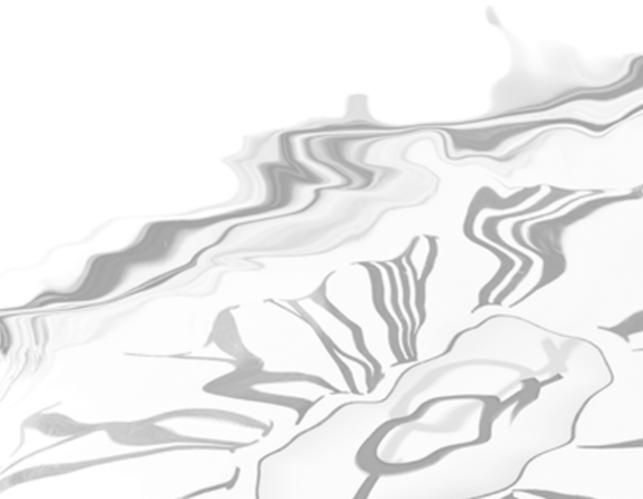
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Abstract

Background

Epoxyeicosatrienoic acids (EETs) are important mediators in vasodilatation, acting as endothelium-derived hyperpolarizing factors. CYP2C enzymes catalyze the metabolism of arachidonic acid to EETs. Genetic variation within the genes encoding these enzymes may result in differences in vascular response, among others in myocardial tissue, and may therefore increase the risk of myocardial infarction (MI). CYP2C8 and CYP2C9 are encoded by the genes of the same name. CYP2C9 polymorphisms have been associated with an increased risk of MI. As CYP2C8 is genetically linked to CYP2C9 and due to its role in EET production, we hypothesized that CYP2C8 polymorphisms are associated with the risk of MI.

Methods

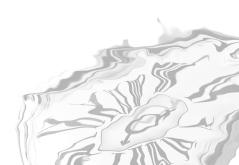
This study was embedded within the Rotterdam study, a prospective population-based cohort study. The study population included all participants with successful genotyping and without prevalent myocardial infarction (N=5,199). Twenty-five single nucleotide polymorphisms (SNPs) within and around the gene-coding areas of CYP2C8 and CYP2C9 were tested for an association with incident MI using survival analysis techniques with multivariable adjustment for potential confounders.

Results

During follow-up, 290 persons developed an incident MI. One SNP in the CYP2C8 gene was associated with incident myocardial infarction after Bonferroni correction, SNP rs1058932C>T (variant genotype HR 1.54; 95% CI 1.22, 1.95). There was a significant gene-sex interaction with a relative excess risk of 1.40 (95% CI 0.33, 2.47) for men.

Conclusion

SNP rs1058932C>T within the CYP2C8 gene is associated with an increased risk of MI, which is, possibly due to a vascular effect of sex steroid hormones, highest in males.



Introduction

The cytochrome P450 system (CYP) is responsible for the metabolism of many xenobiotics and endogenous compounds. Members of the CYP2C family are responsible for the metabolism of 20% of all currently used drugs (e.g., coumarin anticoagulants, phenytoin, various non-steroidal anti-inflammatory drugs, oral antidiabetic drugs, losartan, paclitaxel). CYP2C enzymes are predominantly present in the liver, but CYP2C8 and CYP2C9 are both also located in vascular smooth muscle and endothelial cells: coronary arteries, aorta and myocardial tissue. 4-10

The CYP2C family is also a key player in the generation of epoxyeicosatrienoic acid (EET), an endothelium-derived hyperpolarizing factor (EDHF), which is derived from arachidonic acid (AA). EET plays an important role in the protective mechanism against atherosclerosis. This is processed by inhibition of smooth muscle migration, decrease of inflammation, inhibition of platelet aggregation, contribution to collateral vessel formation and decrease of adhesion molecule expression. Besides these long-term effects, EET also has a role in coronary vasomotor tone and myocardial perfusion. Generation of EET (EDHF) is normally inhibited by nitric oxide (NO). In case of acute coronary disease, EET compensates for nitric oxide loss and induces vasodilation of coronary arteries and arterioles by hyperpolarization of smooth muscle cells. 4,7,11,16-19 Since these data were predominantly derived from animal studies, further research in humans is needed to determine the exact impact on the human cardiovascular system.

Effects of genetic variance in CYP2C on EET production have been investigated in few studies. Polymorphisms in the CYP2C9 gene have been found associated with a decrease in EET biosynthesis. ¹⁸ In Caucasian women, the CYP2C9*3 (allele frequency 11%) and CYP2C9*2 (allele frequency 7%) variants ^{9,20} were found to be associated with a near-significantly increased risk of myocardial infarction (MI) of 30% and 50%, respectively. ²¹ Use of inhibitors and substrates of CYP2C9 seemed to increase the risk of MI. ²² Yasar et al. ²³ found a linkage between CYP2C9*2 and CYP2C8*3. Of the subjects carrying a CYP2C8*3 allele, 96% also carried a CYP2C9*2 allele; 85% of the carriers of a CYP2C9*2 allele, also had a CYP2C8*3 variant. No significant association between CYP2C8 and myocardial infarction has been found yet. ^{21,24,25} Therefore, our primary objective was to study whether polymorphisms in the CYP2C8 gene and CYP2C9 gene are associated with incident MI.

Methods

Setting

The Rotterdam Study is a prospective population-based cohort study of neurological, cardiovascular, locomotor and ophthalmologic diseases. All inhabitants of Ommoord, a suburb of the city of Rotterdam in the Netherlands, aged 55 years or older were invited in 1990 to participate in the study. The medical ethics committee of the Erasmus Medical Centre approved the study and informed consent was obtained from all participants. The rationale and design of the study were described elsewhere. The cohort encompasses 7,983 individuals who were all interviewed and investigated at baseline in the period 1990-1993. Since the start of the Rotterdam Study, cross-sectional surveys have been carried out periodically. In addition, participants are continuously monitored for major events through automated linkage with the files from the general practitioners of the patients. When an event or death has been reported, additional information is obtained from the general practitioner and by scrutinizing information from hospital discharge records in case of admittance or referral.

Cohort definition

All participants with successful genotyping who did not have a history of MI at baseline were included. Prevalent MI was based on medical records from general practitioners or cardiologists, electrocardiography, laboratory measurements and data on hospital admissions. The cohort was followed until the first occurrence of MI, death or end of the study period (January 1, 2005), whichever came first.

Outcome

The study outcome MI was defined based on a hospital discharge diagnosis, or in case a patient was not hospitalized, when signs and symptoms, electrocardiography recordings and cardiac enzyme data were diagnostic of myocardial infarction.²⁹ Two research physicians independently validated all potential cardiac events, according to the International Classification of Diseases, 10th version, as reported earlier.³⁰ In case of disagreement, a cardiologist reviewed the events and decided the definitive validation. The date on which an incident MI was encountered was defined as the index date.

Genotyping

Genomic DNA was extracted from whole blood samples using the salting out method. DNA was extracted with proteinase K and sodium dodecyl sulfate digestion at 37°C overnight and purified with phenol-chloroform extractions. The extracted DNA was then precipitated with NaCl at 4mol/L and two volumes of cold absolute ethanol. DNA was solubilized in double-distilled water and stored at –20°C until used for DNA amplification.

Microarray genotyping was performed in the whole original Rotterdam Study cohort with proper quality DNA samples (N=6,649) using the Infinium II Human-Hap550K Genotyping BeadChip® version 3 (Illumina). Any samples with a call rate of <97.5% (N=209), excess autosomal heterozygosity of > 0.336 (FDR<0.1%) (N=21), mismatch between called and phenotypic gender (N=36), or if there were outliers identified by the IBS clustering analysis clustering > 3 standard deviations away from the population mean (N=102) or IBS probabilities > 97% (N=129) were excluded from the analysis. In total, 5,974 samples met quality control inclusion criteria. Genotyping procedures were followed according to Illumina manufacturer's protocols.

CYP2C9*2 genotype was separately determined in 5-ng genomic DNA with the Taqman allelic discrimination assay (Applied Biosystems, Foster City, California, USA). Primer and probe sequences were optimized by using the single nucleotide polymorphism (SNP) assay-by-design service of applied Biosystems (for details, see http://store.appliedbiosystems.com). Reactions were performed with Taqman prism 7900HT 384 wells format.

Tag SNP selection

SNPs were selected from the gene-coding region of CYP2C8 and CYP2C9. The genes are located on chromosome 10, position 96,787-96,819 kbp and 96,688-96,739 kbp, respectively (Figure 1). We covered a margin of 50 kbp at each end of the gene and the region between the two genes (~50 kbp). All SNPs in this region were combined in one dataset, including CYP2C9*2, CYP2C9*3 and CYP2C8*3 as described at http://www.ncbi.nlm.nih.gov/SNP under identification numbers rs1799853C>T (CYP2C9*2), rs1057910A>C (CYP2C9*3) and rs10509681T>C (CYP2C8*3). The following cut-off points were used: Hardy-Weinberg P-value cut-off: 0.001 (0.05 divided by the number of SNPs withdrawn from the database to correct for multiple testing), minimal successful genotype percentage: 95%, minimal minor allele frequency: 0.01. We included all SNPs that fulfilled the above-mentioned criteria.

Covariables

Information on several potential confounders and effect modifiers, such as age, sex, body mass index (kg/m²) and smoking (classified as never/former/current) were gathered at baseline. Clinical measures were obtained during the visits at the Rotterdam Study research center. In 1990-1993, non-fasting blood samples were obtained, whereas in 1997-2000 blood samples were obtained after an overnight fasting. Diabetes mellitus was defined as the use of blood-glucose lowering medication and/or a non-fasting serum glucose level of 11.1 mmol/l or higher. Serum cholesterol and high-density lipoprotein cholesterol were measured using an automated enzymatic procedure. Systolic and diastolic blood pressures were measured twice from the right upper arm with a random-zero sphygmomanometer with the patient in a sitting position. The mean of the two readings was used to determine blood pressure levels.

Data analysis

The association between CYP2C8 and CYP2C9 genotype and MI was evaluated using survival analysis techniques. We studied the genotype-effect and the allele-effect of the SNPs on the risk of incident MI. Hazard ratios of events were computed using Cox proportional hazard regression analyses. Per SNP, the major allele was used as the referent. Bonferroni correction was applied to correct for multiple testing. As a result of the 25 tagging SNPs tested, P-values <0.002 (0.05/25) were considered statistically significant in testing the association of the tagging SNPs with MI. Cox proportional hazard models were used adjusted for the potential confounding effect of sex, age, total cholesterol, HDL-cholesterol, body mass index (BMI), diabetes mellitus, systolic blood pressure, diastolic blood pressure and smoking habits. Effect modification (multiplicative interaction) of the above-mentioned factors was also tested separately with interaction terms, with special notice for the modifying effect of sex. The modifying effect of smoking, which has been shown for CYP2C8*3,25 was tested for current, past and never smoking. These statistical analyses were performed with SPSS software (version 15.0; SPSS Inc., Chicago, Illinois, USA).

Haplotype analyses were performed to assess the influence of haplotype on the association between genotype and MI by using Haploview. Haploblocks were composed manually, based on linkage disequilibrium, and Chi-square tests were used to measure associations.

As vasodilation is believed to be more profound in women than in men due to the combination of hormones and the EET effect, 32-35 we studied the potential

differences in effect between the sexes for the polymorphism(s) that were found to be associated with MI. Besides testing for statistical interaction between sex and genotype (as described above), we tested for biological (additive) interaction and a stratified analysis was performed. The analysis of biological (additive) interaction between sex and genotype was performed using the Excel sheet, created by Andersson et al.^{36, 37} SAS Enterprise Guide 3.0 (2004) was used to calculate covariance matrices by proportional hazards models. The covariance matrices were used to calculate the relative excess risk due to interaction (RERI), as best reflecting value of the joint effects of two factors by an additive pattern in proportional hazards models. Significant interaction is considered if the value of zero is not enclosed by the 95% confidence interval.³⁶⁻³⁹ This analysis was not adjusted for other covariates.

Results

Of the total population of the Rotterdam Study of 7,983 participants, 2,009 participants were excluded, because blood samples for genotype analysis had not been drawn or genotyping had not been possible. Of the remaining 5,974 patients, 701 were excluded because of a history of MI at baseline. Seventy-four participants were excluded because of missing data on MI. After exclusion, 5,199 participants were left for analysis.

Forty-three SNPs were selected based on location, of which seven SNPs outside cut off levels were excluded. Eleven SNPs had an r-squared value of 0.98 or higher with one of the other selected SNPs. They were excluded from the analysis. Twenty-five SNPs were included in the analysis, seven in the CYP2C9 gene, seven in the CYP2C8 gene, two in the chromosomal area between the two genes. Three and six SNPs were selected around the borders of CYP2C9 and CYP2C8, respectively (Figure 1). Within CYP2C9, also the *2 and *3 polymorphisms (rs1799853C>T and rs1057910A>C respectively) were included in the analysis which had been analyzed in earlier studies. ^{21, 22} For similar reasons, we also included *3 polymorphism (rs10509681T>C) within CYP2C8.

Subject characteristics

The population consisted of 1,984 men (38.2%) and 3,215 women (61.8%). Mean age at baseline was 69 years of age (range 55-99 years). Table 1 shows baseline characteristics of our study population. During follow-up, 290 persons developed MI, 172 men (8.7% of men) and 118 women (3.7% of women).

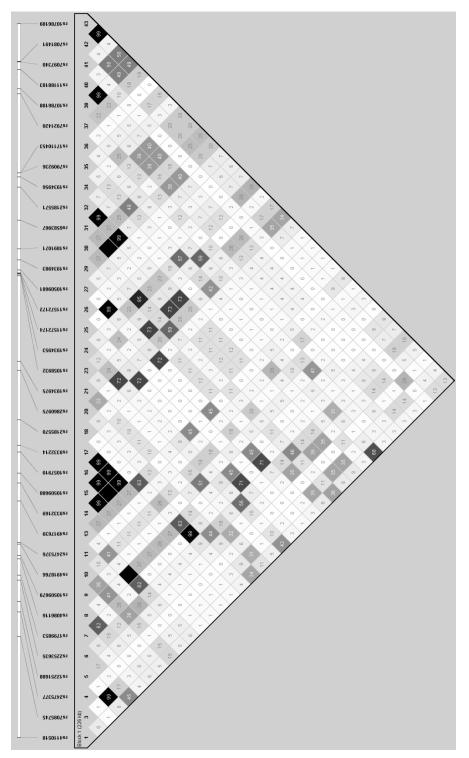


Figure 1. Location of SNPs within CYP2C9 and CYP2C8 on chromosome 10 (position 96.688-96.819 (kbp)) and r-squared value.31

Table 1. Baseline characteristics of the study population

Characteristic		N=5,199		Quartiles		
			1st	2nd	3rd	4th
Sex	Men	1,984 (38.2%)				
	Women	3,215 (61.8%)				
Age (yrs ± SD)		69.0 ± 9.1	55.0-61.7	61.7-67.8	67.8-75.1	75.1-99.2
Cholesterol	Total	6.60 ± 1.20	2.60-5.80	5.80-6.50	6.50-7.30	7.30-13.73
$(mmol/l \pm SD)$	HDL	1.36 ± 0.36	0-1.10	1.10-1.30	1.30-1.60	1.60-3.00
Body mass index (kg/m² ± SD)		26.3 ± 3.6	14.2-23.8	23.8-25.9	25.9-28.4	28.4-49.9
Smoking status	Current	1,166 (23.0%)				
	Past	2,047 (40.4%)				
	Never	1,859 (36.6%)				
Diabetes mellitus		492 (9.5%)				
Blood pressure	Systolic	139 ± 22	74-123	123-138	138-153	153-245
(mmHg ± SD)	Diastolic	74 ± 11	24-66	66-73	73-81	81-139

CYP2C and myocardial infarction

One SNP was found to be significantly associated with the occurrence of MI (Table 2). This concerns the polymorphism rs1058932C>T, within CYP2C8, with a minor allele frequency of 17.8%. The C-allele is replaced by a T-allele in the variant genotype. Sixty-eight percent of the study population had a wild type genotype. Twenty-eight percent was carrier of a heterozygous variant genotype and four percent of a homozygous variant genotype (Table 3). Data of this SNP were missing in 4 participants. They were excluded from the further analysis.

Persons carrying the CT genotype were found to have a hazard ratio of 1.54 for MI (95% CI 1.21, 1.96). The HR for persons, homozygous for the T-allele, was 1.55 (95% CI 0.90, 2.67). The effect on disease risk was dominant in the additive model. Therefore, we compared carriers of one or more variant alleles with subjects carrying the wild type. After adjustment for age and sex, subjects carrying a variant allele had a HR of 1.54 (95% CI 1.22, 1.95) compared with the wild type genotype. Table 4 gives an overview of the HRs with and without adjustment for all potential confounders. Multivariate adjustment for CYP2C9*2, CYP2C9*3 and CYP2C8*3 did not result in a significant change in HR (data not shown). No significant effect modification of smoking status, cholesterol level, BMI, diabetes mellitus and blood pressure was seen (data not shown). For the SNPs discussed in the literature (CYP2C9*2, CYP2C9*3 and CYP2C8*3), linkage between CYP2C8*3 and CYP2C9*2 was seen, with an r-squared value of 0.71 between the involved SNPs (Figure 1). None of these SNPs were found to be associated with MI in this analysis. Tables 5-7 present the results of crude and multivariate analyses with

Table 2. SNPs within CYP2C8 and CYP2C9 genes and association with myocardial infarction

SNP	Change	Location	MAF	Function	Gene	HR for MI (99.8% CI)*
rs4110518	C>T	96,640	0.226			1.03 (0.77, 1.37)
rs2475377	G>A	96,680	0.034			0.49 (0.19, 1.27)
rs12251688	G>T	96,683	0.217			0.99 (0.73, 1.34)
rs2253635	A>G	96,690	0.391	intron	CYP2C9	1.03 (0.80, 1.33)
rs1799853	C>T	96,692	0.129	exon	CYP2C9	0.95 (0.65, 1.39)
rs4086116	C>T	96,697	0.189	intron	CYP2C9	0.90 (0.65, 1.25)
rs10509679	A>G	96,698	0.166	intron	CYP2C9	1.24 (0.91, 1.68)
rs4918766	A>G	96,701	0.459	intron	CYP2C9	1.06 (0.82, 1.37)
rs2475376	C>T	96,702	0.161	intron	CYP2C9	1.04 (0.74, 1.45)
rs1057910	A>C	96,731	0.059	exon	CYP2C9	0.84 (0.47, 1.49)
rs2860975	G>T	96,757	0.427			1.11 (0.86, 1.42)
rs1934975	T>C	96,759	0.310			0.79 (0.60, 1.04)
rs1058932	C>T	96,787	0.178	3'-UTR	CYP2C8	1.39 (1.04, 1.86)
rs1934953	A>G	96,787	0.357	intron	CYP2C8	1.00 (0.77, 1.29)
rs11572172	A>C	96,787	0.051	intron	CYP2C8	1.54 (0.94, 2.52)
rs10509681	T>C	96,788	0.107	exon	CYP2C8	0.95 (0.64, 1.42)
rs1934983	C>T	96,792	0.295	intron	CYP2C8	0.86 (0.65, 1.13)
rs1891071	A>G	96,795	0.392	intron	CYP2C8	0.92 (0.71, 1.20)
rs1934956	C>T	96,818	0.146	intron	CYP2C8	0.89 (0.61, 1.28)
rs7909236	G>T	96,819	0.245			0.96 (0.72, 1.29)
rs17110453	A>C	96,819	0.126			1.31 (0.94, 1.83)
rs7921420	A>G	96,844	0.290			1.20 (0.92, 1.57)
rs10786188	A>G	96,846	0.390			0.96 (0.74, 1.24)
rs7097340	G>A	96,854	0.058			0.74 (0.40, 1.37)
rs7081491	C>T	96,855	0.444			1.14 (0.89, 1.46)

HRs represent the allele-effect; dominance of the alleles was not determined in this measurement of effect; * Confidence Intervals represent the Bonferroni adjusted intervals for the Hazard Ratios for MI.

the functional polymorphisms known from the literature. Haplotype analysis did not yield any additional information regarding increased risk of MI (results not shown).

Effect modification by sex

Considering sex differences, effect modification of sex on gene effect was tested separately for the SNP rs1058932C>T. No significant statistical interaction was found. Stratified analyses were performed to show the effects of the gene per sex. Adjusted for age, men with a variant genotype had a HR of 1.75 (95% CI 1.29, 2.36), compared with the wild type, and women carrying a variant genotype had a HR of 1.27 (95% CI 0.87, 1.84) (Table 4). Biological interaction of sex and

Table 3. Numbers of participants and myocardial infarctions per genotype and sex for the polymorphism associated with MI from the analysis and the functional polymorphisms known from the literature

SNP	Tota	al	1	Men	Wo	men
	N	MI (%)	N	MI (%)	N	MI (%)
rs1058932	(CYP2C8)					
CC	3,551	172 (4.8)	1,352	97 (7.2)	2,199	75 (3.4)
CT	1,463	104 (7.1)	550	67 (12.2)	913	37 (4.1)
TT	181	14 (7.7)	80	8 (10.0)	101	6 (5.9)
rs10509681	(CYP2C8*3)					
TT	4,142	234 (5.6)	1,593	139 (8.7)	2,549	95 (3.7)
CT	980	52 (5.3)	356	30 (8.4)	624	22 (3.5)
CC	65	4 (6.2)	31	3 (9.7)	34	1 (2.9)
rs1799853	(CYP2C9*2)					
CC	3,895	220 (5.6)	1,504	134 (8.9)	2,391	86 (3.6)
CT	1,152	63 (5.5)	421	35 (8.3)	731	28 (3.8)
TT	84	4 (4.8)	36	2 (5.6)	48	2 (4.2)
rs1057910	(CYP2C9*3)					
AA	4,600	262 (5.6)	1,765	157 (8.9)	2,835	105 (3.7)
AC	557	28 (5.0)	203	15 (7.4)	354	13 (3.7)
CC	27	0	9	0	18	0

Table 4. Hazard ratios (HRs) for incident myocardial infarction associated with SNP rs1058932C>T

rs1058932C>T	Total	Men§	Women§
	HR (95%CI)	HR (95%GI)	HR (95%CI)
Model 1*			
CC	1.00	1.00	1.00
CT or TT	1.55 (1.22, 1.96)	1.75 (1.29, 2.37)	1.29 (0.89, 1.87)
Model 2 [†]			
CC	1.00	1.00	1.00
CT or TT	1.54 (1.22, 1.95)	1.75 (1.29, 2.36)	1.27 (0.87, 1.84)
Model 3 [‡]			
CC	1.00	1.00	1.00
CT or TT	1.48 (1.16, 1.89)	1.68 (1.23, 2.30)	1.22 (0.82, 1.80)

^{*} Crude results;

genotype was tested separately. A relative excess risk due to interaction (RERI) was found of 1.40 (95% CI 0.33, 2.47), suggesting significant biological interaction between sex and genotype.

[†] Adjusted for age (and sex);

[‡] Adjusted for age, sex, total cholesterol, HDL cholesterol, systolic blood pressure, diastolic blood pressure, body mass index, diabetes mellitus, smoking;

[§] Relative excess risk due to sex-genotype interaction of 1.40 (95%CI 0.33, 2.47).

Table 5. Hazard ratios (HRs) for incident myocardial infarction associated with SNP CYP2C8*3

rs10509681T>C	Total	Men	Women
	HR (95%CI)	HR (95%CI)	HR (95%CI)
Model 1*			
TT	1.00	1.00	1.00
CT	0.92 (0.68, 1.25)	0.96 (0.64, 1.42)	0.93 (0.58, 1.48)
CC	1.09 (0.40, 2.92)	0.99 (0.32, 3.12)	0.86 (0.12, 6.19)
Model 2 [†]			
TT	1.00	1.00	1.00
CT	0.95 (0.70, 1.28)	0.96 (0.65, 1.43)	0.93 (0.58, 1.48)
CC	0.93 (0.35, 2.51)	0.98 (0.31, 3.07)	0.84 (0.12, 6.03)
Model 3 [‡]			
TT	1.00	1.00	1.00
CT	1.01 (0.74, 1.37)	1.09 (0.73, 1.63)	0.90 (0.56, 1.46)
CC	1.00 (0.37, 2.70)	1.03 (0.33, 3.24)	0.95(0.12, 6.88)

^{*} Crude results;

Table 6. Hazard ratios (HRs) for incident myocardial infarction associated with SNP CYP2C9*2

rs1799853C>T	Total	Men	Women
	HR (95%CI)	HR (95%CI)	HR (95%CI)
Model 1*			
CC	1.00	1.00	1.00
CT	0.95 (0.72, 1.26)	0.91 (0.63, 1.32)	1.06 (0.69, 1.62)
TT	0.84 (0.31, 2.24)	0.56 (0.14, 2.27)	1.23 (0.30, 5.00)
Model 2 [†]			
CC	1.00	1.00	1.00
CT	0.98 (0.74, 1.29)	0.92 (0.63, 1.33)	1.06 (0.69, 1.63)
TT	0.78 (0.29, 2.09)	0.56 (0.14, 2.27)	1.28 (0.31, 5.19)
Model 3‡			
CC	1.00	1.00	1.00
CT	0.98 (0.74, 1.32)	1.01(0.69, 1.47)	0.94 (0.60, 1.47)
TT	0.90 (0.33, 2.41)	0.67 (0.17, 2.72)	1.41 (0.35, 5.76)

^{*} Crude results;

[†] Adjusted for age (and sex);

[‡] Adjusted for age, sex, total cholesterol, HDL cholesterol, systolic blood pressure, diastolic blood pressure, body mass index, diabetes mellitus, smoking.

[†] Adjusted for age (and sex);

[‡] Adjusted for age, sex, total cholesterol, HDL cholesterol, systolic blood pressure, diastolic blood pressure, body mass index, diabetes mellitus, smoking.

Table 7. Hazard ratios (HRs) for incident myocardial infarction associated with SNP CYP2C9*3

rs1057910A>C	Total	Men	Women
	HR (95%CI)	HR (95%CI)	HR (95%CI)
Model 1*			
AA	1.00	1.00	1.00
AC	0.87 (0.59, 1.29)	0.84 (0.49, 1.42)	0.97 (0.55, 1.73)
CC	N.a.	N.a.	N.a.
Model 2†			
AA	1.00	1.00	1.00
AC	0.90 (0.61, 1.32)	0.84 (0.49, 1.42)	0.98 (0.55, 1.74)
CC	N.a.	N.a.	N.a.
Model 3‡			
AA	1.00	1.00	1.00
AC	0.97 (0.66, 1.44)	0.88 (0.52, 1.50)	1.11 (0.62, 1.98)
CC	N.a.	N.a.	N.a.

N.a.=not applicable;

Discussion

We found a significant association between MI and the variant genotype of rs1058932C>T, located in the 3'-UTR of the CYP2C8 gene, which was most substantial in males. This SNP is in LD with rs10882518, rs1934951, rs10882520, rs1934980, rs1341162, rs1113129, rs11572101 in CYP2C8, which are all located in intronic regions. The SNP rs1934951 was earlier found to be associated with bisphosphonate-related osteonecrosis of the jaw in multiple myeloma. 40 As far as we know, no other associations were described in the literature. Whether one of the intronic SNPs is responsible for splice variants that create early stop codons and thus whether they are SNPs of interest should be further investigated. The 3'-UTR is rich in regulatory elements and responsible for transcript cleavage and stability, polyadenylation, nuclear export, level of translation and mRNA targeting. 41-44 Mutations or variation in this part of one allele may lead to a dominant negative effect because of loss of control over one or more genes.⁴² SNPs in this region have been described to be associated with certain diseases, such as cancer, inflammatory disease, and thalassemia. 41, 42, 44 However, vascular disease and angiogenesis seem to be influenced by this mechanism also.45 Possibly, also microRNAs (miRNAs) play a role in this process, as miRNAs bind the 3'-UTR of the gene, and play a role in gene expression post-transcriptionally. 44-46

^{*} Crude results:

[†] Adjusted for age (and sex);

[‡] Adjusted for age, sex, total cholesterol, HDL cholesterol, systolic blood pressure, diastolic blood pressure, body mass index, diabetes mellitus, smoking.

