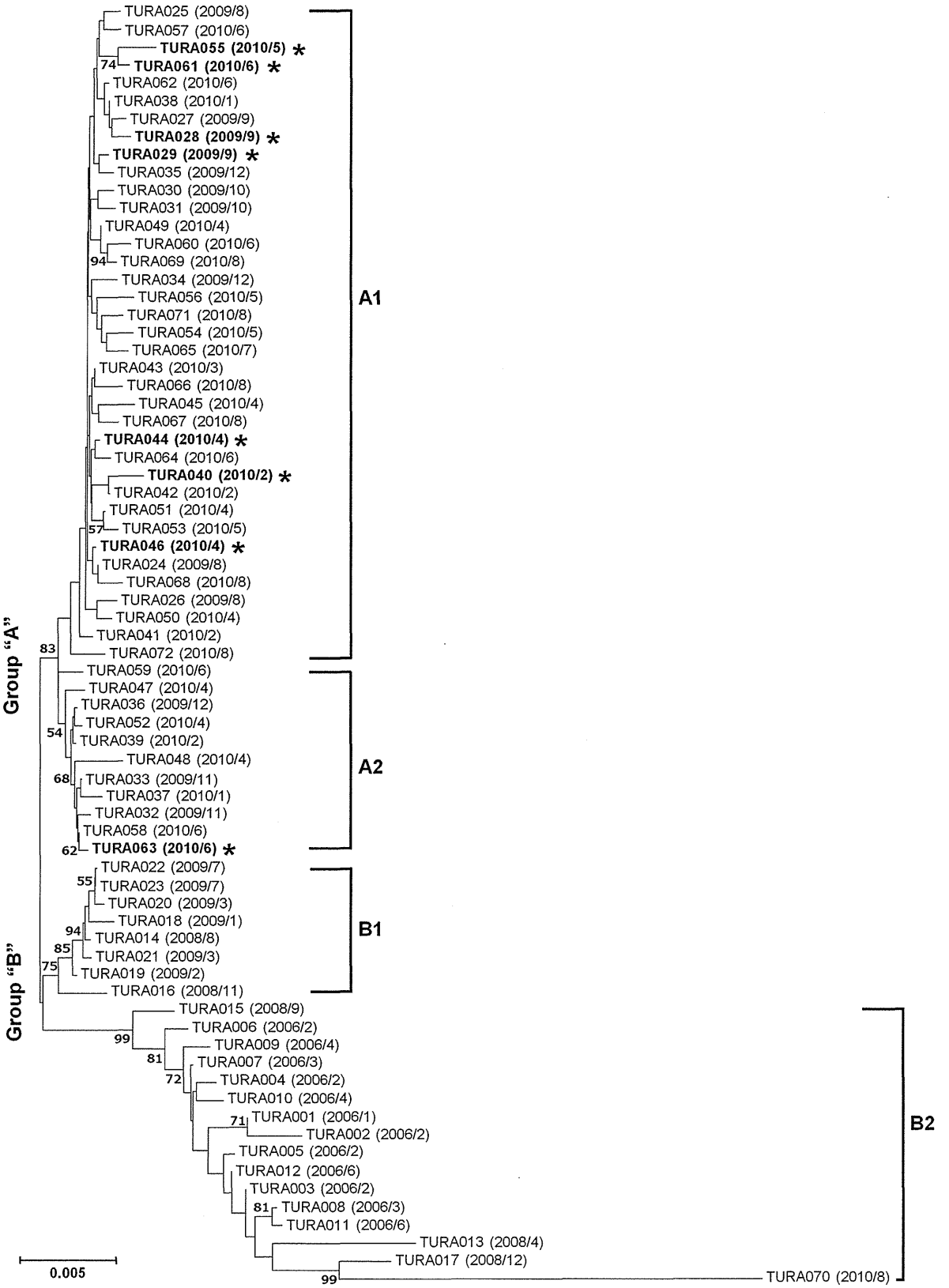

Fig. 1. Evolutionary relationships of *A. baumannii* strains included in this study. The evolutionary history was inferred using the *Neighbor-Joining method*. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. Bootstrap values indicate the reproducibility of certain clustered branches. Bootstrap values lower than 50 were not shown. The evolutionary distances were computed using the Kimura 2-parameter method. All positions with less than 5% site coverage were eliminated. Numbers between two parentheses indicate year and month of isolation, respectively. Taxa with asterisks denote the isolates used in MLST study.

Other highly variable regions, i.e., which include more than 95% of site coverage were considered as characteristics of genotypes.

Molecular based epidemiological analyses are usually affected by compared base sequences, therefore, optimal sets of marker genes for reliable genotyping are still under exploring and evaluating. The NGS technology-based genotyping using HAI kit, generated grouping pattern with fine differentiation of *A. baumannii* strains from same periods, reflecting high discriminatory power. Therefore, it is a promising tool for future epidemiological studies. Accordingly, the phylogeny produced here will be used as a reference database for our prospective studies in order to keep a good management at Teikyo University Hospital and other hospitals in Japan.

In conclusion, the large-scale sequencing genotyping of *A. baumannii* strains provides a comprehensive genetic data that are useful in molecular epidemiology. It shows a discriminatory power comparing MLST. Nevertheless, it creates clustering patterns that can be used as a database and hopefully would help in management of future nosocomial infections, allowing an early and appropriate monitoring on molecular level.

Conflict of interest

K. M. has received research grants from Japan Space Forum and World Geno Matrix. In addition, this study was financially supported by Daiichi Sankyo Co. Ltd. The authors alone are responsible for the content and writing of the paper and declare no conflicts of interest.

References

[1] Peleg AY, Seifert H, Paterson DL. *Acinetobacter baumannii*: emergence of a successful pathogen. Clin Microbiol Rev 2008;21:538–82.
[2] Lambiase A, Piazza O, Rossano F, Del Pezzo MA, Tufano R, Catania MR. Persistence of carbapenem-resistant *Acinetobacter baumannii* strains in Italian intensive care unit during a forty-six month study period. New Microbiol 2012;35:199–206.
[3] Villalón P, Valdezate S, Medina-Pascual MJ, Rubio V, Vindel A, Saez-Nieto JA. Clonal diversity of nosocomial epidemic *Acinetobacter baumannii* strains isolated in Spain. J Clin Microbiol 2011;49:875–82.
[4] Diancourt L, Passet V, Verhoef J, Grimont PAD, Brisse S. Multilocus sequence typing of *Klebsiella pneumoniae* nosocomial isolates. J Clin Microbiol 2005;43:4178–82.
[5] Arena F, Rolfe AP, Doran G, Conte V, Gruszka S, Clarke T, et al. Rapid resistome fingerprinting and clonal lineage profiling of carbapenem-resistant *Klebsiella pneumoniae* isolates by targeted next-generation sequencing. J Clin Microbiol 2014;52:987–90.
[6] Leonard JT, Adesokan A, Bruso S, Clarke IV T, Doran G, Gruszka S, et al. Broad active surveillance enabled by Pathogenica's HAI biodetection kit. (n.d.). Retrieved February 23, 2015, from http://www.bioinnova.ch/uploads/3/0/5/6/30561055/active_surveillance_2013.pdf.
[7] Yuji K, Oiso G, Matsumura T, Murashige N, Kami M. Police investigation into multidrug-resistant *Acinetobacter baumannii* outbreak in Japan. Clin Infect Dis 2011;52:422.
[8] Bartual SG, Seifert H, Hippler C, Luzon MA, Wisplinghoff H, Rodríguez-Valera F. Development of a multilocus sequence typing scheme for characterization of clinical isolates of *Acinetobacter baumannii*. J Clin Microbiol 2005;43:4382–90.
[9] *Acinetobacter baumannii* MLST databases. (n.d.). Retrieved February 23, 2015, from http://pubmlst.org/abaumannii/.
[10] Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 2013;30:2725–9.

International Journal of clinical pharmacology
Original

ISSN 0946-1965

DOI 10.5414/CP202195
e-pub: June 24, 2015

Pitavastatin therapy in polymedicated patients is associated with a low risk of drug-drug interactions: analysis of real-world and phase 3 clinical trial data

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Key words
cytochrome P450 – organic anion-transporting polypeptide – drug-drug interactions – biguanide – pitavastatin – statin

Abstract. Objectives: Medications that interact with the pathways responsible for statin metabolism may increase the risk of statin-associated myalgia. Pharmacokinetic studies show that pitavastatin is carried into the liver by a range of transporters and is minimally metabolized by cytochrome P450 in healthy volunteers, indicating a reduced potential for drug-drug interactions (DDIs). This post hoc analysis investigates the incidence of adverse events in patients receiving pitavastatin with concomitant medication in two large data sets. Methods: The largest pitavastatin patient data sets are the LIVALO Effectiveness and Safety (LIVES) postmarketing surveillance study in Japan (n = 19,925) and the European phase 3 clinical trial program (n = 2,396). Adverse events were classified according to the Medical Dictionary for Regulatory Activities (MedDRA) and whether they occurred in patients taking medications that interact with hepatocyte organic anion-transporting polypeptide or cytochrome P450 (CYP) isoenzyme pathways. Results: Concomitant administration of pitavastatin with other medications was not associated with clinically significant increases in the incidence of adverse drug reactions (ADRs), even when given with medications that interact with CYP2C9, responsible for the minimal CYP metabolism of pitavastatin. There was a significant interaction with biguanides in LIVES, but this was associated with a reduced risk of muscle ADRs. Conclusion: In clinical trials, pitavastatin is associated with a low incidence of adverse events related to DDIs, consistent with data from healthy volunteers. Prescribing a metabolically stable statin, such as pitavastatin, may improve patient adherence to medication, thus facilitating the attainment of lipid targets and reducing cardiovascular risk.

Received
June 16, 2014;
accepted
December 11, 2014

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Introduction

Statins – 3-hydroxy-3-methylglutaryl coenzyme A inhibitors – have a central role in the management of dyslipidemia and in reducing the risk of cardiovascular events, such as myocardial infarction and stroke [1, 2]. More than 25% of all people aged 45 years and older in the USA are receiving a statin [3], while evidence that the benefits of statin therapy extend to patients at a relatively low risk of cardiovascular disease has led to suggestions that statins should be more widely used [2, 4, 5]. The number of people receiving a statin is therefore likely to increase, particularly with the growing recognition that some statins may confer additional benefits through elevation of high-density lipoprotein cholesterol and other pleiotropic effects, in addition to reducing blood concentrations of low-density lipoprotein cholesterol [6, 7].

