行政院國家科學委員會補助專題研究計畫成果報告

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Long-Term Arsenic Exposure and Incidence of Non–Insulin-Dependent Diabetes Mellitus: A Cohort Study in Arseniasis-Hyperendemic Villages in Taiwan

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Diabetes prevalence in arseniasis-hyperendemic villages in Taiwan has been was reported to be significantly higher than in the general population. The aim of this cohort study was to further evaluate the association between ingested inorganic arsenic and the incidence of non–insulin-dependent diabetes mellitus in these villages. A total of 446 nondiabetic residents in these villages were followed biannually by oral glucose tolerance test. Diabetes is was defined as a fasting plasma glucose level ≥ 7.8 mmol/L and/or a 2-hr post-load glucose level ≥ 11.1 mmol/L. During the follow-up period of 1499.5 person-years, 41 cases developed diabetes, showing an overall incidence of 27.4/1,000 person-years. The incidence of diabetes correlated with age, body mass index, and cumulative arsenic exposure. The multivariate-adjusted relative risks were 1.6, 2.3, and 2.1 for an age ≥ 55 versus < 55 years, a body mass index ≥ 25 versus < 25 kg/m^2, and a cumulative arsenic exposure ≥ 17 versus < 17 mg/L-years, respectively. The incidence density ratios (95% confidence intervals) between the hyperendemic villages and the two nonendemic control townships were 3.6 (3.5–3.6), 2.3 (1.1–4.9), 4.3 (2.4–7.7), and 5.5 (2.2–13.5), respectively, for the age groups of 35–44, 45–54, 55–64, and 65–74 years. The findings are consistent with our previous cross-sectional observation that ingested inorganic arsenic is diabetogenic in human beings. *Key words:* diabetes mellitus, incidence, ingested arsenic, oral glucose tolerance test, water pollutant. *Environ Health Perspect* 108:000–000 (2000). [Online _____]
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The atherogenic and carcinogenic effects of arsenic have long been observed among individuals those who had been exposed to a high level of inorganic arsenic from drinking artesian well water in the villages located at the southwestern coast of Taiwan (*1*).

In our recent study, the prevalence of diabetes mellitus was 2-fold higher in these areas than in Taipei City and the Taiwan area in general (*2*). A dose–response relation between cumulative arsenic exposure (CAE) and the prevalence of diabetes mellitus was also demonstrated after adjustment for multiple risk factors (*2*). In that study, Rahman and Axelson (*3*) have also carried out a small case–control study on Swedish copper smelter workers; using the death records for 1960–1976 from the parish register, they compared by comparing three arsenic exposure categories with an unexposed group. from the death records for the years 1960–1976 in the parish register. (***AU: Correct?***) They have observed an increased increasing risk of dying from diabetes mellitus with increasing arsenic exposure; showing odds ratios were of 2.0, 4.2, and 7.0 (p = 0.03 for the trend) for exposure to arsenic in the air of < 0.5, 0.5, and > 0.5 mg/m^3 arsenic in the air, respectively, as compared to with an unexposed control group. In another similar but larger study carried out among art glass workers, the odds ratio of dying from diabetes mellitus was 1.8 [95% confidence interval (CI), 1.1–2.8] for the exposed glass workers as compared to with the unexposed ones (*4*). In According to a community-based survey of diabetes mellitus in Bangladesh, Rahman et al. (*5*) observed a dose–response trend was observed between the prevalence of diabetes mellitus and the arsenic level in drinking water. (*5*).

Prevalence data provide the information for the generation of hypotheses, but suffer from some drawbacks in evaluating a causal relationship. Because the prevalence of a given disease is a function of its incidence and duration (*6*), an increase of prevalence could be attributable due to the increase in disease incidence and/or duration. If the long-term arsenic exposure increases the incidence and decreases the survivalship of diabetes mellitus, the dose–response manner between arsenic and diabetes mellitus will be less striking when by using prevalence is used rather than incidence data. (***AU: Correct?***) Therefore, it is more appropriate to look further into the association between a long-term arsenic exposure and the incidence of diabetes mellitus. In this paper, we describe our study in which we used a biannual 75 g oral glucose tolerance test to that follow ed a cohort of subjects who were free from diabetes mellitus for a period of 4 years. using 75 g oral glucose tolerance test biannually. (***AU: Correct?***)

Materials and Methods

The study area, study subjects, original community-based survey, and estimation of CAE have been previously described in detail (*2*). In brief, the study area included three villages located on at the southwestern coast of Taiwan where arseniasis was hyperendemic. Because of the high salinity in the water of shallow wells, residents in these villages used artesian well water for drinking and cooking (*7*). The median arsenic concentration of artesian well water ranged from 0.70 to 0.93 mg/L (*7,8*). A tap water supply system using surface water was implemented in the 1960s, but the few people had access to this water coverage remained low until the late 1970s. (***AU: Correct?***) The standard for arsenic in drinking water set by the U.S. Environmental Protection Agency is 0.05 mg/L (*9*).

In Taiwan, the household registration system is effective and efficient because of the completeness and accuracy of the registration information. To recruit a cohort of residents for a long-term follow-up study on health hazards associated with long-term arsenic exposure, we selected as eligible only those residents who lived at least 5 days a week in the study villages. A total of 2,258 residents older than over 30 years of age were registered in the study villages, but only 1,571 of them were eligible for the recruitment in as our cohort. Most of the other 687 registered

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residents worked in Chiayi City and its suburban area, returning to the study villages during weekends. All eligible subjects were interviewed at home from September 1988 through June 1989. A standardized personal interview based on a structured questionnaire was carried out by two public health nurses who were well trained in interview techniques and questionnaire details. Information obtained from the interviews included history of high-arsenic artesian well water consumption, residential history, socioeconomic and demographic characteristics, alcohol intake, cigarette smoking, physical activities, as well as a personal and family history of hypertension and diabetes. A total of 1,081 eligible subjects interviewed between September and December 1988 were invited to participate in a health examination in January and February 1989 on a voluntary basis. No incentives were offered to the subjects. Another 490 eligible subjects who were interviewed after December 1988 were not invited to participate in the examination. A total of 941 residents, including 408 men and 533 women, participated in the community-based health examination from January to February 1989. Among them, 381 (93.4%) men and 510 (95.7%) women agreed to be tested for diabetes mellitus by oral glucose tolerance test. Lai et al. (*2*) analyzed these data and have found that the prevalence of diabetes mellitus was significantly associated with CAE. In order To further clarify the diabetogenic effect of arsenic, we decided to follow the subjects who were not found to be diabetic and whose CAE data were available in the prevalence study for the development of diabetes mellitus. After exclusion of known cases of diabetes mellitus identified during the baseline health examination (86 cases) and residents whose status of CAE status was unknown (173 cases), a total of 632 subjects were eligible for the evaluation of the association between the incidence of diabetes mellitus and CAE. In the years 1991 and 1993, 446 of them agreed to participate in the follow-up examination by oral glucose tolerance test.

Some study subjects had moved from one village to another, and the arsenic concentrations were different in the artesian well water of these villages. were different. We derived an index of CAE was derived to reflect the overall exposure to arsenic for each study subject, taking into account both the duration and the arsenic level of artesian well water. This CAE index is It was defined as the sum of products derived by multiplying the arsenic concentration in well water (in milligrams per liter) by the duration of water consumption (in years) during consecutive periods of residence in living at different villages. (***AU: Correct?***) Thus, the In other words, CAE is was derived by the following formula: $\Sigma(C_i \times D_i)$, where C_i is was the median arsenic concentration in the well water of the village where a given study subject lived during the period i, and D_i is was the duration of drinking well water in the village during the period i. We calculated cumulative arsenic exposure was calculated only for those subjects for whom there was had complete information on arsenic exposure from drinking water throughout the subject's his or her lifetime. (***AU: Correct?***) In other words, the arsenic exposure index of a given subject was classified as unknown if the arsenic concentration of well water in any village where the subject had lived during his or her lifetime was not available. We excluded the se subjects with unknown CAEs from the were excluded for follow-up study of diabetes incidence.

For the determination of fasting plasma glucose, we collected blood samples were collected in the morning after overnight fasting for more than -> 12 hr. We then conducted an oral glucose tolerance test was then conducted by administering 75 g glucose dissolved in 300 mL water. Post-load blood samples were taken 2 hr after the glucose loading. Plasma glucose levels were determined on-site with a glucose analyzer (LM4 analyzer; Analox Instruments Ltd., London, UK) using a glucose oxidase method. We define diabetes mellitus was defined as a fasting glucose level ≥ 7.8 mmol/L and/or a 2-hr post-load glucose level ≥ 11.1 mmol/L according to the criteria set by the World Health Organization (WHO).

We recorded the follow-up person-time for each individual was recorded and calculated diabetes incidence was calculated under the assumption that diabetes mellitus is a lifelong disease. We calculated the overall incidence rate of diabetes mellitus in the study population was calculated as the total number of incident cases divided by the sum of follow-up person-time in all subjects. The incidence rate in specific subgroups of age, sex, body mass index (BMI), and CAE was calculated as the number of newly diagnosed cases divided by the sum of person-time of individuals in the subgroup. To clarify the effect of arsenic exposure on the incidence of diabetes mellitus adjusting for age, sex, and BMI, we compared the incidences between the lower and higher arsenic exposure groups were compared at different strata of these possible confounders.