In the literature, some studies concerned the association between the CYP2C9 gene and MI^{21, 22, 24, 25} Yasar et al.²¹ showed a slight increase in risk of MI in women carrying a CYP2C9*2 (OR 1.3; 95% CI, 1.0, 1.9) or CYP2C9*3 allele (odds ratio (OR) 1.5; 95% CI, 1.0, 2.2). For men, the only significant finding was the higher incidence of genotype combination CYP2C8*1*3/CYP2C9*1*1 in the group with MI. Other studies did not show an association between polymorphisms in genes encoding the epoxygenases and MI. ^{24,25} Marciante et al. ²⁴ studied different SNPs and haplotypes of the CYP2C genes. One of the studied SNPs was rs10882520G>C, which is in LD with rs1058932C>T, but no significant association with any of the studied SNPs and MI was found. Visser et al. 22 found a higher risk of MI only in women with allelic variants of CYP2C9, who simultaneously used drugs metabolized by this enzyme, of which some were enzyme inhibitors (HR 3.86, 95% CI: 1.93, 7.75). A protective effect of CYP2C9 variants was also suggested by others.⁴⁷⁻⁴⁹ Production of reactive oxygen species (ROS), which is also CYP2C mediated, leads to reduced NO bioavailability and may therefore contribute to vascular injury and dysfunction. So, in case of an occurring MI, inhibition of CYP2C seems to decrease infarct size.4,17,50 Inhibition of CYP2C9 showed reduced MI size in a mouse model.47 Funk et al.⁵¹ showed a protective effect with an odds ratio of 0.56 (95% CI, 0.33, 0.95; P=0.03) for the risk of MI in men carrying a variant CYP2C9 genotype (CYP2C9*3 or CYP2C9*2). No similar effect has been studied in CYP2C8 specifically.

Differences between the sexes in effect on vasodilation differ per part of the circulation, as shown in animal data.35 In some vascular beds, compensation for the loss of NO by EDHF is more prone in female than in male animals (e.g. rat mesenteric and tail arteries, rabbit genital arteries). In other biological circulatory systems (e.g. rat cerebral arteries and rat kidney), contribution of EDHF to relaxation was more profound in males or similar in both sexes, respectively. However, EDHF-mediated hyperpolarization and relaxation were significantly greater in (middle-aged) females than in males in a study by Sakuma et al.34 A modifying effect of circulating estrogens on the vascular actions of EDHF was suggested. This was supported by others⁵² who showed a larger effect of NO blockade on bradykinin-induced relaxation of human coronary micro arteries in men than women. In NO and COX-1 knockout mice, EDHF compensates with vasodilation to a great extent, but more importantly, predominantly in females.53 These results imply a sex difference in effect following vascular stress. Interestingly, in our study the HR of 1.75 (95% CI 1.29, 2.36) in men differed from the HR of 1.27 (95% CI 0.87, 1.84) in women, with a significant relative excess risk of 1.40 (95% CI 0.33, 2.47) for men. In case of cardiac distress, one can imagine that the back-up mechanism of the EET-dependent vasodilation is more effective in women than in men, due to the more pronounced activity of the EDHF

mechanism and possibly due to the modifying effect of sex steroid hormones. Decreased activity of the enzyme would therefore have more consequences for back-up in men and result in a higher risk of MI, as shown in our results. The significant RERI between sex and genotype as shown in our study supports this. The combined presence of the variant gene and male gender result in a significantly higher risk of MI as would have been expected from the two separate risks.

A potential limitation of our study is that the population included in the analysis is a subset of the Rotterdam Study. Participants for whom no blood could be drawn were excluded, as well as those for whom genotyping was unsuccessful. However, it is highly probable that non-participation was independent of the genotype and the study population was in Hardy-Weinberg equilibrium. The observed association can therefore not be explained by occurrence of selection among genotypes. Random misclassification of the outcome might have occurred due to measurement errors but MI is a fairly specific diagnosis and therefore misclassification is unlikely, and, if present, probably non-differential. Anyhow, such random misclassification would have resulted in underestimation rather than overestimation of the risk. Furthermore, data on genotype and MI were prospectively recorded without knowledge of the research question. Therefore, information bias is not likely. Confounding is unlikely, since we adjusted for the most relevant confounders. Unfortunately, no data on endothelial function and EETs were available in our study population to confirm the direct association between the CYP2C enzymes, EETs and MI.

In conclusion, there is evidence of a relation between CYP2C8 and MI; variant rs1058932C>T, located in the 3'-UTR, is associated with an increased risk of MI. A sex-difference was noticed, suggesting an effect of sex on the pathway of vascular dilation, as earlier described in the literature. Replication of our results by others is desirable to confirm this association.

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Chapter 3.2

Sex-specific differences in effects of local androgen metabolism in the heart as indicator for the risk of myocardial infarction



Abstract

Background

Testosterone influences the risk of myocardial infarction (MI) in women opposite to men. The more potent androgen 5α -dihydrotestosterone (DHT) can be formed through conversion of androstenedione by 17β -hydroxysteroid dehydrogenase types 3 and 5 (encoded by HSD17B3 and AKR1C3) and 5-alpha reductase (encoded by SRD5A1 and SRD5A2). We hypothesized that, due to the presence of DHT in coronary and myocardial tissues, a sexually dimorphic effect can be seen if genetic variance or differences in expression exist in androgen-metabolizing enzymes.

Methods

mRNA levels of steroidogenic enzymes and the androgen receptor (AR) were investigated in normal and diseased human heart samples. Subsequently, all participants within the first cohort of the Rotterdam Study (RS-I) with successful genotyping and without prevalent MI (N=5,199) were used to study the association between single nucleotide polymorphisms (SNPs) within SRD5A1, SRD5A2 and AKR1C3 and incident MI using Cox regression models. Significant results were replicated within the Atherosclerosis Risk in Communities (ARIC) cohort, and a second cohort of the Rotterdam Study (RS-II).

Results

Expression of SRD5A1, AKR1C3 and the AR was found in all myocardial samples, whereas HSD17B3 and SRD5A2 expression levels were low and undetectable, respectively. Myocardial SRD5A1 expression was higher in women than in men. Within SRD5A1, SNP rs248805G>A was significantly associated with incident MI in Western-European women (HR 1.49; 95% CI 1.19, 1.87). This SNP is tightly linked to the Hinfl polymorphism in SRD5A1 (rs248793G>C), of which the minor allele has been associated with a higher DHT/T ratio.

Conclusion

Genetic variation in SRD5A1 appears to be associated with an increased risk of MI in Western-European women, possibly due to the sex-specific potential of local androgen conversion and effect.



Introduction

Both androgens and estrogens influence the risk profile for cardiovascular diseases. An example of this is the increasing risk of developing a myocardial infarction (MI) in women after menopause, when sex steroid hormone levels decrease dramatically. Hormone replacement or suppression therapy also alters the risk of cardiovascular diseases. So Within the heart, sex steroid hormones have opposite effects on ventricular remodelling and repolarisation. Hen have increased left-ventricular mass and a decreased QTc-interval compared with women and changes occur during puberty and after menopause. Sex-specific tissue and cellular differences might partly mediate sex-specific responses but the precise mechanisms are still not completely understood.

Androgens mediate vascular processes and their effect on endothelium is sexually dimorphic. Testosterone has an anti-atherogenic effect in males. ¹⁴⁻¹⁶ Low testosterone levels increase the risk of MI and androgen replacement therapy in men is considered to be protective. ¹⁶⁻¹⁹ In women, testosterone has a stimulating effect on atherosclerotic plaque formation. ^{5, 14, 20} Moreover, myocardial androgen receptor (AR) activation leads to cardiac fibrosis and ventricular hypertrophy. ²¹

Testosterone can have both non-genomic and genomic effects, of which the latter are mediated by the AR. Besides the fact that circulating testosterone levels are higher in men than in women, vascular effects of androgens differ between the sexes. It is likely that factors different from serum testosterone levels alone contribute to this sex difference on vasculature. In non-gonadal tissues, testosterone can be formed from circulating androstenedione through local intracrine conversion by the type 5 17β-hydroxysteroid dehydrogenase (HSD), encoded by AKR1C3 (=HSD17B5), and possibly also by 17β-HSD type 3 (HSD17B3), the principal testosterone-forming enzyme in the testis.²² Dihydrotestosterone (DHT) is a more active androgen and is produced by conversion of testosterone by the enzyme 5-alpha reductase. DHT binds to the AR with a two to three times higher affinity than testosterone and has a slower dissociation rate.23 There are two types of 5-alpha reductase: type I, encoded by the SRD5A1 gene, and type II, encoded by SRD5A2. Expression of the type II enzyme is mainly confined to the male reproductive tract, whereas SRD5A1 expression is widely distributed throughout the body.^{24, 25} Both genes are involved in testosterone metabolism and single nucleotide polymorphisms (SNPs) in both genes have been associated with polycystic ovary syndrome (PCOS) and hirsutism-related traits.²⁶ These findings originate from a candidate gene study of modest size, which lacks replication.

We hypothesized that sex-dependent differences in effects of testosterone on the heart may be caused by SNPs in genes encoding androgen-metabolizing enzymes. Presence of these enzymes in human coronary arteries and myocardial tissue can modulate local conversion of androstenedione and testosterone to DHT and variation in these conversions can result in differences in vascular response and ventricular remodelling. If present in the heart, local androgen conversion could therefore modify the risk of MI or heart failure progression.

In this study, we first investigated the potential of intracrine conversion of sex steroid hormones in physiological and pathological states, by measuring the presence of steroidogenic enzymes and AR mRNA in human hearts. Second, we studied whether variations in genes encoding the androgen-metabolizing enzymes are associated with a change in the risk of MI.

Methods

Measurement of steroidogenic enzymes

Human heart samples

In order to study mRNA expression of androgen-metabolizing enzymes and the AR, human heart tissues were obtained as previously described.²⁷ Ventricular samples were taken from 7 heart-beating organ donors (median age 37, range 7-47 years, 4 women), from 8 patients with hypertrophic cardiomyopathy (median age 43, range 22-59 years, 5 women), and from 5 patients with end-stage dilated heart failure (median age 38, range 30-52, 1 woman). Left and right atrial specimens were taken from 11 different heart-beating organ donors (median age 48, range 5-63, 6 women). Control samples from organ donors were provided by the Rotterdam Heart Valve bank after removal of heart valves; all donors died because of cerebral hypoxia or haemorrhage and did not take any cardiovascular medication. Within 24 hours of death or operation, myocardial pieces were dissected and frozen and stored at -70°C. These studies were approved by the Medical Ethics Committee of the Erasmus Medical Center.

Quantitative real time-polymerase chain reaction (RT-PCR) of androgenmetabolizing enzymes and androgen receptor

Heart samples were homogenized and RNA was isolated and reverse transcribed as described previously.²⁷ Primer and probe sequences have been specified previously.²⁸ The HSD17B3 assay (Hs00970002_m1) was purchased from Applied Biosystems (Nieuwerkerk aan den IJssel, The Netherlands). Quantitative RT-PCR

for expression of the genes AKR1C3, SRD5A1, SRD5A2, AR and hypoxanthine ribosyl transferase 1 (HPRT1) was performed as previously reported.²⁷ All assays displayed >90% efficiency and did not detect genomic DNA. The quantity of mRNA of steroidogenic enzymes and the AR was expressed relative to that of the housekeeping gene HPRT1 using the Δ Ct method.

Genetic analysis

Cohort definition

Data for genetic analysis were obtained from participants of the Rotterdam Study (RS-I), a prospective population-based cohort study of neurological, cardiovascular, locomotor and ophthalmologic diseases of inhabitants of Ommoord, a suburb of the city of Rotterdam in the Netherlands, aged 55 years or older.²⁹ All participants with successful genotyping who did not have a history of MI at baseline were included. The medical ethics committee of the Erasmus Medical Center approved the study and informed consent was obtained from all participants. Prevalent MI was based on medical records from general practitioners and cardiologists, electrocardiography data, laboratory measurements and data on hospital admissions. The cohort was followed until the first occurrence of a MI, death or end of the study period (January 1, 2007), whichever came first.

Outcome

The study outcome MI was defined based on a hospital discharge diagnosis, or in case a patient was not hospitalized, when signs and symptoms, electrocardiography recordings and cardiac enzyme data were diagnostic of MI.³⁰ Two research physicians independently validated all potential cardiac events according to the International Classification of Diseases, 10th version, as previously reported.³¹ In case of disagreement, a cardiologist reviewed the events and decided on the definite validation.

Genotyping

Genomic DNA was extracted from whole blood samples using the salting out method and microarray genotyping was performed using the Infinium II Human-Hap550K Genotyping BeadChip® version 3 (Illumina inc., San Diego, California, USA), as previously described.³²

Tag SNP selection

SNPs were selected from the gene-coding regions of SRD5A1, SRD5A2 and AKR1C3. The first two genes are located on chromosomes 5p15 and 2p23 respectively,

position 6,685-6,722 kbp and 31,603-31,659 kbp, respectively. The AKR1C3 gene is located on chromosome 10p15, position 5,127-5,140 kbp. We selected all SNPs within the gene-coding regions, plus 10kbp at each end of the gene. A minimum of 90% coverage of alleles within these areas was aimed for, with a threshold of an r-squared of 0.75. Using this selection, 95% and 92% allele coverage was reached for SRD5A1 and SRD5A2, respectively. For AKR1C3, 3 extra SNPs were selected between 10-50 kbp at both sides of the gene to reach 92% coverage. The following cut-off points were used: Hardy Weinberg P-value cut off 0.05 divided by the number of SNPs drawn from the database to correct for multiple testing, minimal genotype percentage 95%, and minimal minor allele frequency 0.05. SNPs that did not fulfill the above-mentioned criteria were excluded from the analysis.

Covariables

Information on several potential confounders and effect modifiers, such as age, sex, body mass index (BMI; kg/m²), serum cholesterol, blood pressure, diabetes mellitus, and smoking (classified as never/former/current) were gathered at baseline and non-fasting blood samples were drawn.²⁹ Diabetes mellitus was defined as the use of blood-glucose lowering medication and/or a non-fasting serum glucose level of 11.1 mmol/l or higher.

Testosterone measurement

Serum levels of testosterone and sex hormone-binding globulin (SHBG) were estimated in a random sample of 1,035 participants using coated-tube or double-antibody radioimmunoassay respectively (Diagnostic Systems Laboratories, Webster, TX, USA).³³ As measure of bioavailable testosterone, non-SHBG-bound testosterone was calculated on the basis of hormone, SHBG, and albumin levels, and respective affinity constants according to the method described previously.^{34, 35}

Data analysis

mRNA expression levels in the human heart samples were logarithmically converted and analyzed using either unpaired t-test or one-way analysis of variance, followed by post-hoc evaluation according to Tukey. Correlations between gene expressions were studied using Pearson's correlation coefficient, followed by Bonferroni correction for multiple testing.

The association between SRD5A1, SRD5A2 and AKR1C3 genotype and MI was evaluated using survival analysis techniques, stratified for sex. Subsequently, we tested whether significant effect modification was present between sex and genotype using interaction terms. Per SNP, the major allele was used as the

reference. We studied the genotype-effect and the allele-effect of the SNPs on the risk of incident MI. Cox proportional hazard models were used, adjusted for the potential confounding effect of age, total cholesterol, HDL-cholesterol, body mass index (BMI), diabetes mellitus, systolic blood pressure, diastolic blood pressure and smoking habits, and, additionally, for bioavailable testosterone. Covariates which were univariately associated with MI (with P-value < 0.1) were included in the regression analyses if they changed the point estimate of the association between genotype and MI by more than 10%.36 Bonferroni correction was applied to adjust for multiple testing. Haplotype analyses were performed to assess the influence of haplotype on the association between genotype and MI by using Haploview. Haploblocks were composed manually, based on linkage disequilibrium, and Chi-square tests were used to measure associations. All statistical analyses were performed with SPSS software (version 15.0; SPSS Inc., Chicago, Illinois, USA) with the exception of the Atherosclerosis Risk in Communities (ARIC) replication analyses, which were performed with R software (version 2.7.1; 2008; The R Foundation for Statistical Computing).

Replication

Significant results from the genetic analysis were replicated within two cohorts. First, within the ARIC Study, a prospective study, set up to investigate the etiology of atherosclerosis and variation in cardiovascular disease in four US communities, in adults aged 45-64 years.³⁷ Second, replication was performed within the second cohort of the Rotterdam Study (RS-II)²⁹, according to the methods described above.

Results

Measurement of mRNA expression of steroidogenic enzymes and AR in hearts

The mRNA expression levels of HPRT1, HSD17B3, AKR1C3, SRD5A1, SRD5A2, and AR were studied in a series of normal left and right atria and in different types of left ventricular tissues, i.e. from normal hearts, and hearts from patients with hypertrophic cardiomyopathy and end-stage dilated heart failure. SRD5A2 expression was negative in all but one sample, derived from a right atrium (threshold cycle of 35.7). All other genes tested positive for all samples. Individual gene expression levels were not significantly different between groups of normal left ventricles, and left and right atria (data not shown). The ventricular samples of

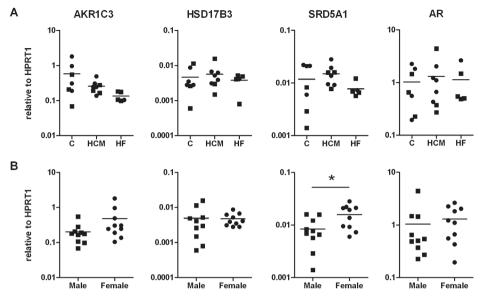


Figure 1. mRNA expression of steroidogenic enzymes 17β -hydroxysteroid types 3 and 5 (HSD17B3 and AKR1C3 respectively), 5α -reductase type I (SRD5A1) and the androgen receptor (AR) in human left ventricular tissues, stratified by tissue type (A) or sex (B). C: control, derived from heart-beating organ donors, HCM: hypertrophic cardiomyopathy, HF: end-stage dilated heart failure. Squares represent male subjects, circles female subjects. Subsequent p-values: A: P=0.074, P=0.677, P=0.236, P=0.894; B: P=0.071, P=0.379, P=0.024*, P=0.336.

patients with dilated heart failure showed a trend towards lower AKR1C3 expression (P=0.07) compared with controls (Figure 1). Overall, expression of AKR1C3 was ~100-fold higher than that of HSD17B3. The expression of SRD5A1, HSD17B3 and AR did not differ between the groups of left ventricular tissues (Figure 1). Within the left ventricle group, however, tissues from female patients showed higher SRD5A1 expression than those from male patients (P<0.05). The mRNA levels of AKR1C3 and SRD5A1 (r=0.47, P=0.04) and of HSD17B3 and AR (r=0.60, P=0.005) were positively correlated.

Genetic analysis

Subject characteristics

Of the 5,974 subjects for whom results of genotyping were available, 701 were excluded because of a history of MI. One-hundred and eight participants were excluded because of missing data on MI. After exclusion, 5,165 participants remained for analysis.

The population consisted of 1,974 men (38%) and 3,191 women (62%). Mean age at baseline was 67.7 years of age (range 55.0-97.8 years) in men and 69.8 years of age (range 55.0-99.2) in women. Table 1 shows baseline characteristics of

Table 1. Baseline characteristics of the study population for the genetic analysis

Characteristic		Men	Women
N (%)		1,974 (38%)	3,191 (62%)
Age (yrs ± SD)		67.7 ± 8.2	69.8 ± 9.5
Cholesterol	Total	6.27 ± 1.13	6.81 ± 1.20
$(mmol/l \pm SD)$	HDL	1.23 ± 0.31	1.44 ± 0.36
Body mass index (kg/m² ± SD)		25.6 ± 2.9	26.7 ± 4.0
Smoking status	Current	590 (30%)	569 (18%)
	Past	1,180 (61%)	857 (28%)
	Never	170 (9%)	1,672 (54%)
Diabetes mellitus		162 (8%)	327 (10%)
Blood pressure	Systolic	139 ± 22	139 ± 23
(mmHg ± SD)	Diastolic	75 ± 11	73 ± 11

the study population. During follow-up, 358 persons developed an MI: 209 men (10.6%) and 149 women (4.7%).

Analysis of polymorphisms within SRD5A1, SRD5A2 and AKR1C3

Eleven SNPs were selected in the area coding for the SRD5A1 gene, of which three SNPs were located outside SRD5A1. In the SRD5A2 gene coding area, six SNPs were selected, of which two SNPs were located around the SRD5A2 gene. In the AKR1C3 gene coding area, ten SNPs were selected, of which four were within the borders of the gene.

SRD5A1, SRD5A2 and AKR1C3 and risk of MI

All polymorphisms were analyzed for their association with MI (Supplementary tables 1-3). One tagging SNP was found to be significantly associated with the occurrence of MI in women: SNP rs248805G>A in SRD5A1 (Figure 2). The effect of this SNP was significantly different between men and women (P-value for interaction <0.001; data not shown). For women carrying two variant alleles of rs248805G>A, the hazard ratio (HR) for MI was 2.12 (95% CI 1.36, 3.32) (Table 2). Adjustment for bioavailable testosterone increased the HR to 5.00 (95% CI 1.42, 17.61). In men, the association was not significant, but the effect of the polymorphism tended to be protective: homozygous variant genotype showed an HR of 0.84 (95% CI 0.56, 1.27) and after adjustment for bioavailable testosterone an HR of 0.84 (95% CI 0.33, 2.13). We tested the cardiovascular risk factors for potential confounding effects. However, none of them resulted in a 10% change in point estimate. Adjustment for all included risk factors only resulted in subtle changes in hazard ratios (Table 2). SNP rs248805G>A was not significantly associated with

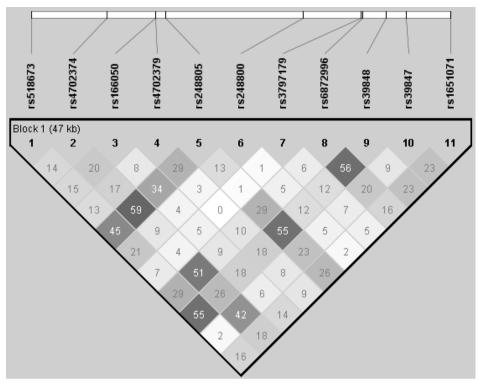


Figure 2. Location of SNPs within SRD5A1 (chr 5; 6,686-6,722 kbp) and r-squared value.38

bioavailable testosterone levels. Testosterone and SHBG levels in participants with and without MI are summarized in Table 3. Analyses using haplotypes did not show an increased association with MI (data not shown).

For the polymorphisms within SRD5A2 and AKR1C3 no significant associations with MI were found, with and without adjustment for the enzyme substrate.

Replication

Within the ARIC study population, SNP rs248805G>A was also associated with MI in women only, although in the opposite direction, only for allele effect (HR 0.84; 95% CI 0.71, 0.99) (Table 4). Adjustment for the included cardiovascular risk factors did not change point estimates and yielded similar results (data not shown).

Within RS-II, carriage of one or more variant alleles of SNP rs248805G>A showed an effect on MI risk in the same direction as in the first cohort of the Rotterdam Study, although not statistically significant (Table 4). The number of MI cases was low due to limited follow-up time. The risk estimate of MI was increased in women (HR 1.44; 95% CI 0.65, 3.22); no effect was seen in men. Adjustment for covariates did not significantly change the point estimate (data not shown).

Table 2. Hazard ratios (HRs) for incident myocardial infarction associated with SNP rs248805G>A

rs248805G>A		Men			Wom	en
	N	MI (%)	HR (95%CI)	N	MI (%)	HR (95%CI)
Model 1a*						
Per copy allele	1,974		0.93 (0.76, 1.13)	3,191		1.48 (1.18, 2.86)
GG	528	54 (10.2)	1.00	915	31(3.4)	1.00
AG	996	105 (10.5)	1.03 (0.74, 1.43)	1,545	67 (4.3)	1.28 (0.83, 1.95)
AA	450	40 (8.9)	0.84 (0.56, 1.27)	731	51 (7.0)	2.12 (1.36, 3.32)
Model 1b*‡						
Per copy allele	411		0.92 (0.58, 1.45)	624		2.68 (1.42, 5.08)
GG	101	10 (9.9)	1.00	175	3 (1.7)	1.00
AG	219	21 (9.6)	0.94 (0.44, 2.00)	304	7 (2.3)	1.30 (0.34, 5.04)
AA	91	8 (8.8)	0.84 (0.33, 2.13)	145	13 (9.0)	5.00 (1.42, 17.61)
Model 2a†						
Per copy allele	1,852		0.88 (0.72, 1.08)	2,917		1.44(1.14, 1.82)
GG	488	50 (10.2)	1.00	836	29 (3.5)	1.00
AG	935	98 (10.5)	0.95 (0.68, 1.34)	1,419	63 (4.4)	1.32 (0.85, 2.06)
AA	429	39 (9.1)	0.77 (0.50, 1.17)	662	47 (7.1)	2.03 (1.28, 3.24)
Model 2b†‡						
Per copy allele	393		0.88 (0.55, 1.42)	579		2.18 (1.14, 4.17)
GG	95	10 (10.5)	1.00	163	3 (1.8)	1.00
AG	209	18 (8.6)	0.83 (0.38, 1.82)	284	7 (2.5)	1.39 (0.35, 5.50)
AA	89	8 (9.0)	0.78 (0.31, 2.01)	132	12 (9.1)	3.92 (1.07, 14.42)

^{*} Adjusted for age;

Table 3. Steroid levels in men and women, with and without MI

Steroid levels	N	MI	N	No MI
Men				
Testosterone	55	11.4 ± 3.2	515	11.5 ± 4.1
SHBG	55	35.0 ± 12.2	502	36.6 ± 14.9
Bioavailable testosterone	40	6.7 ± 2.2	374	6.8 ± 2.9
Women				
Testosterone	33	1.5 ± 1.2	758	1.3 ± 0.8
SHBG*	35	39.2 ± 14.7	740	45.6 ± 18.0
Bioavailable testosterone	23	0.7 ± 0.6	603	0.7 ± 0.4

Values represented are mean serum levels \pm SD;

SHBG=sex hormone binding globulin; MI=myocardial infarction;

 $^{^{\}dagger}$ Adjusted for age, total cholesterol, HDL cholesterol, systolic blood pressure, diastolic blood pressure, diabetes mellitus, smoking, body mass index;

[‡] Adjusted for bioavailable testosterone;

^{*} P-value=0.038

Table 4. Hazard ratios (HRs) for incident myocardial infarction associated with SNP rs248805G>A; replication within ARIC and RS-II cohort

rs248805G>A	Men			Women		
	N	MI (%)	HR (95%CI)	N	MI (%)	HR (95%CI)
ARIC						
Per copy allele*	4,056		0.99 (0.87, 1.12)	4,915		0.84 (0.71, 0.99)†
GG	1,123	133 (11.8)	1.00	1,331	90 (6.8)	1.00
AG	2,022	231 (11.4)	0.94 (0.76, 1.17)	2,478	137 (5.5)	0.81 (0.62, 1.05)
AA	911	105 (11.5)	0.98 (0.76, 1.27)	1,106	54 (4.9)	0.71 (0.51, 1.00)
RS-II						
Per copy allele*			0.86 (0.54, 1.36)			1.44 (0.65, 3.22)
GG	256	10 (3.9)	1.00	302	2 (0.7)	1.00
AG	447	19 (4.3)	1.08 (0.50, 2.32)	561	6 (1.1)	1.65 (0.33, 8.19)
AA	215	6 (2.8)	0.69 (0.25, 1.89)	291	4 (1.4)	2.16 (0.40, 11.82)

^{*} Adjusted for age

Discussion

In this study, we showed that SRD5A1 has a significantly higher cardiac expression level in women than in men. Furthermore, we found that genetic variation within SRD5A1 is associated with an increased risk of MI in Western-European women. This genetic effect was opposite to that found in an American population, where the same SNP appeared to be cardioprotective. We found that SRD5A1 mRNA was locally expressed in normal and pathological human myocardial tissues, whereas SRD5A2 was not expressed. A previous semi-quantitative study found increased SRD5A1 mRNA in hypertrophic cardiomyopathy compared with normal samples.²¹ We could not replicate this finding in a larger series, but did detect a difference in ventricular SRD5A1 expression between men and women. A potential explanation for this difference might be that the upregulated SRD5A1 expression in women results from lower local androgen concentration, as we have described earlier for expression of SRD5A1 in the prostate.28 In this tissue, AKR1C3 was also upregulated in the absence of androgens, supporting our present observation of a positive correlation between the levels of expression of SRD5A1 and AKR1C3. In agreement with results of animal studies³⁹, no sex-dependent difference was shown in cardiac AR expression in the present study. As androgen concentrations, including DHT levels, are lower in women than in men, the effect of an increased SRD5A1 enzyme activity, and thus a higher conversion of testosterone to DHT, might be relatively more pronounced in women.

[†] P-value=0.042

The results from our cohort data analysis indicate that there is an increased risk of MI in Western European women carrying at least one variant allele of SNP rs248805G>A within the SRD5A1 gene, with an almost 2.5 times higher risk in carriers of two variant alleles compared with the wild type genotype. These results suggest that there is an increase in enzyme expression or activity in those carrying the variant allele. This will result in increased production of DHT and therefore increased AR activation and steroid-related effects. In women, this effect is positively associated with an increased risk of atherosclerosis.5, 14, 40 A nested case control study performed by Rexrode et al. 41 showed higher androgen levels in women with a cardiovascular event. In our study, a non-significant protective effect was noticed in men. This remarkable dimorphic effect corresponds with the known effects of testosterone on the cardiovascular system in both sexes.^{3, 14, 16} In our study, adjustment for bioavailable testosterone in a subgroup with sex steroid hormone level measurements increased the risk of MI. As discussed above, lower substrate androgen levels might stimulate SRD5A1 expression, which could account for this increased risk. In the ARIC cohort, an effect of the SNP was observed in women, although in the opposite direction. Analyses in the second cohort of the Rotterdam Study, RS-II, however, revealed the same effect as in the original cohort of the Rotterdam Study, although statistically not significant. One explanation could be that there are differences between Dutch Caucasian and American Caucasian populations with consequences for sex steroid hormone effects. Unfortunately, we only obtained myocardial samples from Western-Europeans, which showed increased expression of SRD5A1 in women. Possibly, this increase could be absent in female hearts from other populations, leading to different effects of genetic variation in the SRD5A1 gene. So far, no definitive conclusions can be drawn on this point.