Statins are prescribed as part of an overall approach to cardiovascular risk management, and many patients have comorbidities that require further pharmacological therapy [8]. Polypharmacy is increasingly common, particularly among elderly patients [9]. It is also associated with an increased risk of potentially harmful drug-drug interactions (DDIs) that may increase plasma statin concentrations and the incidence of muscle-associated adverse drug reactions (ADRs), such as myalgia and rare, but potentially fatal, rhabdomyolysis [10, 11, 12]. For example, although the reported incidence of rhabdomyolysis is low in patients receiving standard-dose statin monotherapy (3.4 cases

per 100,000 person-years [13]), the risk of experiencing muscle-related adverse events with some statins has been shown to increase six-fold in patients receiving cytochrome P450 (CYP) inhibitors [14]. Moreover, polypharmacy is not confined to prescription medications; many patients use over-the-counter products, vitamin or mineral supplements, or herbal medications. Some dietary components, notably grapefruit juice, may also interact with certain statins [11].

Not all statins have the same pharmacokinetic profile, as they are metabolized by different routes, and the interactions between individual statins and the pathways involved in their absorption, distribution, metabolism, and elimination directly influence their DDI potential. Also, statins are metabolized to different degrees, from prodrugs, such as lovastatin and simvastatin, to more metabolically stable drugs, such as pravastatin and rosuvastatin. Thus, statins that are minimally metabolized by CYP isoenzymes or uridine 5′-diphosphate glucuronosyltransferase (UGT), or that are not dependent on restricted numbers of hepatic uptake (e.g., organic anion-transporting polypeptide [OATP]) or efflux transporters for biliary clearance, may have a lower risk of being associated with DDIs than other statins [10, 11, 15, 16].

Pitavastatin is a potent, partially lipophilic statin that is subject to minimal metabolism by CYP isoenzymes and is mainly excreted unchanged in bile [11, 17]. Its major metabolite is an inactive lactone generated through the activity of UGT1A3 and UGT2B7 [18]. Pitavastatin is a substrate for several hepatic transporters and may be less susceptible to inhibition of OATP-mediated uptake into hepatocytes than some other statins [10, 11]. Similarly, pitavastatin appears to be a substrate for both multidrug resistance-associated protein 2 (MRP2) and breast cancer resistance protein (BCRP), and seems to be less susceptible to drug interactions during hepatic efflux transport [19]. The effect of coadministration of a range of commonly prescribed medications, including lipid-lowering therapies (bezafibrate, fenofibrate, gemfibrozil, and ezetimibe), antifungal or antimicrobial agents (itraconazole, erythromycin, and rifampicin), or cyclosporine, on pitavastatin pharmacokinetics has been investigated in healthy volunteers [11]. Only coadministration of erythromycin and cyclosporine, known inhibitors of multiple transporter proteins, produced clinically significant increases in pitavastatin exposure. Although no pharmacokinetic interaction with fibrates was identified, the pitavastatin label contains a warning about a possible interaction with fibrates, as both classes of drug have been associated with myotoxicity. The extent to which the promising pharmacokinetic profile of pitavastatin is associated with a reduced risk of patients experiencing clinically important DDIs compared with other statins is difficult to assess, as the European phase 3 clinical trial program for pitavastatin [20, 21, 22, 23, 24], which included commonly used first-line statins as comparators, excluded drugs known to interact with these drugs, such as CYP3A4 inhibitors.

The LIVALO Effectiveness and Safety (LIVES) study was a 2-year postmarketing surveillance study of the effect of pitavastatin in Japanese patients with primary hypercholesterolemia or familial hypercholesterolemia in a real-world clinical setting. Initial analysis of data from the 2-year LIVES study, and its 3-year extension study, indicated that pitavastatin therapy is associated with a low incidence of adverse events (6.3%, mean follow-up of 5.3 years) [25, 26]. Moreover, concomitant administration of pitavastatin with a range of common medications, including antidiabetic, antihypertensive, or antiplatelet therapies, did not have a significant effect on the incidence of ADRs ($p = 0.39$, $p = 0.59$, and $p = 0.91$, respectively) [25].

The aim of this post hoc analysis was to investigate the incidence of ADRs, categorized according to metabolic pathway, in patients receiving pitavastatin and at least one other medication who were enrolled in the LIVES study or the European phase 3 clinical trial program for pitavastatin.

Methods

Data sources

LIVES study

Full details of the LIVES study have been published previously [25]. Briefly, the LIVES study was a 2-year postmarketing surveillance study of Japanese patients with

primary hypercholesterolemia or familial hypercholesterolemia who received pitavastatin at daily doses of 1, 2, or 4 mg [25]. The study was started in December 2003, and patients were enrolled at 2,811 facilities across Japan using a central registration system. Data on patient demographics, treatment regimen (pitavastatin and concomitant therapies and clinical investigations), and ADRs were collected for the duration of the study. The safety population comprised all enrolled patients with completed survey sheets who completed at least one follow-up clinic visit. At the end of the 2-year follow-up period in the LIVES study, patients were able to enroll in a 3-year extension study [26].

Phase 3 clinical trial program

The pitavastatin phase 3 clinical trial program compared the efficacy and safety of pitavastatin at daily doses of 2 and 4 mg with atorvastatin (10 and 20 mg/day) and simvastatin (20 and 40 mg/day) and of pitavastatin at daily doses of 1, 2, and 4 mg with pravastatin (10, 20, and 40 mg/day) in patients with hypercholesterolemia and/or combined (mixed) dyslipidemia. In this analysis, we used the pooled data set from the following five prospective, randomized, double-blind, double-dummy, controlled trials after 12 weeks of treatment. Full details of the individual trials have been published previously [20, 21, 22, 23, 24]; in brief, all studies enrolled patients with hypercholesterolemia or combined (mixed) dyslipidemia. In addition to trial-specific criteria, all studies excluded patients with homozygous familial hypercholesterolemia, conditions with potential to cause secondary dyslipidemia, uncontrolled diabetes mellitus, pregnancy, significant cardiovascular disease, cerebrovascular disease, and uncontrolled or poorly controlled hypertension.

Trial 1: 821 patients with hypercholesterolemia or combined (mixed) dyslipidemia were included. Patients with previous contraindications or intolerance to statin therapy, and those with conditions or receiving treatments that would interact with the pharmacokinetics of statins, were excluded, as were those with symptomatic heart failure, impaired pancreatic function, liver enzyme levels greater than 1.5 times the upper limit of normal, impaired renal function, impaired urinary tract function, uncontrolled hypothyroidism, left ventricular ejection fraction less than 0.25, muscular or neuromuscular disease, or neoplastic disease. Enrolled patients were randomly assigned to 1 of 4 treatments: pitavastatin 2 mg/day (n = 316), atorvastatin 10 mg/day (n = 102), pitavastatin 4 mg/day (n = 300; 2 mg/day force titrated to 4 mg/day after 4 weeks), or atorvastatin 20 mg/day (n = 103; 10 mg/day force titrated to 20 mg/day after 4 weeks) [21].

Trial 2: 848 patients with hypercholesterolemia or combined (mixed) dyslipidemia were included. In addition to the exclusion criteria used in trial 1, patients with serum creatine kinase (CK) activity greater than 5 times the upper limit of the reference range without clinical explanation were excluded. Concomitant medications that were allowed included therapy for hyperthyroidism or hypothyroidism, antihypertensive drugs (except verapamil and amiodarone), estrogen receptor modulators, noncyclic estrogen/progesterone preparations for hormone replacement or sustained contraception, and hypoglycemic agents (except for thiazolidinediones). Enrolled patients were randomly assigned to 1 of 4 treatments: pitavastatin 2 mg/day (n = 311), simvastatin 20 mg/day (n = 107), pitavastatin 4 mg/day (n = 320; 2 mg/day force titrated to 4 mg/day after 4 weeks), or simvastatin 40 mg/day (n = 110; 20 mg/day force titrated to 40 mg/day after 4 weeks) [20].

Trial 3: 352 patients with hypercholesterolemia or combined (mixed) dyslipidemia and at least two coronary heart disease risk factors were randomized to receive pitavastatin 4 mg/day (n = 233; 2 mg/day force titrated to 4 mg/day after 4 weeks) or simvastatin 40 mg/day (n = 119; 20 mg/day force titrated to 40 mg/day after 4 weeks). All other lipid-modifying therapies were prohibited for the duration of the study. Patients with conditions that could have affected drug pharmacokinetics were excluded from the study, as were those with symptomatic heart failure with left ventricular ejection fraction less than 0.25, impaired liver or kidney function, or other serious medical conditions [22].

Trial 4: 412 patients with combined (mixed) dyslipidemia and type 2 diabetes treated with an oral antidiabetic treatment (not including glitazones) or insulin were

Table 1. Inhibitors of CYP isoenzymes analyzed in the LIVES study [28].

CYP isoenzyme	Inhibitors
CYP2C19	Omeprazole, fluvoxamine maleate, fluconazole
CYP3A4	Itraconazole, erythromycin, cimetidine, cyclosporine, clarithromycin, ketoconazole, atazanavir, indinavir, nelfinavir, ritonavir, saquinavir, telithromycin, amiodarone, fluvoxamine
CYP2C8	Thiazolidinediones, trimethoprim
CYP1A2	Quinolone antibiotics, fluvoxamine maleate, cimetidine, amiodarone
CYP2D6	Quinidine, cimetidine, paroxetine, amiodarone, ritonavir
CYP2C9	Trimethoprim-sulphamethoxazole, fluconazole, amiodarone, cimetidine, ketoconazole, fluvoxamine maleate

CYP = cytochrome P450; LIVES = LIVALO Effectiveness and Safety.

randomized to receive pitavastatin 4 mg/day (n = 275; 2 mg/day force titrated to 4 mg/day after 4 weeks) or atorvastatin 20 mg/day (n = 137; 10 mg/day force titrated to 20 mg/day after 4 weeks). Patients with body mass index greater than 35 kg/m^2, neoplastic disease within the last 10 years, or serum CK activity greater than 5 times the upper limit of the reference range without clinical explanation were excluded, as were those with a history of muscular or neuromuscular disease, or of resistance to lipid-lowering therapy, or use of supplements that affect lipid metabolism [23].

Trial 5: 942 elderly patients (≥ 65 years of age) with hypercholesterolemia or combined (mixed) dyslipidemia were randomized to receive pitavastatin 1 mg/day (n = 207), pravastatin 10 mg/day (n = 103), pitavastatin 2 mg/day (n = 224), pravastatin 20 mg/day (n = 96), pitavastatin 4 mg/day (n = 210; 2 mg/day force titrated to 4 mg/day after 4 weeks), or pravastatin 40 mg/day (n = 102; 20 mg/day force titrated to 40 mg/day after 4 weeks). Patients with uncontrolled hypothyroidism, gastrointestinal conditions that may have interfered with drug absorption, impaired liver or renal function, or serum CK greater than 5 times the upper limit of the reference range were excluded. Concomitant medications necessary for the treatment of underlying non-lipid medical conditions were permitted at the discretion of the investigators and included therapies for hyperthyroidism or hypothyroidism (provided that serum thyroid-stimulating hormone was within the normal range), antihypertensive therapy, estrogen receptor modulators for the prevention of osteoporosis, noncyclic (continuous) estrogen/progesterone preparations for hormone-replacement therapy or sustained contraceptive preparations, and glycemic agents (if patients had stable type 2 diabetes mellitus) [24].