For control areas, we used two nonendemic townships for which incidence of non–insulin-dependent diabetes mellitus (NIDDM or Type 2) was recently reported (*10*). was used as control areas. (***AU: Correct?***) We used this published data for ecologic comparison because the two populations (endemic and nonendemic areas) shared many similarities. The study areas are were all rural areas in Taiwan. Most of the residents in these areas are engaged in farming, fishery, and salt production. All of the se were subjects are of the same racial origin (Fukkien Taiwanese) and share d similar socioeconomic status, living environments, lifestyles, dietary patterns, and even medical facilities, and educational levels. The only major differences in environmental exposure among residents in the arseniasis-hyperendemic area appears to be the arsenic level in the drinking water. Moreover, our cohort study shares d many similarities in method and analysis with the study of Wang et al. (*10*). Both studies of them were carried out during similar study periods by using the WHO criteria for diagnosis of diabetes mellitus. The calculation and expression of incidences of diabetes mellitus were similarly based on an incidence density method by calculating the person-years of follow-up of each of the study subject. Moreover, similar confounders such as age, sex, and BMI body mass index were all considered in both studies. The values of these variables were also available for making comparisons. In order To compare our data with those of Wang et al. (*10*), we calculated the age-specific incidences of diabetes mellitus for the arseniasis-hyperendemic villages by categorizing the subjects into similar age groups similar to those used by Wang et al. (*10*) as having been published in the control areas. We also calculated the incidence density ratios between the arseniasis-hyperendemic villages and the control areas. were also calculated.

We used the chi-square test and the Student's *t*-test were used to compare the differences in the baseline data between the subjects who were successfully followed-up successfully and those who were lost to follow-up in our cohort, between our cohort and the external control cohort by Wang et al. (*10*), and between the incident and nonincident cases in our cohort. We used multivariate analysis by Cox's proportional hazards model was used to estimate the relative risks of higher arsenic exposure on the incidence of diabetes mellitus after adjustment for the effects of age, sex, and BMI. We calculated the 95% CIs of the relative risks were calculated from the corresponding regression coefficients and standard errors.

Results

During a follow-up period of 1499.5 person-years, a total of 41 of among 446 subjects developed diabetes mellitus in the arseniasis-hyperendemic villages. The calculated incidence rate was 27.4/1,000 person-years.

The follow-up rate was 70.6%. Table 1 shows a comparison of ~~compares~~ the baseline characteristics between subjects who were ~~successfully~~ followed-up successfully and those who were lost to follow-up in the arseniasis-hyperendemic villages. None of these variables differed significantly between these two groups. In univariate analyses, we found no significant associations with incidence of diabetes mellitus ~~were found~~ for variables such as sex, cigarette smoking, alcohol consumption, physical activity at work, and family history of diabetes mellitus. However, age and BMI ~~body mass index~~ were significantly associated with diabetes incidence.

Table 2 presents the comparison of ~~compares~~ the baseline characteristics in subjects ~~successfully~~ followed-up successfully between the arseniasis-hyperendemic group and the nonendemic external control group. The BMI ~~Body mass index~~ was not significantly different between the two groups. However, our arsenic-exposed cohort ~~with arsenic exposure~~ was younger with an equal number of men and women, and the control group was older and had ~~with~~ more women. Because sex was not found to be a significant risk factor for newly developed diabetes mellitus in both our current study and the ~~reported~~ study by Wang et al. (*10*) and the BMI was similar between the two comparison groups, only age could exert significant confounding effect when ~~we compare~~ the incidence rates in these two groups are compared. We ~~thus~~ further stratified age into subgroups and compared the age-specific incidence density ratios in each subgroups.

Table 1. Comparison of baseline characteristics between subjects who were followed-up successfully ~~followed-up~~ and those who were lost to follow-up in arseniasis-hyperendemic villages.

Variables	Followed-up (n = 446)	Lost (n = 186)
Age (years)	47.4 ± 0.5	47.5 ± 0.9
Sex (% male)	50%	54.3%
Body mass index (kg/m^2)	24.5 ± 0.2	23.9 ± 0.3
CAE	12.1 ± 0.5	13.2 ± 0.8

Values shown are mean ± SE except where indicated. ~~standard error.~~ ~~p~~ NS, Differences are not significant for any ~~all~~ the variables based on the basis of chi-square test or Student's t-test. (~~***~~AU: Changes ok?~~***~~)

Table 2. Comparison of baseline characteristics in subjects who were ~~successfully~~ followed-up successfully between the arseniasis-hyperendemic group and the nonendemic control group.

Variables	Arseniasis-hyperendemic	Nonendemic control	p^a
Age (years)	47.4 ± 0.5	52.3 ± 0.5	<0.005
Sex (% male)	50%	45.4 %	<0.005
BMI (kg/m^2)	24.5 ± 0.1	24.2 ± 0.2	NS

NS, not significant. Values shown are mean ± SE except where indicated.
[a]Based on chi-square test or Student's t-test. (~~***~~AU: Changes ok?~~***~~)

The age-specific incidences of diabetes mellitus in the arseniasis-hyperendemic villages and the two nonendemic control townships are shown in Figure 1. The age-specific incidence density ratios (95% CIs) between the hyperendemic villages and control townships were 3.6 (3.5–3.6), 2.3 (1.1–4.9), 4.3 (2.4–7.7), and 5.5 (2.2–13.5), respectively, for the age groups of 35–44, 45–54, 55–64, and 65–74 years. ~~It is evidenced that~~ The subjects living in arseniasis-hyperendemic villages have ~~has~~ higher incidence rates of diabetes mellitus than subjects living in nonendemic areas.

The baseline data of the newly diagnosed diabetic and nondiabetic subjects living in arseniasis-hyperendemic villages are shown in Table 3. The distribution of sex ~~gender~~ was not ~~different~~ significantly different between these two groups. However, newly diagnosed diabetic cases had a significantly higher mean age, BMI, and CAE than nondiabetic subjects. We used ~~Following~~ cutoff points ~~were used for to~~ categorize ing various continuous variables. We used a BMI ~~Body mass index was~~ cutoff of ~~at~~ 25 kg/m^2 because it is generally used to define ~~as the definition of~~ overweight (*11*). We did not use the ~~a~~ definition of obesity (BMI ≥ 30 kg/m^2) ~~was not used,~~ because in the Taiwanese population, only a small proportion of the diabetic patients met this criterion. We used the age ~~was~~ cutoff of ~~at~~ 55 years because there was an abrupt increase in the incidence of diabetes mellitus in the age cohort ~~above~~ > 55 years of age at the time of recruitment, ~~showed an abrupt increase in the incidence of diabetes mellitus as~~ shown in Figure 1. We used the CAE ~~cumulative arsenic exposure was~~ cutoff of ~~at~~ 17 mg/L-years, the median value of CAE in the newly diagnosed cases, in order to obtain optimal numbers in each subgroup of exposure to assure precise estimates for the relative risks.

Table 4 shows the comparison of ~~compares~~ the incidence rates of diabetes mellitus between groups of CAE < 17 and ≥ 17 mg/L-years by different strata of age, sex, and BMI. Subjects ~~Those~~ with a CAE ≥ 17 mg/L-years consistently had a higher incidence rate ~~s~~ of diabetes mellitus than those with a CAE < 17 mg/L-years.

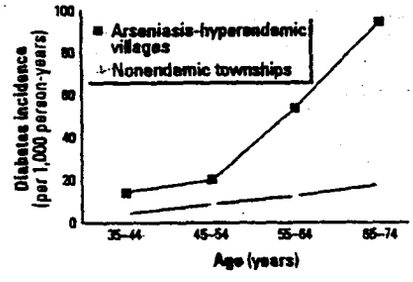

Figure 1. Comparison of age-specific incidence rates (per 1,000 person-years) of diabetes mellitus in arseniasis-hyperendemic villages (■) and in two nonendemic townships (—○—) in Taiwan.

Table 5 shows a comparison of ~~compares~~ the incidence rates and relative risks between different subgroups of age, sex, BMI, and CAE. Age, BMI, and CAE were significantly associated with the development of diabetes. The multivariate-adjusted relative risks (95% CIs) based on the Cox's proportional hazards model were 1.6 (0.8–3.3), 1.1 (0.6–2.1), 2.3 (1.2–4.3), and 2.1 (1.1–4.2), respectively, for groups of old age, male sex ~~gender~~, overweight, and high CAE compared with groups of young age, female sex ~~gender~~, normal weight, and low CAE. When used as a continuous variable, the CAE was associated with incidence of diabetes mellitus with a relative risk of 1.03 for every 1 mg/L-year of exposure after adjustment for age, sex, and BMI ~~body mass index~~ (p < 0.05).

Discussion

The results of this study ~~further~~ support the association between a long-term arsenic exposure and diabetes mellitus, as found in our previous prevalence study (*2*) and in the studies observed by other investigators (*3–5*). However, this is the first ~~ever~~ prospective follow-up study that assessed the incidence of diabetes mellitus in the arseniasis-hyperendemic villages by comparing ~~with~~ the data to data obtained from two nonendemic control areas (external control) (Figure 1) and by comparing a higher arsenic exposure group to ~~with~~ a lower exposure group after adjustment for age, sex, and BMI ~~body mass index~~ in the arseniasis-hyperendemic villages (internal control) (Tables 4 and 5). The incidences rose abruptly in the age groups ~~above~~ > 55 years among villagers in arseniasis-hyperendemic area (Figure 1). These birth cohorts also have an abrupt increase in the prevalence of peripheral vascular disease assessed with Doppler ultrasonography (*12*). They had a ~~higher~~ long-term arsenic exposures than those ~~aged below~~ < 55 years of age. They were ~~Their exposured history~~ to well water for ~~was more than~~ > 30 years, which was deemed to be as an induction period for blackfoot disease (BFD) before ~~the coverage of~~ tap water ~~supply~~ became ~~high~~ commonly accessible. (~~***~~AU: Correct?~~***~~) BFD ~~Blackfoot disease~~ is an endemic peripheral vascular disease confined to the southwestern coast of Taiwan. It is characterized by progressive narrowing of peripheral arteries, especially those involving the lower extremities. Clinically, the patients suffer from coldness, numbness, and intermittent claudication in ~~of~~ the lower legs, which may progress to ulceration, gangrene, and spontaneous amputation (*12,13*).