Genetic polymorphisms in SRD5A1 have been described in earlier studies and variants in 5-alpha reductase have been associated with androgen-related diseases.²⁶ The polymorphism of interest in our study, SNP rs248805G>A, is located in an intronic region of the SRD5A1 gene. This SNP is tightly linked to SNP rs248793G>C (r-squared value of 0.81) and SNP rs566202C>T (r-squared value of 0.94)⁴², which are associated with peripheral arterial disease (PAD). Variant alleles of both rs248793G>C and rs566202C>T, were associated with a protective effect on PAD.⁴³ However, this study mainly consisted of males (111 males versus 34 females) and concerned another vascular system, in which expression patterns of SRD5A1 might differ. SNP rs248793G>C, previously detected as a restriction fragment length polymorphism (RFLP) at position 6,633 kbp, is also referred to as the HinfI RFLP of SRD5A1.⁴³ A study within 62 males showed a significant association between this RFLP and DHT/T ratio.⁴⁴ Carriage of two variant alleles

was associated with a higher DHT/T ratio. This strengthens the suspicion of increased effect of sex steroid hormones, which we expected with the variant alleles of SNP rs248805G>A.

The strength of our study is that it is set up in two parts, indicating sex-specific effects on the expression of SRD5A1, combined with sex-specific effects of a SNP within this candidate gene on the risk of MI. Since one part is focusing on the biological aspects and the other part shows the results from the epidemiological point of view, the combined conclusion that local production of DHT plays a role in MI in women rests on more solid ground. However, since the genetic analysis could not be replicated in an independent cohort, we must be careful in drawing our conclusions.

Adjustment was performed for potential cardiovascular confounders but this revealed only a minor difference with the crude analysis. It is unlikely that selection bias occurred by exclusion of study participants of whom no data on genotype were available, as it is highly improbable that genotyping error or non-participation are dependent of genotype. The outcome, MI, is a fairly specific diagnosis. Therefore, misclassification is unlikely. Also information bias is improbable, since data on genotype and MI were prospectively recorded without knowledge of the research hypotheses. Unfortunately, we were not able to obtain human coronary artery tissues for analyses of steroidogenic enzyme expression in this tissue. Myocardial mRNA levels do not necessarily reflect endothelial expression, but the increased SRD5A1 expression in women does suggest a sex-specific difference. Combined with changes in serum testosterone levels this could explain the association between MI and genetic variation in SRD5A1, which was only significant in women of Western-European descent. Also, we only studied mRNA expression, which may not completely correspond to protein levels. Studying coronary artery samples and local protein and sex steroid hormone levels could further substantiate conclusions concerning local effects of SRD5A1 expression on MI risk profile.

In conclusion, our study suggests an effect of the local conversion of testosterone by SRD5A1 in the heart in Western-European women. High testosterone levels were already known to be atheroprotective in men. This study is the first in suggesting that a local effect of androgens in the heart is associated with an increased risk of MI in women, mediated by the local presence of androgen-metabolizing enzymes.

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Supplementary table 1. Polymorphisms within SRD5A1 and risk of myocardial infarction (allele-effect)

SNP	Change	Location	MAF	Men		Women	1
				HR (95%CI)	P-value	HR (95%CI)	P-value
rs518673	G>A	6,682,930	0.343	1.01 (0.83, 1.25)	0.897	0.72 (0.56, 0.92)	0.009
rs4702374	A>G	6,691,429	0.396	1.09 (0.89, 1.33)	0.396	0.74 (0.58, 0.94)	0.015
rs166050	A>G	6,696,847	0.238	1.05 (0.84, 1.32)	0.663	1.33 (1.04, 1.71)	0.022
rs4702379	G>A	6,696,886	0.210	0.92 (0.72, 1.18)	0.500	1.22 (0.94, 1.59)	0.132
rs248805	G>A	6,698,016	0.476	0.93 (0.76, 1.13)	0.464	1.49 (1.19, 1.87)	5.5×10 ^{-4*}
rs248800	C>A	6,713,400	0.125	0.92 (0.68, 1.25)	0.602	0.74 (0.51, 1.08)	0.119
rs3797179	G>A	6,719,922	0.146	1.13 (0.87, 1.47)	0.357	1.02 (0.74, 1.42)	0.886
rs6872996	G>A	6,720,102	0.286	0.90 (0.71, 1.13)	0.353	0.82 (0.63, 1.07)	0.143
rs39848	A>G	6,722,762	0.422	0.94 (0.77, 1.14)	0.510	0.73 (0.58, 0.92)	0.008
rs39847	A>G	6,724,959	0.315	1.00 (0.81, 1.23)	0.997	0.79 (0.61, 1.01)	0.064
rs1651071	G>A	6,729,931	0.485	0.93 (0.76, 1.12)	0.430	0.87 (0.70, 1.09)	0.232

^{*} P-value statistically significant after Bonferroni correction

Supplementary table 2. Polymorphisms within SRD5A2 and risk of myocardial infarction (allele-effect)

SNP	Change	Location	MAF	Men		Women	
				HR (95%CI)	P-value	HR (95%CI)	P-value
rs2224310	A>G	31,595,971	0.276	1.03 (0.83, 1.28)	0.774	0.98 (0.76, 1.26)	0.875
rs1884722	G>A	31,601,711	0.088	1.22 (0.87, 1.70)	0.253	0.77 (0.50, 1.20)	0.248
rs2281546	A>C	31,610,528	0.110	1.40 (0.84, 1.54)	0.397	0.89 (0.61, 1.29)	0.530
rs12470143	G>A	31,617,062	0.478	1.04 (0.86, 1.26)	0.703	1.20 (0.96, 1.50)	0.117
rs2268796	G>A	31,635,784	0.403	1.09 (0.90, 1.33)	0.382	0.90 (0.72, 1.14)	0.388
rs2300697	A>G	31,640,141	0.446	0.94 (0.77, 1.13)	0.483	0.85 (0.67, 1.07)	0.154

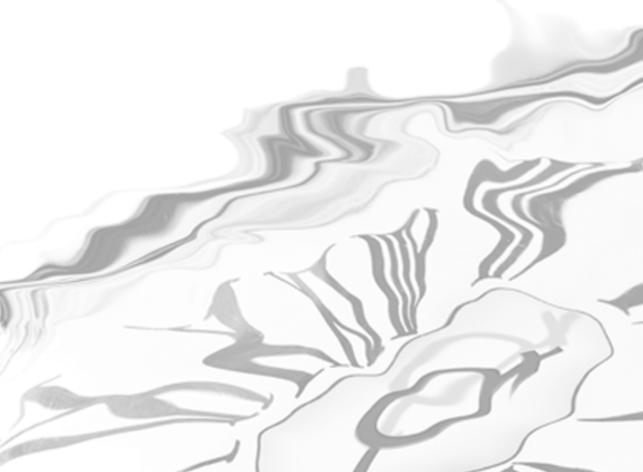
Supplementary table 3. Polymorphisms within AKR1C3 and risk of myocardial infarction (allele-effect)

SNP	Change	Location	MAF	Men		Women	
				HR (95%CI)	P-value	HR (95%CI)	P-value
rs4881395	A>G	5,109,982	0.173	1.10 (0.85, 1.42)	0.472	0.80 (0.57, 1.12)	0.202
rs1937905	G>A	5,120,610	0.169	1.00 (0.77, 1.29)	0.992	0.97 (0.72, 1.31)	0.858
rs11252934	A>G	5,124,416	0.353	1.04 (0.85, 1.27)	0.741	1.27 (1.01, 1.60)	0.044
rs2801883	A>G	5,129,003	0.402	0.90 (0.74, 1.10)	0.305	0.90 (0.72, 1.14)	0.390
rs10508293	A>G	5,131,137	0.175	1.02 (0.79, 1.31)	0.882	0.97 (0.72, 1.31)	0.839
rs4559587	A>C	5,132,855	0.065	0.76 (0.48, 1.20)	0.235	0.89 (0.55, 1.45)	0.645
rs4881400	A>C	5,134,037	0.230	1.13 (0.90, 1.42)	0.291	0.91 (0.70, 1.20)	0.512
rs1937923	G>A	5,149,883	0.171	1.02 (0.79, 1.31)	0.886	0.94 (0.69, 1.27)	0.664
rs1937920	A>G	5,151,955	0.232	0.85 (0.67, 1.09)	0.201	0.90 (0.68, 1.19)	0.466
rs10795250	A>G	5,154,836	0.356	0.99 (0.81, 1.22)	0.958	0.88 (0.69, 1.11)	0.278

Chapter 3.3

CYP1A2 and coffee intake, and the modifying effect of sex, age and smoking

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Abstract

Background

The CYP1A2 enzyme is involved in the metabolism of certain drugs and caffeine, and its activity can be influenced by factors such as sex, age, and smoking. The Single Nucleotide Polymorphism (SNP) rs762551A>C, which has also been studied for its modifying effect on cardiovascular disease, has been reported to alter enzyme activity. The objective was to study the effect of CYP1A2, sex, age and smoking on coffee intake.

Methods

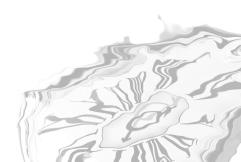
Within the Rotterdam Study, a population-based cohort, all coffee drinkers for whom genome-wide association data were available were selected. Since SNP rs762551A>C was not on the Illumina 550 platform, SNP rs2472299G>A was used as a proxy, with the A allele of SNP rs762551A>C linked to the G allele of SNP rs2472299G>A. Linear regression analyses were used to determine the effect and interaction of SNP rs2472299G>A, sex, age and smoking on coffee intake. Adjusted geometric means of coffee intake were calculated per genotype for the different smoking and sex strata using multivariable general linear models. A combined analysis, using a 'risk score', was performed to determine the contribution of each separate factor.

Results

SNP rs2472299G>A, female sex and non-smoking were significantly inversely related SNP to coffee intake. Coffee intake was lowest in non-smoking women homozygous for SNP rs2472299G>A (3.49 cups/day). All factors contributed almost linearly to the intake of coffee, with highest coffee intake in smoking men without A allele (5.32 cups/day).

Conclusion

SNP rs2472299G>A, linked to SNP rs762551A>C, sex, age and smoking significantly contribute to coffee intake.



Introduction

Cytochrome 1A2 (CYP1A2) accounts for 13% of the total hepatic content of cytochrome isoenzymes and plays a role in drug metabolism of various drugs, such as clozapine, olanzapine, omeprazole, erythromycin, propranolol and paracetamol.^{1,2} CYP1A2 is encoded by the CYP1A2 gene located on chromosome 15q24.1. Polymorphism (SNP) rs762551A>C, has been reported to alter the enzyme activity. The C-allele, the variant allele in Caucasians, is associated with a decrease in enzyme activity.^{2,3} Single nucleotide polymorphisms within the CYP1A2 gene have been associated with coffee intake recently.⁴⁻⁶ The pharmacological effect of these variants remains to be proven with experimental studies.

The activity of CYP1A2 can be influenced by factors such as sex, age, and smoking. Sex has an influence that mainly seems to be caused by the effect of the sex steroid hormones. Overall, women have lower enzyme activity than men.⁷ Fluctuations in estradiol levels cause fluctuations in enzyme activity; higher estradiol levels result in a lower metabolic rate.⁸ Increasing age was associated with a decrease in enzyme activity previously, although not consistently.^{2,9} Smoking is responsible for induction of CYP1A2 enzyme activity.^{10,11}

Caffeine is a commonly used substance to measure enzyme activity of CYP1A2 by urinary caffeine metabolic ratios.^{3, 12, 13} Caffeine is almost completely metabolized via CYP1A2 to paraxathine (82%), theobromine (11%) and theophylline (5%).¹⁴ Caffeine may have several negatively experienced effects on the body, such as tachycardia, tremor, and flushing. These adverse effects are influenced by CYP1A2 activity, sex, and smoking.¹⁵ Coffee is one of the most widely consumed, caffeine containing beverages. Certain diseases or health effects have been related to coffee intake, such as a decrease in bone mineral density (BMD), decreased risk of hormone related cancer in women, and protection against Parkinson's disease and development of diabetes mellitus (DM).¹⁶⁻²⁰ Regarding cardiovascular effects, coffee is associated with an increased risk of hypertension,²¹ modified by SNP rs762551A>C.²² The effect of coffee on coronary heart disease (CHD) is multi-factorial and has been inconclusive.²³

We conducted a study to investigate the effects of CYP1A2 on coffee intake and the modifying effects of sex, age and smoking. We hypothesized that all of these factors, possibly by influencing enzyme activity, are related to coffee intake. The aim of our study was to confirm the results from small experimental studies with population-based data.

Methods

Cohort

This study was embedded in the Rotterdam Study, a prospective population-based cohort study of neurological, cardiovascular, locomotor and ophthalmologic diseases. All inhabitants of Ommoord, a suburb of the city of Rotterdam in the Netherlands, aged 55 years or older were invited in 1990 to participate in the study. The medical ethics committee of the Erasmus Medical Center approved the study and informed consent was obtained from all participants. The rationale and design of the study were described elsewhere. The first cohort included 7,983 individuals who were all interviewed and investigated at baseline in the period 1990-1993 (RS-I). In addition, in 2000 a second cohort (RS-II) was enrolled. All inhabitants of Ommoord aged 55 years and above at that time and not yet participating in RS-I were invited. This cohort encompasses 3,011 individuals, who entered the study after consent. We included all participants of RS-I and RS-II with successful genotyping and positive coffee intake at baseline.

Outcome

Dietary intake data were collected at baseline between 1990 and 1993 for RS-I and between 2000 and 2001 for RS-II. Participants were interviewed by a trained dietician at the study centre, using a validated semi-quantitative food frequency questionnaire.²⁵ In the interview, information on amount and consumption frequency of food items was collected. Participants reported their habitual coffee and tea intake as number of cups per day or week, which was recalculated into cups per day (one cup of coffee is assumed to contain 125mL). Intake of non-caffeine containing coffee was defined as non-coffee intake. One cup of tea was considered to contribute the caffeine content of 0.5 cups of coffee.^{26,27}

Genotyping

Microarray genotyping was performed in the whole original Rotterdam Study cohort with proper quality DNA samples using the Infinium II HumanHap550K Genotyping BeadChip® version 3 (Illumina). These methods have been described in detail previously.²⁸ Since SNP rs762551A>C was not on the Illumina 550 platform, we extracted SNP rs2472299G>A, which was in complete linkage disequilibrium (LD) with SNP rs762551A>C, according to HapMap data (release 22).²⁹ The A allele of SNP rs762551A>C was linked to the G allele of SNP rs2472299G>A.³⁰ In

addition, SNP rs2472297G>A and SNP rs2470893G>A were extracted to include in the analysis as covariables. These SNPs are from previous GWAs and were associated to coffee intake.⁴⁻⁶

Covariables

Information on potential confounders and effect modifiers, namely genotype, sex, age, and smoking were gathered at baseline. Smoking status was classified as current and non-current smoking, because of its direct induction of the CYP1A2 enzyme.¹⁰

Data analysis

The association of SNP rs2472299G>A, sex, age, and smoking on coffee intake (in cups/d) was assessed cross-sectional at baseline using linear regression models, including 95% confidence intervals. The major allele G was used as the reference to study the genotype-effect (GG, GA, or AA) of the SNP. In the analyses, we adjusted for cohort (RS-I or RS-II) and the other covariables (sex, age, smoking, and the SNPs rs2472297G>A and rs2470893G>A). Effect modification was tested for genotype, sex, age and smoking by using interaction terms, adjusted and unadjusted for the other covariables. To illustrate the differences between the strata, adjusted geometric means were calculated per genotype, sex and smoking stratum, using multivariable general linear models. In order to study the contribution of tea in the association with coffee (tea containing half of the amount of caffeine compared with coffee), we performed an additional analysis where coffee and tea were combined as outcome. To study the contribution of the factors genotype, sex and smoking on the intake, we created a 'risk model'. Different variables were created of all possible combinations of the contributing factors genotype, sex and smoking. The combination associated with lowest coffee intake was considered 'baseline risk'. P-values < 0.05 were considered statistically significant. All statistical analyses were performed with SPSS software (version 15.0; SPSS Inc., Chicago, Illinois, USA).

Results

Of the population of RS-I and RS-II, data on genotype were available for 8,126 participants. Within this group, coffee intake was known for 6,698 of the participants. After exclusion of participants who do not drink coffee (N=410), 6,288

Table 1. Baseline characteristics of the participants1

	Total	cohort	Study pop	oulation
	RS-I	RS-II	RS-I	RS-II
N	7,983	3,011	4,473	1,815
Female sex (N (%))	4,878 (61)	1,693 (56)	2,608 (58)	966 (53)
Age (years)	70.6 ± 9.8^2	65.2 ± 8.4	67.5 ± 7.6	64.6 ± 7.9
Smoking (N (%))	1,725 (22)	942 (31)	1,079 (24)	607 (33)
Genotype rs2472299G>A (N (%))				
GG (%)	3,202 (54)	1,123 (52)	2,383 (53)	950 (52)
GA (%)	2,359 (40)	862 (40)	1,790 (40)	721 (40)
AA (%)	409 (7)	171 (8)	300 (7)	144 (8)
Coffee intake (cups/day)	3.8 ± 1.9	3.8 ± 2.9	4.0 ± 1.8	4.5 ± 2.5
Tea intake (cups/day)	2.9 ± 2.0	3.0 ± 2.4	2.9 ± 2.0	2.8 ± 2.3

 $^{^1}$ Differences in the characteristics sex, age and smoking between the study population and total cohort, and between RS-I and RS-II were significant (p<0.05). Differences in coffee and tea intake were significant between all groups, except for RS-I and RS-II in the total cohort for coffee and for RS-I (total cohort and study population) for tea. The analyses were performed using the Chi-square test for binary variables and the Student's t-test for continuous variables. Allele frequencies: G=73%, A=27%; Hardy-Weinberg equilibrium: χ^2 =0.96. RS=Rotterdam Study;

participants were included in the study. Mean age was slightly lower in RS-II, and RS-II contained more current smokers than the first cohort. Compared with the total cohort of the Rotterdam Study, the study population was slightly younger, consisted of relatively more men and more smokers (Table 1). There was no significant difference in genotype pattern between the coffee drinkers and the participants who did not drink coffee (data not shown).

Genetic effect

SNP rs2472299G>A had a minor allele (A) frequency of 27.0%. The distribution of the SNP did not deviate from Hardy-Weinberg equilibrium. Intake of coffee was 0.19 cup/day lower (95%CI –0.29, –0.09; P<0.0002) in carriers of one minor allele (GA), compared with carriers of two major alleles (GG). Carriers of two minor alleles (AA) drank 0.34 cup/day less (95% CI –0.53, –0.15; P<0.0005). In a univariate analysis, SNP rs2472297G>A and rs2470893G>A were significantly associated with coffee intake in our population (P=1.7x10-8 and P=1.1x10-7, respectively). In a multivariate analysis, adjusting for the individual effects of the SNPs, the SNP of interest, rs2472299G>A, remained significantly associated with coffee intake (P=7.7x10-6), but the effect of SNP rs2472297G>A and rs2470893G>A was not significant (P=0.14 and P=0.10, respectively). The results for the total study population and stratified for sex, age and smoking are shown in Table 2. Including the

² Mean ± SD (all such values).

Table 2	Effect of	rs2472299G>A on	coffee intake

		Change in coffee intake (cup/day (95% CI))*						
		GG		GA		AA	P-value	
	N	Cups/d	N	Cups/d	N	Cups/d	interaction	
Total	3,315	1.00	2,505	-0.19 (-0.29, -0.09)	441	-0.34 (-0.52, -0.16)		
Men	1,449	1.00	1,079	-0.30 (-0.46, -0.13)	176	-0.50 (-0.82, -0.17)	0.049	
Women	1,866	1.00	1,426	-0.10 (-0.23, -0.02)	265	-0.24 (-0.47, -0.02)		
Non-smoking	2,426	1.00	1,836	-0.18 (-0.28, -0.08)	312	-0.33 (-0.53, -0.13)	0.79	
Smoking	889	1.00	669	-0.21 (-0.45, 0.03)	129	-0.37 (-0.81, 0.08)		
Age ≤65 [†]	1,640	1.00	1,232	-0.20 (-0.37, -0.04)	233	-0.44 (-0.74, -0.14)	0.16	
Age >65†	1,675	1.00	1,273	-0.15 (-0.26, -0.03)	208	-0.25 (-0.47, -0.02)		

SNP rs2472299G>A was used as proxy for rs762551A>C genotype; smoking data missing in N=27;

non-coffee drinkers in the analysis showed similar results (GA: -0.20 cup/day; 95% CI -0.30, -0.09 and AA: -0.40 cup/day; 95% CI -0.60, -0.40).

Sex, age and smoking effect

Women drank less coffee than men with a difference of 0.38 cup/day (95% CI –0.48, –0.28). Age was associated with a significantly decreased coffee intake in both men and women. Per year of age, coffee intake declined with 0.07 cup/day (95% CI –0.07, –0.06). In men, coffee intake declined with 0.08 cup/day (95% CI –0.09 –0.07); in women 0.06 cup/day (95% CI –0.06 –0.05) per year of age. Smoking was associated with a higher coffee intake compared with non-smoking. Smokers drank almost one cup of coffee (0.90 cup) per day more than non-smokers (95% CI 0.79, 1.01).

Effect modification

Men showed a greater decline in coffee intake per variant allele; statistical interaction between sex and genotype was significant (P=0.049; Table 2). This difference was more pronounced within the non-smoking stratum, but this higher-level interaction could not be statistically confirmed (P=0.05). There was no significant interaction between genotype and age (P=0.16), or genotype and smoking (P=0.79).

The intake of coffee calculated per stratum is shown in Figure 1. Smoking men without a variant allele drank ~50% more coffee than non-smoking women with two variant alleles ($5.32 \pm 0.11 \text{ cups/day}$) versus $3.49 \pm 0.11 \text{ cups/day}$). To illustrate the effects after inclusion of tea to total caffeine intake, the analysis was also

^{*} Adjusted for sex, age, smoking and cohort; † Age categories based on median; lower half and upper half; Analyses were performed using the linear regression analysis.

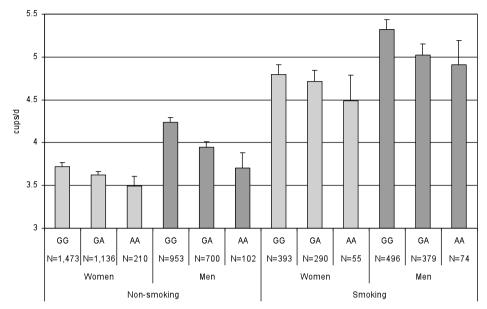


Figure 1. rs2472299G>A and coffee intake, per sex and smoking status. Values represent mean coffee intake (± SE), adjusted for age and cohort; rs2472299G>A is used as proxy for rs762551A>C; smoking data missing in N=27. Adjusted geometric means were calculated using multivariable general linear models.

performed for coffee and tea combined. The effects of the separate determinants were less pronounced in this analysis, meaning differences in effect between the strata were less, although the effects show similar patterns (data not shown). The genotype effect was significant for coffee and tea combined (GA: -0.11 cup/day; 95% CI -0.21, -0.01 and AA: -0.41 cup/day; 95% CI -0.60, -0.21), but in the stratified analyses not in all strata. Effect modification by sex was not significant (P=0.70).

Of the studied factors, age was responsible for 54% of variation, followed by smoking (28%), sex (11%) and genotype (8%). The effects of every additional determinant on coffee intake, adjusted for age because of its continuous value, are shown in Figure 2. Carriage of two variant alleles, female sex and non-smoking, were considered 'baseline risk'. Carriage of one or no variant alleles, male sex and smoking were considered as contributing to coffee intake. A linear effect could be distinguished in contribution of determinants.

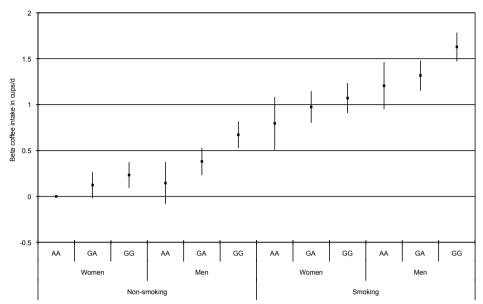


Figure 2. 'Risk' score for rs2472299G>A, sex and smoking in relation with coffee intake. Values represent betas (± SE), adjusted for age and cohort; rs2472299G>A is used as proxy for rs762551A>G. Betas were calculated using linear regression analyses.

Discussion

Our study showed the contributing effects of genetic variance in the CYP1A2 gene, sex, age and smoking on coffee intake. Intake was lowest in non-smoking women with two variant alleles of SNP rs2472299G>A (AA genotype), and increased with addition of each of these factors. Smoking and sex were responsible for the largest difference in coffee intake, followed by genetic effect. Activity of CYP1A2, which is involved in the metabolism of caffeine, explains only part of these results, since the effect of sex, age and smoking was independent of genotype and had a larger effect on the intake than this genotype.

Influence of SNP rs762551C>A on caffeine metabolism was shown previously in experimental studies.² In a small study (N=146) in a Turkish population, the metabolite ratio of caffeine after coffee intake was significantly influenced by carriage of the A allele in smokers (explaining 19% of the variation in the paraxanthine/caffeine metabolite ratio). Effect of age was only seen in non-smoking women. Smoking and sex explained the majority of the variation (24% and 10%, respectively) whereas SNP rs762551C>A genotype and age explained less than 1%. Our study shows similar results regarding the contribution of sex, smoking and genotype. This is also in agreement with a previous experimental study

on toxicity of caffeine. Females and non-smokers had the highest risk of toxic symptoms within a group of 120 healthy volunteers. 15

The relationship between SNP rs762551A>C genotype and caffeine intake was studied in a case-control study previously.³¹ No significant association was found between coffee intake and carriage of variant alleles. Three recent genome wide association studies (GWAS) on coffee consumption identified a relation between SNPs within the gene coding area of CYP1A1 and CYP1A2 and coffee intake. Two SNPs were significantly associated with coffee intake (rs2472297G>A and rs2470893G>A).⁴⁻⁶ These SNPs were not linked to SNP rs762551C>A, but nonetheless this suggests a role of this gene-coding area in coffee intake. The relation between SNP rs762551C>A and coffee intake was present, but was not genome-wide significant.⁶ Our additional analysis showed that the effect of rs2472297G>A and rs2470893G>A was not independent of SNP rs762551C>A. We showed the additional role of sex, age and smoking within our population. A significant effect of SNP rs2472299G>A was present in all strata; in both men and women, and both smokers and non-smokers. Significant effect modification was present between SNP rs2472299G>A and sex (P<0.05).