The analysis population for this pooled data set was defined as patients who were randomized and had at least one safety observation.

Concomitant medications

Three broad classes of concomitant medications were assessed (CYP- and OATP-interacting medications, and biguanides). Medications that interact with the CYP metabolic pathway were categorized according to individual CYP isoenzymes (Table 1). Medications that interact with OATPs were assessed as a class.

Despite restrictions on concomitant medication in the phase 3 clinical trial program, data on adverse events were available for patients who received concomitant medications known to interact with OATPs and CYP2C19. The incidence of adverse events in patients receiving pitavastatin and biguanides (most of whom had diabetes mellitus) was also assessed.

Classification of adverse events

In the LIVES study, the terminology for ADRs was based on the system organ class and preferred terms of the ICH Medical Dictionary for Regulatory Activities (MedDRA; version 10.1). Table 2 shows the specific set of muscle-related adverse events – myopathy-associated ADRs (maADRs) – that were

Table 2. Muscle-related adverse events identified in the initial LIVES analysis and the post hoc analysis using validated criteria.

Adverse events identified by		
LIVES maADR criteria only	Both the LIVES maADR and the R/M SMQ criteria	R/M SMQ criteria only
• Muscle spasms • Musculoskeletal stiffness • Pain in the extremities • Neck pain • Decreased grip strength • Malaise • Asthenia	• Myalgia • Muscular weakness • Myopathy • Musculoskeletal pain • Rhabdomyolysis • Chromaturia • Renal failure • Increased blood creatine phosphokinase • Increased blood myoglobin • Urine myoglobin present	• Increased blood creatinine

LIVES = LIVALO Effectiveness and Safety; maADR = myopathy-associated adverse drug reaction; R/M SMQ = Rhabdomyolysis/Myopathy Standard Medical Query.

assessed in the initial LIVES data set [25]. Post hoc analysis of muscle-related adverse event data in the LIVES study was conducted by reclassifying adverse events using a validated set of preferred reporting terms – the MedDRA (version 10.1) Rhabdomyolysis/Myopathy Standard Medical Query (R/M SMQ). Table 2 highlights the substantial overlap in the muscle-related ADRs classified by the initial LIVES maADR and the R/M SMQ criteria.

In the phase 3 trials [20, 21, 22, 23, 24], adverse events of interest were identified using a standard set of preferred terms, comprising 3 MedDRA (version 8.1) SMQs: R/M SMQ, acute renal failure SMQ, and a subset of hepatic disorders SMQ (possible drug-related hepatic disorders – comprehensive search).

Statistical analysis

Data were summarized in terms of the number of patients taking a particular class of concomitant medication and the percentage reporting an adverse event or ADR over the duration of the trial. Differences in the incidence of adverse events for patients receiving or not receiving a concomitant medication with pitavastatin were assessed using a χ^2-test in the LIVES study and Fisher's exact test in the pooled data set of the phase 3 program. All tests were two-sided and had a significance level of 0.05. Statistical analyses were carried out using SAS 9.3 software (SAS Institute Inc., Cary, NC, USA).

Table 3. Demographic characteristics of the 19,925 patients in the safety population for the LIVES study [25].

Characteristic	n (%)
Age	
< 65 years	10,311 (51.7)
≥ 65 years	9,614 (48.3)
Male	6,535 (32.8)
Diagnosis	
Familial hypercholesterolemia	313 (1.6)
Comorbidities	
Hypertension	9,436 (47.4)
Diabetes mellitus	5,133 (25.8)
Hepatic disease	1,580 (7.9)
Renal disease	720 (3.6)
Concomitant medication	
Antihypertensive agents	10,549 (52.9)
Antidiabetic drugs	3,966 (19.9)
Lipid-lowering drugs	1,368 (6.9)

LIVES = LIVALO Effectiveness and Safety.

Results

Patients

The demographic characteristics of the 19,925 patients in the LIVES safety population are summarized in Table 3. Overall, 10,311 (51.7%) of patients were younger than 65 years, and ~ 1/3 were male. The most common comorbidities were hypertension (47.4% of patients), diabetes mellitus (25.8%), and liver disease (7.9%) [25].

The demographic characteristics of the 2,396 patients in the pooled data set of the phase 3 program are shown in Table 4. Overall, 1,246 (52.0%) of the patients were younger than 65 years, and 1,109 (46.3%) were male.

Table 4. Demographic characteristics of the 2,396 patients treated with pitavastatin in the safety population for the phase 3 clinical trials.

Characteristic	n (%)
Age	
< 65 years	1,246 (52.0)
≥ 65 years	1,150 (48.0)
Male	1,109 (46.3)
Primary diagnosis	
Primary hypercholesterolemia	1,752 (73.1)
Combined dyslipidemia	627 (26.2)
Heterozygous familial hypercholesterolemia	17 (0.7)
Comorbidities	
Hypertension	1,454 (60.7)
Diabetes mellitus	413 (17.2)
Concomitant medication	
Antihypertensive agents	1,290 (53.8)
Antidiabetic drugs	366 (15.3)
Lipid-lowering drugs	0 (0.0)

Concomitant medications that interact with CYP isoenzymes

LIVES

Post hoc analysis of the 2-year LIVES study data revealed no significant differences in the incidence of ADRs between patients who received concomitant treatment with CYP inhibitors and those who did not (Table 5). Moreover, there were no significant increases in the incidence of ADRs in patients receiving pitavastatin and omeprazole, cyclosporine, or erythromycin ($p = 0.56$, $p = 0.81$, and $p = 0.23$, respectively; data not shown).

The incidence of R/M SMQ muscle-associated ADRs in patients receiving a concomitant inhibitor of CYP2C9, the CYP isoenzyme responsible for the minimal CYP metabolism of pitavastatin, was not significantly different from the incidence in patients not receiving a CYP2C9 inhibitor (Figure 1) (3.2% vs. 4.1%; $p = 0.44$).

Phase 3 clinical trial program

Adverse event data are available for 81 of the 2,396 patients in the phase 3 clinical trial program who were receiving at least 1 of the following concomitant medications known to inhibit CYP2C19: the proton pump inhibitor omeprazole, the antifungal agent fluconazole, or the antidepressant fluvoxamine maleate.

Table 6 shows that the overall incidence of adverse events in patients receiving daily pitavastatin (2 or 4 mg) was significantly higher in those who were receiving concomitant CYP2C19 inhibitors (pitavastatin 2 mg, 66.7% vs. 33.3%, $p = 0.004$; pitavastatin 4 mg, 60.0% vs. 36.3%, $p = 0.002$); no such difference was seen in patients receiving the lowest dose of pitavastatin (1 mg; $p = 0.79$). Almost all the adverse events seen in patients receiving a CYP2C19 inhibitor were observed in patients receiving concomitant omeprazole; only 1 patient (taking pitavastatin 2 mg) did not receive omeprazole.

Concomitant treatment with CYP2C19 inhibitors was not associated with a significant increase in the incidence of muscle-associated adverse events, assessed using the R/M SMQ

Table 5. Incidence of adverse drug reactions in patients receiving pitavastatin with concomitant medications in the LIVES study, categorized according to the metabolic pathway affected.

Metabolic pathway affected by concomitant medication	Number of reported events, n (%)		p-value for difference in incidence of adverse events
	Patients not receiving concomitant medication	Patients receiving concomitant medication	
CYP isoenzymes			
CYP1A2	2,018 (10.4)	51 (10.9)	0.69
CYP2C19	2,023 (10.4)	46 (9.7)	0.62
CYP2D6	2,040 (10.4)	29 (10.2)	0.92
CYP2C9	2,044 (10.4)	25 (9.9)	0.79
CYP3A4	2,009 (10.3)	60 (11.9)	0.26
CYP2C8	1,987 (10.5)	82 (9.0)	0.15
OATP	1,995 (10.3)	74 (12.8)	0.057
UGT	2,069 (10.4)	0 (0.0)	–

CYP = cytochrome P450; LIVES = LIVALO Effectiveness and Safety; OATP = organic anion-transporting polypeptide; UGT = uridine 5'-diphosphate glucuronosyltransferase.

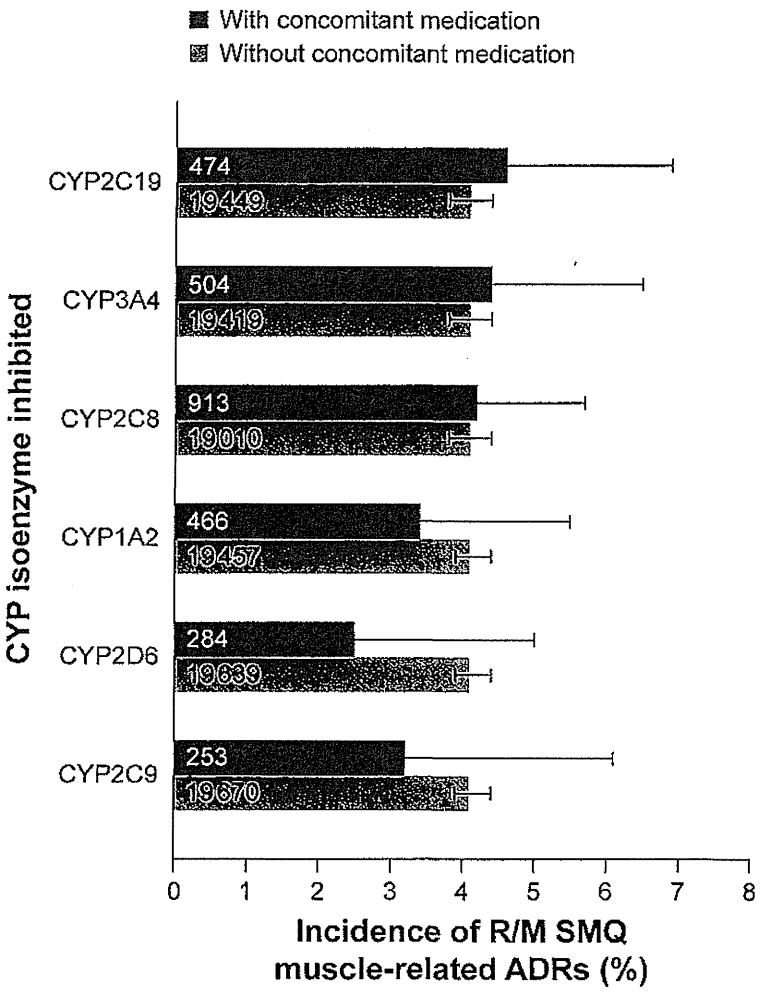

Figure 1. Incidence of R/M SMQ muscle-related ADRs in patients receiving or not receiving concomitant CYP inhibitors in the LIVES study [28]. Bars show mean adverse event incidences and 95% confidence intervals in the total patient population. Patient numbers are indicated inside each bar. No significant differences in ADR incidence were observed between patients receiving concomitant medication and those who were not. ADR = adverse drug reaction; CYP = cytochrome P450; LIVES = LIVALO Effectiveness and Safety; R/M SMQ = Rhabdomyolysis/Myopathy Standard Medical Query.

criteria, at any dose of pitavastatin, and there was no increase in possible drug-related hepatic SMQ adverse events except at the 1 mg dose, where there were few patients (2 out of 15 patients taking omeprazole compared with one out of 200 patients not taking omeprazole) (Table 6). The imbalance between the groups in overall adverse events was accounted for by a larger number of gastrointestinal events reported by patients taking omeprazole. These gastrointestinal events were not accompanied by an increase in other typical statin-associated adverse events, suggesting a confounding effect of the indication for a proton-pump inhibitor.