Pathologically, BFD is compatible with thromboangiitis obliterans and arteriosclerosis obliterans (*12,13*). The cause of the disease has been ascribed to the drinking of artesian well water containing high arsenic concentrations (*12,13*).

Age and BMI are known risk factors for the development of diabetes mellitus (*10,14*). We also observed this association in the present study. However, the diabetes incidence for the higher arsenic exposure group (CAE ≥ 17 mg/L-years) remained 2 times higher after multivariate adjustment (Table 5). In the comparison between the higher arsenic exposure group (≥ 17 mg/L-years) and the lower exposure group (< 17 mg/L-years) in our cohort, the relative risk for the high exposure group would have been underestimated because a large proportion of the referent group had also been exposed to arsenic. (***AU: Correct?***) They were not in a group with truly low exposure levels. This can be supported in part by the observation that the age-specific incidence density ratios between the arseniasis-hyperendemic group and the external control group without arsenic exposure had a risk > 2 times higher (Figure 1). (***AU: Correct?***) According to the continuous data analysis, a CAE difference of 50 mg/L-years will result in a relative risk of 4.4.

We observed NIDDM because all of the study subjects were > 30 years of age. None of them developed diabetic ketoacidosis or required insulin treatment during the period of follow-up. Moreover, all of the subjects were diagnosed by an oral glucose tolerance test without significant clinical symptoms.

There are only a few reports on the incidence of NIDDM. According to the Minnesota study of Bender et al. (*15*) in Minnesota, the incidence rate of NIDDM in the Caucasians is 1.2/1,000 person-years. In Nauruans, the incidence rate is 16.0/1,000 person-years in subjects > 20 years of age (*16*), and in the Pima Indians, the incidence rate is 18.5/1,000 person-years for all ages combined and 46/1,000 person-years in subjects > 25 of age (*17*). After standardization to the white population in the United States in 1970, the incidence rates of NIDDM are 1.34/1,000 and 26.5/1,000 person-years for the Caucasians and Pima Indians, respectively (*17*). Wang et al. (*10*) recently followed a cohort for up to 5 years; this cohort, from two townships in Taiwan, was aged 35–74 years of age and free from diabetes. (***AU: Correct?***) The crude incidence rates of NIDDM in men and women were 9.8/1,000 and 9.0/1,000 person-years, respectively. After age-standardization to the United States population in 1970, the incidence rate was calculated to be 9.3/1,000 person-years. In our present study carried out in a cohort with arsenic exposure, the incidence of diabetes mellitus standardized to the United States population in 1970 was approximately 2 times higher than that in the nonendemic areas.

The administration of arsenic has been demonstrated to cause hyperglycemia in experimental animals and to affect the functions of insulin receptor and glucose transportation (*18–24*). Arsenic has been found to cause mitochondrial damage, degeneration, and necrosis of β cells in the islets of mice after intraperitoneal injection of arsenite plus hydroxylamine, with a consequence of transient hyperglycemia (*18*). Sulfhydryl groups play important structural and functional roles in both insulin receptors (*25*) and glucose transporters (*26*). Phenylarsine oxide, a trivalent arsenical, forms stable cyclic thioarsenite complexes with vicinal or paired thio groups of cellular proteins. This compound has been shown to inhibit glucose transport in adipocytes stimulated by

Table 3. Comparison of baseline characteristics between newly diagnosed incident diabetic and nondiabetic subjects living in arseniasis-hyperendemic villages in Taiwan.

Variables	Newly diagnosed cases (n = 41)	Noncases (n = 405)	p^a
Sex (% male)	58.5	49.1	NS
Age (% ≥ 55 years)	36.6	18.5	< 0.01
BMI (% ≥ 25 kg/m²)	58.5	36.3	< 0.01
CAE (% ≥ 17 mg/L-years)	51.2	27.4	< 0.005
Age (years)	52.7 ± 1.6[b]	46.8 ± 0.5	< 0.001
BMI (kg/m²)	25.8 ± 0.7	24.3 ± 0.2	< 0.05
CAE (mg/L-years)	15.6 ± 1.7	11.8 ± 0.5	< 0.05

NS, not significant.
[a]Based on chi-square test or Student's t-test. [b]Mean ± SE.

Table 4. Incidence of diabetes mellitus (per 1,000 person-years) in low and high CAE groups in arseniasis-hyperendemic villages in Taiwan.

	CAE (mg/L-years)					
	< 17			≥ 17		
Variable	Total no.	Newly diagnosed cases (n) no.	Incidence rate	Total no.	Newly diagnosed cases (n) no.	Incidence rate
Age (years)						
≥ 55	28	2	21.1	62	13	64.8
< 55	286	18	18.8	70	8	33.3
Sex						
Male Men	145	11	22.1	78	13	49.2
Female Women	169	9	16.1	54	8	45.2
BMI (kg/m²)						
≥ 25	122	11	26.5	49	13	78.1
< 25	192	9	13.8	83	8	28.2

(***AU: Changes ok?***)

Table 5. Incidence rates (per 1,000 person-years) and relative risks for diabetes mellitus in different subgroups of subjects living in arseniasis-hyperendemic villages in Taiwan.

Variable	Total no.	Newly diagnosed DM cases (n) no.	Incidence rate	RR (95% CI)	ARR (95% CI)
Age (years)					
≥ 55	90	15	50.8	2.4 (1.3–4.5)*	1.6 (0.8–3.3)
< 55	356	26	21.6	1.0	1.0
Sex					
Male	223	24	31.5	1.3 (0.7–2.5)	1.1 (0.6–2.1)
Female	223	17	23.1	1.0	1.0
BMI (kg/m²)					
≥ 25	171	24	42.1	2.3 (1.2–4.3)*	2.3 (1.2–4.3)*
< 25	275	17	18.3	1.0	1.0
CAE (mg/L-years)					
≥ 17	132	21	47.6	2.5 (1.4–4.7)*	2.1 (1.1–4.2)*
< 17	314	20	18.9	1.0	1.0

Abbreviations: DM, diabetes mellitus; RR, relative risk (based on Cox models with each variable singly); ARR, adjusted relative risk (based on Cox model with all variables simultaneously).
* p < 0.05.
(***AU: Is 1.3 (0.7–2.5) correct for Male RR?***)

the insulin mimickers vanadate and hydrogen peroxide (*22*). Phenylarsine oxide has also been shown to ~~be an~~ inhibit ~~or of~~ protein internalization, and it exhibits an inhibitory effect on the internalization of insulin receptor complexes in rat hepatocytes (*27*) and CT3-C2 fibroblasts (*28*). Jhun et al. (*29*) ~~has~~ demonstrated the existence of a phenylarsine oxide sensitive GLUT4 degradation in rat adipocytes, which might have pathophysiologic ~~al~~ significance, giving rise to clinical problems of insulin resistance. Phenylarsine oxide ~~is has also been found to~~ inhibits the stereospecific uptake of D-glucose in basal and insulin-stimulated rat adipocytes (*19–22*) in a dose–response pattern. **(***AU: Correct?***)** Phenylarsine oxide also inhibits insulin binding at a higher concentration and insulin internalization (*19*). ~~The study by~~ Douen and Jones (*19*) suggested that phenylarsine oxide has a direct inhibitory ~~effect of phenylarsine oxide~~ on both the receptor system and the transporter system, possibly by reacting with sulfhydryl groups at or near the receptor or transporter sites. This ~~But it~~ does not exclude the possibility of a reaction with a component of the coupling system between receptor and transporter, as suggested by Frost and Lane (*22*).

Vicinal sulfhydryls also play important roles in the activation of glucose transport by insulin and insulin-like agents in ~~the~~ skeletal muscle (*24*). Phenylarsine oxide exhibits an inhibitory effect on insulin-stimulated or hypoxia-stimulated glucose transport in rat skeletal muscle (*24*). Denervation-induced postreceptor resistance of glucose transport to insulin and insulin-like growth factor-I also involves primarily a phenylarsine oxide-sensitive pathway in rat skeletal muscle (*25*).

Deficiencies of trace elements such as copper and zinc have been suggested to play some role in the pathogenesis of diabetes mellitus (*30*). On the other hand, administration of cadmium has been shown to cause hyperglycemia (*31*). Arsenic has been reported to interact with these chemicals. Arsenic exposure can lead to a significant increase in renal copper excretion and can potentiate the effects of cadmium when arsenic and cadmium are used together (*32*). Arsenic may also compete with zinc in metal-binding proteins that display ~~ing~~ vicinal dithiols contained in zinc fingers of DNA binding and repair proteins. This competitive binding causes ~~the proteins'~~ conformational change and altered biologic function in proteins (*33*). ~~These above~~ effects of arsenic may explain some of the possible mechanisms of its diabetogenic effect.

In conclusion, the diabetogenic effect of arsenic in humans ~~beings~~ has been reported in different ethnic groups via different exposure routes (*2–5*). To the best of our knowledge, this is ~~was~~ the first study to evaluate ~~evaluating~~ the association between high arsenic exposure from drinking water and the development of diabetes mellitus by following the incidence of diabetes in a cohort exposed to arsenic. We also performed an ecologic comparison ~~was also performed~~ by comparing the incidences of diabetes mellitus in our cohort and an external control population studied ~~carried out~~ by other investigators (*10*). **(***AU: Correct?***)** Our ~~The~~ findings support the hypothesis that arsenic is diabetogenic in humans, ~~beings~~. Although the pathophysiologic ~~al~~ mechanisms of arsenic require further investigation, ~~s~~ the health hazards of arsenic exposure should ~~need to~~ be amended and remedial measures should be taken.