Differences in CYP1A2 activity have been attributed to effects of sex steroid hormones.^{8,32} Our study only included postmenopausal women, resulting in less difference in sex steroid hormone levels. Therefore, it is hard to explain the role of these influences, which might be overruled by other factors. In non-smokers, genetic effects seemed larger in men than in women, although the interaction between sex and genotype within the non-smoking stratum was not significant (P=0.05). Previous findings suggest that the influence of genetic variation on caffeine metabolism is larger in smokers.³ Smoking induces CYP1A2 by polycyclic aromatic hydrocarbons (PAHs) in cigarette smoke.¹¹

One of the strengths of our study is the size of the population with data on genotype and coffee intake. A potential limitation is that the population is a subset of the Rotterdam Study. Participants for whom no blood could be drawn were excluded, as well as those for whom genotyping was unsuccessful. However, it is highly probable that non-participation was independent of genotype as we showed in comparing our study population with the total population. Selection bias is less likely, since genotype distribution did not differ between coffee drinkers and participants without coffee intake. Random misclassification of the outcome might have occurred due to the subjectivity of the intake questions in the questionnaire. The additional value of caffeine from decaffeinated coffee and tea could have led to, most probably non-differential, misclassification due to our definition used. Besides, there is a possibility of habits being temporary. These issues were most probably non-differential. Caffeinated sodas were not included

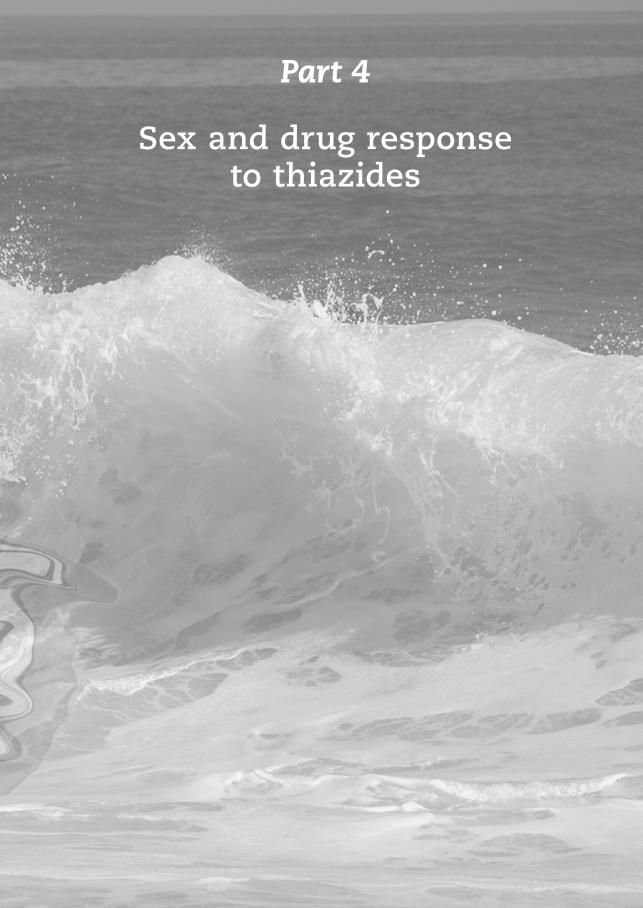
in the analyses, but regarding the age of the population we do not expect regular consumption. Data on genotype and coffee and tea intake were prospectively recorded without knowledge of the research question. Therefore, information bias is unlikely. Drug use of CYP1A2 inducers, such as omeprazole and carbamazepine, was not taken into account. This is most probably non-differential, since use of these drugs might have resulted in either an increase or decrease in coffee intake due to indication and effect of these drugs. In conclusion, genotype of CYP1A2 SNP rs2472299G>A, sex, age and smoking affect coffee intake.

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Chapter 4.1

Sex as a risk factor for thiazide-induced hyponatremia. A population-based study

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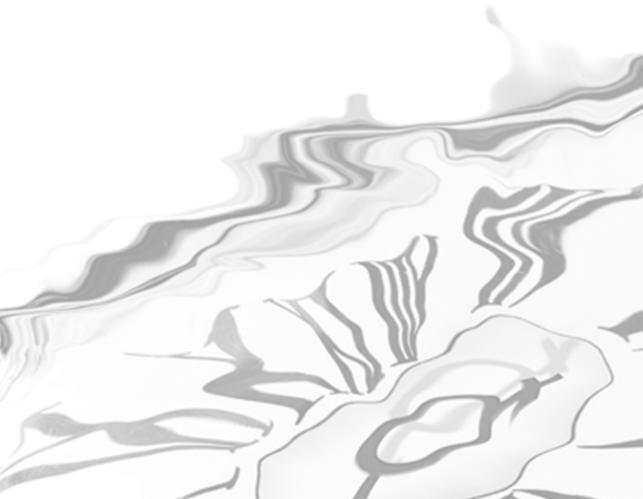
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Abstract

Background

Hyponatremia is one of the most common adverse reactions to thiazide diuretics. Women have a more than three times higher risk to be hospitalized due to diuretic-induced hyponatremia than men. In addition, risk factors such as age and body mass index (BMI) have been described. The aim of this study was to analyze whether sex is associated with an increased risk of thiazide-induced hyponatremia or a fall in serum sodium concentration.

Methods

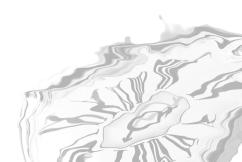
Within a population-based cohort study, the association between thiazide exposure and hyponatremia (sodium level ≤135 mmol/L; moderate to severe: <130 mmol/L) was studied in a period covering more than 10 years. This was performed by Cox proportional hazard regression analyses with thiazide use as time varying exposure. Repeated measures analyses were used to assess the effect of thiazides on serum sodium levels.

Results

Thiazide exposure was associated with an almost five times higher risk of hyponatremia than no exposure (RR 5.31; 95% CI 4.32, 6.53 in women and RR 3.71; 95% CI 2.38, 5.77 in men). In all thiazide-exposed participants, women had a 35% lower risk of hyponatremia than men. Thiazide exposure decreased serum sodium levels by 1.20 mmol/L (95% CI –1.43, –0.97) in women versus 0.90 mmol/L (95% CI –1.24, –0.55) in men. Age and BMI significantly modified thiazide effect.

Conclusion

Thiazide use is a risk factor for hyponatremia in both sexes, but female sex is no independent risk factor for hyponatremia in thiazide users. Age and BMI significantly influenced thiazide-associated sodium decline and the risk of hyponatremia.



Introduction

Diuretics are a common cause of hyponatremia, as was first reported almost 50 years ago. ¹⁻³ Thiazide diuretics account for the majority of cases of diuretic-induced hyponatremia. ^{4,5} Various mechanisms have been identified for thiazide-induced hyponatremia; ^{6,7} however, the two main causes are sodium deficiency and water overload. Thiazides inhibit sodium chloride reabsorption in the distal convoluted tubule, which leads to a direct inhibition of urinary dilution capacity. Furthermore, due to direct diuretic action and an increase in vasopressin secretion, sodium loss may be relatively higher than water loss. ^{6,8} After initiation of thiazide therapy, a new equilibrium in sodium-water balance is reached, usually within a time period of two weeks. ⁹ Hyponatremia is most likely to occur within this first period, ⁴ but may occur at any time during treatment as a consequence of physiological or environmental changes. ^{10,11}

Women have a more than three times higher risk to be hospitalized due to diuretic-induced hyponatremia than men.^{12, 13} The reason why hyponatremia is more frequently observed in women is unclear, but higher age and lower body mass have been implied.^{4, 8, 10, 14-16} Furthermore, the effect of thiazides may be modified by sex steroid hormones.¹⁷ Thiazides are more frequently prescribed in women,¹⁸ and there is ongoing debate whether a higher risk of hyponatremia in women is genuine or whether this can be explained by more frequent use of these drugs.

In the present study, conducted in a population-based cohort, we analyzed the association of sex with thiazide-associated hyponatremia and changes in serum sodium concentration. In addition, we studied the contribution of other potential effect modifiers, including age and body mass index (BMI). Because of the pivotal role of the kidneys in the thiazide effect, we also assessed the influence of renal function in the analysis (using the estimated glomerular filtration rate (eGFR)).

Methods

Cohort

This study was embedded in the Rotterdam Study, an ongoing prospective population-based cohort study of chronic diseases in the elderly. All inhabitants of Ommoord, a suburb of the city of Rotterdam in the Netherlands, aged 55 years or older were invited in 1990 to participate in the study. The medical ethics com-

mittee of the Erasmus Medical Center approved the study and informed consent was obtained from all participants. The rationale and design of the study were described elsewhere. The first cohort encompassed 7,983 individuals who were all interviewed and examined at baseline in the period 1990-1993 (Rotterdam Study-1 (RS-I)). In 2000, all inhabitants of Ommoord aged 55 years and above at that time and not yet participating in RS-I were invited to participate in the extended cohort (RS-II). This cohort encompassed 3,011 individuals, who entered the study with consent. In 2006, a further extension of the cohort (second extended cohort, RS-III) was initiated, in which 3,932 subjects, aged 45 years and over were included.

Follow-up examinations are carried out periodically, while all participants are continuously monitored for major morbidity and mortality through linkage with general practitioner and municipality records. All available serum sodium levels of participants from the study population were gathered from a general practitioner's laboratory serving the area of the Rotterdam Study. Data were available for the period from May 1, 1997 up to March 31, 2010.

Drug exposure is continuously monitored since January 1, 1991, through computerized pharmacy records of the pharmacies in the Ommoord district. The pharmacy data include the Anatomical Therapeutical Chemical (ATC) - code, the dispensing date, the total amount of drug units per prescription, the prescribed daily number of units, and product name of the drugs.

Outcome

For the first part of the study ('risk analysis') we followed all participants from RS-I, RS-II and RS-III from May 1, 1997 until January 1, 2008, because follow-up data on survival status were complete up to that date. Participants, who were referred to the laboratory for a serum sodium level assessment by their general practitioner, were defined as having hyponatremia if they had a serum sodium level of ≤135 mmol/L or as having moderate to severe hyponatremia when serum sodium levels were <130mmol/L. The date of first hyponatremia (either mild or severe) was defined as the index date. For the assessment of severe hyponatremia, the index date was the date of first severe hyponatremia.

For the second part ('level analysis'), for all participants who had two or more serum sodium level assessments between May 1, 1997 and March 31, 2010, the difference between subsequent measurements was calculated and thiazide exposure between these measurements was assessed. The change in serum sodium levels between measurements was defined as a continuous outcome (delta Na (Δ Na)).

Drug Exposure

The exposure of interest was defined as use of a thiazide diuretic (sole or in combination) or related agent (chlorothalidone, mefruside, indapamide) at the day of sodium measurement. The exposure period started at the prescription filling date and was calculated by dividing the number of units issued per prescription by the prescribed daily number of units. Participants who had hyponatremia on a date within an exposure period were considered as 'current users'.

Covariables

The following covariables were assessed as potential confounders or effect modifiers: sex, age, BMI and eGFR. BMI was expressed as kg/m² and measurements (height and weight) were obtained during examination at the center visits. Serum creatinine concentration was obtained at the moment of the serum sodium measurement. The eGFR was calculated using the four-variable MDRD formula²0 and was expressed in mL/min/1.73m². For the analyses in strata, the continuous variables BMI and eGFR were divided into quartiles. For age we used pre-defined categories (\leq 55 yrs, > 55 - 65 yrs, > 65 - 75 yrs, > 75 - 85 yrs and > 85 yrs). Missing values of eGFR were mainly participants without a serum sodium measurement and missing BMI data were random.

Statistical analysis

In the 'risk analysis', the association between thiazides and hyponatremia was evaluated using Cox proportional hazard regression analyses with exposure to thiazides as time-varying determinant, matched on follow-up time since May 1, 1997.²¹ In this model, thiazide exposure in those with hyponatremia was compared with all other participants within the cohort who did not have hyponatremia, with the same duration of follow-up. Risks were expressed as relative risks (RR) plus 95% confidence limits (95% CI). Models were adjusted for the confounding effect of sex and age, both associated with a more than 10% change of the estimate. These analyses were also performed within separate strata of sex, age, defined daily dosage (DDD), BMI and eGFR. Effect modification (multiplicative interaction) was tested separately with interaction terms. Additionally, a sensitivity analysis was performed, in which cases were compared with all participants with a sodium measurement between a week before and a week after the index date. Statistical analyses were performed by SPSS software (version 17.0; SPSS Inc., Chicago, Illinois, USA).

In the 'level analysis', the association between thiazide exposure (newly initiated) and serum sodium concentration was studied by use of linear regression for repeated measurements as implemented in the PROC MIXED function (SAS, version 9.2, SAS Institute Inc., Cary, NC, USA) to account for correlation between serum sodium level measurements within individuals. For these calculations, deltas were excluded from participants who had been exposed at the first of the two measurements or who continued exposure since the first of the two measurements. These analyses were also performed within separate strata of sex, age, DDD, BMI, eGFR, and were adjusted for sex and age. Effect modification was formally tested by use of interaction terms. P-values <0.05 were considered statistically significant.

Results

'Risk analysis'

During the follow-up period, 522 participants developed hyponatremia. Of these, 32.4% was exposed to thiazide diuretics at the time of hyponatremia. At baseline, participants who were exposed to thiazides were slightly older, had a higher BMI and a lower eGFR than non-exposed participants (Table 1).

Thiazide exposure was associated with an almost five times higher risk of hyponatremia than non-exposure (RR 4.95, 95% CI 4.12, 5.96). In women, this risk was slightly, but not significantly higher than in men (RR 5.31; 95% CI 4.32, 6.53 and RR 3.71; 95% CI 2.38, 5.77; Table 2). Exposure with a minimum dosage of 1 defined daily dosage (DDD) was associated with a higher risk of hyponatremia than exposure below 1 DDD (RR 5.72; 95% CI 4.67, 7.00 and RR 3.42; 95% CI 2.46, 4.77, respectively). The mean DDD did not differ significantly between men (0.85 \pm 0.38) and women (0.86 \pm 0.35; P=0.221). Within the group of participants that were exposed to thiazides, women had a lower risk of hyponatremia than men (RR 0.74; 95% CI 0.60, 0.92). In the non-exposed group, men and women had a similar risk (RR 0.92; 95% CI 0.82, 1.03).

The sensitivity analyses showed slightly lower risks, but the results were comparable with those from our main analyses (women: RR 3.40; 95% CI 2.70, 4.29; men: RR 2.23; 95% CI 1.38, 3.60).

Of the 522 cases of hyponatremia that occurred during the study period, 80 were moderate to severe (serum sodium <130 mmol/L). The risk of severe hyponatremia was almost eight times higher in subjects exposed to thiazides than in non-exposed subjects (RR 7.99; 95% CI 5.79, 11.04). Sex did not significantly

Table 1. Baseline characteristics of subjects with and without exposure to thiazide diuretics; hyponatremia analysis

	Thiazide exposure		
	None	Current	P-value
N	12,698	627	
N women (%)	7,402 (58.3)	468 (74.6)	< 0.001
Age (mean ±SD)	62.6 ±13.9	72.2 ±11.3	< 0.001
BMI (mean ±SD)	26.9 ±4.1	28.5 ±4.1	< 0.001
eGFR (mean ±SD)	73.3 ±18.2	65.0 ±15.7	< 0.001
Diabetes mellitus (%)	872 (7.3)	55 (9.2)	NS
Prevalent MI (%)	705 (8.1)	44 (7.6)	NS
Prevalent HF (%)	174 (2.0)	17 (2.9)	NS
C01 (cardiac therapy)	828 (6.6)	60 (9.6)	0.004
C02 (antihypertensives)	684 (5.4)	101 (16.1)	< 0.001
C04 (peripheral vasodilators)	66 (0.5)	6 (1.0)	NS
C05 (vasoprotectives)	52 (0.4)	7 (1.1)	0.021
C07 (beta-blocking agents)	1746 (13.8)	170 (27.2)	< 0.001
C08 (calcium channel blockers)	698 (5.5)	56 (8.9)	< 0.001
C09 (RAS acting agents)	1,165 (9.2)	84 (13.4)	< 0.001
C10 (lipid modifying agents)	1,356 (10.7)	61 (9.7)	NS

Abbreviations: SD=standard deviation; BMI=body mass index (kg/m²); eGFR= estimated glomerular filtration rate (mI/min/1.73m²); MI=myocardial infarction; HF=heart failure; RAS=renin-angiotensin system; 'C+number' represents the anatomical therapeutic chemical (ATC) code for the drug group mentioned between brackets;

Percentages are not for all factors the percentages of the total number of participants, due to missing values.

modify the effect of thiazides on the risk of moderate to severe hyponatremia (P=0.10). Women exposed to thiazides had a more than nine times higher risk of severe hyponatremia than non-exposed women (RR 9.07; 95% CI 6.23, 13.21). Exposed men had a more than five times higher risk than non-exposed men (RR 5.33; 95% CI 2.69, 10.56). There was no significant sex-effect in thiazide-exposed participants (RR 0.81; 95% CI 0.59, 1.12; women compared with men).

Across the age strata, the risk of hyponatremia due to thiazide exposure decreased with higher age (Table 2). The lowest age category was not analyzed, since it contained only one exposed case. In the exposed stratum, age was associated with a 7% increase in risk per year of age (RR 1.07; 95% CI 1.06, 1.09). For the non-exposed, this risk was 1.10 (95% CI 1.09, 1.11) per year of age. Regarding BMI, the risk of thiazide-induced hyponatremia was highest in the lowest stratum: almost eight times higher in the exposed group than in the non-exposed (RR 7.91; 95% CI 5.31, 11.79). The risk was lowest in the highest BMI stratum (RR 2.98; 95% CI 2.05, 4.32). In the eGFR strata, the highest risk of hyponatremia with thiazide exposure was seen within the highest eGFR quartile (suggesting better

Table 2. Risk of thiazide-induced hyponatremia; per stratum

	Exposed cases	RR (95% CI) *
Sex		
Men	24/135	3.71 (2.38, 5.77)
Women	125/387	5.31 (4.32, 6.53)
Thiazide dose		
DDD <1.0	39	3.42 (2.46, 4.77)
DDD 1.0 and higher	130	5.72 (4.67, 7.00)
Age (yrs)†		
≤ 55	2/5	n.a.
> 55 – 65	10/35	7.88 (3.77, 16.48)
> 65 – 75	33/96	6.18 (4.03, 9.48)
> 75 – 85	77/221	4.52 (3.41, 5.99)
> 85	47/165	3.83 (2.73, 5.37)
BMI (kg/m²)†		
1st quartile (<25)	36/115	7.91 (5.31, 11.79)
2nd quartile (25-<27)	35/109	5.93 (3.95, 8.92)
3rd quartile (27-29)	40/98	5.77 (3.82, 8.70)
4th quartile (>29)	40/133	2.98 (2.05, 4.32)
eGFR (mL/min/1.73m²)		
1st quartile (<61)	67/193	3.19 (2.36, 4.31)
2nd quartile (61-<71)	30/95	3.75 (2.43, 5.81)
3rd quartile (71-81)	32/102	4.06 (2.66, 6.19)
4th quartile (>81)	38/126	5.06 (3.45, 7.43)

 $Abbreviations: DDD=defined\ daily\ dosage;\ BMI=body\ mass\ index\ (kg/m^2);\ eGFR=estimated\ glomerular\ filtration\ rate\ (mL/min/1.73m^2);$

renal function), but confidence intervals overlapped. Both age and BMI significantly modified thiazide effect (P<0.05).

'Level analysis'

In this analysis, 668 individuals had a newly initiated thiazide exposed sodium measurement (937 measurements in total). A number of 727 participants had subsequent measurements that were not exposed to thiazides (14,706 measurements).

In non-thiazide users, serum sodium levels were significantly lower in women than in men, with a difference of 0.35 mmol/L (95% CI -0.50, -0.20).

^{*} Relative risks (RRs) represent the risk of hyponatremia associated with thiazide exposure, adjusted for sex and age;

 $^{^{\}dagger}$ P-value<0.05 for multiplicative interaction with thiazides (calculated using interaction terms in the regression model);

Hyponatremia is defined as serum sodium level ≤135 mmol/L.

Table 3. Effect of thiazide diuretics (initiation) on change in sodium level; per stratum

	Exposed measurements	Beta (95% CI)*
Sex		
Men	266	-0.90 (-1.24, -0.55)
Women	671	-1.20 (-1.43, -0.97)
Thiazide dose		
DDD <1.0	382	-0.96 (-1.25, -0.67)
DDD 1.0 and higher	555	-1.21 (-1.46, -0.97)
Age (yrs)†		
<55	65	-0.20 (-1.20, 0.86)
55-65	137	-0.44 (-1.13, 0.26)
65-75	277	-0.84 (-1.36, -0.32)
75-85	327	-1.23 (-1.71, -0.75)
≥ 85	131	-1.42 (-2.38, -0.46)
BMI (kg/m²)**		
1st quartile (<25)	170	-1.31 (-1.76, -0.86)
2nd quartile (25-28)	207	-1.55 (-1.96, -1.14)
3rd quartile (28-31)	219	-1.15 (-1.53, -0.78)
4th quartile (>31)	276	-0.61 (-0.97, -0.26)
eGFR (mL/min/1.73m²)		
1st quartile (<55)	215	-0.99 (-1.42, -0.57)
2nd quartile (55-68)	260	-1.06 (-1.42, -0.70)
3rd quartile (68-80)	245	-1.16 (-1.53, -0.80)
4th quartile (>80)	211	-1.09 (-1.50, -0.68)

Abbreviations: DDD=defined daily dosage; BMI=body mass index (kg/m²); eGFR=estimated glomerular filtration rate (mL/min/1.73m²);

Initiation of thiazide exposure was associated with a lower serum sodium level of 1.11 mmol/L (95% CI -1.30, -0.92) between subsequent measurements. Serum sodium levels decreased in all strata of sex, age, BMI, and eGFR (Table 3). The decrease in serum sodium after initiation of thiazides was not significantly greater in women than in men (-1.20 mmol/L; 95% CI -1.43, -0.97 in women and -0.90 mmol/L; 95% CI -1.24, -0.55 in men, respectively). Significant effect modification of the sodium lowering effect of thiazides was seen for the factors age and BMI (P<0.001). Thiazide exposure was associated with a greater decrease in serum sodium with higher age and lower BMI.

^{*} Betas represent \(\Delta \) Na between measurements with initiation of thiazide exposure between measurements, compared to non-exposure, adjusted for sex and age;

 $^{^{\}dagger}$ P-value <0.0001 for multiplicative interaction with thiazides (calculated using interaction terms in the regression model);

^{**} P-value <0.001 for multiplicative interaction with thiazides (calculated using interaction terms in the regression model).

Discussion

Our study showed that thiazide exposure was associated with an almost five times higher risk of hyponatremia than non-exposure. The risk estimate and the decrease in sodium level after newly initiated thiazide exposure was higher in women than in men, especially for moderate to severe hyponatremia, but this difference was not statistically significant. Within the group with participants exposed to thiazides, men were at a higher risk of hyponatremia.

Female sex is often considered to be an independent risk factor for hyponatremia. Women are overrepresented in case series and case-control studies concerning thiazide-induced hyponatremia.^{4, 10, 13, 22, 23} However, this has mainly been observed in hospital-based studies and not all studies have taken into account other covariables. Population-based data are scarce.¹⁶ A recent nationwide study on hospital admissions related to adverse drug reactions showed that women had a more than three times higher risk than men to be hospitalized for diuretic-induced hyponatremia.¹² Previously, a similar risk of diuretic-induced hyponatremia was observed in elderly hypertensive women.¹³ In this study, we observed a high risk of severe hyponatremia in women, but not different from men.

Thiazide diuretics exert their effect through inhibition of the renal thiazide-sensitive sodium-chloride co-transporter. This co-transporter mediates sodium and chloride reabsorption in the distal convoluted tubule of the kidney and its expression is increased with higher estradiol levels. He and women differ in the expression of sex steroid hormone receptors, organic anion transporters and vasopressin V2 receptors along the nephron. In rats, the effect of thiazide diuretics was greater in (ovariectomized) females than in males. Following this, one would expect a higher risk of hyponatremia in women in response to thiazides.

In our study, the risk of hyponatremia was highest in the lower age strata and in the lowest BMI quartiles. An increasing trend in risk was also observed in the higher eGFR quartiles. In the 'level-analysis', age and BMI were also significant effect modifiers. Thiazide use was associated with a greater serum sodium decline within the highest age categories and two lowest quartiles of BMI. The decrease in serum sodium was similar across eGFR strata.

Higher age and lower BMI were previously reported risk factors for severe thiazide-associated hyponatremia.^{10, 13, 15, 16} The literature suggests underlying illness as a possible explanation, which can be associated with low body mass or muscle wasting. Second, fluctuations in serum sodium concentration occur to a greater extent in subjects with less total body water, since the serum sodium level is determined by total solute and body water ratio.³⁰

We observed a greater serum sodium decline with thiazide exposure from age 75 and older than in the younger age groups. At higher age, the ability to maintain sodium-water homeostasis is reduced.³¹ Fluctuations in intravascular volume may more easily cause electrolyte changes in the elderly. Moreover, renal function decreases with age and this may reduce the ability to clear water and drugs. An explanation for the discrepancy between the change in serum sodium level and the risk of hyponatremia across the age strata is that the risk of thiazide-induced hyponatremia at higher age is diluted by other causes. This is supported by the fact that increasing age was associated with a higher risk of hyponatremia. Another explanation could be that more severe hyponatremias are not captured in the laboratory data we used, because these participants may have been directly hospitalized.

As far as we are aware, this is the first population-based study in which the effect of thiazides on hyponatremia and serum sodium levels was investigated, adjusting for the most relevant cofactors. One of the strengths of our study is that multiple laboratory measurements were available over a period of approximately 10 years of follow-up in a population-based cohort. The combination of laboratory data with drug use made it possible to compare these measurements during on-off periods of drug use. A potential limitation of these laboratory measurements may be that they were requested by the general practitioner and may have led to information bias. This bias is most likely non-differential for the effect of thiazides within the separate strata. In addition, most serum sodium measurements were usually combined with serum potassium and serum creatinine levels, so these other parameters may have also been the indication for measurement. BMI was not measured at exactly the date of serum sodium measurement, which could have resulted in some misclassification, but we assumed it to be relatively stable over time.

There were differences in baseline characteristics of the exposed and non-exposed participants, and residual confounding may have been present, even after adjustment for the most relevant covariables. Furthermore, data were collected independently and missing values were random, which is unlikely to have influenced our results.

In conclusion, thiazide use is a risk factor for hyponatremia in both sexes, but female sex is not an independent risk factor for hyponatremia in thiazide users. The risk of thiazide-induced hyponatremia is influenced by age and BMI. Besides the risk factors that were evaluated in this study, the effects of other factors, such as the effect of drugs used simultaneously and the impact of different sex steroid hormones and levels should be further evaluated. We illustrated that the risk of hyponatremia, including milder forms, is relatively high with thiazide use.

Mild hyponatremia is not necessarily accompanied by symptoms, but can have serious consequences if it is aggravated and not monitored correctly. Recent data suggest that even mild hyponatremia may have adverse long-term outcomes, including a higher risk of osteoporosis, fractures, and mortality.³²⁻³⁴ Monitoring of serum sodium levels, especially for those at risk, is therefore recommended.

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Chapter 4.2

Drug interactions with thiazides and the risk of hyponatremia

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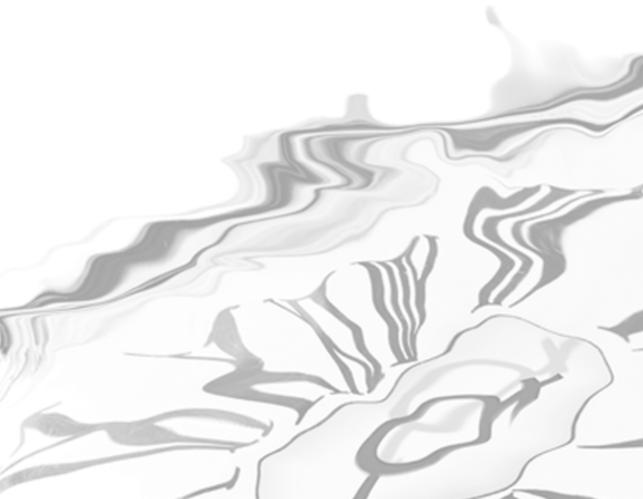
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Abstract

Background

Thiazide-induced hyponatremia is a common adverse drug reaction (ADR). Drug interactions with thiazides occur frequently and might increase the risk of an ADR. The aim was to study the risk of hyponatremia associated with drug-drug interactions with thiazides.

Methods

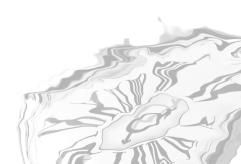
Within a population-based cohort study, the association was investigated between drug interactions with thiazides and hyponatremia (serum sodium level ≤135 mmol/L), using Cox proportional hazards models with drug use as time varying exposure. The selected drugs were loop diuretics, antiepileptics, reninangiotensin system (RAS) inhibitors, non-steroidal anti-inflammatory drugs (NSAIDs) and antidepressants. Repeated measures analyses were used to assess the effect of drug interactions with thiazides on serum sodium levels.

Results

The risk of hyponatremia substantially increased if thiazides were used in combination with a loop diuretic or RAS-inhibitor. Subjects exposed to these interacting drugs had a thirty and ten times higher risk of hyponatremia than non-exposed subjects, respectively, and a 5.3 and 1.6 times higher risk compared with thiazide use solely (RR 5.32; 95% CI 2.30, 12.27 and RR 1.63; 95% CI 1.10, 2.41, respectively). Use of thiazides in combination with at least two of the selected drug groups was associated with a three times higher risk of hyponatremia (RR 3.06; 95% CI 1.97, 4.76). In women, serum sodium levels were significantly lower in users of thiazides plus a RAS-inhibitor than thiazides only.

Conclusion

The combination of thiazides with a RAS-inhibitor or loop diuretic was associated with a substantially increased risk of hyponatremia.