Concomitant medications that interact with OATPs

LIVES

Concomitant administration of medications with the potential to interact with OATPs in the LIVES study (enalapril, rifampicin, and cyclosporine) did not significantly increase the incidence of ADRs in patients receiving pitavastatin (Table 5). The overall incidence of ADRs was 12.8% for patients who were receiving an OATP-interacting medication and 10.3% for those who were not ($p = 0.057$). Similarly, none of these medications individually increased the incidence of ADRs when administered with pitavastatin ($p > 0.05$ for all comparisons).

Phase 3 clinical trial program

300 of the 2,396 patients in the phase 3 clinical trial program were receiving at least 1 of the following concomitant medications known to be a substrate for, or to inhibit, OATPs: the antimicrobial agents erythromycin, clarithromycin, azithromycin, telithromycin, and rifampicin, and the angiotensin-converting enzyme inhibitor enalapril.

The overall incidence of adverse events was significantly lower among patients receiving pitavastatin (2 mg or 4 mg) who were also receiving a medication that interacts with OATPs than in those who were not receiving such a medication (pitavastatin 2 mg, 21.3% vs. 36.2%, $p = 0.001$; pitavastatin 4 mg, 21.1% vs. 39.3%, $p < 0.001$) (Table 6). There was no significant difference in the incidence of adverse events between patients receiving pitavastatin 1 mg and a concomitant OATP-interacting medication and those not receiving a concomitant medication ($p = 1.00$). Concomitant treatment with an OATP-interacting medication was also not associated with an increased rate of muscle- or hepatic-related adverse events at any dose of pitavastatin.

Of the 300 patients who received a concomitant therapy that interacts with OATPs, only 12 (4.0%) were not receiving enalapril, which is an OATP substrate rather than an

Table 6. Incidence of adverse events in the phase 3 clinical trial program for patients receiving pitavastatin and concomitant medications with the potential to interact with OATPs, CYP2C19, and biguanides.

	Daily pitavastatin dose	Number of reported events n (%)		p-value
		No concomitant medication	Concomitant medication	
Concomitant CYP2C19 inhibitor				
Any AE	1 mg	104 (54.2)	9 (60.0)	0.79
	2 mg	**276 (33.3)**	**14 (66.7)**	**0.004**
	4 mg	**469 (36.3)**	**27 (60.0)**	**0.002**
R/M SMQ AEs	1 mg	4 (2.1)	0 (0.0)	1.00
	2 mg	36 (4.3)	1 (4.8)	0.61
	4 mg	47 (3.6)	2 (4.4)	0.68
Possible drug-related hepatic SMQ AEs	**1 mg**	**1 (0.5)**	**2 (13.3)**	**0.01**
	2 mg	12 (1.4)	0 (0.0)	1.00
	4 mg	9 (0.7)	0 (0.0)	1.00
Concomitant OATP inhibitor				
Any AE	1 mg	106 (54.4)	7 (58.3)	1.00
	2 mg	**264 (36.2)**	**26 (21.3)**	**0.001**
	4 mg	**461 (39.3)**	**35 (21.1)**	**< 0.001**
R/M SMQ AEs	1 mg	4 (2.1)	0 (0.0)	1.00
	2 mg	33 (4.5)	4 (3.3)	0.81
	4 mg	45 (3.8)	4 (2.4)	0.51
Possible drug-related hepatic SMQ AEs	1 mg	2 (1.0)	1 (8.3)	0.16
	2 mg	8 (1.1)	4 (3.3)	0.08
	4 mg	8 (0.7)	1 (0.6)	1.00
Concomitant biguanide				
Any AE	1 mg	110 (53.9)	3 (100.0)	0.25
	2 mg	283 (34.3)	7 (28.0)	0.67
	4 mg	407 (36.6)	89 (39.6)	0.41
R/M SMQ AEs	1 mg	4 (2.0)	0 (0.0)	1.00
	2 mg	36 (4.4)	1 (4.0)	1.00
	4 mg	36 (3.2)	13 (5.8)	0.08
Possible drug-related hepatic SMQ AEs	1 mg	3 (1.5)	0 (0.0)	1.00
	2 mg	12 (1.5)	0 (0.0)	1.00
	4 mg	7 (0.6)	2 (0.9)	0.65

Bold values highlight pitavastatin doses where the incidence of AEs is significantly different between patients receiving concomitant interacting medications and those not receiving interacting medications. AE = adverse event; CYP = cytochrome P450; OATP = organic anion-transporting polypeptide; R/M SMQ = Rhabdomyolysis/Myopathy Standard Medical Query.

inhibitor. Overall, 11 of these patients, who were all taking antimicrobial agents, reported an adverse event. However, only 1 of these was a muscle event included in the R/M SMQ and none was included in the possible drug-related hepatic disorders SMQ, suggesting that there had been confounding by indication.

Concomitant biguanide medications

LIVES

The initial LIVES analysis showed that overall ADRs and maADRs were significantly reduced when patients with diabetes mellitus were treated concomitantly with biguanides and pitavastatin [25]. The present post hoc analysis was performed on 5,133 patients (25.8%) from the LIVES study who had diabetes mellitus, 3,783 (73.7%) of whom were being treated with an antidiabetic agent [27]. The incidence of R/M SMQ ADRs was not significantly different for patients receiving or not receiving concomitant thiazolidine derivatives, α-glucosidase inhibitors, and phenylalanine derivatives (Figure 2). In contrast, the incidence of R/M SMQ ADRs was significantly lower for patients receiving concomitant sulphonylureas or biguanides ($p < 0.05$ and $p < 0.001$, respectively) compared with individuals who were not receiving these classes of medication (Figure 2).

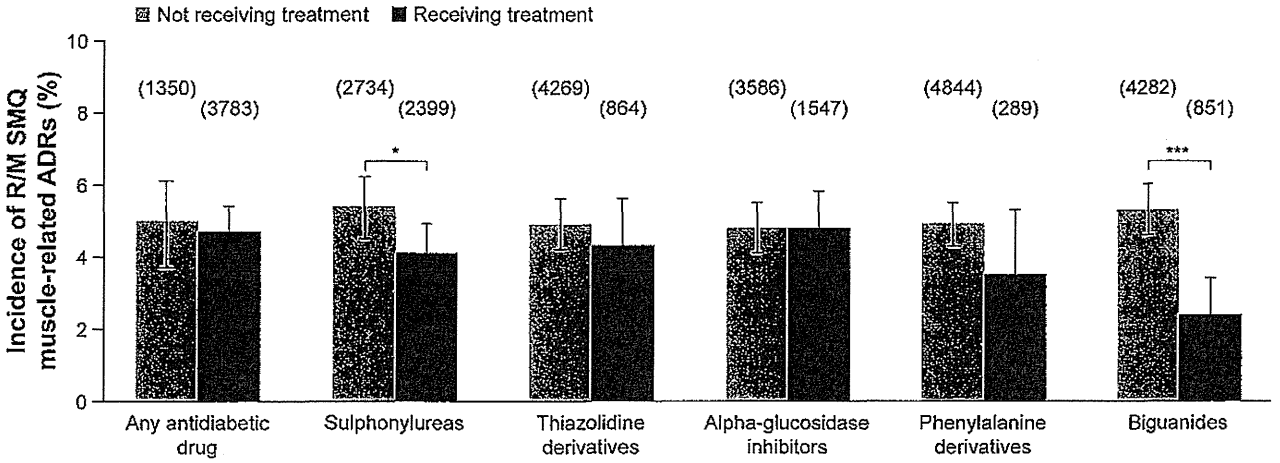

Figure 2. Incidence of R/M SMQ muscle-related ADRs in patients with diabetes mellitus enrolled in the LIVES study. Bars show ADR incidences and 95% confidence intervals in the population of patients with diabetes mellitus. Patient numbers are shown in parentheses. *p < 0.05; ***p < 0.001. ADR = adverse drug reaction; LIVES = LIVALO Effectiveness and Safety; R/M SMQ = Rhabdomyolysis/Myopathy Standard Medical Query.

The incidence of such adverse events was 2.4% in patients receiving pitavastatin and biguanides compared with 5.3% ($p < 0.001$) in those who were not receiving biguanides. The decreased risk in individuals treated with biguanides was irrespective of the level of glycemic control (glycated hemoglobin $< 6.5\%$ vs. $\geq 6.5\%$).

Phase 3 clinical trial program

In total, 253 of the 2,396 patients in the phase 3 clinical trial program were receiving concomitant pitavastatin and biguanide therapy. Of these, 250 patients had a diagnosis of diabetes mellitus.

As shown in Table 6, there were no significant differences in the incidence of adverse events between patients receiving any dose of pitavastatin who were also receiving a biguanide therapy and those who were not. Concomitant treatment with biguanides was also not associated with an increased rate of muscle- or hepatic-related adverse events at any dose of pitavastatin. Similar results were seen for an analysis including only the 413 patients in the phase 3 clinical trial program who had a diagnosis of diabetes mellitus; of these, 250 were receiving biguanide therapy. Of the 3 patients receiving pitavastatin and biguanides who were not diabetic, 1 reported an adverse event, which was not included in the R/M SMQ or the possible drug-related hepatic disorders SMQ.

Discussion

This post hoc analysis of data from the LIVES postmarketing surveillance study and the pitavastatin phase 3 clinical trial program confirms the low potential for DDIs with pitavastatin that was anticipated from its promising pharmacokinetic profile. In the phase 3 program, patients taking drugs known to inhibit the comparator were excluded, so it is not possible to make a comparison of DDI risk. In the LIVES study, only data on ADRs, rather than adverse events, were collected. Nevertheless, concomitant administration of pitavastatin with other medications was not associated with clinically significant increases in the incidence of ADRs typically associated with statins, irrespective of the interacting metabolic pathway (CYP isoenzyme or OATP).