References and Notes

1. Chen CJ, Lin LJ. Human carcinogenicity and atherogenicity induced by chronic exposure to inorganic arsenic. In: Arsenic in the Environment, Part II: Human Health and Ecosystem Effects (Nriagu JO, ed). New York:John Wiley & Sons, Inc., 1994;109–131.
2. Lai MS, Hsueh YM, Chen CJ, Shyu MP, Chen SY, Kuo TL, Wu MM, Tai TY. Ingested inorganic arsenic and prevalence of diabetes mellitus. Am J Epidemiol 139:484–492 (1994).
3. Rahman M, Axelson O. Diabetes mellitus and arsenic exposure: a second look at case-control data from a Swedish copper smelter. Occup Environ Med 52:773–774 (1995).
4. Rahman M, Wingren G, Axelson O. Diabetes mellitus among Swedish art glassworkers—an effect of arsenic exposure? Scand J Work Environ Health 22:146–149 (1996).
5. Rahman M, Tondel M, Ahmad SA, Axelson O. Diabetes mellitus associated with arsenic exposure in Bangladesh. Am J Epidemiol 148:198–203 (1998).
6. Kleinbaum DG, Kupper LL, Morgenstern H. Epidemiologic Research: Principles and Quantitative Methods. New York:Van Nostrand Reinhold Company, 1982;121.
7. Chen KP, Wu HY, Wu TC. Epidemiologic studies on blackfoot disease in Taiwan: III. Physicochemical characteristics of drinking water in endemic blackfoot disease areas. Mem Coll Med Natl Taiwan Univ 8:115–123 (1962).
8. Kuo TL. Arsenic content of artesian well water in endemic area of chronic arsenic poisoning. ~~Reports of Institute of Pathology, Taipei, Taiwan:National Taiwan University College of Medicine~~ Ren Inst Pathol TMU 20:7–13 (1964). **(***AU: Correct?***)**
9. Smith AH, Hopenhayn-Rich C, Bates MN, Goeden HM, Hertz-Picciotto I, Duggan HM, Wood R, Kosnett MJ, Smith MT. Cancer risks from arsenic in drinking water. Environ Health Perspect 97:259–267 (1992).
10. Wang SL, Pan WH, Hwu CM, Ho LT, Lo CH, Lin SL, Jong YS. Incidence of NIDDM and the effects of gender, obesity and hyperinsulinaemia in Taiwan. Diabetologia 40:1431–1438 (1997).
11. WHO Expert Committee. Physical Status: The Use and Interpretation of Anthropometry. WHO Technical Report Series no. 854. Geneva:World Health Organization, 1995.
12. Tseng CH, Chong CK, Chen CJ, Tai TY. Dose-response relationship between peripheral vascular disease and ingested inorganic arsenic among residents in blackfoot disease endemic villages in Taiwan. Atherosclerosis 120:125–133 (1996).
13. Tseng CH, Chong CK, Chen CJ, Tai TY. Lipid profile and peripheral vascular disease in arsenosis-hyperendemic villages in Taiwan. Angiology 48:321–335 (1997).
14. Zimmet P. Type-2 (non-insulin-dependent) diabetes-an epidemiological overview. Diabetologia 22:399–411 (1982).
15. Bender AP, Sparks JM, Jagger HG, Muckala KH, Martin CP, Edwards TR. Incidence, prevalence and mortality of diabetes mellitus in Wadena, Marshall and Grand Rapids, Minnesota: the Three-City Study. Diabetes Care 9:343–350 (1986).
16. Balkau B, King H, Zimmet P, Raper LR. Factors associated with the development of diabetes in the Micronesian population of Nauru. Am J Epidemiol 122:594–605 (1985).
17. Knowler WC, Bennett PH, Hamman RF, Miller M. Diabetes incidence and prevalence in Pima Indians: a 19-fold greater incidence than in Rochester, Minnesota. Am J Epidemiol 108:497–505 (1978).
18. Boquist L, Boquist S, Ericsson I. Structural beta-cell changes and transient hyperglycemia in mice treated with compounds inducing inhibited citric acid cycle enzyme activity. Diabetes 37:89–98 (1988).
19. Douen AG, Jones MN. The action of phenylarsine oxide on the stereospecific uptake of D-glucose in basal and insulin-stimulated rat adipocytes. Biochim Biophys Acta 968:109–118 (1988). **(***AU: Small cap correct?***)**
20. Douen AG, Kacem R, Jones MN. Direct interaction of phenylarsine oxide with hexose transporters in isolated adipocytes. Biochim Biophys Acta 944:444–450 (1988).
21. Frost SC, Kohanski RA, Lane MD. Effect of phenylarsine oxide on insulin-dependent protein phosphorylation and glucose transport in 3T3-L1 adipocytes. J Biol Chem 262:9872–9878 (1987).
22. Frost SC, Lane MD. Evidence for the involvement of vicinal sulfhydryl groups in insulin-inactivated hexose transport by 3T3-L1 adipocytes. J Biol Chem 260:2646–2652 (1985).
23. Sowell MO, Robinson KA, Buse MG. Phenylarsine oxide and denervation effects on hormone-stimulated glucose transport. Am J Physiol 255:E139–165 (1988).
24. Henriksen EJ, Holloszy JO. Effects of phenylarsine oxide on stimulation of glucose transport in rat skeletal muscle. Am J Physiol 258:C848–853 (1990).
25. Pike LJ, Eakes AT, Krebs EG. Characterization of affinity-purified insulin receptor/kinase. Effects of dithiothreitol on receptor/kinase function. J Biol Chem 261:3782–3789 (1986).
26. May JM. The inhibition of hexose transport by permeant and impermeant sulfhydryl agents in rat adipocytes. J Biol Chem 260:462–467 (1985).
27. Draznin B, Trowbridge M, Ferguson L. Quantitative studies of the rate of insulin internalization in isolated rat hepatocytes. Biochem J 218:307–312 (1984).
28. Knutsen VP, Ronnett GV, Lance MD. Rapid, reversible internalization of cell surface insulin receptors. Correlation with insulin-induced down-regulation. J Biol Chem 258:12139–12142 (1983).
29. Jhun BH, Hah JS, Jung CY. Phenylarsine oxide causes an insulin-dependent, GLUT4-specific degradation in rat adipocytes. J Biol Chem 268:22280–22285 (1993).
30. WHO. Diabetes Mellitus. Technical Report Series no. 727. Geneva:World Health Organization, 1985.
31. Bell RR, Early JL, Nonavinakere VK, Mallory Z. Effect of cadmium on blood glucose level in the rat. Toxicol Lett 54:199–205 (1990).
32. Mahaffey KR, Capar SG, Gladen BC, Fowler BA. Concurrent exposure to lead, cadmium, and arsenic. Effects on toxicity and tissue metal concentrations in the rat. J Lab Clin Med 98:463–481 (1981).
33. Engel RR, Hopenhayn-Rich C, Receveur O, Smith AH. Vascular effects of chronic arsenic exposure: a review. Epidemiol Rev 16:184–209 (1994).

The epidemiology of lower extremity amputations in centres in Europe, North America, and East Asia: results from the Global LEA Study

The Global Lower Extremity Amputation Study Group[a,b]

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Abstract

Background: This study was established to enable the comparison of lower extremity amputation incidence rates between different centres around the world.

Methods: Ten centres, all with populations greater than 200,000, in Japan, Taiwan, Spain, Italy, United States and England collected data on all amputations occurring between July 1995 and June 1997. Cases were identified from at least two data sources (to allow checks on ascertainment); denominator populations were based on census figures.

Results: The highest rates were in the Navajo population (e.g. 43.9/100,000/ yr for first major amputations in men) and the lowest in Tochigi, Japan (e.g. 3.8/100,000/yr). The incidence of amputation rose steeply with age, with most amputations occurring over the age of 60, in most centres the incidence was higher in men than women, and the incidence of major was greater than that of minor amputations. Diabetes was associated with between 25% and 90% of amputations.

Conclusion: Apart from the Navajo centre, differences in the known prevalence of diabetes can not account for the differences in overall incidence. Differences in the prevalence of peripheral vascular disease are likely to be important but this and the role of other factors, including health care, are worthy of further investigation.

INTRODUCTION

The study described in this paper was established to overcome some of the difficulties encountered in comparing amputation rates from previously published studies. For example, inconsistencies between published studies related to the inclusion or not of people with diabetes in the overall rates, the inclusion of first ever or all amputations in the rates, and differences in the level of amputation presented. In addition age specific rates tended not to be presented making comparison between populations with different age structures difficult. Finally, different studies often used different data sources and numbers of sources to identify amputations. This means it is possible that differences exist in the level of ascertainment of amputations between studies[1].

Thus the main aim of establishing this study was to enable the comparison of the incidence of lower extremity amputations between populations by using a standard approach to data collection and analysis.

METHODS

Recruitment and characteristics of the study centres

Most centres were recruited to the study following publication of the study protocol[1]. Other centres became involved once the study was established - mainly through professional contact with investigators at participating centres. A detailed "Methods of Operation" manual was written and distributed to each centre.

Sufficient data were received from 10 centres, in 5 countries, to be presented in this paper. Data were collected on all amputations occurring between July 1st 1995 and June31st 1997. The names, population sizes and sources of data for LEAs for each of the centres are shown in table 1. In order to participate each centre had to have access to up to date demographic data for their population in order to allow the calculation of age and sex specific rates. Tochigi in Japan was only able to collect data on first ever major and minor amputations.

Definition of LEA and collection of data on amputations

The definition of a lower extremity amputation used in this study is the complete loss of any part of the lower limb, for any reason, in the following anatomical planes: in the transverse anatomical plane proximal to and including the subtalar joint, and in the frontal anatomical plane distal to the subtalar joint. For the purposes of this paper we define a "major amputation" as through or proximal to the tarsometatarsal joint and a "minor amputation" as one distal to this joint.

Centres were asked to use more than one source to identify cases - ideally at least three, but where this was not possible two were accepted. Sources from which cases were identified were dependent on local circumstances and so differed between centres. They are shown in Table 1. As far as possible, medical records were traced for each case and a standard data abstraction form completed. With the exception of some aspects of personal details the data abstraction form was identical for each of the centres. The data collected included the date and level of the most recent amputation, and conditions associated with it (including diabetes,

infection, peripheral vascular disease and trauma); and the presence and level of any previous amputations.