Introduction

Thiazide diuretics are often used in the first line treatment of hypertension.^{1,2} Adverse drug reactions (ADRs) related to thiazides are common, with electrolyte disorders being one of the most frequently described. Thiazide-induced hyponatremia can cause serious health problems, such as nausea, delirium, hospitalization and even death. ^{3,4} Risk factors have been described, such as age and low body mass.^{4,5} Female sex has also been indicated as a risk factor for hyponatremia,⁴⁻⁷ possibly related to the effect of sex steroid hormones on sodium transport and water balance, or due to the presence of accompanying risk factors, such as a low body mass index (BMI) or advanced age.^{4,5,8}

Besides thiazides, a variety of other drugs may cause hyponatremia. Some of these drugs interact with thiazides and may increase this risk of adverse reactions. Since the mean age of the population increases, treatment periods and total number of used drugs might increase. In 2008, a report was published by the Dutch Health authorities, in which recommendations were made for extra safety measures to prevent harmful effects of drugs in high risk patients, such as drug-drug interactions. In thiazide users of older age, who start one of the interacting drugs (loop diuretics, carbamazepine, non-steroidal anti-inflammatory drugs (NSAIDs), selective serotonin re-uptake inhibitors, or venlafaxine or related agents), or increase dosage, it was recommended to perform extra serum sodium level measurements. Similar measures were recommended for users of one of these interacting drugs starting a thiazide.

Drug interactions with thiazides occur frequently¹⁸ and the effect of drug interactions with thiazides on hyponatremia has not been studied in the general population. It is important to gain more insight into the consequences of these drug interactions. Hereto, we conducted a population-based study to investigate the association between hyponatremia and use of thiazides and known interacting drugs simultaneously.

Methods

Cohort

This study was embedded in the Rotterdam Study, an ongoing prospective population-based cohort study of chronic diseases in the elderly. All inhabitants of Ommoord, a suburb of the city of Rotterdam in the Netherlands, aged 55 years or older were invited in 1990 to participate in the study. The medical ethics com-

mittee of the Erasmus Medical Center approved the study and informed consent was obtained from all participants. The rationale and design of the study were described elsewhere. The first cohort encompassed 7,983 individuals who were all interviewed and examined at baseline in the period 1990-1993 (Rotterdam Study-1 (RS-I)). In 2000, all inhabitants of Ommoord aged 55 years and above at that time and not yet participating in RS-I were invited to participate in the extended cohort (RS-II). This cohort encompassed 3,011 individuals, who entered the study with consent. In 2006, a further extension of the cohort (second extended cohort, RS-III) was initiated, in which 3,932 subjects, aged 45 years and over were included.

Follow-up examinations are carried out periodically, while all participants are continuously monitored for major morbidity and mortality through linkage with general practitioner and municipality records. All available serum sodium levels of participants from the study population were gathered from a general practitioner's laboratory serving the area of the Rotterdam Study. Data were available for the period from May 1, 1997 up to March 31, 2010.

Drug exposure is continuously monitored since January 1, 1991, through computerized pharmacy records of the pharmacies in the Ommoord district. The pharmacy data include the Anatomical Therapeutical Chemical (ATC) - code, the dispensing date, the total amount of drug units per prescription, the prescribed daily number of units, and product name of the drugs.

Outcome

In the first analysis, the association between exposure and hyponatremia was evaluated. We followed all participants from RS-I, RS-II and RS-III from May 1, 1997 until January 1, 2008, because follow-up data on survival status was complete up to that date. Participants, who were referred to the laboratory for a serum sodium level assessment by their general practitioner, were defined as having hyponatremia if they had a serum sodium level of ≤135 mmol/L. The date of first hyponatremia was defined as the index date. For the second analysis (the association between exposure and serum sodium levels), serum sodium levels were assessed as a continuous outcome using the measurements up to March 31, 2010 (7,089 participants with 27,416 sodium measurements).

Drug Exposure

Drug exposure was calculated for thiazide diuretics (sole or in combination) or related agents (chlorothalidone, mefruside, indapamide). We selected drug

groups, known to interact with thiazides and which could increase the risk of hyponatremia: anti-epileptics, antidepressants, loop diuretics, non-steroidal anti-inflammatory drugs (NSAIDs) and renin-angiotensin system (RAS) inhibitors. The exposure of interest was defined as use of the combination of one of the selected drugs and a thiazide at the index date. The exposure period started at the prescription filling date and was calculated by dividing the number of units issued per prescription by the prescribed daily number of units. Participants who had hyponatremia on a date within an exposure period were considered as 'current users'.

Covariables

Sex, age, body mass index (BMI) and renal function (expressed as estimated glomerular filtration rate (eGFR)) were assessed as baseline variables. BMI was expressed in kg/m² and measurements (height and weight) were obtained at the center visits. Serum creatinine concentration was obtained at the moment of the serum sodium measurement. The eGFR was calculated using the four-variable MDRD formula²0 and expressed in mL/min/1.73m².

Statistical analysis

In the first analysis, the association between the exposure and hyponatremia was evaluated using Cox proportional hazard regression analyses with time-varying exposure.²¹ In this model, thiazide exposure in those with hyponatremia was compared with all other participants within the cohort who did not have hyponatremia, with the same duration of follow-up. Risks were expressed as relative risks (RR) plus 95% confidence limits (95% CI). Cox proportional hazard models were adjusted for the potential confounding effect of sex and age.

First, dummies were created for thiazide use, with or without use of one or more of the additional drug groups. Risks were calculated for all exposure categories, compared with non-exposure and compared with thiazide exposure only. Effect modification by sex was formally tested by use of interaction terms (multiplicative interaction). Additionally, a sensitivity analysis was performed, in which cases were compared with all participants with a sodium measurement between a week before and a week after the index date, also adjusted for eGFR. Statistical analyses were performed by SPSS software (version 17.0; SPSS Inc., Chicago, Illinois, USA).

In the second analysis, the association between exposure and serum sodium levels was studied by use of linear regression for repeated measurements as

implemented in the PROC MIXED function (SAS, version 9.2, SAS Institute Inc.) to account for correlation between sodium level measurements within individuals. Effect modification by sex was formally tested by use of interaction terms (multiplicative interaction). P-values <0.05 were considered statistically significant.

Results

During the study period, 522 participants developed hyponatremia. Differences in covariables between exposed and non-exposed participants varied per drug. Except for exposure to anti-epileptics, participants were older in the exposed group than in the non-exposed. BMI was generally higher in exposed groups; glomerular filtration rate was slightly lower in the majority of the exposed groups, except for anti-epileptics and antidepressants (Table 1).

Table 1. Baseline characteristics on drug use for the laboratory measurements during the study period

Drug use			Age	BMI	eGFR
		N (% women)	Mean (±SD)	Mean (±SD)	Mean (±SD)
Thiazides	Exposed	627 (74.6)	72.2 ± 11.3 [†]	28.5 ± 4.1 [†]	65.0 ± 15.7 [†]
	Non-exposed	12,698 (58.3)	62.6 ± 13.9	26.9 ± 4.1	73.3 ± 18.2
Loop diuretics	Exposed	453 (64.5)	$78.2 \pm 9.9^{\dagger}$	$28.0 \pm 4.5^{\dagger}$	$58.9 \pm 28.0^{\dagger}$
	Non-exposed	12,872 (58.9)	62.5 ± 13.7	27.0 ± 4.1	73.7 ± 16.5
Anti-epileptics	Exposed	40 (55.0)	$69.4 \pm 11.2^{\dagger}$	27.2 ± 4.6	78.0 ± 22.3
	Non-exposed	13,285 (59.1)	63.0 ± 13.9	27.0 ± 4.1	72.5 ± 18.2
RAS-inhibitors	Exposed	957 (56.2)	$71.4 \pm 10.8^{\dagger}$	$28.1 \pm 4.2^{\dagger}$	$65.9 \pm 18.9^{\dagger}$
	Non-exposed	12,368 (59.3)	62.4 ± 13.9	26.9 ± 4.1	73.6 ± 17.8
NSAIDs	Exposed	1,205 (71.6)	$65.6 \pm 13.4^{\dagger}$	27.6 ± 4.4	$72.4 \pm 17.1^{\dagger}$
	Non-exposed	12,120 (57.8)	62.8 ± 13.9	26.9 ± 4.1	72.5 ± 18.3
Antidepressants	Exposed	165 (76.4)	62.1 ± 14.1	27.6 ± 4.8	72.7 ± 18.1
	Non-exposed	13,160 (58.8)	63.1 ± 13.9	27.0 ± 4.1	72.5 ± 18.2

Abbreviations: BMI=body mass index (kg/ m^2); eGFR=estimated glomerular filtration rate (mL/min/1.73 m^2); N=number; SD=standard deviation; RAS=renin-angiotensin system; NSAIDs=non-steroidal anti-inflammatory drugs;

Drug exposure and risk of hyponatremia

Thiazide exposure, without exposure to any of the other selected drugs, was associated with an almost seven times higher risk of hyponatremia than non-exposure (RR 6.97; 95% CI 5.25, 9.24; Table 2). Simultaneous exposure to loop diuretics was associated with a thirty times higher risk than non-exposure; this

[†] Statistically significantly different between exposed and non-exposed group.

Drug combination	Total		Men		Women	
	N	RR (95% CI)*	N	RR (95% CI)*	N	RR (95% CI)*
None of the selected drugs	132	Reference	37	Reference	95	Reference
Sole thiazide diuretics	79	6.97 (5.25, 9.24)	11	6.90 (3.50, 13.59)	68	7.09 (5.19, 9.70)
+ Loop diuretics	6	33.55 (14.72, 76.46)	0	N.a.	6	39.97 (17.41, 91.74)
+ RAS-inhibitors	38	11.04 (7.67, 15.88)	4	4.36 (1.55, 12.26)	34	12.23 (8.91, 19.64)
+ NSAIDs	13	7.05 (3.98, 12.49)	2	9.35 (2.23, 39.15)	11	6.90 (3.70, 12.90)
$+ \ge 2$ drug groups	27	21.03 (13.87, 31.89)	4	17.81 (6.31, 50.27)	23	22.26 (14.09, 35.17)

Table 2. Drug-combinations and risk of hyponatremia in the total population; men and women

Abbreviations: N=number of exposed cases; RAS=renin-angiotensin system; NSAIDs=non-steroidal anti-inflammatory drugs

risk was more than five times higher than thiazide exposure solely (RR 5.32; 95% CI 2.30, 12.27). Simultaneous use of a RAS-inhibitor was associated with a more than ten times higher risk of hyponatremia than exposure to none of these drugs, and with an almost two times higher risk compared with thiazide exposure solely (RR 1.63; 95% CI 1.10, 2.41).

Exposure to NSAIDs in combination with thiazides showed a similar risk of hyponatremia (RR 6.90; 95% CI 3.70, 12.90) as exposure to thiazides solely. For simultaneous use of anti-epileptics or antidepressants, numbers of exposed cases were too low (<5 exposed cases) for accurate assessment (data not shown).

Use of two or more selected drug groups in combination with thiazides was associated with a more than twenty times higher risk than non-exposure, and with a more than three times higher risk than thiazide exposure solely (RR 3.06; 95% CI 1.97, 4.76).

In the sensitivity analysis, exposure to thiazides only was associated with a more than three times higher risk of hyponatremia compared with non-exposure (RR 3.16; 95% CI 2.37, 4.22), and adjustment for eGFR even increased this risk (RR 3.30; 95% CI 2.46, 4.42). Any of the drug combinations with thiazides showed an increased risk of hyponatremia, compared with no exposure, but there was no significant additional effect of any drug combination with thiazides. The highest risk was for thiazides plus two or more drug groups in combination with an almost six times increased risk compared with non-exposure (RR 5.67; 95% CI 3.66, 8.78).

Hyponatremia risk; men and women

Risk estimates for hyponatremia were higher in women than in men (Table 2). However, the number of exposed cases in men was low and differences in risk of hyponatremia between men and women were not significant for any combination of drugs.

^{*}Adjusted for sex and age.

Drug exposure and serum sodium levels

In the serum sodium level analysis, 11,031 measurements were available with non-exposure to any of the studied drug groups. Of the exposed measurements, 2,425 were exposed to thiazides solely, 110 to thiazides and loop diuretics, 1,447 to thiazides and RAS-inhibitors, 346 to thiazides and NSAIDs and 606 to thiazides plus more than one drug of interest. Thiazides were significantly associated with lower serum sodium levels than non-exposure (serum sodium –0.83 mmol/L; 95% CI –0.97, –0.68). RAS-inhibitors and NSAIDS were associated with a further lower serum sodium level of –0.57 mmol/L (95% CI –0.82, –0.33) and –0.44 mmol/L (95% CI –0.80, –0.08) compared with thiazide exposure, respectively. Simultaneous exposure to more than one drug plus thiazides was associated with a serum sodium level of –1.37 mmol/L (95% CI –1.64, –1.10) compared with non-exposure.

Serum sodium levels; men and women

In women, serum sodium levels were all significantly lower during exposure to the selected drug combinations, compared with non-exposure (Figure 1). Additional exposure to a RAS-inhibitor was associated with a greater effect in women than in men, compared with exposure to thiazides only (P=0.016).

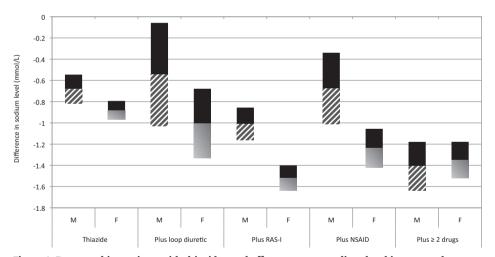


Figure 1. Drug combinanations with thiazides and effect on serum sodium level in men and women separately. Drug response to thiazides and separate drug combinations is compared to non-exposure to any of the studied drugs. * P-values compared to non-exposure, respectively: <0.001, <0.001, 0.264, 0.002, <0.001, <0.001, <0.001, <0.001, <0.001.

Discussion

Our study showed that the combination of a thiazide diuretic and a RAS-inhibitor or a loop diuretic was associated with a higher risk of hyponatremia and with a lower serum sodium level. Although these drugs are known to interact with thiazide diuretics, little is known about the occurrence of these adverse drug reactions under everyday circumstances in community-dwelling elderly. As far as we know, this is the first prospective, observational study in a non-clinical population-based setting.

In the treatment of hypertension, RAS-inhibitors are often prescribed in combination with thiazides to increase the antihypertensive effect.^{22, 23} RAS-inhibitors counteract the RAS stimulation, induced by thiazides. Inhibition of RAS leads to less sodium reabsorption in renal distal tubules and therefore induces a maximum effect of sodium depletion by diuretics.^{22, 24} A review of case reports of hyponatremia following RAS-inhibitor therapy showed a combination of diuretic use in the majority of cases, mainly including thiazides.²⁵ The syndrome of inappropriate antidiuretic hormone (ADH) is also proposed as a causal factor.

In our study, the combination of a RAS-inhibitor with thiazides showed its main effect in women. Thiazide diuretics in combination with a RAS-inhibitor showed significantly lower serum sodium levels in women than in men, compared with thiazides only. However, the risk of hyponatremia was not statistically significantly higher in one of the sexes. The RAS is stimulated by lower estrogen levels and higher testosterone levels, leading to an increased susceptibility to hypertension in postmenopausal women. ^{26,27} Clinical trials have shown a greater blood pressure reduction in women than in men by angiotensin receptor blocker (ARBs) and plasma concentrations of the drug were higher in women. ^{28,29} For angiotensin-converting enzyme inhibitors (ACE-I), blood pressure reduction was similar in men and women. ³⁰ Effects in heart failure patients seem to be less beneficial in women than in men. ^{31,32} However, also in subjects without ventricular dysfunction, ³³ results are inconclusive, due to the low number of women enrolled in these studies. Regarding adverse events, the frequency of cough following ACE-I therapy was more frequent in women. ^{34,35}

Loop diuretics mainly act on the thick ascending limb of Henle and are less often causing hyponatremia than thiazides. 4.36 The combination of thiazides with loop diuretics can be prescribed in the treatment of heart failure and hyponatremia due to this combination has been described as well. 12 This drug interaction effect could possibly be partly explained by confounding by indication, since heart failure itself is also associated with hyponatremia due to water overload. 37

Additional exposure to NSAIDs with thiazides was not associated with a higher risk of hyponatremia in our study compared with thiazides solely. The problem with NSAIDs is that they can be obtained over the counter and used without prescription. This information was not available for our study, so the effect may probably be diluted.

In previous studies, sex differences were seen for severe diuretic-induced hyponatremia.^{4,7} In our study, however, numbers were too small to analyze severe hyponatremia separately.

One of the strengths of our study is that multiple laboratory measurements were available over a period of approximately 15 years of follow-up time in a population-based study population. The combination with drug exposure made it possible to compare these measurements during on-off periods of drug use. A potential limitation of these laboratory measurements may be that they are requested by the general practitioner and might have led to information bias, as suggested by the results of the sensitivity analysis. One of the reasons could be that the use of an interacting drug with a thiazide has been a reason for sodium measurement, as recommended by the guidelines of health authorities. 15

We demonstrated that certain drug interactions with thiazides increase the risk of hyponatremia. RAS-inhibitors are not included in the recommendations of the report by the Dutch Health authorities, 15 but they are known for their interaction with thiazides and have shown to cause hyponatremia in combination with thiazides. 13, 14, 25 This finding should be further explored and, if necessary, taken into account in safe drug prescribing as well.

In conclusion, the results of our study show that combined use of a thiazide diuretic with a RAS-inhibitor, loop diuretics, or one or more of the known interacting drugs, was associated with an increased risk of hyponatremia and a lower serum sodium level. This is clinically important in the management of drug interactions and potentially preventable adverse reactions following polypharmacy. In addition to previous literature, on which guidelines from health authorities are based, our results show the importance of acknowledging the risk of ADRs caused by drug-drug interactions.

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Chapter 4.3

Genetic variation of the organic anion transporters 1 and 3 modifies the risk of thiazide-induced hyponatremia



Abstract

Background

Hyponatremia is a common and potentially serious adverse reaction of thiazide diuretics. Before thiazides can act in the distal tubule, they are secreted in the proximal tubule through the organic anion transporters (OAT) 1 and 3. We hypothesized that genetic variation in the genes encoding OAT1 and OAT3, SLC22A6 and SLC22A8, modified the risk of thiazide-induced hyponatremia.

Methods

Within thiazide users in the population-based Rotterdam Study, the association between 'single nucleotide polymorphisms' (SNPs) in and around (±50 kbp) SLC22A6 and SLC22A8 and hyponatremia was analyzed using Cox proportional hazard regression analyses. Analyses were adjusted for age, sex, renal function and simultaneously used medication. Effect modification by sex was tested with multiplicative interaction terms. For SNPs that were statistically significantly associated with hyponatremia, mRNA expression levels were investigated.

Results

During the study period, 131 cases of hyponatremia occurred in 479 thiazide-exposed subjects. Of the 41 SNPs tested, six SNPs were associated with the risk of thiazide-induced hyponatremia. Three SNPs (rs4592425T>G, rs6591722T>A, rs2187383C>A) reduced this risk (79%, 64% and 54%, respectively, per minor variant allele in the adjusted model; all P-values <0.006). Conversely, three other SNPs (rs4149172T>C, rs11231294T>C, rs955434G>A) increased the risk of thiazide-induced hyponatremia by 54%, 62% and 57%, respectively (all P-values <0.005). Sex did not modify these effects. SNP rs4592425T>G and rs6591722T>A were associated with mRNA expression levels of probes in the genes BSCL2, EEF1G and NXF1 (false-discovery rate (FDR) <0.001).

Conclusion

SNPs in the genetic region of OAT1 and OAT3 are associated with an increased or reduced risk of thiazide-induced hyponatremia. This suggests that the risk of hyponatremia depends on the degree of delivery of the drug to its target. Ultimately, these findings may possibly lead to more tailored pharmacotherapy.



Introduction

Hyponatremia is a common and potentially serious adverse reaction to thiazide diuretics that often leads to hospitalization.¹⁻³ The complications of thiazide-induced hyponatremia range from the development of cerebral edema if hyponatremia is acute⁴ to the development of osmotic demyelination if chronic hyponatremia is corrected too rapidly.^{5,6}

Thiazides are extensively bound to plasma proteins and secreted largely in the proximal tubule of the kidney.³ This is largely mediated by the organic anion transporters type 1 (OAT1) and type 3 (OAT3).7,8 OAT1 and OAT3 are located in the basolateral plasma membrane and transport various anions from the blood into the tubular fluid.9 Animal studies have shown that OAT1 and OAT3 knockout mice have higher blood and lower urinary concentrations of both loop and thiazide diuretics, which illustrates the crucial role of these OATs in diuretic metabolism.8,9 OAT1 and OAT3 are encoded by the genes SLC22A6 and SLC22A8, respectively, which are members of the solute carrier family 22. Single nucleotide polymorphisms (SNPs) have been identified in both genes, 10, 11 and their functionality has been shown, as they are associated with the therapeutic response to antiviral drugs.¹² OAT3 has also been associated with the regulation of blood pressure in mice. 13 Recently, the results of two independent case-control studies showed an association between an intergenic SNP of SLC22A6 and SLC22A8 (rs10792367G>C) and the blood pressure response to hydrochlorothiazide.14

Here, we hypothesized that genetic variation in the region encoding these transporters is associated with the risk of thiazide-induced hyponatremia. In addition, we investigated if there was a modifying effect of sex, because hyponatremia is more common in women^{2, 5} and because sex differences in OAT expression have been reported.¹⁵

Methods

Cohort

This study was embedded in the Rotterdam Study, an ongoing prospective population-based cohort study of chronic diseases in Caucasian elderly. All inhabitants of Ommoord, a suburb of the city of Rotterdam in the Netherlands, aged 55 years or older were invited in 1990 to participate in the study. The medical ethics committee of the Erasmus Medical Center approved the study and

informed consent was obtained from all participants. The rationale and design of the study were described elsewhere. 16

Follow-up examinations are carried out periodically while all participants are continuously monitored for major morbidity and mortality through linkage with general practitioner and municipality records. All available serum sodium levels of participants from the study population were gathered from a general practitioner's laboratory serving the area of the Rotterdam Study. Data were available for the period from May 1, 1997 up to March 31, 2010.

Drug exposure is continuously monitored since January 1, 1991, through computerized pharmacy records from all pharmacies in the Ommoord district. The pharmacy data include the Anatomical Therapeutical Chemical (ATC) - code, the dispensing date, the total amount of drug units per prescription, the prescribed daily number of units, and product name of the drugs. This provides us with information on start and duration of use of all prescribed medication.

Outcome

We followed all participants from May 1, 1997 until January 1, 2008, because follow-up data on survival status were complete up to that date. Participants, who were referred to the laboratory for a serum sodium level assessment by their general practitioner, were defined as a case of hyponatremia if they had a serum sodium level of \leq 135 mmol/L. The date of hyponatremia was defined as the index date.

Drug Exposure

We selected all participants who used a thiazide diuretic (as a single agent or in a combination preparation) or related agent (chlorothalidone, mefruside, indapamide) during the study period to calculate the exposure period. For each participant, the exposure period started at the prescription filling date and the exposure duration was calculated by dividing the number of units issued per prescription by the prescribed daily number of units. Participants who had hyponatremia (or were analyzed as part of the rest of the cohort) on a date within an exposure period were considered as exposed.

Genotype data

'Genome-wide association' genotyping and imputation was performed following standard methods, as have been described elsewhere. 17 SNPs were selected

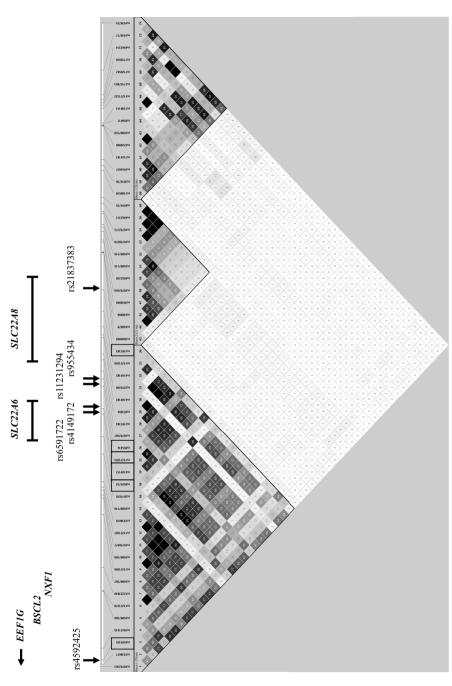


Figure 1. Location and r-squared values of single nucleotide polymorphisms (SNPs) in the area of SLC22A6 and SLC22A8, located on chromosome 11. The shown region covers position 65,500-62,540 kbp.18

from the gene-coding regions of SLC22A6 and SLC22A8. These genes are located on chromosome 11q12.3, position 65,500-62,509 kbp and 62,516-62,540 kbp, respectively (Figure 1). We covered a margin of 50 kbp at each end of the gene and the region between the two genes (~8 kbp). The following cut-off points were used: minimal genotype percentage: 95%, minimal minor allele frequency: 0.10. We included all SNPs that fulfilled the above-mentioned criteria.

Covariables

Sex, age and renal function (using the estimated glomerular filtration rate (eGFR) were included as covariables, plus drugs which act on the renal system and interact with thiazides (loop diuretics, potassium sparing agents and renin-angiotensin system (RAS) inhibitors). Serum creatinine concentration was obtained at the moment of the serum sodium measurement. The eGFR was calculated using the four-variable MDRD formula¹⁹ and was expressed in mL/min/1.73m². The use of loop diuretics, potassium sparing agents and RAS-inhibitors was determined as described above for exposure to thiazide diuretics. Data on body mass index (BMI), diabetes mellitus, and cardiovascular history were assessed at baseline.

Statistical analysis

The association between thiazide use and hyponatremia was evaluated using Cox proportional hazard regression analyses with time-varying exposure of thiazides.²⁰ In this model, thiazide exposure in participants with a hyponatremia on the index date was compared with the exposure in the remainder of the cohort at the same date of follow-up. These results are presented elsewhere.²¹ For this study, we analyzed only those participants (cases as well as non-cases) who were using thiazides on the index date, in which the risk factor of interest was defined as the presence of one or more variant alleles in the genes SLC22A6 and/or SLC22A8. Per SNP, having two major alleles (AA) was used as the referent while the risk was assessed according to an allele-dose-effect model (AA=0, aA=1, aa=2). Bonferroni correction was applied to adjust for multiple testing. Based on an r-squared value of <0.2 for independency, only seven SNPs were independent. Following, P-values <0.007 (0.05 / 7) was considered statistically significant in testing the association of the tagging SNPs with hyponatremia. Risks were expressed as relative risks (RR) plus 95% confidence limits (95% CI).

Additional analyses were performed for SNPs that were statistically significantly associated with hyponatremia. Models were adjusted for the potential confounding effect of sex, age, renal function and simultaneous drug use of RAS-

inhibitors, loop diuretics and potassium sparing diuretics. Effect modification (multiplicative interaction) by sex was tested separately with interaction terms.

Two sensitivity analyses were performed. First, the effect of the SNPs was tested in all non-thiazide exposed participants from the original source population. Second, only participants were included with a sodium measurement between a week before and a week after the index date.

All statistical analyses were performed by SPSS software (version 17.0; SPSS Inc., Chicago, Illinois, USA).

Expression quantitative trait loci (eQTL) analysis

For the eQTL analysis, the RS-III cohort was used. Whole blood cells were collected (PAXgene Tubes-Becton Dickinson) and total RNA was isolated (PAXgene Blood RNA kit-Qiagen). RNA was amplified, labelled (Ambion TotalPrep RNA), and hybridized to the Illumina Whole-Genome Expression Beadchips (HT-12v4). The RS-III expression dataset is available at GEO (Gene Expression Omnibus) public repository under the accession GSE 33828. The total number of RS-III samples with both imputed genotypes and Whole-Genome Expression data equals 762.

Expression and eQTL analyses in RS-III were performed using quantile-normalized, log²-transformed, and standardized gene expression data. We tested the most significant SNPs out of the candidate gene study against all probes within 250 kilobases (kb) from the SNP position, using Spearman rank correlation. Significance of findings was determined by controlling the false-discovery rate (FDR) at 0.05, determined by generating a null-distribution based upon permuted data. The analyses were adjusted for the first 40 eigenvectors of the principal component analysis to account for possible confounding effects.

Results

The study population consisted of 479 participants, who were either exposed to thiazides at the index date (i.e., at the moment they developed hyponatremia), or at the moment they acted as a control in the rest of the cohort. Baseline characteristics of the study population showed a higher proportion of women, higher age, lower eGFR and a higher proportion of simultaneous use of RAS-inhibitors compared with the total cohort (Table 1). During the study period, 131 cases of hyponatremia occurred, of whom 22 (16.8%) were men and 109 (83.2%) were women.

Table 1. Baseline characteristics of the study population, compared to the total population

	Total population	Study population*	P-value
N (% women)	13,325 (59.1)	479 (74.7)	
Age (mean ± SD)	63.1 ± 13.9	72.7 ± 10.6	< 0.001
BMI (mean ± SD)	27.0 ± 4.1	28.3 ± 4.0	< 0.001
eGFR (mean ± SD)	72.5 ± 18.2	64.9 ± 16.0	< 0.001
Diabetes Mellitus	927 (7.4)	41 (8.6)	NS
Prevalent MI	749 (8.0)	34 (7.1)	NS
Prevalent HF	191 (2.0)	15 (3.1)	NS
RAS-inhibitors	957 (7.2)	86 (18.0)	< 0.001
Loop diuretics	453 (3.4)	14 (2.9)	NS
Potassium-sparing diuretics	73 (0.5)	3 (0.6)	NS

 $Abbreviations: SD=standard\ deviation;\ BMI=body\ mass\ index\ (kg/m^2);\ eGFR=estimated\ glomerular\ filtration\ rate\ (mL/min/1.73m^2);\ MI=myocardial\ infarction;\ HF=heart\ failure;\ RAS=renin-angiotensin\ system;$

Percentages may correspond to lower total numbers due to missing values.