As pitavastatin is not a substrate for CYP2C19, it was surprising that there appeared to be more adverse events overall in patients taking pitavastatin and the CYP2C19 inhibitor omeprazole in the phase 3 program. This was accounted for by more gastrointestinal adverse events, such as dyspepsia, esophagitis, upper abdominal pain, and gastritis, being reported by these patients than by those not taking omeprazole. Although gastrointestinal events may be reported by patients receiving statin alone, the lack of imbalance in muscle- or hepatic-related adverse events suggests that these events are

the result of confounding by the indication for omeprazole rather than due to omeprazole-induced inhibition of CYP2C19 or an omeprazole-pitavastatin DDI.

Importantly, the results of the post hoc analyses in the phase 3 data set are consistent with the analyses in the separate LIVES data set in Japanese patients under more real-life conditions. Furthermore, the use of validated R/M SMQ criteria to determine muscle events confirmed the findings of a low DDI potential for pitavastatin using the maADR criteria applied in the initial LIVES analysis, showing that this is unlikely to be an artefact of the reporting criteria [28].

One of the findings in the original LIVES analysis was that patients taking pitavastatin with biguanides had a reduced risk of muscle ADRs compared with patients not taking them. Although this effect was not seen in the analysis of the phase 3 trial program, perhaps due to the small number of muscle ADRs reported, the present post hoc analysis, confined to patients known to have diabetes, has confirmed this finding. A similar, but less marked, reduction in risk was seen in patients in LIVES taking sulphonylureas. This interesting observation does not seem to have been reported in other statin trials. Although the mechanism is unknown, it has previously been suggested that metformin may be useful in preventing or countering the detrimental effects of statins in muscle [29], which may be due to the contrasting effects of statins and biguanides on mitochondrial function [30]. It appears not to be related to better glycemic control in patients taking these medications.

DDIs in patients receiving statin therapy are an increasing concern for prescribing clinicians. Statins are likely to be among the first drugs prescribed for a patient at risk of cardiovascular disease [11]; it is therefore important to choose an agent with a low potential for DDIs to prevent possible interactions when further drugs are prescribed. The incidence of DDIs in patients aged over 75 years receiving 6 or more medications is almost directly proportional to the number of medications prescribed [31], and studies in a number of clinical settings have shown that a large proportion of patients receiving a statin are coprescribed a potentially interacting therapy [9, 11]. Moreover, approximately half of all patients taking statins stop their therapy within 1 year of starting treatment, potentially leading to an increased risk of individuals experiencing a cardiovascular event. Adherence continues to be an issue during long-term therapy, and adverse events are the most common reason given for discontinuation [32]. The favorable safety profile of pitavastatin might be expected to promote adherence to therapy, thereby facilitating the attainment of lipid targets and hence potentially reducing the risk of cardiovascular events.

Conclusions

It is increasingly recognized that differences in pharmacokinetics among statins influence their potential for DDIs and the consequent risk of an adverse event. Pitavastatin has a distinctive metabolic profile among statins, in that it is partially lipophilic, is a substrate for multiple hepatic influx and efflux transporters, and CYP isoenzymes play only a minor role in its metabolism; thus, the potential for interactions with inhibitors or inducers of these enzymes and transporters is low. Caution is advised when pitavastatin is coadministered with other myotoxic drugs, like fibrates, or with drugs that inhibit multiple hepatic transporters. Use with macrolide antibiotics is therefore not advised, and use with cyclosporine is contraindicated.

Post hoc analysis of adverse events in a real-world, postmarketing surveillance study and a large phase 3 clinical trial program confirm previous pharmacokinetic evidence from healthy volunteers that pitavastatin would be associated with a low incidence of drug-related adverse events in patients receiving concomitant medications. This analysis suggests that pharmacokinetic data in healthy volunteers is predictive of the risk of DDIs in patients and that the different metabolic pathways for different statins is likely to translate into different adverse event profiles in patients in the clinic.

Acknowledgments

The authors take responsibility for the content of the paper and would like to thank

Dr. Nick Leach (Oxford PharmaGenesis Ltd) for providing editorial assistance in developing the manuscript.

Conflicts of interest

All studies in this article were sponsored by Kowa Company, Ltd., or Kowa Research Europe Ltd. Dr Gosho was an employee of Kowa Company, Ltd., from April 2001 to December 2012. Mr Tanahashi is an employee of Kowa Company, Ltd., and Dr Hounslow is an employee of Kowa Research Europe Ltd. Dr Teramoto has received honoraria as a speaker's fee from Kowa Company, Ltd., and Kowa Pharmaceutical Company Ltd.

References

[1] *Baigent C, Blackwell L, Emberson J, Holland LE, Reith C, Bhala N, Peto R, Barnes EH, Keech A, Simes J, Collins R; Cholesterol Treatment Trialists' (CTT) Collaboration.* Efficacy and safety of more intensive lowering of LDL cholesterol: a meta-analysis of data from 170,000 participants in 26 randomised trials. Lancet. 2010; *376:* 1670-1681.

[2] *Mihaylova B, Emberson J, Blackwell L, Keech A, Simes J, Barnes EH, Voysey M, Gray A, Collins R, Baigent C; Cholesterol Treatment Trialists' (CTT) Collaborators.* The effects of lowering LDL cholesterol with statin therapy in people at low risk of vascular disease: meta-analysis of individual data from 27 randomised trials. Lancet. 2012; *380:* 581-590.

[3] *National Center for Health Statistics.* Health, United States, 2010: With special feature on death and dying. Available from: http://www.cdc.gov/nchs/hus/hus10.pdf. Accessed 5 November 2013.

[4] *Ebrahim S, Casas JP.* Statins for all by the age of 50 years? Lancet. 2012; *380:* 545-547.

[5] *Stone NJ, Robinson JG, Lichtenstein AH, Bairey Merz CN, Blum CB, Eckel RH, Goldberg AC, Gordon D, Levy D, Lloyd-Jones DM, McBride P, Schwartz JS, Shero ST, Smith SC Jr, Watson K, Wilson PW, Eddleman KM, Jarrett NM, LaBresh K, Nevo L, et al; American College of Cardiology/American Heart Association Task Force on Practice Guidelines.* 2013 ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. Circulation. 2014; *129 (25 Suppl 2):* S1-S45.

[6] *Yamashita S, Tsubakio-Yamamoto K, Ohama T, Nakagawa-Toyama Y, Nishida M.* Molecular mechanisms of HDL-cholesterol elevation by statins and its effects on HDL functions. J Atheroscler Thromb. 2010; *17:* 436-451.

[7] *Davignon J.* Pleiotropic effects of pitavastatin. Br J Clin Pharmacol. 2012; *73:* 518-535.

[8] *Perk J, De Backer G, Gohlke H, Graham I, Reiner Z, Verschuren WM, Albus C, Benlian P, Boysen G, Cifkova R, Deaton C, Ebrahim S, Fisher M, Germano G, Hobbs R, Hoes A, Karadeniz S, Mezzani A, Prescott E, Ryden L, et al; Fifth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice; European Association for Cardiovascular Prevention and Rehabilitation.* European Guidelines on cardiovascular disease prevention in clinical practice (version 2012): The Fifth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of nine societies and by invited experts). Atherosclerosis. 2012; *223:* 1-68.

[9] *Linton A, Garber M, Fagan NK, Peterson MR.* Examination of multiple medication use among TRICARE beneficiaries aged 65 years and older. J Manag Care Pharm. 2007; *13:* 155-162.

[10] *Catapano AL.* Statin-induced myotoxicity: pharmacokinetic differences among statins and the risk of rhabdomyolysis, with particular reference to pitavastatin. Curr Vasc Pharmacol. 2012; *10:* 257-267.

[11] *Corsini A, Ceska R.* Drug-drug interactions with statins: will pitavastatin overcome the statins' Achilles' heel? Curr Med Res Opin. 2011; *27:* 1551-1562.

[12] *Elsby R, Hilgendorf C, Fenner K.* Understanding the critical disposition pathways of statins to assess drug-drug interaction risk during drug development: it's not just about OATP1B1. Clin Pharmacol Ther. 2012; *92:* 584-598.

[13] *Law M, Rudnicka AR.* Statin safety: a systematic review. Am J Cardiol. 2006; *97 (8A):* 52C-60C.

[14] *Rowan C, Brinker AD, Nourjah P, Chang J, Mosholder A, Barrett JS, Avigan M.* Rhabdomyolysis reports show interaction between simvastatin and CYP3A4 inhibitors. Pharmacoepidemiol Drug Saf. 2009; *18:* 301-309.

[15] *Corsini A, Bellosta S.* Drug-drug interaction with statins. Expert Rev Clin Pharmacol. 2008; *1:* 105-113.

[16] *Custodio JM, Wang H, Hao J, Lepist EI, Ray AS, Andrews J, Ling KH, Cheng A, Kearney BP, Ramanathan S.* Pharmacokinetics of cobicistat boosted-elvitegravir administered in combination with rosuvastatin. J Clin Pharmacol. 2014; *54:* 649-656.

[17] *Fujino H, Yamada I, Shimada S, Nagao T, Yoneda M.* Metabolic fate of pitavastatin (NK-104), a new inhibitor of 3-hydroxy-3-methyl-glutaryl coenzyme A reductase. Effects on drug-metabolizing systems in rats and humans. Arzneimittelforschung. 2002; *52:* 745-753.

[18] *Fujino H, Yamada I, Shimada S, Yoneda M, Kojima J.* Metabolic fate of pitavastatin, a new inhibitor of HMG-CoA reductase: human UDP-glucuronosyltransferase enzymes involved in lactonization. Xenobiotica. 2003; *33:* 27-41.

[19] *Hirano M, Maeda K, Matsushima S, Nozaki Y, Kusuhara H, Sugiyama Y.* Involvement of BCRP (ABCG2) in the biliary excretion of pitavastatin. Mol Pharmacol. 2005; *68:* 800-807.

[20] *Ose L, Budinski D, Hounslow N, Arneson V.* Comparison of pitavastatin with simvastatin in primary

hypercholesterolaemia or combined dyslipidaemia. Curr Med Res Opin. 2009; *25:* 2755-2764.

[21] *Budinski D, Arneson V, Hounslow N, Gratsiansky N.* Pitavastatin compared with atorvastatin in primary hypercholesterolemia or combined dyslipidemia. Clin Lipidol. 2009; *4:* 291-302.

[22] *Eriksson M, Budinski D, Hounslow N.* Comparative efficacy of pitavastatin and simvastatin in high-risk patients: a randomized controlled trial. Adv Ther. 2011; *28:* 811-823.

[23] *Gumprecht J, Gosho M, Budinski D, Hounslow N.* Comparative long-term efficacy and tolerability of pitavastatin 4 mg and atorvastatin 20-40 mg in patients with type 2 diabetes mellitus and combined (mixed) dyslipidaemia. Diabetes Obes Metab. 2011; *13:* 1047-1055.

[24] *Stender S, Budinski D, Gosho M, Hounslow N.* Pitavastatin shows greater lipid-lowering efficacy over 12 weeks than pravastatin in elderly patients with primary hypercholesterolaemia or combined (mixed) dyslipidaemia. Eur J Prev Cardiol. 2013; *20:* 40-53.