Centres undertook a pilot study to test the feasibility of abstracting data from the different sources, and sent the completed forms to Newcastle (the co-ordinating centre) in order to check that the data collection forms from each centre were completed to a comparable and adequate standard.

Data analysis

Most centres entered their data locally and all data were entered using a standard data base format designed only to allow valid values for each variable. Age and sex denominator population sizes were taken from the most recent census figures for each population. Age specific, crude and age adjusted rates are presented for men and women separately. As all the centres (with the exception of Navajo Area) have relatively old population age structures the standard European population[2] was used as the population for direct age standardisation.

The aim of this study was to measure the incidence of amputations in whole geographically defined populations and not samples of larger populations hence we do not present confidence intervals or use p values. What is relevant is the level of ascertainment of amputations. Estimates of the level of ascertainment were derived using capture-recapture methods[3,4]. These derive their name from their development and use in estimating the size of wildlife populations. They have been increasingly used in epidemiology. With more than one source of cases it is possible to estimate the total number from the numbers of cases identified on one source only and those identified on more than one source[5]. With only two sources the capture-recapture method is based on the assumption that the sources are independent. This assumption is rarely met with health care data, but if the direction of dependency is known it is still possible to derive useful information[6], and this approach has been used here in the situation where only two sources were available. With three or more sources log-linear modelling[5] and a confidence interval for the total number of cases computed using a goodness of fit based method[7]. The numbers of cases were too small to provide robust age specific estimates of ascertainment but it was felt important to examine ascertainment for first

and all amputations separately as experience suggested ascertainment amongst these categories may differ. The ascertainment of minor and major amputations was also examined separately. This was because experience also suggested that ascertainment between these two is likely to differ but in addition because some sources, in particular, limb fitting centres, provide data on major but not on minor amputations. In order to have reasonable numbers of cases for these different categories in the analyses men and women were combined.

Population diabetes prevalence figures were not available for all centres, therefore the numbers of people with known diabetes are presented simply as a proportion of all cases of lower extremity amputations in each centre. The proportions of cases noted to be associated with peripheral vascular disease, infection and trauma are also presented.

RESULTS

Overall incidence of amputations

There were marked differences in the overall incidence of both first and all amputations between the study centres (tables 2a&b and 3a&b) with over a 10 fold difference between the highest and lowest areas. The data for first ever major and minor amputations are illustrated in figures 1 and 2. The highest rates for men and women, first and all, major and minor amputations, were in the Navajo area. The lowest rates for first amputations were in the Japanese population of Tochigi. Between the Western European centres there were also marked differences. Rates in Madrid (Spain), Vicenza (Italy) and Leicester (UK) all being substantially lower than those were in the North of England i.e. Leeds, Middlesborough, Newcastle.

Estimated levels of ascertainment

Table 4 shows capture-recapture estimated levels of case ascertainment. Even with the figures for men and women combined (as done here) the confidence intervals on the estimated number of amputations tend to be broad. For minor amputations in Leicester and Ilan the numbers were either too small or the overlaps between the data sources inadequate to enable meaningful capture-recapture estimates to be made. For first amputations estimates of ascertainment were low (less than 70%) in two centres, Tochigi and Vicenza. In three (Leeds, Middlesborough and Montgomery County) they were above 95% for first major and minor amputations. All the rest fell somewhere above 75% and less than 95%. The interpretation and utility of these estimates is addressed in the discussion.

Age and sex distribution of amputations

Although the overall rates varied greatly between centres the age and sex distribution of amputations were very similar. Thus in all centres the incidence of both major and minor amputations in both men and women rose steeply with age, with the largest increases tending to come between the age groups of 40-59 and 60-79 (tables 2a&b and 3a&b). Around two

thirds or more of amputations occurred in the ages above 60 with the exception of minor amputations in men and women in the Navajo area and first major amputations in men in Japan. Overall amputation rates were substantially higher in men than in women, from 17% higher crude rates for first major amputations in men compared to women in Leicester, to over 400% higher for all major and minor amputations in Madrid. The exceptions to this were first and all major amputations in Vicenza, where crude and age adjusted rates were higher in women than men (entirely accounted for by high rates in women aged 80 and over), and for first minor amputations in Leeds where the rates in men and women were similar.

Relationship of major to minor and all to first amputations

In most centres the incidence of both first and all major amputations was greater than the incidence of first and all minor amputations respectively (tables 2a&b and 3a&b). The most extreme exception to this is the Navajo area where the incidence of major amputations was between 50% (all, men) and 79% (first, women) of the incidence of minor. As would be expected the incidence of all amputations was greater than the incidence of first amputations in almost all centres. The only exception was for minor amputations in Ilan where the rates were similar. In the other centres rates of all amputations were higher than rates of first amputations from an order of around 10% (major amputations in men in Ilan) up to 251% (major amputations in women in Montgomery county). Most were in the order of 20 to 40% higher.

Conditions associated with amputations

Table 5 shows the percentage of first major and minor amputations associated with trauma, diabetes, infection and peripheral vascular disease (PVD) for men and women in each of the centres. Note that more than one condition may be present per case and so the percentages in the rows can add up to more than 100%.

The percentage of amputations associated with diabetes varied from between 25 and 30% (minor amputations in women in Leeds and major and minor in women in Newcastle) up to 80% and above, such as in men and women in the Navajo area. These are illustrated in figures 3 and 4. Note that these figures provide no information on the incidence of amputations in

people with diabetes. In general there is a strong positive relationship between the percentage of amputations associated with diabetes and the percentage associated with infection. Obvious exceptions to this are major amputations in men in Montgomery county and major and minor amputations in men and major amputations in women in Tochigi, Japan.

In the 9 of the 10 centres the percentage of major amputations associated with trauma in men was 10% or less. The exception to this was Ilan in Taiwan where 50% of major amputations were associated with trauma. The highest percentages of major amputations associated with trauma in women were in Newcastle and Ilan where 15 to 16% were associated with trauma. The percentage of major amputations associated with peripheral vascular disease in men ranged from 51.3% in the Navajo Area to 93.3% in Madrid. The percentages were similar in women with the exception of Tochigi where only 29.6% were associated with peripheral vascular disease.

DISCUSSION

In this paper we have presented data on lower extremity amputation from 10 centres in 5 countries. There are some striking contrasts between the centres. There are, for example, marked differences between centres in the incidence of amputations, in the proportion associated with diabetes and in the ratio of first to all amputations. Equally noteworthy are the similarities between the centres in the distribution of amputations by age and sex despite differences in overall rates. The methodology used in this study was designed, within the limits of using routinely available data sources, to provide valid comparisons between the centres. Amputation case definitions and presentation of rates are identical for each centre. Where greater scepticism must exist is whether the level of ascertainment of amputations between centres is comparable and of the comparability of some specific data items from medical records.

In order to both maximise ascertainment and to allow checks on ascertainment level each centre used more than one data source. Estimates of ascertainment using capture recapture methods suggested that there are indeed some differences in levels of ascertainment. Here capture-recapture estimates of ascertainment are used as an aid to interpretation in comparing the results rather than providing robust estimates of the "true" rates. The confidence intervals of many of the estimates in table 4 illustrate the uncertainty around the point estimates and indeed the known difficulty in modelling this type of data[5]. In addition, where only two data sources were available these tended to be positively dependent, meaning that the capture recapture estimate will over estimate the level of ascertainment[6]. Nonetheless the use of this technique provides useful information that can not otherwise be obtained. Thus, the capture recapture estimates suggest that in two of the centres with the lowest rates, Tochigi and Vicenza, there may be substantial underascertainment, in the order of around 50%. However, even doubling the rates of these centres they remain substantially below those in the north of England and North America, particularly those of Japan. We conclude that there remain substantial differences in amputation rates between the centres even when allowing for potential differences in ascertainment, with the lowest rates in Japan and Taiwan and the highest rates the Navajo area. Within Western Europe and to a lesser extent England there are also marked differences in rates between some of the centres.

The high levels of lower extremity amputations in Native American populations are well known[8] and are associated with a high prevalence of diabetes[9,10]. The low incidence of amputations in the centres in Japan, Taiwan, Spain and Italy contrast with the higher rates in the North of England and Montgomery, USA. Recent data and estimates suggest that the prevalence of diabetes in Spain, Italy, Japan and Taiwan is at least twice as high as in England and similar to that in the USA[11]. Clearly therefore a higher prevalence of diabetes is unlikely to be part of the explanation for these differences in amputation incidence. Differences in the prevalence peripheral vascular disease may be, although data are scarce. For example, in the Edinburgh Artery Study the prevalence of peripheral vascular disease was 30% in men and women aged 55 to 74 years[12]. In a study of Italian male and female elderly[13] the prevalence was 10%, and in a Japanese study of village residents aged 60-79 years a prevalence of 0.6% was reported[14]. The WHOinternational study of vascular disease in people with diabetes (ages 35-54 years) found a huge variation in the prevalence of leg vascular disease, with the lowest prevalence in Tokyo and Hong Kong[15]. The relatively low incidence of amputations in Leicester compared to the other English centres fits with previous findings from Leicester[16], in both its general and Indo-Asian populations. Within England data, on the epidemiology of peripheral vascular disease are also scarce but there is evidence for a south to north gradient[17] and for differences by ethnic group[18].