Fifty-four SNPs fulfilled the inclusion criteria, of which thirteen were in complete linkage disequilibrium with another SNP, leaving 41 SNPs for analysis. Three SNPs were located in the gene coding area of OAT1 (SLC22A6) and five SNPs were located in the gene coding area of OAT3 (SLC22A8) (Figure 1). The remaining SNPs were selected on the basis of the predetermined extra margins. After adjustment for multiple testing, six of these SNPs were statistically significantly associated with hyponatremia (all SNPs shown in supplementary table). SNP rs4592425T>G, rs6591722T>A, rs4149172T>C, rs11231294T>C, rs955434G>A, rs2187383C>A had a minor allele frequency of 31%, 38%, 29%, 25%, 25%, and 43%, respectively. All these SNPs were located in one haploblock that covered both SLC22A6 and SLC22A8 (Figure 1), and thus these associations probably represent the same signal. The minor allele of rs4149172T>C, rs11231294T>C and rs955434G>A was associated with an increased risk of hyponatremia, whereas for the other three SNPs, the minor allele was associated with a decreased risk of hyponatremia (Table 2). SNP rs4592425T>G was associated with the largest effect size with a 79% decreased risk of thiazide-induced hyponatremia (RR 0.56; 95% CI 0.42, 0.76). The effect of the six SNPs was not different in men and women (all P-values for interaction >0.05).

^{*}All thiazide exposed participants with available genetic data;

Table 2. SNPs in SLC22A6 and SLC22A8 associated with hyponatremia in thiazide users

SNP	Exposed cases	Crude model	Exposed cases	Adjusted model‡	
	N	RR (95% CI)*	N	RR (95% CI)*	
rs4592425	131	0.63 (0.47, 0.84)†	130	0.56 (0.42, 0.76)†	
TT	78	1.00	78	1.00	
TG	48	0.69 (0.47, 0.97)	47	0.59 (0.41, 0.85)	
GG	5	0.31 (0.13, 0.77)	5	0.28 (0.11, 0.69)	
rs6591722ª	131	0.67 (0.51, 0.88) [†]	130	0.61 (0.47, 0.81)†	
TT	67	1.00	67	1.00	
TA	51	0.61 (0.43, 0.88)	51	0.57 (0.39, 0.82)	
AA	13	0.50 (0.28, 0.91)	12	0.42 (0.23, 0.78)	
rs4149172ª	131	1.42 (1.11, 1.82) [†]	130	1.54 (1.20, 1.98) [†]	
TT	57	1.00	56	1.00	
TC	55	1.35 (0.93, 1.95)	55	1.49 (1.02, 2.17)	
CC	19	2.10 (1.25, 3.53)	19	2.43 (1.44, 4.11)	
rs11231294	131	1.52 (1.18, 1.96)†	130	1.62 (1.26, 2.09) [†]	
TT	60	1.00	59	1.00	
TC	55	1.47 (1.02, 2.12)	55	1.61 (1.11, 2.34)	
CC	16	2.37 (1.37, 4.12)	16	2.64 (1.52, 4.61)	
rs955434	131	1.47 (1.14, 1.90) [†]	130	1.57 (1.21, 2.03) [†]	
GG	62	1.00	61	1.00	
GA	54	1.42 (0.99, 2.05)	54	1.53 (1.06, 2.22)	
AA	15	2.24 (1.27, 3.93)	15	2.51 (1.42, 4.44)	
rs2187383 ^b	131	0.69 (0.54, 0.90)†	130	0.65 (0.50, 0.85)†	
CC	59	1.00	59	1.00	
CA	55	0.63 (0.43, 0.90)	55	0.56 (0.43, 0.81)	
AA	17	0.53 (0.31, 0.91)	16	0.50 (0.31, 0.87)	

SNP=single nucleotide polymorphism; N=number of exposed cases;

Sensitivity analyses

To assess potential confounding, we tested the association between the selected SNPs and the risk of hyponatremia in non-thiazide users. In non-thiazide users, none of the SNPs was associated with hyponatremia (data not shown). The second sensitivity analysis, in which only participants were included with a serum sodium measurement between one week before and one week after the index date, showed similar results as the primary analysis, with only a subtle change of effect (maximal difference in RR 0.06, data not shown).

^a Gene region SLC22A6 (OAT1); ^b Gene region SLC22A8 (OAT3);

^{*} Relative risks represent the risk of hyponatremia for variant genetic models compared to homozygous wild type (genotype-effect model (aa vs AA, aA vs AA));

[†] allele-effect model (AA=0, aA=1, aa=2)

[‡] Adjusted models are adjusted for age, sex, eGFR and concomitant use of RAS-inhibitors, loop and potassium-sparing diuretics

Expression levels

One of the six SNPs that was associated with the risk of hyponatremia, SNP rs4592425T>G, was also associated with increased expression levels of two probes (probe center position 62,334,904 \pm 25 bp and 62,214,393 \pm 25 bp; FDR<1). These probes were located at a distance of 119 kbp and 240 kbp and correspond to the genes EEF1G and BSCL2, respectively. In the stratified analysis by sex, this SNP showed a significant association with the BSCL2 probe in both men and women (FDR=9.1·10⁻¹⁷). In men, an association was also identified between SNP rs6591722T>A and the expression of a probe located on NXF1 (position 62,214,393 \pm 25 bp; FDR<0.001).

Discussion

In thiazide users, six SNPs in and around the gene-coding region of SLC22A6 and SLC22A8 were associated with the risk of hyponatremia, while no association was present in non-users. Two of these SNPs, rs4592425T>G and rs6591722T>A, were also associated with mRNA expression of three different probes, located near these genes. Since these genes encode OAT1 and OAT3, the results of this study suggest a role for these transporters in the risk of hyponatremia in thiazide users.

The involvement of OAT1 and OAT3 in the effect of natriuresis and drug elimination by thiazide diuretics was shown previously.^{8, 9} Because OAT1 and OAT3 mediate the secretion of thiazide diuretics in the proximal tubule, genetic variation in these transporters probably determines the amount of drug delivered to the target of thiazide diuretics, the sodium chloride cotransporter in the distal convoluted tubule, and therefore also the associated adverse effects. Further research is needed to investigate how these SNPs alter the function of these transporters.

Genetic variation in OAT1 was recently associated with interindividual differences in thiazide response in two independent case-control studies in China. ¹⁴ The C allele of SNP rs10792367G>C was associated with a lower reduction in systolic blood pressure in response to hydrochlorothiazide. This SNP was also included in our analysis, but was not significantly associated with hyponatremia in our Caucasian study population. In a previous clinical study, none of the SNPs in the OAT1 and OAT3 region were associated with renal clearance of the loop diuretic torsemide. ²² Torsemide, however, is eliminated for two thirds by extrarenal routes, namely through metabolism by the enzyme cytochrome P450 (CYP) 2C9. ²³

As far as we are aware, this is the first cohort study associating genetic variation in the OAT1 and OAT3 transporter with the risk of thiazide-induced hyponatremia. The SNPs we identified in our study are located within and between the two genes encoding OAT1 and OAT3, SLC22A6 and SLC22A8. However, none of these SNPs is located in an exonic region.²⁴ In an animal model, expression levels of OAT1 transporters in the kidney varied with age and sex.15 At young age, expression levels were similar between males and females, whereas older females had a lower expression levels than men. Other studies also showed higher mRNA expression in kidneys of male animals. 25, 26 The secretion of paminohippurate, a marker for this secretory pathway, was significantly faster in male than in female rats, possibly because this transport route was affected by testosterone. 27, 28 These results suggest that this transport pathway is partly under hormonal control. In our study, we did not observe effect modification of the SNPs by sex, although this could have been due the postmenopausal status of the women in the study population or due to a lack of power by the lower number of male cases.

In the part of our study analyzing mRNA expression levels, the following SNPs were associated with a probe of a certain gene area: SNP rs4592425T>G with BSCL2 (which encodes the multi-pass transmembrane protein seipin)²⁹ and with EEF1G (which encodes the eukaryotic translation elongation factor 1)²⁹, and rs6591722T>A with NXF1 (which encodes the nuclear RNA export factor)²⁹. Both eukaryotic translation elongation factor 1 and nuclear RNA export factor play a central role in the nuclear export of RNA for protein synthesis.^{30, 31} Therefore, mutations in these proteins could impair proper delivery of OAT to the plasma membrane.³² For nuclear RNA transport factor, transcription binding sites were previously identified in the murine OAT3 5' flanking region and OAT1-3 intergenic region (OAT1 5' flanking region).³³ Of interest, the association with NXF1 was only identified in men.

One of the strengths of this study is the large amount of data available over a broad period of time in a population-based study population, and the availability of genome-wide association data on DNA and mRNA expression. A potential limitation of our analysis was that we may have missed cases of hyponatremia in thiazide users in whom no serum sodium measurement was performed. To address this potential limitation, a sensitivity analysis was performed within only those users who had at least one serum sodium measurement within the same two weeks of follow-up; this analysis yielded similar results. Moreover, as such misclassification would probably be random with regard to genotype, it would lead to conservative risk estimates. The results regarding mRNA expres-

sion levels could have been influenced by the fact that expression levels have been assessed in blood samples, rather than from kidney tissues.

In conclusion, SNPs in and around the gene coding areas of OAT1 and OAT3, are associated with hyponatremia in thiazide users. This suggests a role for OAT1 and OAT3 transporters in thiazide-induced hyponatremia, namely that the risk of hyponatremia depends on the degree of delivery of the drug to its target. Ultimately, these findings may possibly lead to more tailored pharmacotherapy.

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Supplementary table. Single nucleotide polymorphisms in and around the gene-coding area of SLC22A6 and SLC22A8 and the risk of hyponatremia in thiazide users

SLC22A6 and SLC22A	Change	Location	MAF	RR (95% CI)†	P-value
rs10792363	C>T	62,452,993	0.313	1.37 (1.08, 1.75)	0.011
rs1938677	G>A	62,453,527	0.474	0.78 (0.61, 0.995)	0.046
rs4592425	T>G	62,454,389	0.314	0.63 (0.47, 0.84)	0.002
rs10431145	T>C	62,456,935	0.384	1.32 (1.04, 1.68)	0.021
rs10897306	T>C	62,468,805	0.484	1.19 (0.93, 1.52)	0.176
rs11231279	T>C	62,468,991	0.291	1.32 (1.03, 1.70)	0.028
rs10897307	C>T	62,484,988	0.338	1.34 (1.05, 1.71)	0.017
rs11231286	G>A	62,488,467	0.288	1.40 (1.09, 1.80)	0.008
rs10897310	T>C	62,497,752	0.36	1.38 (1.08, 1.75)	0.009
rs3017670 ^a	G>A	62,501,475	0.13	1.18 (0.86, 1.63)	0.311
rs6591722 ^a	T>A	62,506,256	0.38	0.68 (0.52, 0.88)	0.004
rs4149172 ^a	T>C	62,507,434	0.288	1.42 (1.11, 1.82)	0.005
rs11231294	T>C	62,512,095	0.252	1.51 (1.18, 1.94)	0.001
rs955434	G>A	62,513,689	0.249	1.47 (1.14, 1.90)	0.003
rs10792367	C>G	62,515,375	0.423	1.21 (0.95, 1.55)	0.120
rs2187384 ^b	C>T	62,517,737	0.207	1.35 (1.03, 1.78)	0.033
rs953894 ^b	C>T	62,518,998	0.226	1.37 (1.05, 1.79)	0.021
rs2276299 ^b	A>T	62,523,007	0.146	1.28 (0.94, 1.73)	0.115
rs11231299 ^b	G>A	62,527,964	0.205	1.39 (1.06, 1.82)	0.019
rs2187383 ^b	C>A	62,532,474	0.425	0.70 (0.54, 0.90)	0.006
rs3809069	A>G	62,540,348	0.165	0.98 (0.71, 1.35)	0.884
rs948979	G>T	62,540,402	0.239	1.27 (0.97, 1.66)	0.080
rs3948869	G>C	62,542,574	0.276	1.26 (0.98, 1.62)	0.078
rs10792369	T>C	62,543,011	0.311	1.16 (0.91, 1.49)	0.237
rs4963228	C>T	62,544,782	0.436	1.12 (0.88, 1.43)	0.346
rs10897315	G>A	62,545,707	0.499	0.85 (0.67, 1.07)	0.167
rs10897316	A>G	62,546,339	0.434	1.11 (0.87, 1.41)	0.390
rs10750978	G>C	62,548,586	0.292	0.84 (0.63, 1.11)	0.219
rs1108539	A>C	62,557,724	0.32	1.09 (0.84, 1.41)	0.515
rs3935736	G>C	62,558,824	0.461	1.11 (0.87, 1.41)	0.409
rs1944037	C>G	62,560,101	0.423	1.00 (0.78, 1.28)	0.979
rs7124143	C>T	62,567,039	0.456	1.09 (0.85, 1.39)	0.511
rs2226868	A>G	62,567,134	0.27	1.16 (0.89, 1.51)	0.275
rs956617	C>T	62,567,455	0.291	0.93 (0.71, 1.22)	0.611
rs11231322	C>T	62,570,810	0.19	0.98 (0.72, 1.33)	0.890
rs17157693	A>G	62,571,447	0.239	1.00 (0.75, 1.33)	0.983
rs7126542	C>T	62,572,000	0.406	0.96 (0.75, 1.24)	0.776
rs7119028	T>C	62,572,656	0.269	1.08 (0.82, 1.42)	0.577
rs4963334	T>C	62,573,645	0.451	1.04 (0.82, 1.33)	0.754
rs1939777	G>A	62,578,857	0.19	0.98 (0.72, 1.33)	0.890
rs1939754	T>C	62,588,695	0.427	1.02 (0.80, 1.31)	0.863

Abbreviations: SNP=single nucleotide polymorphism; MAF=minor allele frequency;

- ^a Gene region SLC22A6 (OAT1); ^b Gene region SLC22A8 (OAT3);
- † Relative risks (RRs) represent the risk of hyponatremia (according to an allele-effect model (AA=0, aA=1, aa=2))..

Linkage: rs11231279 was in complete LD with rs12223849; rs11231286 was in complete LD with rs10897308, rs11231287, rs1938678; rs10897310 was in complete LD with rs10750977; rs953894 was in complete LD with rs4149183 and rs4149182; rs948979 was in complete LD with rs948980; rs10750978 was in complete LD with rs10792372, rs4963331, rs6591725; rs2226868 was in complete LD with rs10897320; rs11231322 was in complete LD with rs7109114

Chapter 4.4

Thiazides and the risk of hypokalemia in the general population

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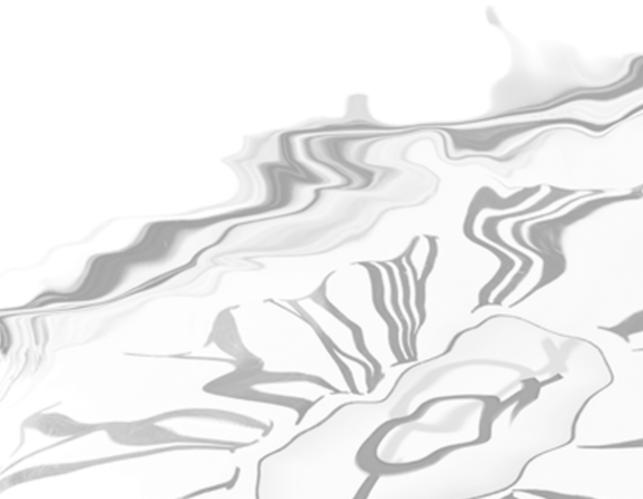
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Abstract

Background

Hypokalemia is a frequent adverse reaction to thiazide diuretics and is often asymptomatic. However, also asymptomatic hypokalemia may contribute to the development of diabetes mellitus and increase cardiovascular morbidity and mortality. The aim of this study was to assess the risk of thiazide-induced hypokalemia in men and women, in the general population.

Methods

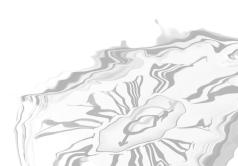
Within a population-based cohort study, the association between thiazide exposure and hypokalemia (serum potassium level <3.5 mmol/L; severe form: ≤3.0 mmol/L) was studied using Cox proportional hazard regression analysis with thiazide use as time-varying exposure variable.

Results

During follow-up, 507 cases of hypokalemia occurred in 13,328 subjects. Thiazide exposure was associated with an eleven times higher risk of hypokalemia than non-exposure (RR 11.18; 95% CI 8.95, 13.96) after adjustment for sex, age and use of a renin-angiotensin system (RAS) inhibitor or separate potassium-sparing diuretic. In users of a thiazide in combination with a potassium-sparing diuretic the risk of hypokalemia was still six times higher (RR 5.93; 95% CI 4.65, 7.55) than in non-users. The risk of thiazide-induced hypokalemia was significantly higher in men than in women (RR 21.58; 95% CI 15.00, 31.06 and RR 8.82; 95% CI 6.73, 11.56, respectively) and changed significantly with age and dosage. The risk of severe hypokalemia was almost five times higher in subjects exposed to thiazides (RR 4.80; 95% CI 2.61, 8.84) than in non-exposed subjects.

Conclusion

The risk of thiazide-induced hypokalemia is high, and in men more than twice as high as in women. Risk of hypokalemia is still increased if used in combination with a potassium-sparing diuretic.



Introduction

Hypokalemia is one of the most frequent adverse reactions associated with thiazide use. The prevalence was varyingly reported in different studies and ranged from 7 to 56% in thiazide users. ¹⁻³ Hypokalemia may lead to severe disorders, such as ventricular arrhythmias, muscle weakness, growth retardation and decreased glucose intolerance. ⁴ In the majority of cases of thiazide-induced hypokalemia, it concerned a mild form with a serum level varying from 3.0-3.6 mmol/L.²

Mild hypokalemia is not associated with specific symptoms and is therefore often not a reason for measurement. This is of clinical importance, since even mild depletion may lead to serious health concerns. Thiazide-induced hypokalemia is associated with the development of diabetes mellitus by decreased insulin-production and insulin-sensitivity.^{5, 6} Furthermore, cardiovascular mortality is higher in diuretic-treated patients with hypokalemia^{7, 8} and in these patients the benefit of diuretic treatment is less for the risk of cardiovascular events.¹

Thiazides are more frequently used in women and the prevalence of thiazide-induced hypokalemia is assumed to be higher in women than in men. In an earlier nationwide study, in chapter 2.2, we demonstrated that women had a more than twice higher risk to be hospitalized for this adverse drug reaction (ADR). The question arose whether women are at greater risk to develop hypokalemia following thiazide exposure or whether a higher proportion may be explained by more frequent use of thiazides in women. Therefore, we conducted a study in a population-based cohort to assess the risk of thiazide-induced hypokalemia in men and women in the general population.

Methods

Cohort

This study was embedded in the Rotterdam Study, an ongoing prospective population-based cohort study of chronic diseases in the elderly. All inhabitants of Ommoord, a suburb of the city of Rotterdam in the Netherlands, aged 55 years or older were invited in 1990 to participate. The medical ethics committee of the Erasmus Medical Center approved the study and informed consent was obtained from all participants. The rationale and design of the study were described elsewhere. The first cohort encompassed 7,983 individuals who were all interviewed and examined at baseline in the period 1990-1993 (Rotterdam Study-I (RS-I)). In 2000, all inhabitants of Ommoord aged 55 years and above

at that time and not yet participating in RS-I were invited to participate in the extended cohort (RS-II). This cohort encompassed 3,011 individuals. In 2006, a further extension of the cohort (second extended cohort, RS-III) was initiated, in which 3,932 subjects, aged 45 years and over were included.

Follow-up examinations are carried out periodically, while all participants are continuously monitored for major morbidity and mortality through linkage with general practitioner and municipality records. All available serum potassium levels of participants from the study population were gathered from a general practitioner's laboratory serving the area of the Rotterdam Study. Data were available for the period from May 1, 1997 up to March 31, 2010.

Drug exposure is continuously monitored since January 1, 1991, through computerized pharmacy records from the pharmacies in the Ommoord district. The pharmacy data include the Anatomical Therapeutical Chemical (ATC) - code, the dispensing date, the total amount of drug units per prescription, the prescribed daily number of units, and product name of the drugs.

Outcome

We followed all participants from RS-I, RS-II and RS-III from May 1, 1997 until January 1, 2009, because follow-up data on survival status were complete up to that date. Participants who were referred by their general practitioner to the laboratory for a serum potassium level assessment, were defined as having hypokalemia if they had a serum potassium level of <3.5 mmol/L, and as severe hypokalemia if the serum potassium level was ≤3.0 mmol/L. The date of the first episode of hypokalemia was defined as the index date.

Drug Exposure

The exposure of interest was defined as use of a thiazide diuretic (as a sole agent or in combination with a potassium-sparing diuretic) or related agent (chlorothalidone, mefruside, indapamide) at the day of sodium measurement. The exposure period started at the prescription filling date and was calculated by dividing the number of units issued per prescription by the prescribed daily number of units. Participants who had hypokalemia on a date within an exposure period were considered as 'current users'. 'Current use' of an angiotensin-converting enzyme (ACE) inhibitor or angiotensin II receptor blocker (both renin-angiotensin system (RAS) inhibitors), or separate potassium-sparing diuretic was assessed to adjust for their possible confounding effect in the analyses.

Covariables

The following covariables were assessed as potential confounders or effect modifiers: sex, age, body mass index (BMI), and renal function (expressed as estimated glomerular filtration rate (eGFR)). BMI was expressed as kg/m² and measurements (height and weight) were obtained during examination at the center visits. Serum creatinine concentration was obtained at the moment of the serum sodium measurement. The eGFR was calculated using the four-variable MDRD formula¹² and was expressed in mL/min/1.73m². Missing values of eGFR were present, but were mainly from participants without a serum potassium measurement and missing BMI data were random. Low potassium levels may result in diabetes mellitus and potassium effects are worse in subjects with a cardiovascular history.^{5, 13} Data on diabetes mellitus, previous myocardial infarction (MI) and prevalent heart failure (HF) were assessed at baseline.

Statistical analysis

The association between thiazides and hypokalemia was evaluated using Cox proportional hazard regression analyses with exposure to thiazides as time-varying determinant, matched on follow-up time since May 1, 1997. In this model, thiazide exposure in those with hypokalemia was compared with all other participants within the cohort who did not have hypokalemia, with the same duration of follow-up. Risks were expressed as relative risks (RR) plus 95% confidence limits (95% CI). Cox proportional hazard models were adjusted for sex and age, use of a RAS-inhibitor and separate potassium-sparing diuretic. Effect modification (multiplicative interaction) of sex, age, eGFR and BMI was tested separately with interaction terms and, if present, stratified analyses were performed.

Additionally, a sensitivity analysis was performed, in which cases were compared with all participants with a potassium measurement between a week before and a week after the index date. This analysis was additionally adjusted for eGFR. Dosage effect was assessed separately, in which thiazide use was divided in low dose (defined daily dose (DDD) <1.0) and high dose (DDD \geq 1.0). Statistical analyses were performed using SPSS software (version 17.0; SPSS Inc., Chicago, Illinois, USA). P-values <0.05 were considered statistically significant.

Results

Baseline characteristics of the cohort (N=13,328) indicated that participants exposed to either a thiazide in combination with a potassium-sparing agent or a thiazide as a sole diuretic were more frequently female, diabetic, had a higher age, a higher BMI and a lower eGFR (Table 1). Previous MI or HF were not more common in subjects using thiazides, although they more often used other antihypertensives, such as RAS-inhibitors, calcium channel blockers and beta-blocking agents.

Table 1. Baseline characteristics of subjects with and without exposure to thiazide diuretics

	None	Thiazide plus*	Thiazide solely	P-value
N	12,680	532	116	
N women (%)	7,386 (58)	410 (77)	76 (66)	< 0.001
Age (mean ± SD)	62.59 ± 13.87	72.39 ± 11.47	69.81 ± 11.00	< 0.001
BMI (mean ± SD)	26.91 ± 4.13	28.47 ± 4.10	28.27 ± 4.14	< 0.001
eGFR (mean ± SD)	73.23 ± 18.30	64.82 ± 15.35	69.62 ± 15.29	< 0.001
Diabetes mellitus (%)	868 (7.3)	40 (7.9)	19 (17.2)	< 0.001
Prevalent MI (%)	706 (8.1)	41 (8.3)	3 (2.9)	NS
Prevalent HF (%)	173 (2.0)	16 (3.2)	2 (1.9)	NS

Abbreviations: SD=standard deviation; BMI=body mass index (kg/m²); eGFR=estimated glomerular filtration rate (mL/min/1.73m²); MI=myocardial infarction; HF=heart failure; NS=non-significant;

Percentages are not for all factors the percentages of the total number of participants, due to missing values; *Thiazide with potassium-sparing agent.

During follow-up, 507 cases of hypokalemia occurred. Of these, 89 cases were exposed to thiazides in combination with a potassium-sparing agent and 122 cases to a thiazide only. Compared with no use of any of these two drugs, exposure to thiazides was associated with a more than fourteen times higher risk of hypokalemia (RR 14.30; 95% CI 11.51, 17.77; Table 2). Adjustment for age and sex showed a twelve-fold higher risk in thiazide users (RR 11.81; 95% CI 9.51, 14.68). Further adjustment for RAS-inhibitors and additional potassium-sparing diuretics showed similar risks (Table 2). Thiazides combined with a potassium-sparing agent were associated with a lower, but still significantly increased risk of hypokalemia (Table 2). Ninety-eight cases of hypokalemia were severe (≤3.0 mmol/L). The risk of severe hypokalemia was almost six times higher with thiazide use than non-use and was similar to use of a thiazide plus potassium-sparing agent (Table 2).

Sex significantly modified the risk of hypokalemia associated with thiazides, either with or without potassium-sparing agent ($P=2.89\cdot10^{-4}$). Men who used

Table 2. Risk of hypokalemia associated with thiazides plus potassium-sparing agent and thiazides solely

Hypokalemia	Thiazide exposure				
	None	Thiazide plus* RR (95% CI)†	Thiazide solely RR (95% CI)†		
All (< 3.5 mmol/L)	N=296‡	N=89‡	N=122 [‡]		
Crude	Ref	8.31 (6.55, 10.53)	14.30 (11.51, 17.77)		
Adjusted§	Ref	5.93 (4.65, 7.55)	11.18 (8.95, 13.96)		
Sensitivity analysis§	Ref	3.26 (2.56, 4.16)	5.84 (4.68, 7.28)		
Sensitivity analysis [¶]	Ref	3.35 (2.62, 4.27)	5.82 (4.65, 7.28)		
Severe (≤ 3.0 mmol/L)	N=71 [‡]	N=14 [‡]	N=13 [‡]		
Crude	Ref	5.93 (3.34, 10.52)	6.45 (3.53, 11.79)		
Adjusted§	Ref	3.89 (2.17, 6.97)	4.80 (2.61, 8.84)		
Sensitivity analysis [§]	Ref	1.92 (1.06, 3.45)	2.51 (1.36, 4.62)		
Sensitivity analysis [¶]	Ref	2.03 (1.12, 3.66)	2.41 (1.28, 4.53)		

^{*} Thiazide with potassium-sparing agent

thiazides solely were at highest risk, which was almost 22 times higher than in men without exposure to thiazides. In women, the risk of hypokalemia in thiazide users was almost nine times higher than in non-users (Table 3). Within all subjects who did not use thiazides, directly comparing the sexes, the risk of hypokalemia was higher in men (RR 0.86; 95% CI 0.76, 0.98); within all subjects using thiazides, direct comparison of the sexes, women compared with men, showed no significant difference (RR 1.04; 95% CI 0.81, 1.33 and RR 1.20; 95% CI 0.995, 1.44 for thiazides plus potassium-sparing agent and thiazides solely, respectively). Sex did not significantly modify the risk of severe hypokalemia (P=0.547).

Age also affected the risk of thiazide-associated hypokalemia (P-value for interaction 1.28·10⁻¹⁸; Table 3). The risk of hypokalemia due to thiazides was lower with higher age, although age was associated with a higher risk of hypokalemia in general, with 8% higher risk per year of age (RR 1.08; 95% CI 1.07, 1.09). Thiazide-associated hypokalemia was not significantly modified by BMI or eGFR (P-values for interaction respectively 0.347 and 0.165).

In the sensitivity analysis, in which only participants were included with a serum potassium measurement, risks of hypokalemia (all and severe) were slightly lower (Table 2). These results were similar with and without adjustment for renal function.