[25] *Kurihara Y, Douzono T, Kawakita K, Nagasaka Y.* A large-scale, long-term, prospective post-marketing surveillance of pitavastatin (LIVALO Tablet): LIVALO Effectiveness and Safety (LIVES) study. Jpn Pharmacol Ther. 2008; *36:* 709-731.

[26] *Teramoto TU, Shimano H, Yokote K, Saito Y; LIVES Study Extension Group.* A large-scale survey on cardio-cerebrovascular events during pitavastatin (LIVALO tablet) therapy in Japanese patients with hypercholesterolemia - LIVALO Effectiveness and Safety Study Extension (LIVES Study Extension). Jpn Pharmacol Ther. 2011; *39:* 789-803.

[27] *Hounslow N, Teramoto T.* Pitavastatin is associated with a low risk of adverse reactions when co-administered with biguanides in patients with diabetes mellitus: 2-year data. Presented at the 9th International Conference on Coronary Artery Disease, 23–26 October 2011, Venice, Italy.

[28] *Teramoto T.* Pitavastatin co-administration with CYP450 inhibitors does not increase the incidence of muscle-related adverse drug reactions: 2-year data from 19 925 patients in the LIVES observational study. Presented at the XVI International Symposium on Atherosclerosis, 25–29 March 2012, Sydney, Australia.

[29] *Hanai J, Cao P, Tanksale P, Imamura S, Koshimizu E, Zhao J, Kishi S, Yamashita M, Phillips PS, Sukhatme VP, Lecker SH.* The muscle-specific ubiquitin ligase atrogin-1/MAFbx mediates statin-induced muscle toxicity. J Clin Invest. 2007; *117:* 3940-3951.

[30] *Finsterer J, Segall L.* Drugs interfering with mitochondrial disorders. Drug Chem Toxicol. 2010; *33:* 138-151.

[31] *Schuler J, Dückelmann C, Beindl W, Prinz E, Michalski T, Pichler M.* Polypharmacy and inappropriate prescribing in elderly internal-medicine patients in Austria. Wien Klin Wochenschr. 2008; *120:* 733-741.

[32] *Maningat P, Gordon BR, Breslow JL.* How do we improve patient compliance and adherence to long-term statin therapy? Curr Atheroscler Rep. 2013; *15:* 291.

J Epidemiol 2015;25(7):489-495
doi:10.2188/jea.JE20140160

Original Article

Changes in Waist Circumference and the Incidence of Type 2 Diabetes in Community-Dwelling Men and Women: The Suita Study

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Received August 21, 2014; accepted February 1, 2015; released online May 23, 2015

ABSTRACT

Backgrounds: The association between weight gain and the incidence of type 2 diabetes is well known. The aim of our study was to investigate the relationship between change in waist circumference (WC) and type 2 diabetes incidence.

Methods: The participants in the Suita Study, a population-based cohort study in an urban area of Japan, underwent a baseline survey between 1989 and 1994 (Exam 1) and were examined at follow-up every 2 years. We performed a 9.3-year cohort study of 946 men and 1327 women with no history of diabetes who underwent Exam 1 and Exam 2 (between 1997 and 1999). Participants were stratified by sex and median WC at Exam 1, and, in each stratum, participants were further classified into three categories by tertile of WC change per year between Exam 1 and Exam 2. Hazard ratios (HRs) and 95% confidence intervals (CIs) for type 2 diabetes incidence were calculated by Cox proportional hazard models. The endpoints were first diagnosis of type 2 diabetes or March 2011.

Results: During follow-up, 287 participants developed type 2 diabetes. In both sexes with median WC or higher, participants in the highest tertile of WC change had a significantly higher risk of developing type 2 diabetes. Multivariable adjusted HRs were 1.84 (95% CI, 1.10–3.08) in men and 2.30 (95% CI, 1.31–4.04) in women. No significant association was observed among participants with WC below median.

Conclusions: Preventing WC gain is important in preventing type 2 diabetes in the Japanese population, especially among individuals with a relatively high WC.

Key words: waist circumference; type 2 diabetes mellitus; prospective cohort study

INTRODUCTION

The worldwide prevalence of type 2 diabetes is alarmingly high. The International Diabetes Federation (IDF) has reported that the global prevalence of diabetes has reached 8.3% (382 million people), and that the prevalence will be 10% by 2035.[1] In particular, of IDF regions, the Western pacific region, which includes China, Indonesia, and Japan, has a high prevalence of diabetes (8.6%) and is home to 36% of the total number of people with diabetes in the world.[2] At the same time, the prevalence of obesity is escalating worldwide. The mean body mass index (BMI) worldwide has increased by 0.4 kg/m^2 per decade in men and 0.5 kg/m^2 per decade in women.[3] Although the prevalence of obesity or overweight in Asia is relatively low compared with other parts of the world, the drastic increase in BMI in Asia is similar to that in other regions. Many studies have reported significant associations between weight gain and the incidence of type 2 diabetes.[4–10] Therefore, it is anticipated that this increase in obesity will lead to increased rates of type 2 diabetes.

It is well known that higher waist circumference (WC), as well as higher BMI, is associated with elevated risks of type 2

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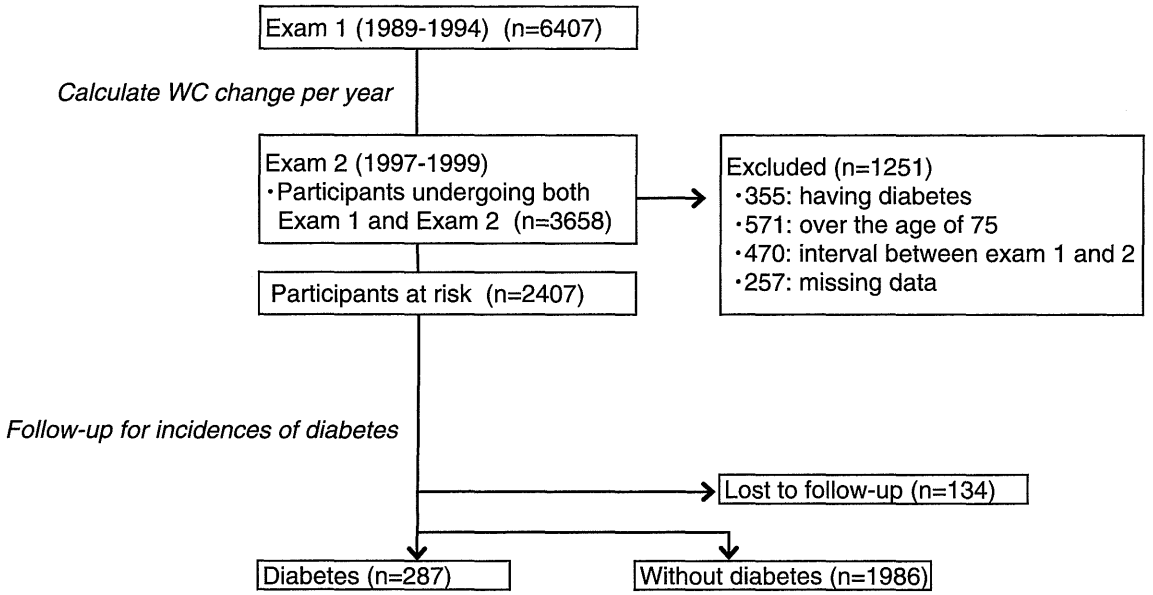

Figure 1. Process of selecting the study participants and overview of analysis of waist circumference (WC) change and the incidence of type 2 diabetes (shown in italics).

diabetes.[11] WC, an index for central obesity, is an important component in the diagnostic criteria for metabolic syndrome.[12,13] Because WC changes over the years within the same individual, in addition to assessment of the risk of type 2 diabetes based on WC at a certain point, it would be also useful to consider subsequent WC change. However, there have been just two studies on the association between WC change with the risk of type 2 diabetes, which were conducted among Iranian community residents and American health professionals.[7,14] However, percentage of visceral adipose tissue in abdominal fat is likely to differ according to race, so the impact of change in WC on type 2 diabetes could also differ from country to country.[15] This association remains unknown among the East Asians, who have a relatively low degree of obesity. In addition, it has recently also been reported that WC change does not necessarily correspond to weight change in a Chinese population.[16]

Accordingly, the purpose of this study is to investigate the association between WC change and the incidence of type 2 diabetes in an urban Japanese population, taking into consideration the influence of BMI change.

METHODS

Subjects and design

The Suita Study, a prospective population-based cohort study in an urban area of Japan, started in 1989. The details of this study have been described elsewhere.[17–19] Briefly, 6407 men and women aged 30–83 years underwent a baseline survey at the National Cerebral and Cardiovascular Center (NCVC) between September 1989 and March 1994 (examination 1 [Exam 1]) and visited the NCVC every 2 years for follow-up examinations, including blood sample testing. Of 6407 participants, 3658 underwent the follow-up examination between April 1997 and March 1999 (examination 2 [Exam 2]). Overall, 1251 participants were excluded for the following reasons: (i) having diabetes at Exam 2 ($n = 355$); (ii) age >75 years at Exam 2 ($n = 571$); (iii) the interval between Exam 1 and Exam 2 was <5 years or >9 years ($n = 470$); or (iv) missing data ($n = 257$). In addition, participants who could not be followed-up ($n = 134$) were excluded. The remaining 2273 participants were followed up from Exam 2 to the end of March 2011 (Figure 1). The Institutional Review Board of the NCVC approved this cohort study.

Data collection

Blood samples were centrifuged immediately upon collection, and a routine blood examination was performed, which included measurement of glucose levels. The Suita Study started to measure HbA_{1c} from Exam 2. The value for HbA_{1c} (%) was estimated as the National Glycohemoglobin Standardization Program equivalent value (%) calculated by the following formula: HbA_{1c} (%) = 1.02 × HbA_{1c} (Japan Diabetes Society, %) + 0.25%.[20] HbA_{1c} values are presented as percentages and SI units (mmol/mol).

Trained physicians measured blood pressure in triplicate on the right arm after 5 minutes of rest using a standard mercury sphygmomanometer. WC was measured at the umbilical level in a standing position. Participants were wearing light clothing during measurement of height and weight. BMI was calculated as weight (kg) divided by the square of height (m). Public health nurses obtained information on cigarette smoking status (current-smoker, ex-smoker, or non-smoker), alcohol drinking status (current-drinker, ex-drinker, or non-drinker) and medical histories.

Endpoint determination

Type 2 diabetes was defined as either a fasting (at least 8 hours) plasma glucose level ≥7.0 mmol/L (126 mg/dL), non-fasting plasma glucose level ≥11.1 mmol/L (200 mg/dL), HbA_{1c} ≥6.5% (48 mmol/mol),[21] or the use of antidiabetic agents. The endpoints of the present study were: (i) first diagnosis of type 2 diabetes, or (ii) March 31, 2011. Individuals not examined during follow-up were censored on the date of their last examination.