In this paper we also present data on the conditions associated with, or potential causes, of amputation. These data are abstracted from medical records and in presenting these we acknowledge that such data can be prone to omissions and inaccuracies[19]. The highest proportions of amputations associated with trauma were in Ilan, Taiwan, where up to 57% of amputations were reportedly associated with trauma. Excluding cases associated with trauma, as is often done in the presentation of amputation rates, makes little difference to the ranking of the centres by incidence. The proportion of amputations associated with diabetes varied from around 20% (major amputations in women in Vicenza) to 90% and over. The highest proportions for men and women were in the Navajo area. For major amputations in men the lowest proportion associated with peripheral vascular disease was 55% in Tochigi, Japan, and in women was 30% in Tochigi. The proportions associated with peripheral vascular disease tended to be lower for minor amputations with the converse being true for the proportions

associated with infection.

In summary, we have demonstrated marked differences in the incidence of amputations between 10 centres in 5 different countries, but with similar distributions by age and sex. Rates were highest in the North American and northern European centres and lowest in Taiwan and Japan. In one population (Navajo) a very high prevalence of diabetes is likely to be the explanation of the high rates. Differences between the other populations do not seem to be related to what are known of differences in diabetes prevalence, and it seems likely that differences in the prevalence of peripheral vascular disease are important. Other factors, such as cultural and medical care differences[20] including access to vascular surgery services may also be important but require further investigation.

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REFERENCES

1. LEA Study Group. Comparing the incidence of lower extremity amputations across the world: the global lower extremity amputation study. *Diabetic Medicine* 1995;**12:**14-18.
2. World Health Organisation. World Health Statistics Annual,. Geneva: WHO, 1993.
3. International Disease Monitoring Group. Capture-recapture: applications in human diseases. *American Journal of Epidemiology* 1995;**142:**1059-68.
4. Laporte RE. Assessing the human condition: capture-recapture techniques. *British Medical Journal.* 1994.;**308**(1 January 1994.):5-6.
5. International Disease Monitoring Group. Capture-recapture and multiple record systems: history and theoretical development. American Journal of Epidemiology. *American Journal of Epidemiology* 1995;**142:**1047-58.
6. Brenner H. Use and limitations of the capture recapture method in disease monitoring with two dependent sources. *Epidemiology* 1995;**6**(1):42-8.
7. Regal RR, Hook EB. Goodness-of-fit based confidence intervals for estimates of the size of a closed population. *Stat Med* 1984;**3:**287-91.
8. Valway SE, Linkins RW, Godhes DM. Epidemiology of lower extremity amputations in the Indian Health Service, 1982-1987. *Diabetes Care* 1993;**16**(Supplement 1):349-353.
9. Sugarman JR, Gilbert TJ, Weiss NS. Prevalence of diabetes and impaired glucose tolerance among Navajo Indians. *Diabetes Care* 1992;**15:**115-120.
10. Gohdes D. Diabetes in North American Indians and Alaska Natives. In: National diabetes data group, ed. Diabetes in America. Second ed. Washington: National Institutes of Health, 1995: 683-701.
11. Amos AF, McCarty DJ, Zimmet P. The rising global burden of diabetes and its complications: estimates and projections to the year 2010. *Diabetic Medicine* 1997;**14**(Suppl 5):S1-85.
12. Fowkes FGR. Epidemiology of peripheral vascular disease. *Atherosclerosis* 1997;**131:**S29-S31.
13. Gallotta G, Iazzetta N, Milan G, Ruocco A, Napoli C, Postiglione A. Prevalence of peripheral arterial disease in an elderly rural population of Southern Italy. *Gerontology* 1997;**43**(5):289-95.
14. Takei H, Ishikawa S, Otaki A, et al. Screening for abdominal aortic aneurysm and

occlusive peripheral vascular disease in Japanese residents. *Surgery Today* 1995;**25**(7):608-11.

15. Diabetes Drafting Group. Prevalence of small vessel and large vessel disease in diabetic patients from 14 centres. *Diabetologia* 1985;**28**:615-640.
16. Gujral JS, McNally BP, O'Malley BP, Burden AC. Ethnic differences in the incidence of lower extremity amputation secondary to diabetes mellitus. *Diabetic Medicine* 1993;**10**:271-274.
17. Coggon D, Winter P, Martyn C, Inskip H. Contrasting epidemiology of aortic aneurysm and peripheral vascular disease in England and Wales. *British Medical Journal* 1996;**312**:948.
18. Lip GYH, Beevers DG. Epidemiology of aortic aneurysm and peripheral vascular disease may show ethnic differences (letter). *British Medical Journal* 1996;**313**:173.
19. Williams DRR, Fuller JH, Stevens LK. Validity of routinuely collected hospital admissions data on diabetes. *Diabetic Medicine* 1989;**6**:320-324.

20. Ebskov LB, Schroeder TV, Holstein PE. Epidemiology of leg amputation: the influence of vascular surgery. *British Journal of Surgery* 1994;**81**:1600-1603.

Table 1 - **The participating centres, population size, and sources from which cases were identified**

Centre	Population	Sources from which cases were identified
Leeds, UK	732055	Operating Theatre Records
		Hospital Discharge Data
		Limb Fitting Centre Records
Leicester, UK	923504	Operating Theatre Records
		Surgical Records
		Hospital Discharge Data
		Diabetic Foot Clinic
		Other
Madrid, Area 7, Spain	569307	Hospital records (Servicio de Cirugia Vascular)
		Theatre Records
		Prescribing Physicians
Middlesborough, UK	288887	Operating Theatre Records
		Hospital Discharge Data
		Limb Fitting Centre Records
		Diabetic Foot Register
Montgomery County, USA	705179	Operating Theatre Records
		Hospital Discharge Data
Navajo Area, Indian Health Service, USA	209147	IHS Hospital Discharge Data
		Operating Theatre Records
		LEA Registries
Newcastle upon Tyne, UK	283000	Operating Theatre Records
		Hospital Discharge Data
		Limb Fitting Centre Records
Ilan, Taiwan	465120	Hospital Discharge Data
		Limb Fitting Centre Records
		Bureau of Social Welfare
Tochigi, Japan	1982565	Orthopaedic and Surgical Records
		Handicap Register
		Physical Therapist records
Vicenza, Italy	279980	Hospital Discharge Data
		Operating Theatre Records
		Limb Fitting Centre Records

Table 2a – Age specific, crude and age adjusted (to a standard European population) incidence (number per 100,000 population per year) of first ever and all major amputations in men. Numbers in parentheses are cases observed over 2 years.

Centre	Level	Age group						
		0-19	20-39	40-59	60-79	80+	All	Age adjusted
Leeds, UK	First	1.1 (2)	0.8 (2)	11.5 (20)	65.1 (70)	97.2 (17)	15.4 (111)	16.4
	All	1.1 (2)	0.8 (2)	15.0 (26)	80.0 (86)	108.6 (19)	18.8 (135)	19.9
Leicester, UK	First	0.0	0.0	3.9 (9)	22.3 (30)	40.0 (9)	5.2 (48)	5.6
	All	0.0	0.0	4.4 (10)	29.7 (40)	48.9 (11)	6.6 (61)	7.2
Madrid, Spain	First	0.0	0.0	0.8 (1)	9.8 (9)	35.3 (5)	2.9 (15)	2.8
	All	0.0	0.0	2.4 (3)	10.9 (10)	49.4 (7)	3.8 (20)	3.7
Middlesborough, UK	First	0.0	2.4 (2)	14.7 (10)	84.1 (36)	77.9 (4)	18.4 (52)	19.8
	All	0.0	2.4 (2)	23.5 (16)	121.5 (52)	77.9 (4)	26.2 (74)	27.8
Montgomery , USA	First	0.0	0.0	14.7 (26)	84.9 (85)	79.4 (17)	18.8 (128)	19.2
	All	0.0	0.0	24.9 (44)	154.0 (154)	159.9 (34)	34.2 (232)	34.9
Navajo Area, USA	First	1.0 (1)	3.2 (2)	34.8 (10)	184.6 (22)	176.8 (4)	18.9 (39)	43.9
	All	1.0 (1)	3.2 (2)	41.8 (12)	260.1 (31)	221.0 (5)	24.8 (51)	58.7
Newcastle, UK	First	1.4 (1)	1.0 (1)	14.9 (9)	61.9 (25)	78.1 (5)	14.8 (41)	16.3
	All	1.4 (1)	2.1 (2)	19.9 (12)	74.3 (30)	93.8 (6)	18.4 (51)	20.2
Ilan, Taiwan	First	0.7 (1)	4.2 (7)	6.1 (6)	32.8 (19)	37.9 (2)	7.2 (35)	9.2
	All	0.7 (1)	4.2 (7)	8.2 (8)	36.2 (21)	75.8 (4)	8.5 (41)	11.3
Tochigi, Japan	First	0.0	0.7 (4)	4.1 (24)	12.1 (38)	24.3 (10)	3.9 (76)	3.8
	All	-	-	-	-	-	-	-
Vicenza, Italy	First	0.0	0.0	8.1 (6)	17.4 (7)	75.5 (5)	6.6 (18)	6.9
	All	0.0	0.0	9.4 (7)	32.3 (13)	75.5 (5)	9.2 (25)	9.6

Table 2b – Age specific, crude and age adjusted (to a standard European population) incidence (number per 100,000 population per year) of first ever and all major amputations in women. Numbers in parentheses are cases observed over 2 years.