[†] Relative risks (RRs) represent the risk of hypokalemia associated with thiazide exposure compared with non-exposure;

[‡] Number of cases;

[§] adjustment for sex and age, use of RAS-inhibitors or separate potassium-sparing diuretics

adjustment for sex and age, use of RAS-inhibitors or separate potassium-sparing diuretics, and eGFR.

Table 3. Thiazide exposure and risk of hypokalemia in men and women and for separate age categories

	N	Io thiazide	Thiazide plus potassium- sparing agent		Thiazide solely	
	\mathbf{N}^{\dagger}	RR (95% CI)*	N [†]	RR (95% CI)*	N^{\dagger}	RR (95% CI)*
Sex						
Men	88	Ref	21	8.96 (5.54, 14.49)	47	21.58 (15.00, 31.06)
Women	208	Ref	69	4.99 (3.78, 6.58)	75	8.82 (6.73, 11.56)
Age (yrs)						
≤ 55	12	Ref	3	24.16 (6.79, 86.01)	13	82.27 (35.96, 188.23)
> 55 – 65	31	Ref	15	18.09 (9.71, 33.70)	24	38.22 (22.02, 66.31)
> 65 – 75	54	Ref	31	13.76 (8.77, 21.59)	30	16.94 (10.70, 26.81)
> 75 – 85	108	Ref	24	3.42 (2.19, 5.35)	39	8.05 (5.52, 11.73)
> 85	91	Ref	17	2.45 (1.44, 4.19)	16	4.69 (2.74, 8.03)

^{*} Relative risks (RRs) represent the risk of hypokalemia related to thiazide exposure compared with non-exposure, adjusted for sex and age;

Table 4. Risk of thiazide-induced hypokalemia for low and high dosage

		Men	Women	
	N [†]	RR (95% CI)*	N†	RR (95% CI)*
No thiazide	88	Ref	208	Ref
Thiazide plus potassium-sparing a	gent			
Low dose (DDD <1.0)	4	5.69 (2.08, 15.55)	14	3.84 (2.23, 6.61)
High dose (DDD ≥1.0)	17	10.37 (6.15, 17.49)	54	5.40 (4.00, 7.31)
Thiazide solely				
Low dose (DDD <1.0)	14	15.00 (8.47, 26.56)	18	4.77 (2.93, 7.78)
High dose (DDD ≥1.0)	33	26.40 (17.56, 39.69)	57	11.89 (8.84, 16.00)

Abbreviations: DDD=defined daily dosage;

The risk of hypokalemia was higher with higher doses of either thiazides in combination with a potassium-sparing agent or thiazides solely (Table 4).

Discussion

Exposure to thiazides was associated with an almost twelve times higher risk of hypokalemia than non-exposure. This risk was significantly higher in men than in women, and was influenced by age. At higher age, more cases of hypokalemia occurred, but the contribution of thiazides was lower. A clear dose effect was observed, as was already shown previously.¹⁵

[†] Number of cases.

^{*} Relative risks (RRs) represent the risk of hypokalemia related to thiazide exposure compared with non-exposure:

[†] Number of cases.

Although hypokalemia is a well-known adverse reaction to thiazides, the risk of this adverse effect has not been investigated in detail in population-based studies. The prevalence of hypokalemia (<3.5mmol/L) with thiazides and related derivatives was reported as varying between approximately 7.2-8.5% at dosages of 12.5-25 mg of chlorthalidone ^{1, 2, 16} and up to 56% with 50 mg hydrochlorothiazide.³ From the results in the ALLHAT trial, the relative risks of hypokalemia could be calculated for chlorthalidone and were 10.61 (95% CI 7.64, 14.73) compared with the lisinopril group and 4.50 (95% CI 3.63, 5.57) compared with the amlodipine group, after 4 years of treatment. However, these risks could not be calculated for men and women separately.

A previous study analyzed the prevalence of hypokalemia in a hypertension clinic, in which benfrofluazide was used as standard treatment. This study showed a prevalence of hypokalemia of 19% among the studied population and the prevalence was higher in exposed women. In a nationwide hospital-based study, hypokalemia due to diuretics was also relatively more common in women than in men. In contrast with these earlier studies, our results showed a significantly higher risk of thiazide-induced hypokalemia in men and no difference between men and women using thiazides, in direct comparison.

We propose two possible explanations for these discrepant findings. Thiazide-induced hypokalemia is thought to be caused by a combination of increased sodium delivery to the collecting duct and secondary aldosteronism due to renal sodium loss. Therefore, sex differences in either of these two mechanisms could contribute to the higher risk of hypokalemia in men. For example, women may restrict dietary sodium more readily than men and this has been shown to reduce the risk of thiazide-induced hypokalemia. 19

Another explanation could be that RAS activation itself leads to a dimorphic effect in men and women. The RAS is a complicated hormone system with multiple interactions and feedback mechanisms. Sex differences are present at multiple levels of this cascade and changes take place after menopause, due to the influence of estrogen.^{20, 21} Renin, angiotensin-converting enzyme (ACE) activity and angiotensin (AT1) receptor density are under hormonal control of estrogen and testosterone, whose specific effects also differ between men and women.^{20, 21} Whether and which of these factors could have played a role in the observed difference, needs further exploration.

Thiazides are often prescribed in combination with a potassium-sparing agent to prevent hypokalemia. Our study shows that, despite this combination, the risk of hypokalemia remains relatively high. For severe hypokalemia, risks were even similar in both thiazide groups. This is in line with previous results, suggesting that inhibition of potassium secretion through the renal outer

medullary potassium channel does not completely prevent diuretic-induced hypokalemia.²² This may be due to flow-dependent potassium secretion that is mediated through so-called Maxi K channels.²³ Potassium suppletion neither completely prevented thiazide-induced hypokalemia.²⁴

Hypokalemia has been associated with worse cardiovascular outcomes¹³ and the beneficial effects of diuretics could be counterbalanced by its harmful effects. Besides predisposing to arrhythmias, hypokalemia also increases the risk of diabetes mellitus.⁵ Although the beneficial effects of thiazides were shown in patients with cardiovascular risk, patients with thiazide-induced hypokalemia favored less from the beneficial effects of thiazide treatment with respect to cardiovascular outcomes.¹ Furthermore, potassium supplementation in patients with diuretic-induced hypokalemia led to a decrease in blood pressure.²⁵

As far as we are aware, this is the first population-based study in which the risk of hypokalemia due to thiazides was investigated, specifically focussing on differences between men and women. One of the strengths of our study is that laboratory measurements were available over a period of approximately 10 years of follow-up. The combination of laboratory data with drug use made it possible to compare these measurements during on-off periods of drug use. Data were collected independently of the research question. However, a potential limitation of these laboratory measurements may be, that they were requested by the general practitioner, which may have led to information bias. However, mild hypokalemia is asymptomatic, and potentially not a direct indication for measurement. Moreover, by analyzing only people with a potassium assessment in the same period, we dealt with this potential information bias.

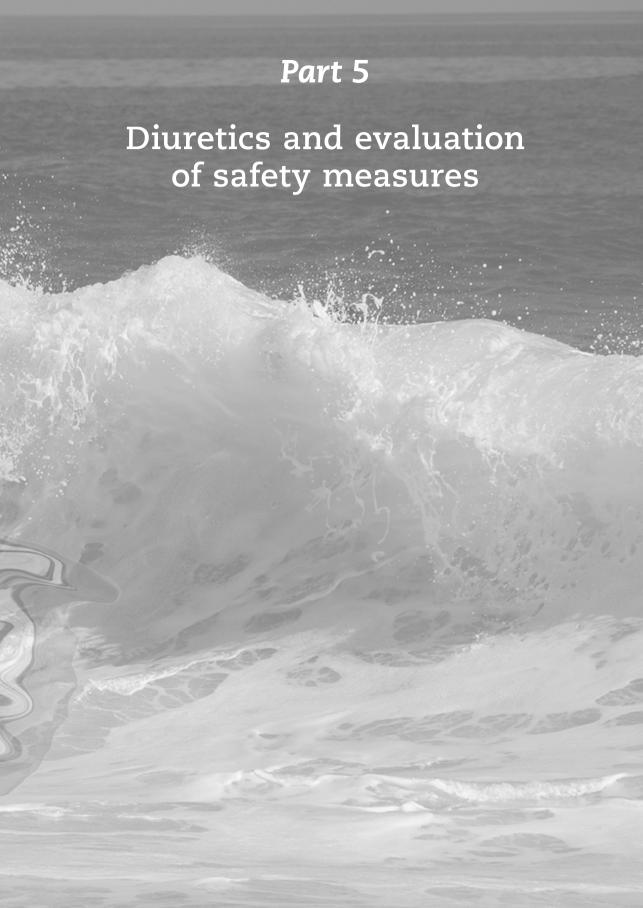
In conclusion, the risk of thiazide-induced hypokalemia is high and combination of thiazides with potassium-sparing agents does not abolish this. The risk is highest in men, which could potentially negatively influence the beneficial effect of thiazides on cardiovascular outcome. Although thiazides remain valuable in the treatment of hypertension, clinicians should be aware of the substantial risk of hypokalemia and its potential consequences.

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Chapter 5.1

Evaluation of drug safety measures to prevent electrolyte disorders by thiazides and other potassium-losing diuretics

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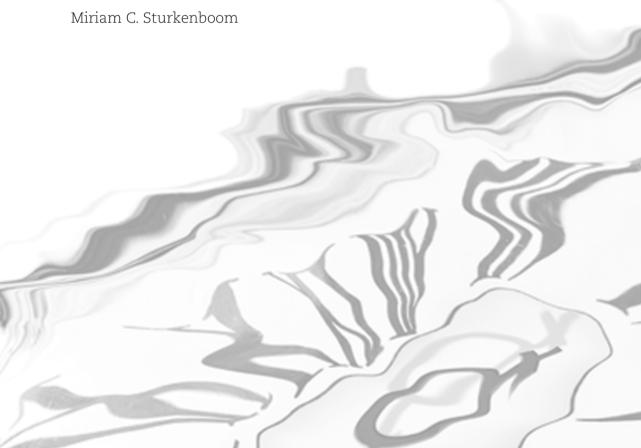
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Abstract

Background

Thiazides and other potassium-losing diuretics are associated with hyponatremia and hypokalemia. The elderly are at increased risk. In 2008, recommendations were made by the Dutch HARM-Wrestling Task Force to perform extra laboratory measurements in those at risk. The aim of this study was to identify to what extent recommended measurements were performed and to gain insight into the frequency of hyponatremia and hypokalemia.

Methods

Within the Integrated Primary Care Information (IPCI) database, which contains patient records of over one million individuals, we identified all patients who were prescribed a potassium-losing diuretic between January 1, 2007 and December 31, 2010. The proportion of measurements, which were recommended by the Dutch HARM-Wrestling Task Force, was assessed for high-risk patients at the moment of (1) an incident thiazide prescription, (2) prevalent thiazide use with an incident interacting drug prescription, and (3) an incident potassium-losing drug prescription.

Results

A recommended sodium measurement was performed after 458 (9.4%) incident thiazide prescriptions. The frequency of hyponatremia in these 458 measurements was 21.0% (moderate to severe hyponatremia: 4.6%). In prevalent users starting an interacting drug, performed measurements varied from 3.9% to 20.2%. Hyponatremia was observed in 19.7% and was more frequent in women. During treatment with a potassium-losing agent, potassium levels and renal function were checked in 12.0% and 16.0% prior to and 6.6% and 7.3% after start, respectively. Hypokalemia was observed in 2.4% (prior) and 4.0% (after) of measurements; this mainly concerned women.

Conclusion

The number of recommended measurements that is performed in the treatment with thiazides and other potassium-losing diuretics is low, and remained low throughout the study period. We conclude that the specific recommendations by the Dutch HARM-Wrestling Task Force are insufficiently abided to.

Introduction

Thiazide diuretics are pivotal in the first line-treatment of hypertension.^{1, 2} Hyponatremia and hypokalemia following thiazide therapy are not uncommon and may have serious consequences, such as coma (hyponatremia) and heart rhythm disorders (hypokalemia).^{3, 4} Both electrolyte disorders are associated with increased mortality.^{5,6}

The elderly are at higher risk to develop adverse drug reactions (ADRs) and/or electrolyte disorders⁷ and this patient group should be treated with extra caution. Drug-drug interactions occur frequently⁸ and may potentially cause adverse reactions.⁹

In 2008, a report was drafted on request of the Dutch Ministry of Health, in which recommendations for safe drug prescribing were made by a task force for reduction of hospital admissions as a consequence of medication use (Dutch HARM-Wrestling Task Force). 10, 11 This report was officially published in 2009. One of the drug groups of interest were potassium-losing diuretics, with special attention for thiazides. These drug groups were one of the most frequently involved in adverse reactions requiring hospital admissions. 12-14 Laboratory measurements of sodium 5-9 days after start of thiazide diuretics in high-risk patients was recommended. These high-risk patients were defined as being 80 years or older, or 70 years or older while using a selective serotonin re-uptake inhibitor (SSRI), venlafaxin or related agent, non-steroidal anti-inflammatory drug (NSAID), carbamazepine or loop diuretic. Additionally, such a measurement was also recommended in prevalent thiazide users starting with concurrent use of one of these potentially interacting drugs (SSRI, venlafaxin or related agent, NSAID, carbamazepine or loop diuretic). For all potassium-losing diuretics, measurements of potassium and renal function were recommended prior to and after starting. Also for these measures, specific risk profiles were defined to select high-risk patients. These included age, use of a potassium-sparing diuretic, prevalent renal function disorder, coronary heart disease or rhythm- or conducting disorder, and use of digoxin without concurrent use of a renin-angiotensin system (RAS) inhibitor or potassium-sparing diuretic.

The objective of this study was to determine to what extent these recommended measurements were actually performed in the high-risk population in the years around the task force recommendations, and to study the occurrence of hyponatremia and hypokalemia in those who had measurements according to the recommendations. Since women were more frequently admitted with electrolyte disturbances related to diuretics than men¹⁴, special attention was given to differences between sexes.

Methods

Setting

A retrospective cohort study was conducted in the Integrated Primary Care Information (IPCI) database. This longitudinal observational general practitioner (GP) research database contains patient records of over one million patients throughout the Netherlands. In the Netherlands, all citizens are registered with a GP practice, which forms the central point of healthcare and acts as a gatekeeper for secondary health care. Therefore, the medical record of each patient can be assumed to contain all relevant medical information, including medical findings and diagnoses from secondary care. To further maximize the completeness of data, GPs contributing data to the IPCI database are not allowed to maintain a system of paper based records separate from the electronic medical records. The electronic records contain coded and anonymous data on patient demographics, symptoms (in free text), diagnoses using the International Classification for Primary Care (ICPC)¹⁵ and free text, clinical findings, referrals, laboratory findings and hospitalizations. Furthermore, there is a complete record of all drug prescriptions, their indications and dosage regimen. Further details of the database have been described elsewhere.16,17

Study population

Within the IPCI database, all patients who were prescribed a potassium-losing diuretic between January 1, 2007 and December 31, 2010 were identified, provided they had at least 12 months of valid database history prior to the date of prescription. This 12-month period was required in order to allow assessment of inclusion criteria and baseline characteristics of all study subjects. Patients were excluded for assessment if less than 4 weeks of valid database history following the date of prescription was present, as this period was required to assess outcomes.

We selected (1) all new prescriptions of thiazides (including related agents such as chlortalidone, indapamide, mefruside, either as sole agent or as combination product), (2) all prevalent thiazide users starting an interacting drug (SSRI, venlafaxine, NSAID, carbamazepine or a loop diuretic), and (3) all new prescriptions of any potassium-losing diuretic (thiazides as described under (1) and loop diuretics, sole or in combination). Prescriptions were defined as new if a patient had not used a potassium-losing diuretic in the 6 months prior to the prescription date.

Exposure definitions

Separate exposure definitions were used for incident thiazide prescriptions (risk of hyponatremia), prevalent thiazide use with an incident interacting drug prescription (risk of hyponatremia), and incident potassium-losing diuretic prescriptions (risk of hypokalemia and renal function disorder). Patients who were prescribed a thiazide were considered at high risk of hyponatremia and therefore candidates for a recommended sodium measurement, if one of the following risk factors was present at the moment of prescription: (1) age 80 years or over; or (2) age 70 years or over and simultaneous use of an SSRI, venlafaxine, NSAID, carbamazepine or a loop diuretic. Patients with prevalent use of a thiazide starting an interacting drug were at risk if they were age 70 years or over.

Patients who were newly prescribed any potassium-losing diuretic were candidate for a potassium and renal function measurement prior to the start, if they were aged 70 or over or had one or more of the following risk factors at the time of prescription: (1) use of a potassium-sparing diuretic; (2) a history of renal failure (defined as renal clearance below 40 mL/min (according to laboratory calculations) or defined as such by the GP); (3) a history of a cardiac rhythm- or conducting disorder; (4) a history of ischemic heart disease (defined as myocardial infarction or angina pectoris); or (5) use of digoxine without concurrent use of a renin-angiotensin system (RAS) inhibitor or a potassium-sparing diuretic. After start, patients were eligible for a control measurement if they were aged 80 years or older or if they were 70 years or older in combination with one of the factors described above.

Outcomes

Primary outcome was the proportion of recommended measurements that were performed in patients who were defined as being at risk. For all thiazide prescriptions (incident and prevalent starting an interacting drug) we studied whether recommended sodium measurements within four weeks after start were performed. For incident potassium-losing diuretics, we studied potassium and renal function measurements within 3 months prior to the start and within four weeks after start. Between the date of prescription and laboratory assessment, a slightly broader period was used than recommended, because the actual filling date of the prescription by the patient and the actual laboratory assessment date could deviate a few days from the dates that are registered in the database. Secondary outcome of interest was the proportion of cases of hyponatremia or hypokalemia. Hyponatremia was defined as a sodium level of

 \leq 135 mmol/L (moderate to severe hyponatremia <130mmol/L); hypokalemia was defined as a potassium level of <3.5mmol/L (moderate to severe \leq 3.0mmol/L).

Data analysis

Proportions of recommended measurements within the specific high-risk patient groups were calculated in total, per year and per sex. Proportions of hyponatremia and hypokalemia, were calculated within the measurements that were performed. Differences were assessed using chi-square tests.

Results

Recommended measurements after starting thiazides

The source population consisted of 807,527 subjects in 2010, increasing from 269,323 in 2007. Within the study period, 30,720 episodes of newly prescribed thiazides were identified in 26,460 subjects. Of these, 4,858 prescriptions were in high-risk subjects. The number of newly prescribed thiazides increased over the years from 408 in 2007 up to 1999 in 2010, in a growing source population. The

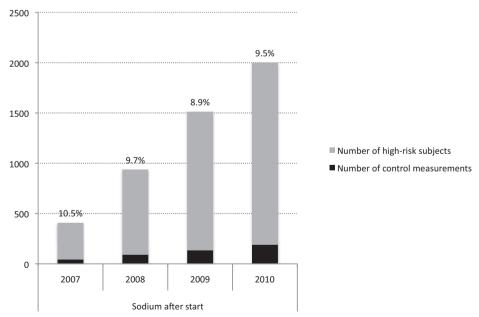


Figure 1. Sodium measurements after initiation of a thiazide diuretic in high-risk subjects; per year.

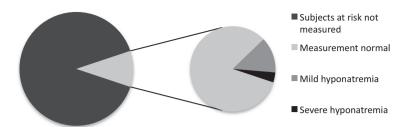


Figure 2. Control sodium measurements and occurrence of hyponatremia after starting a thiazide diuretic in high-risk subjects.

percentage of recommended measurements remained relatively constant over time (Figure 1).

In 458 (9.4%) of the new thiazide prescriptions, a sodium measurement was performed within four weeks after initiation, of which 61.4% was performed in the first two weeks. Of the sodium measurements that were done (N=458), the proportion of hyponatremia was 21.0%, with a severe hyponatremia in 4.6% (Figure 2).

The proportion of women at risk at the start of a thiazide prescription was higher than the proportion of men at risk (19.7% vs 10.4%; P<0.001). No differences were observed between men and women in the proportion of control measurements. In the samples that were taken, hyponatremia was more common in women than in men, although not significantly different (21.7% vs 14.5%; P=0.227; moderate to severe cases 9.0% vs 4.3%; P=0.304).

Measurements in prevalent thiazide-users after starting interacting drugs

We defined 353,389 periods in which subjects were prevalent thiazide user at the start of an interacting drug, of which 146,345 periods were in subjects aged 70 years or older. NSAIDs or loop diuretics were the most frequent newly started drugs during these periods (Table 1). The percentage of recommended sodium measurements performed varied from 3.9% in NSAIDs to 20.2% in loop diuretics. Although the number of measurements was relatively low, hyponatremia was relatively common in the measurements that were taken (19.7%). Four percent concerned a sodium level of 130 mmol/L and lower. The proportions per specific interacting drug are shown in table 1. Measurements were more frequently performed in women and also the majority of hyponatremia cases were women.

Table 1. Thiazide users aged \geq 70 yrs, starting an interacting drug and control sodium measurements after start (<4wks)

Start interacting d	eracting drug Laboratory measurement			Hyponatremia		
Drug	N*	N† (%)	M%:W%	N‡ (%)	M%:W%	Severe (%)
SSRI	291	41 (14.1)	8.5 : 15.9	11 (26.8)	All women	4 (36.4)
Venlafaxin	69	13 (18.8)	8.7 : 23.9	5 (38.5)	All women	2 (40.0)
NSAID	3,945	153 (3.9)	3.7 : 23.9	29 (19.0)	15.6 : 20.4	4 (12.5)
Carbamazepine	37	3 (8.1)	7.7:8.3	2 (66.7)	All women	2 (100)
Loop diuretic	1,301	263 (20.2)	19.6 : 20.5	46 (17.5)	14.5: 18.7	7 (14.9)

Abbreviations: SSRI=selective serotonin reuptake inhibitor; NSAID=non-steroidal anti-inflammatory drug

M% : W%= percentages of either N measurements or N hyponatremia cases within men and women separately.

Measurements before and after starting potassium-losing diuretics

The period between 2007 and 2010 covered 42,232 new prescriptions of potassium-losing diuretics in 35,511 subjects. The high-risk group for recommended measurement before start included 24,720 subjects; for recommended measurement after start, the high-risk group consisted of 13,467 subjects.

A measurement of potassium and/or renal function within 3 months prior to start of a potassium-losing agent was performed in 12.0% and 16.0%, respectively. (Table 2) Within 4 weeks after start, a measurement was done in 6.6% and 7.3%, respectively. About half of these measurements were performed during the first two weeks (55.9 and 57.3%). The proportion of performed measurements increased slightly over the years (Figure 3 and 4).

Of the potassium measurements that were done before start (N=2,973), the proportion of hypokalemia was 2.4% (N=70), and of hypokalemia of 3.0 mmol/L or lower 0.4% (N=13). In case of a hypokalemia prior to the start of the potassium-losing diuretic, the potassium level was re-evaluated in 15.7% (N=11) of these

Table 2. Laboratory measurements before and after start of a potassium-losing diuretic; in total and for men and women separately

Measurements	Total	S	ex	P-value interaction
		Men	Women	-
Potassium before*	2,973 (12.0)	1,219 (12.0)	1,754 (12.1)	NS
Potassium after†	883 (6.6)	297 (5.8)	586 (7.0)	0.007
Renal function before*	3,966 (16.0)	1,649 (16.2)	2,317 (15.9)	NS
Renal function after†	989 (7.3)	337 (6.6)	652 (7.8)	0.010

Abbreviations: NS=non-significant

^{*} N=number of thiazide users who are ≥ 70 yrs of age at the moment of start with the interacting drug;

[†] N=number of performed measurements within four weeks after start of an interacting drug;

^{*} N=number of hyponatremia cases within the performed measurements;

^{* &}lt; 3 months prior to start of a potassium-losing diuretic;

 $^{^{\}scriptscriptstyle \dagger}$ $\! < \! 4$ weeks after start of a potassium-losing diuretic.

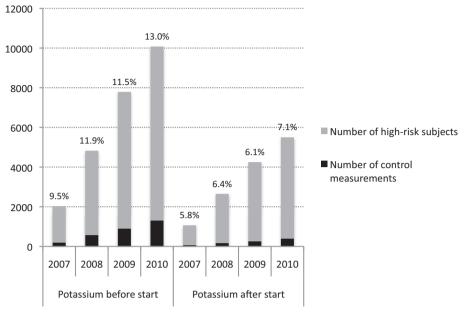


Figure 3. Potassium measurements before and after starting a potassium-losing diuretic in highrisk subjects; per year.

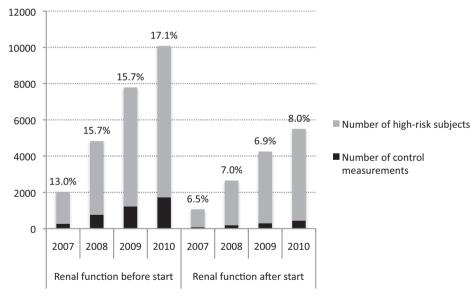


Figure 4. Renal function measurements before and after start of a potassium-losing diuretic in high-risk subjects; per year.

subjects. After start, the proportion of hypokalemia was 4.0% (N=35), of which the majority was in women (N=29; P=0.043 for interaction). The five severe cases were all women.

Discussion

Approximately 5 percent of hospital admissions is attributed to drug-related problems and almost half of these admissions could have potentially been prevented. 18, 19 Following signals from the literature, the national authorities requested recommendations by a task force for reduction of hospital admissions as a consequence of medication use (Dutch HARM-Wrestling Task Force). These recommendations were officially reported in 2009. 10 For the most common drug-ADR combinations, 12, 13 recommendations for safety measurements were made. 11 Diuretics were one of the drug groups receiving extra attention. It was recommended to perform an extra sodium measurement after 5-9 days in subjects starting a thiazide, or in prevalent thiazide users of age over 70 starting an interacting drug.

The results of our study showed that only in a small proportion of the subjects at risk the recommended sodium measurement was performed. In these subjects, about one in five measurements showed hyponatremia. Possibly, these measurements were performed in subjects at the highest risk and therefore this proportion of hyponatremia is potentially an overestimation. However, in a previous study on electrolyte measurements during thiazide exposure, 13.7% of the patients had hyponatremia after start of a thiazide.²⁰ In that study, median age of those with a measurement was 69 years, and the risk of hyponatremia was almost four times higher above 70 years than below that age. This strengthens the need of increased control above this age, as included by the recommendations.

The number of measurements and the proportion of hyponatremia were not significantly different between men and women for incident thiazide users. However, the proportion of women at risk was higher than the proportion of men at risk. This could partly explain the higher prevalence of hyponatremia in women, which is often seen.^{3, 14}

In prevalent thiazide users starting an interacting drug, the frequency of control measurements varied from 3.9% to 20.2% and was higher in women. Also the majority of hyponatremia cases were women. Differences between men and women in response to drug interactions have not been studied in detail for diuretics. Preliminary results show a higher prevalence of drug interactions with thiazides in women, but a higher risk of adverse drug reactions could not be confirmed.²¹

In the report by the task force, separate recommendations were made for starting potassium-losing diuretics, including thiazides. Control measurements of potassium and renal function prior to and one to two weeks after start were recommended. Our results show that measurements for both potassium and

renal function were performed more frequently prior to the start of the drug than afterwards. Hypokalemia was present 72 times (2.4%) prior to start of a potassium-losing diuretic. Only in 15.7% of these hypokalemia cases, the potassium level was re-evaluated within 4 weeks after start. This is relatively low considering the higher risk of a relapse. The measurements after start were more frequently performed in women and 83% of the hypokalemia cases were women. The prevalence of hypokalemia, either thiazide-induced or not, was previously found to be higher in women. 22-24

One of the strengths of this study is the large pool of data available over four years. Risk groups were discernable and drug prescriptions were available, which made it possible to study the specific subjects at risk before and after start of the selected diuretics. Exposure periods were calculated based on prescription data from general practitioners. Misclassification could have been present if a subject did not start the prescribed drug at the date the prescription was filled, or did not fill it at the pharmacy at all. We partly solved this by selecting a broader period for control assessment. Misclassification of the outcome could have been present if a subject had a measurement by a medical specialist. We think this is unlikely, since thiazides and potassium-losing diuretics are frequently prescribed in the first line treatment of hypertension, which is mainly treated by the general practitioner. If so, it would be more plausible that the treatment is started in the hospital and further checked by the general practitioner.

We can conclude that measurement of electrolytes and renal function prior to and after start of thiazides or potassium-losing diuretics is not frequently performed, despite recommendations by the Dutch HARM-Wrestling Task Force. Since the first publication of the report the proportion of measurements has not increased. A possible explanation is that due to the limited time after the publication of the report we were unable to detect an increase, but it could also be due to a lack of publicity given to the recommendations. One way or the other, it is clear that the proportion of recommended measurements is too low to be effective. Regarding the preventable diuretic-induced adverse drug reactions, we can conclude that performing extra measurements in high-risk patients could potentially prevent a great part of unwanted electrolyte disorders in subjects at risk. Considering the widespread use of electronic prescribing systems, implementation of pop-ups in the case of high-risk patients could increase the awareness of this problem, as well as the safety of these frequently prescribed drugs.