Statistical analysis

Participants were stratified by sex and median WC at Exam 1 and were additionally classified into three categories by tertile of WC change per year between Exam 1 and Exam 2. We calculated age-adjusted WC at Exam 1, Exam 2, and endpoint by sex. In addition, we assessed the correlation of changes in WC and BMI per year between Exam 1 and Exam 2.

Cox proportional hazards regression was used to estimate the adjusted hazard ratios (HRs) and 95% confidence intervals (CIs) for the lowest and highest tertiles, with the middle tertile as the reference group. Model 1 was adjusted for age; model 2 was adjusted for age and WC at Exam 1, HbA_{1c}, family history of diabetes, and drinking and smoking status at Exam 2; and model 3 was adjusted for model 2 variables, BMI at Exam 1, and BMI change as a continuous variable. In an additional model, HRs and 95% CIs were adjusted for model 2 variables and estimated without stratification by WC at Exam 1, with the participants falling into both the WC below median and the tertile 2 in WC change groups being set as the reference group. Interactions between WC at Exam 1 (below median or ≥median) and WC change (tertiles) were tested by adding the interaction term to the model 2. All data were analyzed using SPSS statistical software (Version 20.0J; SPSS Japan Inc., Tokyo, Japan). All reported *P*-values are two-tailed; $P < 0.05$ was considered statistically significant.

RESULTS

The median (interquartile range) WCs at Exam 1 were 82.0 (77.0–87.0) cm in men and 75.0 (69.0–82.0) cm in women. The mean (standard deviation) interval between Exam 1 and Exam 2 was 6.8 (0.9) years. Table 1 shows characteristics at Exam 2 for men and women. WC change was considerably larger in women than men (0.51 and 0.17 cm/year, respectively). Table 2 shows age-adjusted WC at Exam 1, Exam 2, and at the endpoint examination. In the lowest tertile of WC change, WC increased from Exam 2 to the endpoint examination, regardless of sex and WC strata (WC <median or WC ≥median). Conversely, the change in WC from Exam 2 to endpoint examination in the highest tertile was stable. Figure 2 shows scatter plots of WC change and BMI change between Exam 1 and Exam 2. The correlation coefficients

Table 1. Characteristics of subjects at examination 2 (*n* = 2273)

	Men	Women
n	946	1327
Age, years	58.8 (10.2)	57.6 (9.7)
WC	83.5 (7.9)	79.7 (8.6)
WC change, cm/year	0.17 (0.74)	0.51 (1.05)
BMI, kg/m^2	23.2 (2.8)	22.2 (2.9)
HbA_{1c}, %, mmol/mol	5.6 (0.3), 38 (3.3)	5.6 (0.3), 38 (3.3)
Plasma glucose level, mmol/L	5.3 (0.5)	5.1 (0.5)
Systolic blood pressure, mm Hg	126.4 (18.0)	125.3 (18.9)
Diastolic blood pressure, mm Hg	80.8 (10.7)	78.6 (10.5)
Hypertension, *n* (%)	341 (36.0)	406 (30.6)
Total cholesterol, mmol/L	5.26 (0.81)	5.60 (0.85)
Hypercholesterolemia, *n* (%)	302 (31.9)	644 (48.5)
Family history of diabetes, *n* (%)	90 (9.5)	148 (11.2)
Smoking status, *n* (%)		
Current	372 (39.3)	100 (7.5)
Former	296 (31.3)	47 (3.5)
Never	278 (29.4)	1180 (88.9)
Drinking status, *n* (%)		
Current	690 (72.9)	411 (31.0)
Former	23 (2.4)	9 (0.7)
Never	233 (24.6)	907 (68.3)

BMI, body mass index; WC, waist circumference.
Continuous data are shown as mean (standard deviation).

with a WC below the median and at the median or higher were 0.72 and 0.71 in men, respectively, and 0.39 and 0.38 in women, respectively (all $P < 0.001$).

During the follow-up periods (mean 9.3 [3.5] years), 287 participants developed type 2 diabetes (Figure 1). Table 3 shows multivariable adjusted HRs for incidences of type 2 diabetes according to WC change tertile. Among participants with the median WC or higher, the highest tertile had significantly higher risk for the incidence of type 2 diabetes in both sexes in model 2 (HR 1.84; 95% CI, 1.10–3.08 in men and HR 2.30; 95% CI, 1.31–4.04 in women). The lowest tertile did not have a significantly lower risk for the incidence of type 2 diabetes in either sex (HR 1.27; 95% CI, 0.73–2.21 in men and HR 1.13; 95% CI, 0.59–2.18 in women). Among participants with WC below the median, there was no significant association between WC change and the incidence of type 2 diabetes in either sex. Interactions between WC at Exam 1 (below median or ≥median) and WC change (tertiles) was not significant in men ($P = 0.395$) but was significant in women ($P = 0.011$).

Even after adjustment of BMI at Exam 1 and BMI change (model 3), these results did not change much, although the HR of the highest tertile of WC change among men with WC median or higher was borderline significant (HR 1.72; 95% CI, 0.98–3.02). In model 3, the HRs for BMI change (per 1.0 kg/m^2/year) were 2.11 (95% CI, 0.49–9.16) in men with the median WC or higher and the highest tertile of WC change and 0.93 (95% CI, 0.31–2.82) in women. In the additional model, HRs of the highest tertile of WC change significantly increased among both men and women with the median WC at Exam 1 or higher.

Table 2. Waist circumference and BMI adjusted by age at Exam 1, Exam 2, and endpoint examination (n = 2273)

	WC change		
	tertile 1	tertile 2	tertile 3
Men with WC <median[a]			
Range of WC change, cm/year	−2.556 to 0.000	0.117 to 0.552	0.558 to 2.648
Age at examination 2	59.2 (10.6)	57.0 (10.0)	57.1 (10.6)
WC, Exam 1/Exam 2/endpoint	76.9/74.2/77.6	76.3/78.4/80.2	75.9/82.9/83.9
BMI, Exam 1/Exam 2/endpoint	20.9/20.5/20.8	21.3/21.7/21.7	21.2/22.6/22.7
Men with WC ≥median[a]			
Range of WC change, cm/year	−3.598 to −0.265	−0.263 to 0.296	0.304 to 2.454
Age at examination 2	60.9 (10.4)	60.0 (8.8)	58.2 (10.1)
WC, Exam 1/Exam 2/endpoint	90.1/84.7/87.4	88.3/88.6/89.4	87.3/92.1/92.6
BMI, Exam 1/Exam 2/endpoint	24.8/23.8/23.8	24.6/24.8/24.6	24.7/25.8/25.7
Women with WC <median[a]			
Range of WC change, cm/year	−2.023 to 0.506	0.509 to 1.378	1.380 to 3.820
Age at examination 2	53.5 (9.8)	55.0 (10.3)	56.9 (8.9)
WC, Exam 1/Exam 2/endpoint	69.5/69.3/73.3	67.7/74.4/77.3	67.5/81.7/81.7
BMI, Exam 1/Exam 2/endpoint	19.7/20.3/21.2	19.6/20.7/22.2	19.7/20.8/21.9
Women with WC ≥median[a]			
Range of WC change, cm/year	−2.363 to −0.341	−0.334 to 0.463	0.472 to 3.160
Age at examination 2	60.0 (9.6)	59.5 (9.3)	60.1 (8.7)
WC, Exam 1/Exam 2/endpoint	85.3/79.4/82.9	83.4/83.8/84.8	80.8/88.4/88.5
BMI, Exam 1/Exam 2/endpoint	23.0/23.4/23.9	22.5/23.5/24.6	22.6/23.1/24.2

BMI, body mass index; WC, waist circumference.
[a]Medians of waist circumference at examination 1 were 82.0 cm in men and 75.0 cm in women. Ages are shown as mean (standard deviation).

Table 3. Multivariable adjusted hazard ratios for the incidence of type 2 diabetes according to change in waist circumference (n = 2273)

	Cases/n	IR[a]	HRs (95% CIs)			
			Model 1	Model 2	Model 3	Additional model
Men with WC <median[b]						
tertile 1	25/169	16.6	1.06 (0.59–1.90)	1.19 (0.66–2.16)	1.01 (0.52–1.96)	1.25 (0.69–2.25)
tertile 2	21/148	15.5	ref	ref	ref	ref
tertile 3	32/158	22.3	1.44 (0.83–2.49)	1.25 (0.71–2.22)	1.34 (0.73–2.46)	1.36 (0.77–2.38)
Men with WC ≥median[b]						
tertile 1	29/157	20.0	1.16 (0.68–1.99)	1.27 (0.73–2.21)	1.40 (0.75–2.58)	1.50 (0.84–2.68)
tertile 2	24/157	16.8	ref	ref	ref	1.18 (0.65–2.15)
tertile 3	39/157	29.3	1.80 (1.08–3.01)	1.84 (1.10–3.08)	1.72 (0.98–3.02)	2.22 (1.28–3.83)
Women with WC <median[b]						
tertile 1	16/213	7.9	1.70 (0.77–3.75)	1.38 (0.60–3.20)	1.98 (0.80–4.91)	1.67 (0.75–3.69)
tertile 2	10/213	4.7	ref	ref	ref	ref
tertile 3	10/213	4.7	0.93 (0.39–2.25)	0.70 (0.28–1.74)	0.52 (0.20–1.38)	0.83 (0.35–2.23)
Women with WC ≥median[b]						
tertile 1	19/229	8.6	0.90 (0.48–1.69)	1.13 (0.59–2.18)	1.24 (0.63–2.44)	1.56 (0.72–3.39)
tertile 2	20/229	9.4	ref	ref	ref	1.24 (0.57–2.69)
tertile 3	42/230	19.9	2.14 (1.26–3.65)	2.30 (1.31–4.04)	2.07 (1.13–3.79)	2.45 (1.22–4.94)

CI, confidence interval; HR, hazard ratio; Ref, reference group; WC, waist circumference.
Model 1: Adjusted by age; Model 2: Adjusted by age, HbA1c, family history of diabetes, smoking and drinking status at examination 2, and WC at examination 1; Model 3: Adjusted by model 2 variables, body mass index at examination 1, and change in body mass index as continuous variables; Additional Model: Without stratification by median of WC at exam 1 and adjusted by model 2 variables except for WC at examination 1;
[a]Incidence rates/1000 person-years; [b]Medians of waist circumference were 82.0 cm in men and 75.0 cm in women at examination 1.

DISCUSSION

The present study demonstrates that, among participants with relatively high WC and regardless of sex, WC gain for 5–9 years was significantly associated with an elevated risk of incidence of type 2 diabetes for almost 10 years following WC gain, after adjustment for baseline HbA_{1c}. On the other hand, WC loss was not associated with a decreased risk of incidence of type 2 diabetes. No significant association between WC change and the incidence of type 2 diabetes was observed among individuals with relatively low WC.