Centre	Level	Age group						
		0-19	20-39	40-59	60-79	80+	All	Age adjusted
Leeds, UK	First	0.6 (1)	1.7 (4)	7.2 (12)	26.7 (34)	56.9 (23)	9.9 (74)	8.3
	All	0.6 (1)	1.7 (4)	8.4 (14)	34.6 (44)	69.3 (28)	12.2 (91)	10.2
Leicester, UK	First	0.0	0.7 (2)	0.9 (2)	17.0 (26)	23.2 (11)	4.5 (41)	3.8
	All	0.0	0.7 (2)	0.9 (2)	19.6 (30)	25.3 (12)	5.0 (47)	4.3
Madrid, Spain	First	0.0	0.0	0.0	2.1 (3)	5.8 (2)	0.8 (5)	0.5
	All	0.0	0.0	0.0	2.1 (3)	5.8 (2)	0.8 (5)	0.5
Middlesborough, UK	First	0.0	1.2 (1)	4.4 (3)	19.5 (10)	60.5 (8)	7.5 (22)	6.2
	All	0.0	1.2 (1)	5.8 (4)	29.2 (15)	68.1 (9)	9.8 (29)	8.4
Montgomery , USA	First	0.0	0.0	9.6 (18)	37.0 (48)	148.5 (62)	17.5 (128)	12.5
	All	0.0	0.0	15.6 (29)	49.4 (64)	186.8 (78)	23.4 (171)	17.0
Navajo Area, USA	First	0.0	0.0	17.7 (6)	95.5 (14)	106.2 (3)	10.5 (23)	22.4
	All	0.0	0.0	17.7 (6)	136.4 (20)	212.5 (6)	14.7 (32)	32.0
Newcastle, UK	First	0.0	0.0	3.3 (2)	29.4 (15)	54.9 (9)	9.0 (26)	7.0
	All	0.0	1.1 (1)	5.0 (3)	31.4 (16)	79.3 (13)	11.4 (33)	8.8
Ilan, Taiwan	First	0.0	0.0	3.2 (3)	27.6 (14)	43.5 (3)	4.5 (20)	6.4
	All	0.0	0.0	3.2 (3)	31.6 (16)	87.0 (6)	5.6 (25)	8.3
Tochigi, Japan	First	0.0	0.0	1.1 (6)	4.4 (17)	7.5 (6)	1.5 (29)	1.2
	All	-	-	-	-	-	-	-
Vicenza, Italy	First	0.0	1.1 (1)	0.0	14.5 (8)	100.9 (16)	8.7 (25)	5.6
	All	0.0	1.1 (1)	0.0	20.0 (11)	119.9 (19)	10.8 (31)	7.0

Table 3a – Age specific, crude and age adjusted (to a standard European population) incidence (number per 100,000 population per year) of first ever and all minor amputations in men. Numbers in parentheses are cases observed over 2 years.

Centre	Level	Age group						
		0-19	20-39	40-59	60-79	80+	All	Age adjusted
Leeds, UK	First	2.7 (5)	2.1 (5)	13.3 (23)	46.5 (50)	57.1 (10)	12.9 (93)	13.6
	All	3.8 (7)	2.9 (7)	17.9 (31)	80.0 (86)	91.4 (16)	20.4 (147)	21.5
Leicester, UK	First	0.0	0.4 (1)	3.0 (7)	6.7 (9)	13.3 (3)	2.2 (20)	2.3
	All	0.0	1.1 (3)	3.9 (9)	11.1 (15)	13.3 (3)	3.2 (30)	3.4
Madrid, Spain	First	0.0	0.0	4.0 (5)	6.5 (6)	14.1 (2)	2.5 (13)	2.4
	All	0.0	0.0	5.6 (7)	9.8 (9)	21.2 (3)	3.6 (19)	3.5
Middlesborough, UK	First	0.0	3.5 (3)	14.7 (10)	42.1 (18)	58.4 (3)	12.0 (34)	13.0
	All	0.0	3.5 (3)	17.7 (12)	65.4 (28)	58.4 (3)	16.3 (46)	17.4
Montgomery , USA	First	0.0	2.0 (5)	19.8 (35)	47.9 (48)	50.5 (11)	14.5 (99)	14.4
	All	0	5.0 (10)	30.1 (53)	88.0 (88)	56.2 (12)	24.0 (163)	24.3
Navajo Area, USA	First	1.0 (1)	11.2 (7)	114.9 (33)	176.2 (21)	88.4 (2)	31.1 (64)	61.8
	All	1.0 (1)	20.9 (13)	174.0 (50)	293.6 (35)	132.6 (3)	49.5 (102)	98.8
Newcastle, UK	First	0.0	2.1 (2)	5.0 (3)	27.2 (11)	93.8 (6)	7.9 (22)	8.8
	All	0.0	2.1 (2)	14.9 (9)	44.6 (18)	109.4 (7)	13.0 (36)	14.4
Ilan, Taiwan	First	0.0	1.2 (2)	1.0 (1)	3.5 (2)	37.9 (2)	1.5 (7)	2.3
	All	0.0	1.2 (2)	1.0 (1)	5.2 (3)	37.9 (2)	1.9 (9)	2.5
Tochigi, Japan	First	0.2 (1)	0.0	0.7 (4)	1.3 (4)	4.9 (2)	0.6 (11)	0.6
	All	-	-	-	-	-	-	-
Vicenza, Italy	First	3.6 (2)	0.0	5.4 (4)	22.4 (9)	0.0	5.5 (15)	5.8
	All	3.6 (2)	0.0	8.1 6 (6)	39.8 (16)	0.0	8.8 (24)	9.2

Table 3b – Age specific, crude and age adjusted (to a standard European population) incidence (number per 100,000 population per year) of first ever and all minor amputations in women. Numbers in parentheses are cases observed over 2 years.

Centre	Level	Age group						
		0-19	20-39	40-59	60-79	80+	All	Age adjusted
Leeds, UK	First	2.8 (5)	3.0 (7)	12.6 (21)	33.0 (42)	54.5 (22)	13.0 (97)	11.5
	All	2.8 (5)	3.5 (8)	16.8 (28)	40.1 (51)	74.3 (30)	16.4 (122)	14.3
Leicester, UK	First	0.0	0.0	0.9 (2)	9.2 (14)	2.1 (1)	1.6 (17)	1.7
	All	0.0	0.0	0.9 (2)	9.8 (15)	2.1 (1)	2.0 (18)	1.8
Madrid, Spain	First	0.0	0.0	0.0	2.1 (3)	5.8 (2)	0.8 (5)	0.5
	All	0.0	0.0	0.0	2.1 (3)	5.8 (2)	0.8 (5)	0.5
Middlesborough, UK	First	1.3 (1)	1.2 (1)	4.4 (3)	21.4 (11)	37.8 (5)	7.1 (21)	6.2
	All	1.3 (1)	1.2 (1)	5.8 (4)	31.1 (16)	37.8 (5)	9.2 (27)	8.1
Montgomery , USA	First	0.0	0	12.9 (24)	30.8 (40)	26.3 (11)	10.3 (75)	8.7
	All	0.0	2.0 (4)	13.5 (25)	37.0 (48)	38.3 (16)	12.8 (94)	10.8
Navajo Area, USA	First	3.0 (3)	0.0	29.6 (10)	102.3 (15)	35.4 (1)	13.3 (29)	25.1
	All	3.0 (3)	0.0	56.2 (19)	163.7 (24)	35.4 (1)	21.5 (47)	41.2
Newcastle, UK	First	1.4 (1)	1.1 (1)	1.7 (1)	15.7 (8)	18.3 (3)	4.9 (14)	4.1
	All	1.4 (1)	1.1 (1)	1.7 (1)	21.6 (11)	30.5 (5)	6.6 (19)	5.4
Ilan, Taiwan	First	0.7 (1)	0.0	0.0	4.0 (2)	14.5 (1)	0.9 (4)	1.2
	All	0.7 (1)	0.0	0.0	4.0 (2)	14.5 (1)	0.9 (4)	1.2
Tochigi, Japan	First	0.0	0.0	0.2 (1)	0.8 (3)	1.3 (1)	0.3 (5)	0.2
	All	-	-	-	-	-	-	-
Vicenza, Italy	First	0.0	0.0	2.7 (2)	7.3 (4)	12.6 (2)	2.8 (8)	2.2
	All	0.0	0.0	2.7 (2)	10.9 (6)	12.6 (2)	3.5 (10)	2.7

Table 4 – Estimated (using capture mark recapture methods) total number of first and all major and minor amputations and estimated ascertainment of amputations in each centre. Figures for men and women combined.

Centre	Level	First ever amputations				All amputations			
		N identified from overlapping sources	Estimated N (95% CIs)	Total N identified	Estimated Ascertainment (%)	N identified from overlapping sources	Estimated N (95% CIs)	Total N identified	Estima Ascertain (%)
Leeds, UK	major	175	189 (181,202)	185	97.9	213	226 (216,241)	226	100
	minor	172	178 (173,183)	190	93.7	233	241 (236,248)	269	100
Leicester, UK	major	82	102 (89,207)	89	87.3	108	153 (119,297)	108	70.6
	minor	a	a	37	a	a	a	48	A
Madrid, Spain	major	12	19 (16,25)	20	100	21	25 (21,37)	25	100
	minor	18	23 (19,38)	18	78.3	24	32 (25,56)	24	75.0
Middlesborough, UK	major	74	81 (75,94)	74	91.4	99	101 (99,104)	103	100
	minor	51	53 (52,56)	55	100	69	71 (69,74)	73	100
Montgomery , USA	major	256	256 (255,258)	256	100	403	403 (402,403)	403	100
	minor	174	174 (173,175)	174	100	257	257 (256,258)	257	100
Navajo Area, USA	major	61	69 (62,91)	62	89.9	80	93 (83,110)	89.2	66.9
	minor	92	128 (106,193)	93	72.7	145	184 (159,243)	149	81.0
Newcastle, UK	major	69	78 (71,89)	69	88.5	86	118 (93,212)	86	72.9
	minor	35	39	36	92.3	54	59 (55,69)	55	93.2
Ilan, Taiwan	major	55	57 (55,60)	55	96.5	66	68 (66,73)	66	97.1
	minor	a	a	11	a	a	a	13	A
Tochigi, Japan	major	103	156 (128,209)	105	67.3	-	-	-	-
	minor	16	30 (17,381)	16	53.3	-	-	-	-
Vicenza, Italy	major	37	71 (46,161)	43	60.6	54	76 (61,108)	56	73.7
	minor	24	63 (33,214)	23	36.5	30	78 (44,214)	34	43.6

a In these cases there was insufficient overlap between the different sources to use capture recapture.
In some cases the numbers identified from overlapping sources are different (in all cases by no more than 5%) to the numbers in tables 2 and 3. This is for two reasons. Firstly a few cases were identified from non-overlapping sources, and secondly for two cases in Newcastle age could not be determined from the data available and hence they were not included in the age specific rates.