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Introduction

Traditionally, and unless a drug was tested for gynecological indications, women used to be excluded from clinical studies for simplicity (e.g., because hormonal variation might complicate interpretation) and protection from harmful drug effects to women and their fetuses.¹ Despite the fact that already in 1976, Davies pointed out that women have a higher risk of adverse reactions to drugs,² it was thought that study results in men could be easily extrapolated to women. Since it is recognized that pathophysiology, disease symptoms and drug response are often not similar in men and women, clinical trials have focused more on the distinction between both sexes.

The aim of this thesis was to gain more insight into sex differences in drug response, notably the different risk of adverse events. Therefore, first an overview was obtained of the differences in adverse drug reactions (ADRs) requiring hospitalization. Subsequently, the focus was directed towards cardiovascular drugs, because these were most frequently involved in hospital admissions by adverse events. Furthermore, we studied the effect of genetic variation in drug and androgen metabolizing enzymes on the cardiovascular system, with myocardial infarction as an outcome. Thereafter, we concentrated our research activities on the cardiovascular adverse effects of thiazide diuretics. The main reason was that this is one of the most frequently prescribed drug groups acting on the cardiovascular system and showed the most prominent differences in ADR-related hospitalizations between the sexes. Moreover, it is one of the adverse reactions that led to special measures by the Dutch Ministry of Health, which requested a task force of experts (Dutch HARM-Wrestling Task Force) to make recommendations to limit the occurrence of preventable ADRs. Therefore, the occurrence of hyponatremia and hypokalemia was assessed with special attention for individual factors, such as sex, age, body mass index, drug-drug interactions and genetic variation of the organic anion transporters (OATs). Last, it was studied whether the extra safety measures, as recommended by the task force, were actually performed.

Main Results

Sex differences in adverse drug reactions

Sex differences in cardiovascular drug response have been described from both pharmacodynamic and pharmacokinetic perspectives.³⁻⁵ Pharmacodynamics

tells us what the drug does with the body, while pharmacokinetics tells what the body does with the drug. The response of the body and the drug are both important in determining the drug's beneficial effects, but also in the risk of ADRs. Differences between men and women in adverse drug reactions were shown previously.⁶⁻⁸

The first part of this thesis demonstrates that there are sex differences in severe ADRs, requiring hospitalization. All registered, nationwide hospitalizations attributed to an ADR were studied, for all possibly related drug groups. Sex differences were observed for antineoplastic and immunosuppressive drugs, antirheumatics, anticoagulants and salicylates, cardiovascular and neurological drugs, steroids and antibiotics. The most pronounced differences were observed for cardiovascular drug groups. Low-ceiling diuretics showed the highest relative risk of a severe ADR in women compared with men, i.e. four times higher in women. Hyponatremia was by far the most frequent ADR. Besides this, highceiling diuretics and glycosides also showed a higher risk of ADR-related hospitalizations in women. The risk of an ADR attributed to coronary vasodilators was higher in men than in women. ADRs due to diuretics have been well documented.9, 10 Although the predominance of hyponatremia and hypokalemia in women seems to be explained by the fact that women use diuretics more frequently than men, 11 also pharmacodynamic and pharmacokinetic differences between the sexes may modify the risk. 12, 13 This seems partly be due to age and body composition¹⁴⁻¹⁶ and the differences in expression of renal transporters.^{12,17}

Sex steroid hormones influence the expression of transporters, ^{18, 19} but also of drug metabolizing enzymes (DME)²⁰ and therefore, fluctuating hormone levels may play a role in drug response. In contrast to chapter 2.1 and 2.2, chapter 2.3 presents an overview of severe ADRs in hospitalized children. In this study, the majority of ADRs was attributed to drug exposure of the fetus during pregnancy or breast-feeding. Differences between boys and girls were not consistently present. This could be partly due to the fact that before adolescence, sex steroid hormones do not play a major role in the development of adverse reactions.²¹ Of course, it may also relate to the fact that overall drug exposure is much lower in children than in elderly. Consequently, any difference in ADRs may be less prominent.

A sex-related difference, that is often discussed, is that women use more drugs and suffer more from co-morbidity.²² Drug-drug interactions may therefore be more frequent in women. It was not possible to evaluate this due to the ecological design of these three studies.

Sex and genetic variation in metabolizing pathways

Gene expression and activity of DMEs and transporters is partly dependent on several epigenetic factors, such as DNA methylation.²³ Gene expression is also partly under hormonal control. 12, 20, 24 These factors could potentially lead to sex differences in drug response. Single nucleotide polymorphisms (SNPs) within the coding genes for several of these enzymes and transporters were associated with inter-individual variation in drug response.²⁵⁻²⁷ In this thesis, sex differences in the effect of genetic variation were shown for one of the DMEs and for one of the androgen metabolizing enzymes in cardiac tissue. SNP rs1058932C>T, located in the 3'-UTR region of cytochrome P450 (CYP) 2C8, was associated with an increased risk of myocardial infarction (MI). This risk was significantly higher in men than in women. CYP2C8 is involved in the metabolism of xenobiotics, but also mediates the metabolism of arachidonic acid in the endothelium of arterial vasculature,28 which is involved in atherosclerosis formation and vasodilation.29 Therefore, variation in CYP2C8 activity and subsequently its enzymatic response could involve an increased risk of MI, such as has been described for other members of the CYP2C enzyme group.30

Carriage of one or more variant alleles of SNP rs248805G>A was also significantly associated with an increased risk of MI. This SNP is located in an intronic region in the SRD5A1 gene, which encodes the enzyme 5-alphareductase (SRD5A1). This enzyme converts testosterone to its more active form, dihydrotestosterone (DHT). Genetic variants in SRD5A1 have been associated with androgen-related diseases³¹ and a tightly linked SNP was earlier associated with peripheral artery disease.³² Due to the different effects of testosterone on atherosclerosis formation and endothelial function in the vascular system,^{33, 34} genetic variation in this androgen metabolizing enzyme leads to a difference in the risk of MI between men and women.

Coronary heart disease (CHD) is multi-factorial and is influenced by genetic, hormonal and environmental factors. One of the environmental factors, which have been brought forward, is coffee/caffeine intake. However, the causal role of coffee has been inconclusive and now seems questionable.³⁵ It was shown previously that the effect of coffee on MI was modified by variation in the gene CYP1A2, encoding the enzyme with the same name.³⁶ CYP1A2 is involved in the metabolism of several drugs, but also of caffeine.^{37, 38} Chapter 3.3 described a study, showing that also the intake of coffee is influenced by genetic variation in CYP1A2 (rs2472299G>A), sex, age and smoking, and that the genetic effect is also modified by sex. The C allele of SNP rs762551A>C (in the literature often referred to as CYP1A2*1F) was associated with a decrease in enzyme activity.^{39, 40}

We demonstrated that coffee intake was significantly lower in carriers of the variant (A) allele, with use of the genetic proxy rs2472299G>A of rs762551A>C. These results are in line with the assumption that despite similar genotype, hormonal differences between men and women may modify the way in which pharmacologically active substances are handled.

Sex and drug response: thiazides

Hyponatremia

The sex differences in ADRs attributed to thiazide diuretics, as shown in chapter 2, were further evaluated. It was shown that although the risk of hyponatremia attributed to thiazides was higher in women than in men, sex did not significantly modify this effect. However, age and BMI significantly modified thiazide-induced hyponatremia. These factors were previously associated with hyponatremia. 15, 41

Since age and BMI did not explain all variation, further study was performed to assess the role of drug-drug interactions and renal transporters. Drug-drug interactions may occur without causing adverse events. However, they may also cause serious health damage. Drugs interacting with thiazides in the causal pathway of sodium depletion showed an increased risk of hyponatremia. Loop diuretics and renin-angiotensin system (RAS) inhibitors in combination with thiazides were associated with a significant 5.3 and 1.6 times higher risk of hyponatremia than single thiazide use, respectively. Additional use of a RAS-inhibitor was associated with a greater effect in women than in men.

Secretion of thiazides by the kidneys is mainly facilitated by the organic anion transporters (OAT) 1 and 3.^{42, 43} We found six SNPs in the region covering the transporter-coding genes SLC22A6 and SLC22A8 that were associated with hyponatremia in thiazide-exposed subjects (rs4592425T>G, rs6591722T>A, rs4149172T>C, rs11231294T>C, rs955434G>A, rs2187383C>A). No significantly different genetic effects between men and women were observed, but due to the postmenopausal status of the women in the Rotterdam Study, it is possible that any differences between men and women were no longer demonstrable. SNP rs4592425T>G and rs6591722T>A were associated with expression levels of genes encoding nuclear export factors (EEF1G and NXF1), of which the association with NXF1 was only present in men. These nuclear factors could possibly play a role in the expression of OAT.⁴⁴

Hypokalemia

Besides hyponatremia, sex differences in hypokalemia were observed, with a higher risk of hospital admissions attributed to diuretic-induced hypokalemia in women. Hypokalemia is a frequently reported adverse reaction associated with thiazides, with a reported prevalence between 7-56%. ^{15, 45, 46} In this thesis, a population-based study showed that exposure to thiazides was associated with a more than twenty times higher risk of hypokalemia compared with no exposure. Men had a significantly higher risk of thiazide-induced hypokalemia than women, but also in non-thiazide-exposed subjects, the risk of hypokalemia in men was higher than that in women. The appearing contradictory results with the chapters regarding hospital admissions might be partly explained by detection bias. Both thiazides as a single agent and in combination with potassium-sparing diuretics significantly increased the risk of hypokalemia. This strengthens the previous observation that potassium suppletion or inhibition of potassium secretion through potassium channels does not completely prevent diuretic-induced hypokalemia. ^{47, 48}

Evaluation of drug safety measures

Although electrolyte disorders are well-known adverse reactions to thiazides, and our results show that the risks are relatively high, recommended electrolyte measurements are not frequently performed. In 2009, recommendations were published to perform extra serum sodium or potassium measurements in high-risk patients. From a large database containing data from a selection of general practitioners, it was concluded that recommended measurements after newly prescribed thiazides, were performed in only 9.4% of incident users. Measurements before and after start of a potassium-losing diuretic were performed in 12.0% and 6.6% (for serum potassium) and 16.0% and 7.3% (for renal function) of the subjects at risk. The proportion of cases of hyponatremia and hypokalemia was relatively high in the measurements that were performed, although this is most likely overestimated by diagnostic bias of the general practitioner. However, awareness of the necessity for stricter surveillance of thiazide therapy could be improved.

Potential bias and confounding

In this thesis, various outcomes were used. Regarding hospital admissions in the nationwide database, we observed lower numbers of hospitalizations related to an ADR than described in the literature. It is likely that ADRs have been missed, either because they were not recognized or not coded as such, or that they were not the major reason for admission. For many of our comparisons, however,

underrecognition or underreporting was not different between the groups, and under such circumstances relative risk assessments may remain fairly robust.

The collection of MIs was performed by continuous follow-up of the cohort and prospectively validated by medical doctors, based on standard criteria^{50, 51} without knowledge of the research hypotheses. Misclassification of the outcome is unlikely. Although silent infarction without ECG abnormalities may rarely occur, we do not expect that misclassification of MI was a source of non-validity.

The definition of hyponatremia was based on laboratory measurements. The assumption that when there was no serum sodium assessment, this meant that there was no hyponatremia is of course not always correct. However, it is highly likely that in most people in a community-dwelling population, sodium levels are within normal range. Moreover, sensitivity analyses using only data with available laboratory measurements, gave comparable results.

Sex as determinant, effect modifier and confounder

In this thesis, sex was either studied as determinant ('exposure'), effect modifier or confounder. Although misclassification of the determinant sex is unlikely, it could be debated whether sex should be regarded as a single dichotomous variable, or as part of a semi-quantitative risk profile, including multiple factors. In a population with great hormonal variability, female sex could be subdivided in multiple groups based on one's hormonal profile. Potential effect modification by hormonal profile within the sexes could be of importance in drug response. This is probably less relevant in postmenopausal women, as studied in the majority of the studies presented in this thesis. However, it should be taken into account that similar hormonal patterns in men do not necessarily have the same implications in women. Sex as a determinant is difficult to study, because of the multiple factors acting as confounders and effect modifiers. This was a limitation of the first part of this thesis, since hospitalization data lacked information on comorbidity and individual drug prescriptions.

In genetic studies analyzing drug response, sex can either be a confounder, an effect modifier, or both. Analyses are often adjusted for sex, as also in this thesis. Epigenetics, affecting gene expression, influence the eventual effect of a gene. Since men and women do not differ in the autosomal chromosomes, which determine drug response, but may differ in the extent of gene expression and functionality, it is rather likely that sex is an effect modifier for genetic factors affecting drug response. This probably accounts for both pharmacokinetic and pharmacodynamic factors in drug response. The expression and activity of

drug metabolizing enzymes and drug transporters is influenced by sex steroid hormones and differ between men and women, 19, 20, 24, 52 leading to differences in drug response. To assess these differences, effects should therefore be additionally studied within each sex stratum.

Future perspectives

Differences between the sexes in pharmacokinetics and pharmacodynamics have been demonstrated on multiple occasions^{3-5,53} and the intriguing difficulty to judge sex differences in drug response indicates the need for individual drug assessment. Although there are differences between men and women in drug response, some are only slight or inconclusive. The relevance of knowing all factors that could influence drug response should be balanced against the risk of adverse effects, the indication, and necessity of prescription of the drug. A proper evaluation of drug treatment must include the individual patient profile, including the presence of risk factors for ADRs. For studies assessing drug response or ADRs, the use of individual patient data is also essential, to take into account these potentially influencing factors.

The attention for genetic factors in the pathophysiology of diseases and ADRs has become more prominent during the last years. It is good to realize that effects of common genetic variants in genes encoding DMEs and transporters, and their expression, can be affected by the patient's sex and hormonal levels of, among others, estrogen and androgens.

It is surprising to realize that hyponatremia – which was an already known adverse effect shortly after the discovery of benzothiazides in 1958 – is still one of the most important causes of ADR-attributed hospital admissions. Apparently neither this general knowledge nor recommendations suffice to prevent this. Maybe, risk prediction models in more advanced drug prescription applications might help preventing such common and sometimes severe adverse reactions. Results from this thesis might help to build and test such models.

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Summary

Drug response is not similar in men and women. Physiological variation causes differences in pharmacokinetics and pharmacodynamics. This may lead to differences in efficacy and effectiveness of drugs, and to a different risk of adverse drug reactions (ADRs).

In the first part of this thesis, in **Part 2**, sex differences were studied in hospital admissions attributed to ADRs. Major differences were observed for cardiovascular drugs ('chapter 2.1'). These differences were further explored in 'chapter 2.2'. This chapter showed that 'anticoagulants and salicilates' accounted for more than half of all cardiovascular drug related admissions (63%). Admissions due to diuretics were in second place (16%) and showed a much higher risk in women than in men. The adverse reactions mainly concerned hyponatremia and hypokalemia. 'Chapter 2.3' showed that ADRs in children were not consistently different between boys and girls, and it was shown that the majority of the ADR-related admissions in children were due to effects through placenta and breast milk of the mother.

In Part 3, sex differences were studied in the cardiovascular system, focusing on the effect of genetic variation in genes encoding drug and androgen metabolizing enzymes on myocardial infarction (MI). We showed that several polymorphisms (SNPs) were associated with the risk of MI, and that these associations were modified by sex. These included SNP rs1058932C>T in CYP2C8, a gene encoding the drug-metabolizing enzyme CYP2C8, and rs248805G>A in SRD5A1, a gene encoding an androgen-metabolizing enzyme ('chapter 3.1' and 'chapter 3.2'). CYP2C8 is involved in the metabolism of arachidonic acid in the endothelium, which in involved in atherosclerosis development. Men with a variant allele of this SNP rs1058932C>T had a higher risk of an MI than women. SRD5A1 converts testosterone to the more active form dihydrotestosterone (DHT). Women with a variant allele of SNP rs248805G>A had an increased risk of MI. Additionally, mRNA expression of SRD5A1 was also different between male and female cardiac tissues. This strengthens the observation that the local effect of testosterone on the vascular system is different between women and men,

Another drug-metabolizing enzyme, CYP1A2, which also metabolizes caffeine, was previously shown to modify the association between coffee and MI. A known functional variant, in linkage disequilibrium with SNP rs2472299G>A, was studied for its direct effect on coffee intake ('chapter 3.3'). This SNP was significantly associated with the amount of coffee intake, and the association was modified by sex. Sex, age and smoking were all related to coffee intake.

The observed sex differences in hyponatremia and hypokalemia due to thiazide diuretics from chapter 2 were further studied in detail in **Part 4**. Age and body mass index were found as significant effect modifiers of the association between thiazides and hyponatremia. No significant differences were observed between men and women (**chapter 4.1**).

Drug interactions of thiazides with loop diuretics or renin-angiotensin system (RAS) inhibitors increased the risk of hyponatremia. These results were presented in **chapter 4.2**. No clear sex differences were found in the effect of drug interactions with thiazides on hyponatremia, although concurrent use of a RAS-inhibitor with a thiazide had a greater effect on sodium levels in women than in men.

Further genetic research in **chapter 4.3** was performed on the organic anion transporter (OAT) type 1 and 3 (encoded by the genes *SLC22A6* and *SLC22A8*), which secrete thiazides in the tubulus of the kidney. Six SNPs in and around the region of *SLC22A6* and *SLC22A8* were associated with thiazide-induced hyponatremia (rs4592425T>G, rs6591722T>A, rs4149172T>C, rs11231294T>C, rs955434G>A and rs2187383C>A). These associations were not modified by sex. SNP rs4592425T>G and SNP rs6591722T>A were also associated with mRNA expression levels of *BSCL2*, *EEF1G*, and *NXF1*, of which the latter association was only present in men. *EEF1G* and *NXF1* are involved in nuclear export of products, which might influence the amount of OAT on the plasma membrane.

Thiazides were associated with an eleven times higher risk of hypokalemia in users than in non-users. In men, the risk of hypokalemia was more than twenty times higher in the exposed subjects than in the non-exposed. This risk was significantly higher than in women, who had a nine times higher risk of a thiazide-induced hypokalemia. Both thiazides solely and thiazides in combination with a potassium-sparing agent, showed an increased risk of hypokalemia (chapter 4.4).

Although the risks of hyponatremia and hypokalemia during exposure to thiazides were relatively high, the results presented in **Part 5** (**chapter 5.1**) revealed that extra laboratory measurements in subjects at risk were not performed as frequently as recommended by the 'Dutch HARM-Wrestling Task Force'.

In conclusion, to optimize safety in drug response, all inter-individual differences should be taken into account as far as possible, and the sex of the patient is one of importance. Even for well-known drugs and frequent or expected adverse reactions, we should remain vigilant.

Samenvatting

De respons op geneesmiddelen is niet hetzelfde in mannen als in vrouwen. Fysiologische variatie zorgt voor verschillen in farmacokinetica en farmacodynamica. Dit kan leiden tot een verschillende werking en effectiviteit van geneesmiddelen en tot verschillen in het risico op bijwerkingen.

In het eerste deel van dit proefschrift, in 'Part 2', werden geslachtsverschillen in bijwerking-gerelateerde ziekenhuisopnames bestudeerd. Hierin werden grote verschillen gevonden voor cardiovasculaire geneesmiddelen ('chapter 2.1'). Deze verschillen werden nader onderzocht in 'chapter 2.2'. Hier werd gezien dat bloedverdunners bij de meerderheid van de bijwerking-gerelateerde ziekenhuisopnames betrokken waren (63%). Opnames ten gevolge van diuretica kwamen op de tweede plaats (16%) en lieten een aanzienlijk hoger risico zien in vrouwen zien ten opzichte van mannen. De bijwerkingen die hier gevonden werden, betroffen met name hyponatriëmie en hypokaliëmie. In 'chapter 2.3' zagen we dat bijwerkingen in kinderen niet consistent verschilden tussen jongens en meisjes. Hier was tevens te zien dat de meerderheid van de bijwerking-gerelateerde opnames was ten gevolge van geneesmiddel effecten tijdens de zwangerschap en geven van borstvoeding door de moeder.

In 'Part 3' werden geslachtsverschillen bestudeerd in het cardiovasculaire systeem, gericht op het effect van genetische variatie op het risico op een hartinfarct. Hierbij werd gekeken naar genen die vertalend zijn voor enzymen, betrokken bij het metabolisme van geneesmiddelen en androgenen. We toonden aan dat een paar veel voorkomende DNA varianten (SNPs) geassocieerd waren met het risico op een hartinfarct. Deze associaties werden beïnvloed door geslacht. Het betrof hier SNP met nummer rs1058932C>T in CYP2C8, een gen dat codeert voor een enzym, dat betrokken is in het metabolisme van geneesmiddelen, en SNP met nummer rs248805G>A in SRD5A1, een gen dat codeert voor een enzym dat betrokken is bij het metabolisme van androgenen ('chapter 3.1' and 'chapter 3.2'). CYP2C8 metaboliseert 'arachidonzuur' in de vaatwand, wat een rol speelt in de vorming van atherosclerose. Mannen met een variant allel van SNP rs1058932C>T hadden een hoger risico op een hartinfarct dan vrouwen. SRD5A1 zet testosteron om in de actievere vorm 'dihydrotestosteron' (DHT). Vrouwen met een variant allel van SNP rs248805G>A hadden een verhoogd risico op een hartinfact. Daarbij was de expressie van SRD5A1 mRNA ook verschillend in mannelijk en vrouwelijk hart weefsel. Dit versterkt het idee dat het plaatstelijke effect van testosteron op het hartvaatsysteem anders is bij mannen dan bij vrouwen.

Een ander enzym dat betrokken is bij geneesmiddel metabolisme, CYP1A2, is tevens betrokken bij het metabolisme van caffeine, en beïnvloedde de associatie

tussen koffie drinken en het risico op een hartinfact in eerder onderzoek. Een bekende functionele genetische variant, welke gelinked is ('linkage disequilibrium') met SNP rs2472299G>A, werd hier bestudeerd voor zijn directe effect op de inname van koffie ('chapter 3.3'). Deze SNP was significant geassocieerd met de hoeveelheid koffie die iemand dronk en we zagen dat deze associatie werd beïnvloed door geslacht. Geslacht, leeftijd en roken waren alledrie gerelateerd aan de inname van koffie.

In 'Part 4' werden de geslachtsverschillen in hyponatriëmie en hypokaliëmie door diuretica, die in 'chapter 2' waren gevonden, verder bestudeerd. Hier vonden we dat leeftijd en 'body mass index' de associatie tussen thiazides en het optreden van hyponatriëmie beïnvloedden. Er werden geen significante verschillen gevonden tussen mannen en vrouwen ('chapter 4.1').

Geneesmiddelinteracties tussen thiaziden en een lisdiureticum of een renine-angiotensine-systeem (RAS) remmer verhoogden het risico op hyponatriëmie. Deze resultaten werden in 'chapter 4.2' gepresenteerd. Er werden geen geslachtsverschillen gevonden in het risico op hyponatriëmie, alhoewel het effect van gelijktijdig gebruik van een RAS-remmer met een thiazide op de natrium spiegels groter was in vrouwen dan in mannen.

Verder genetisch onderzoek werd uitgevoerd in 'chapter 4.3' naar de 'organic anion transporters' (OAT) type 1 en 3 (gecodeerd door de genen SLC22A6 en SLC22A8). Deze transporters scheiden thiazides uit in de tubulus van de nier. Zes SNPs in en om de regio waar de genen SLC22A6 en SLC22A8 liggen, waren geassocieerd met hyponatriëmie ten gevolge van thiazides (rs4592425T>G, rs6591722T>A, rs4149172T>C, rs11231294T>C, rs955434G>A and rs2187383C>A). Deze associaties werden niet door geslacht beïnvloed. SNP rs4592425T>G en SNP rs6591722T>A waren tevens geassocieerd met mRNA expressie van de genen BSCL2, EEF1G en NXF1, waarvan de laatste associatie alleen in mannen werd gevonden. EEF1G en NXF1 zijn betrokken bij export van producten uit de celkern en kunnen hierdoor mogelijk de hoeveelheid transporters op de celmembraan beïnvloeden.

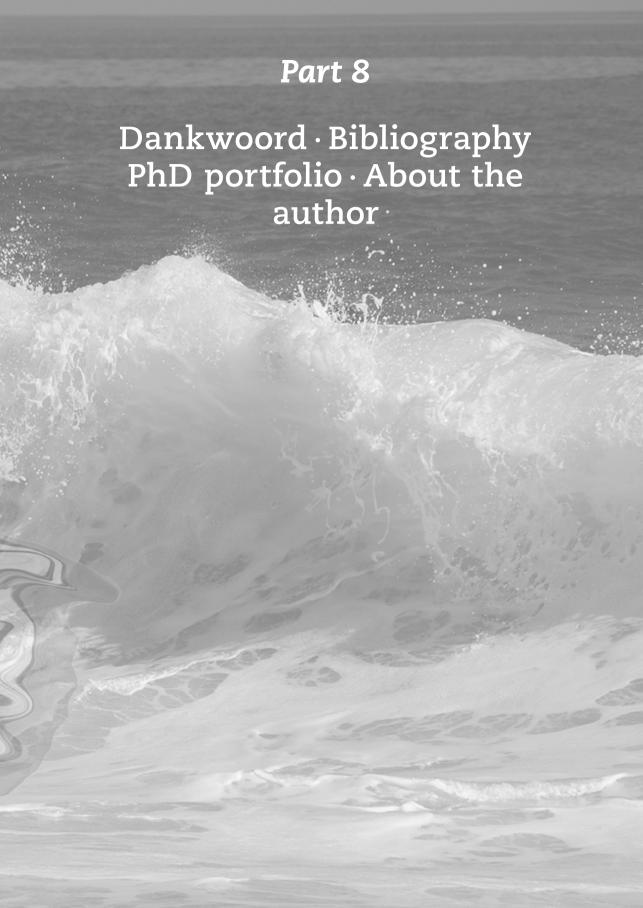
Gebruik van thiaziden was geassocieerd met een elf maal hoger risico op hypokaliëmie in vergelijking met mensen die geen thiaziden gebruikten. In mannen was dit risico meer dan twintig keer hoger dan niet-gebruikers. Dit risico was significant hoger dan in vrouwen, waar het risico op een thiazidegeinduceerde hypokaliëmie negen keer hoger was. Zowel in thiazides los als in thiazides in combinatie met een kalium-sparend middel werd een verhoogd risico gevonden op hypokaliëmie ('chapter 4.4').

Het risico op een thiazide-geinduceerde hyponatriëmie of hypokaliëmie was betrekkelijk hoog. Ondanks dat lieten de resultaten in 'Part 5' ('chapter 5.1') zien,

dat extra laboratorium metingen in hoog-risico patiënten niet zo vaak werden uitgevoerd als werd aanbevolen door de werkgroep voor veilig geneesmiddelgebruik ('HARM-Wrestling').

Concluderend, om veilig geneesmiddelgebruik te optimaliseren, moeten alle inter-individuele verschillen in acht worden genomen. Het geslacht van de patient is hierbij een belangrijke factor. Ook voor goedbekende geneesmiddelen en veelvoorkomende of te verwachten bijwerkingen, moet waakzaam gebleven worden.





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About the author

Eline Rodenburg was born on March 16th 1982 in Leiden, The Netherlands. She attended College Leeuwenhorst (atheneum-beta) in Noordwijkerhout and graduated in 1999. In the same year, she started her study Medicine at Leiden University. In 2006, she obtained her medical degree. She performed her graduation research in the department of Cardiology in the Leiden University Medical Center on 'Radofrequent catheter ablation in ventricular tachycardia', under supervision of Prof.dr. M.J. Schalij.

In 2006, she started working at the department of Internal Medicine at the Reinier de Graaf hospital. During her work as a medical physician, she performed research on the survival of patients with large B-cell lymphoma after introduction of rituximab, under supervision of Dr. E. Maartense and Dr. E.F.M. Posthuma.

From 2008 onwards she worked on her PhD research in Phamaco-epidemiology (head: Prof.dr. B.H.Ch. Stricker), as part of the department of Epidemiology (head: Prof.dr. A. Hofman). The research, as presented in this thesis, was performed under supervision of Prof.dr. B.H.Ch. Stricker (Pharmaco-epidemiology), Prof.dr. A.G. Uitterlinden (Internal Medicine and Genetics laboratory) and Dr. L.E. Visser (Hospital Pharmacy, Internal Medicine, Pharmaco-epidemiology).

In the same period of her PhD research, she also worked as an Inspectorate Officer at the Drug Safety Unit at the Dutch Health Care Inspectorate.

After the defense of her thesis, she will start her residency at the Sint Franciscus Gasthuis, where she will perform her specialty training in Internal Medicine (head: Drs. A.P. Rietveld), as part of her specialty training at the Erasmus Medical Center (head: Prof.dr. J.L.C.M. van Saase).