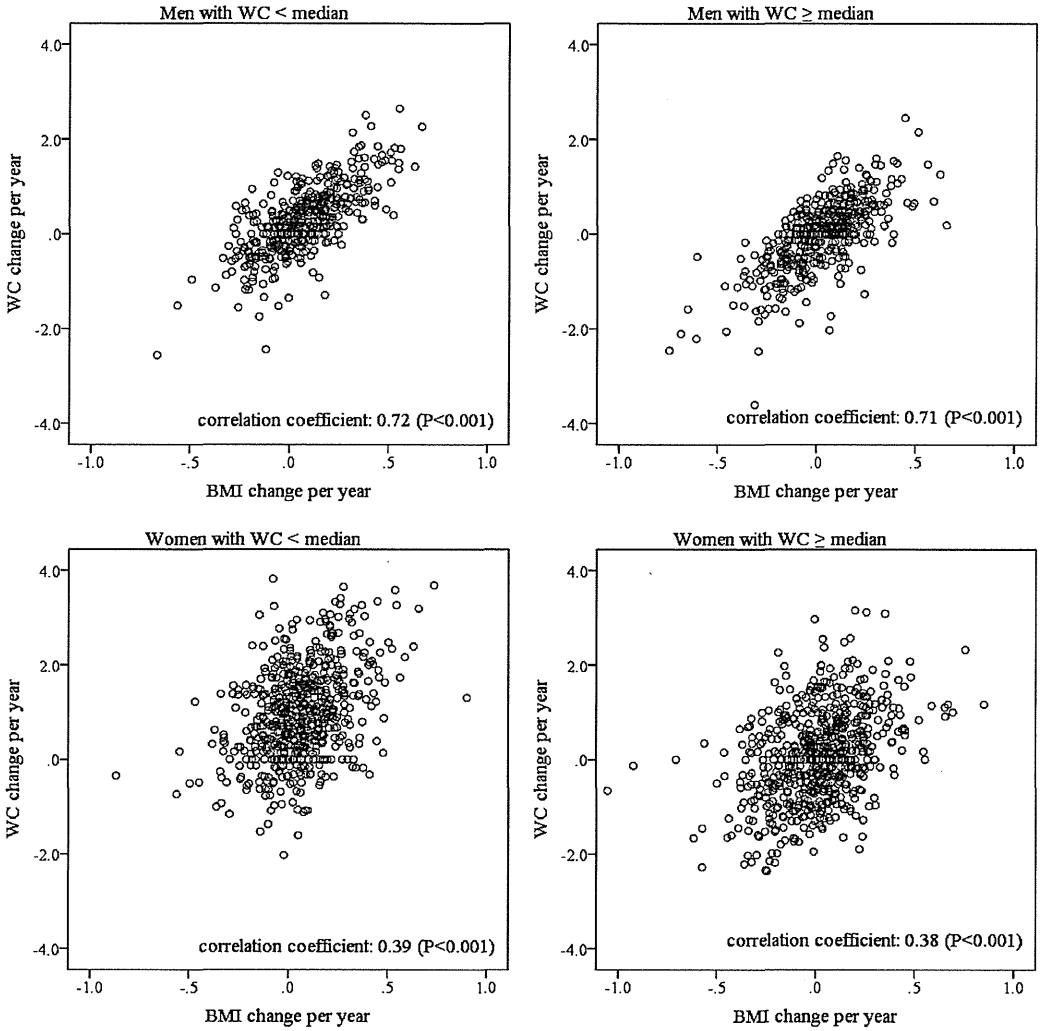

Figure 2. Scatter plots of waist circumference (WC) change and body mass index (BMI) change between examination 1 and examination 2 by sex and WC.

To our knowledge, only two studies have reported the association between WC change and type 2 diabetes. Hadaegh et al reported that WC change during 6 years of follow-up was positively associated with the elevated risk of type 2 diabetes among 4029 community residents in Iran.[14] Odds ratios were 1.6 in men and 1.5 in women per 1 standard deviation increase in WC change (5.2 cm in men and 7.7 cm in women). Koh-Banerjee et al assessed the influence of WC change in 9 years on type 2 diabetes incidences for 4 years after WC change among 22 171 male health professionals in the United States.[7] They demonstrated that men with WC gain ≥14.6 cm (1.6 cm/year) had a higher risk of developing type 2 diabetes than men who had a stable WC (relative risk 2.4; 95% CI, 1.5–3.7). Participants in these studies had higher average WC than those in the present study.[14,22] Because we also observed results similar to previous studies among participants with relatively high WC, the present results would be likely to support the previous ones, although it is difficult to compare the results between the studies because of different observation periods for WC change and ethnicities of participants.

The additional model using the six combined categories of initial WC and subsequent WC change also showed similar results. Compared to individuals with relatively low initial WC levels and subsequent mild WC gain (tertile 2), regardless of sex, WC gain was significantly associated with increased risk of type 2 diabetes only among individuals with relatively high WC levels, while no significant association was observed in other categories. This indirectly suggests that it might be important to combine WC at a certain point and subsequent WC change for estimation of risk of diabetes.

However, an observational study has reported that WC loss was not associated with a decreased risk of type 2 diabetes incidence.[7] In the present study, the effect of WC loss among participants with relatively high WC was not observed, although it is not clear whether WC loss was caused by participants' effort, such as lifestyle changes. Among individuals in the lowest tertile whose WC decreased from Exam 1 to Exam 2 (a 5.4-cm decrease in men with relatively high WC and a 5.9-cm decrease women with relatively high WC), WC increased after Exam 2 (a 2.7-cm increase in men

with a relatively high WC and a 2.5-cm increase in women with relatively high WC) (Table 2). In other words, WC showed a U-shaped change from Exam 1 to the endpoint examination, and it can be inferred that the risk of type 2 diabetes might not decrease merely because of WC gain after Exam 2.

Koh-Banerjee et al reported that men with WC gain had a higher risk for type 2 diabetes incidence after adjustment for weight change.[7] Similarly, in the present study, WC change was associated with the incidence of type 2 diabetes almost independently of BMI change among both men and women with relatively high WC levels, although this relationship was more evident in women. On the other hand, BMI change was not associated at all. The present study suggests that WC change should be considered prior to weight change to estimate the risk of type 2 diabetes, especially in Japanese women. In addition, the results of the correlation analyses showed that WC change was much more strongly correlated with BMI change in men than women. This difference in correlation could be involved in the sex difference. Because body fat distribution differs considerably between age groups, sexes, and ethnicities,[23,24] it would be necessary to take these factors into consideration when combining WC and BMI to estimate the risk of type 2 diabetes.

The present study has several limitations. First, 62.0% of participants did not undergo Exam 2 under fasting conditions (≥8 hours), although almost all underwent Exam 1 under fasting conditions. However, there was no significant difference in fasting status among the tertiles of WC change regardless of sex and WC strata, and the time after a meal was ≥5 hours in 87% of participants. Such random misclassifications by measurement error might lead to underestimation of the real relationship (toward the null) between WC changes and the risk of diabetes. Second, the correlation coefficients between change in WC and BMI were high in men (0.71–0.72), so the presence of co-linearity might influence the adjusted HRs in model 3 of Cox proportional hazards regression. However, since the HRs did not change much after adjustment for BMI changes, we think the influence of co-linearity was likely to be limited. Third, single assessment of WC change may lead to underestimation of the relationship between WC change and type 2 diabetes incidences due to regression dilution bias.[25]

In conclusion, WC gain was significantly related to an increased risk of type 2 diabetes in both sexes with a higher WC. In terms of diabetes prevention, it is important to avoid WC gain, especially among men and women with relatively high WC. In addition, assessing WC change may be important than assessing BMI change in estimating the risk of type 2 diabetes, especially in the Japanese women.

ONLINE ONLY MATERIAL

Abstract in Japanese.

ACKNOWLEDGEMENTS

The present study was supported by the Intramural Research Fund of the National Cerebral and Cardiovascular Center (22-4-5), a grant-in-aid from the Ministry of Health, Labour and Welfare (H26–Junkankitou [Seisaku]–Ippan–001 and H25–Junkankitou [Seisyu]–Ippan–005), and a grant-in-aid for scientific research (C) from the Japan Society for the Promotion of Science (Grant number: 24590837). We sincerely appreciate the members of the Suita Medical Foundation and the Suita City Health Center. We thank all researchers and co-medical staff in the Department of Preventive Cardiology, the National Cerebral and Cardiovascular Center, for their excellent medical examinations and follow-up surveys. We also thank the Satsuki-Junyukai, the society members of the Suita study.

Conflicts of interest: None declared.

REFERENCES

1. International Diabetes Federation. IDF Diabetes Atlas 6th ed. [cited 2014 April 17]. Available from: http://www.idf.org/diabetesatlas/data-visualisations.
2. International Diabetes Federation. IDF Diabetes Atlas 6th ed. [cited 2014 July 13]. Available from: http://www.idf.org/sites/default/files/EN_6E_Ch3_Regional_Overviews.pdf.
3. Finucane MM, Stevens GA, Cowan MJ, Danaei G, Lin JK, Paciorek CJ, et al; Global Burden of Metabolic Risk Factors of Chronic Diseases Collaborating Group (Body Mass Index). National, regional, and global trends in body-mass index since 1980: systematic analysis of health examination surveys and epidemiological studies with 960 country-years and 9.1 million participants. Lancet. 2011;377:557–67.
4. Ford ES, Williamson DF, Liu S. Weight change and diabetes incidence: findings from a national cohort of US adults. Am J Epidemiol. 1997;146:214–22.
5. Wannamethee SG, Shaper AG. Weight change and duration of overweight and obesity in the incidence of type 2 diabetes. Diabetes Care. 1999;22:1266–72.
6. Resnick HE, Valsania P, Halter JB, Lin X. Relation of weight gain and weight loss on subsequent diabetes risk in overweight adults. J Epidemiol Community Health. 2000;54:596–602.
7. Koh-Banerjee P, Wang Y, Hu FB, Spiegelman D, Willett WC, Rimm EB. Changes in body weight and body fat distribution as risk factors for clinical diabetes in US men. Am J Epidemiol. 2004;159:1150–9.
8. Wannamethee SG, Shaper AG, Walker M. Overweight and obesity and weight change in middle aged men: impact on cardiovascular disease and diabetes. J Epidemiol Community Health. 2005;59:134–9.
9. Mishra GD, Carrigan G, Brown WJ, Barnett AG, Dobson AJ. Short-term weight change and the incidence of diabetes in midlife: results from the Australian Longitudinal Study on Women's Health. Diabetes Care. 2007;30:1418–24.
10. Jacobs-van der Bruggen MA, Spijkerman A, van Baal PH, Baan CA, Feskens EJ, Picavet HS, et al. Weight change and incident diabetes: addressing an unresolved issue. Am J Epidemiol.