Table 5 - Conditions associated with first ever major and minor amputations. Figures are percentages. More than one condition may be present per case.

		Men				Women			
		Trauma	**Diabetes**	**Infection**	**PVD**	**Trauma**	**Diabetes**	**Infection**	**PVD**
Leeds, UK	major	4.5	39.6	56.8	82.9	9.5	36.5	58.1	70.3
	minor	9.7	46.2	60.2	57	0	28.9	33	25.8
Leicester, UK	major	0	47.9	33.3	87.5	2.4	35.7	16.7	81
	minor	4.8	57.1	42.9	61.9	0	41.2	11.8	58.8
Madrid, Spain	major	0	80	60	93.3	0	60	40	100
	minor	7.7	69.2	76.9	100	0	80	80	80
Middlesborough, UK	major	9.6	44.2	53.8	76.9	9.1	36.4	50	72.7
	minor	0	58.8	70.6	64.7	4.8	42.9	38.1	61.9
Montgomery,USA	major	2.9	64	14.0	71.6	2.3	62	8.7	60.1
	minor	2.0	60	31.7	89.1	1.2	51	26.5	68.3
Navajo Area, USA	major	10.3	90	84.6	51.3	0	91	100	65.2
	minor	6.3	81.3	87.5	15.6	10.3	86.2	89.7	17.2
Newcastle, UK	major	9.8	33.3	36.6	70.7	15.4	23.1	30.8	61.5
	minor	13.6	36.4	54.5	63.6	0	28.6	28.6	50
Ilan, Taiwan	major	50.0	41.2	52.8	55.6	15.8	52.6	84.2	73.7
	minor	57.1	37.5	42.9	42.9	25.0	75	75.0	75.0
Tochigi, Japan	major	2.6	32.9	48.7	55.3	0	40.7	22.2	29.6
	minor	0	63.6	27.3	36.4	20.0	100	40.0	20.0
Vicenza, Italy	major	0	61.1	22.2	77.8	4	20	4	88
	minor	6.7	60	60	60	12.5	50	37.5	62.5

Figure 1 Age adjusted incidence of first ever major amputations (per 100,000 population per year)

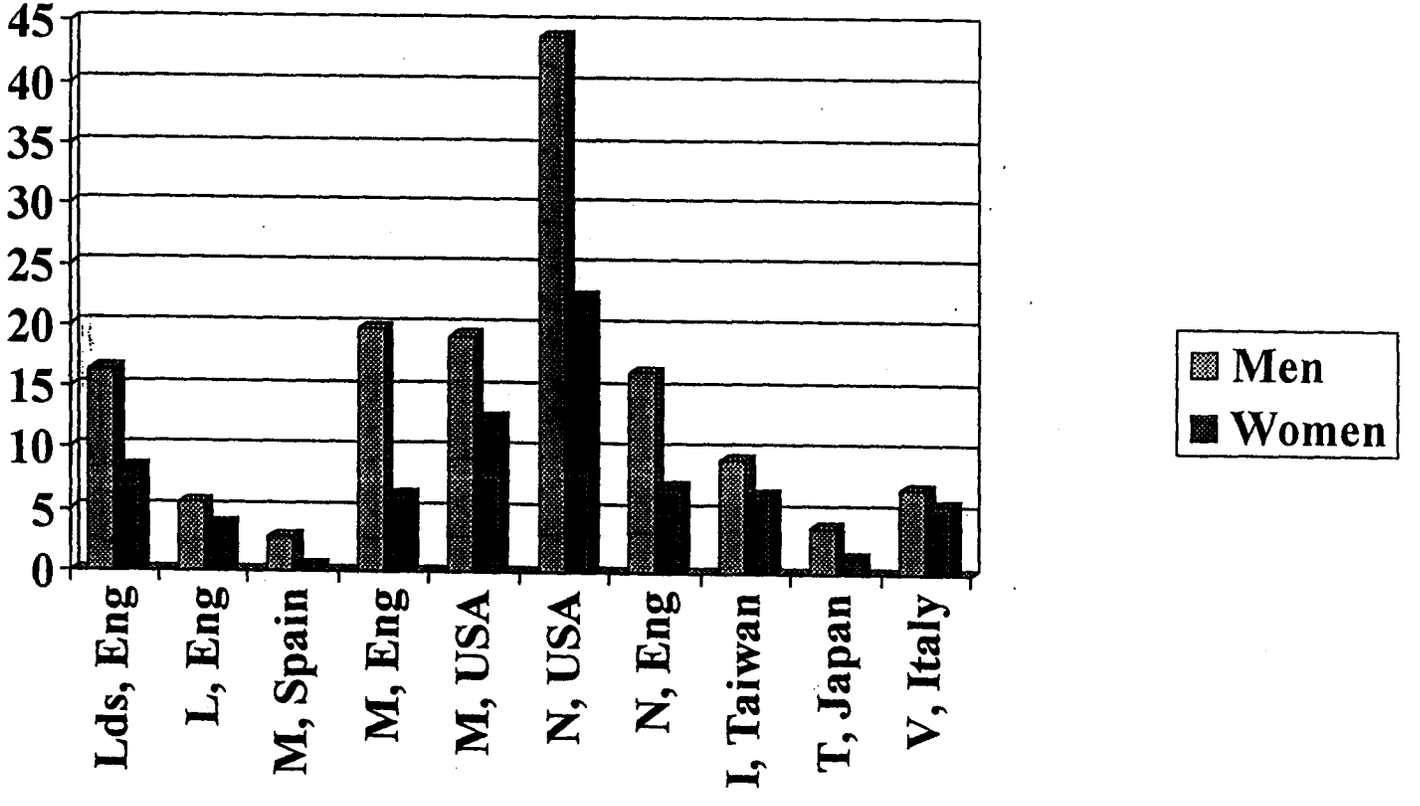

Figure 2 Age adjusted incidence of first ever minor amputations (per 100,000 population per year)

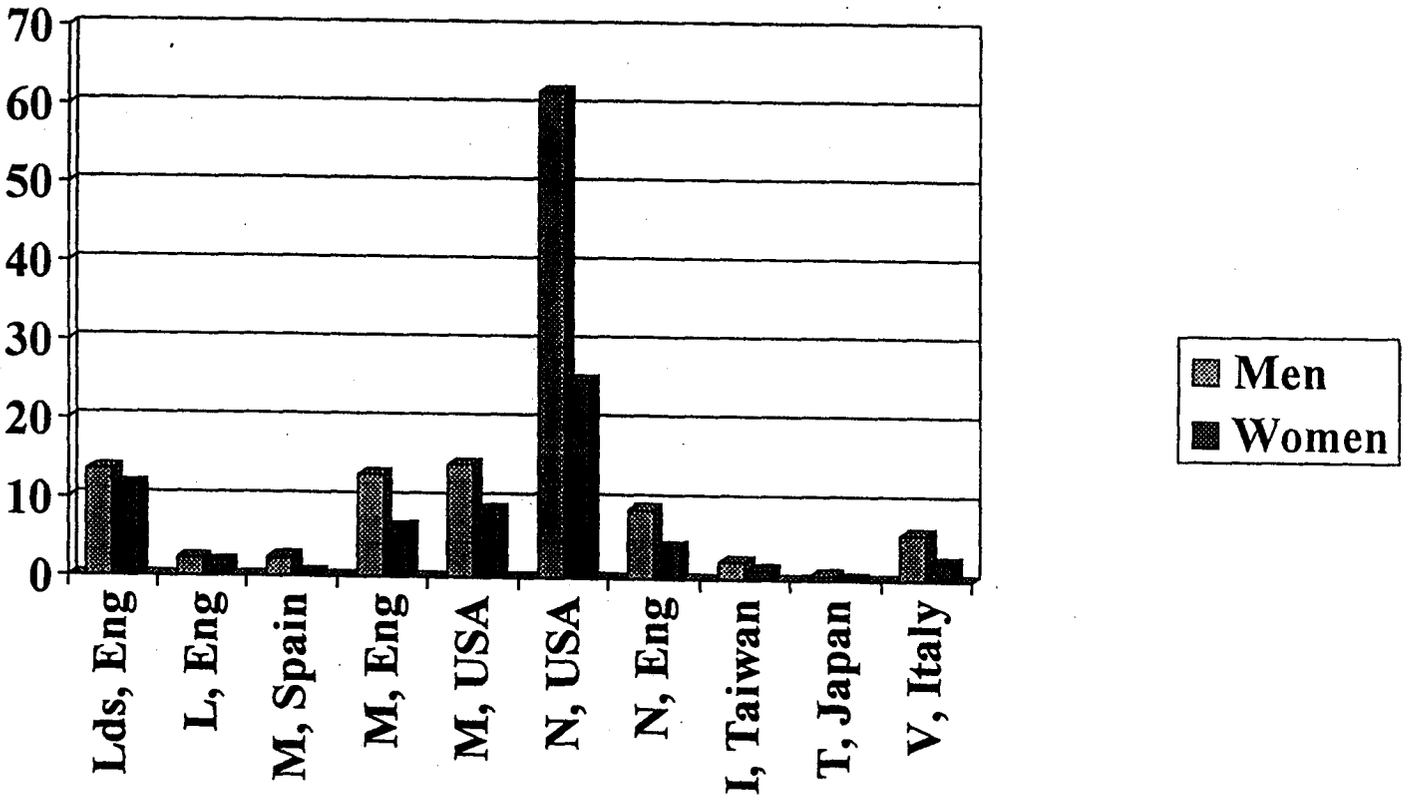

Figure 3 Percentage of first ever major amputations associated with diabetes

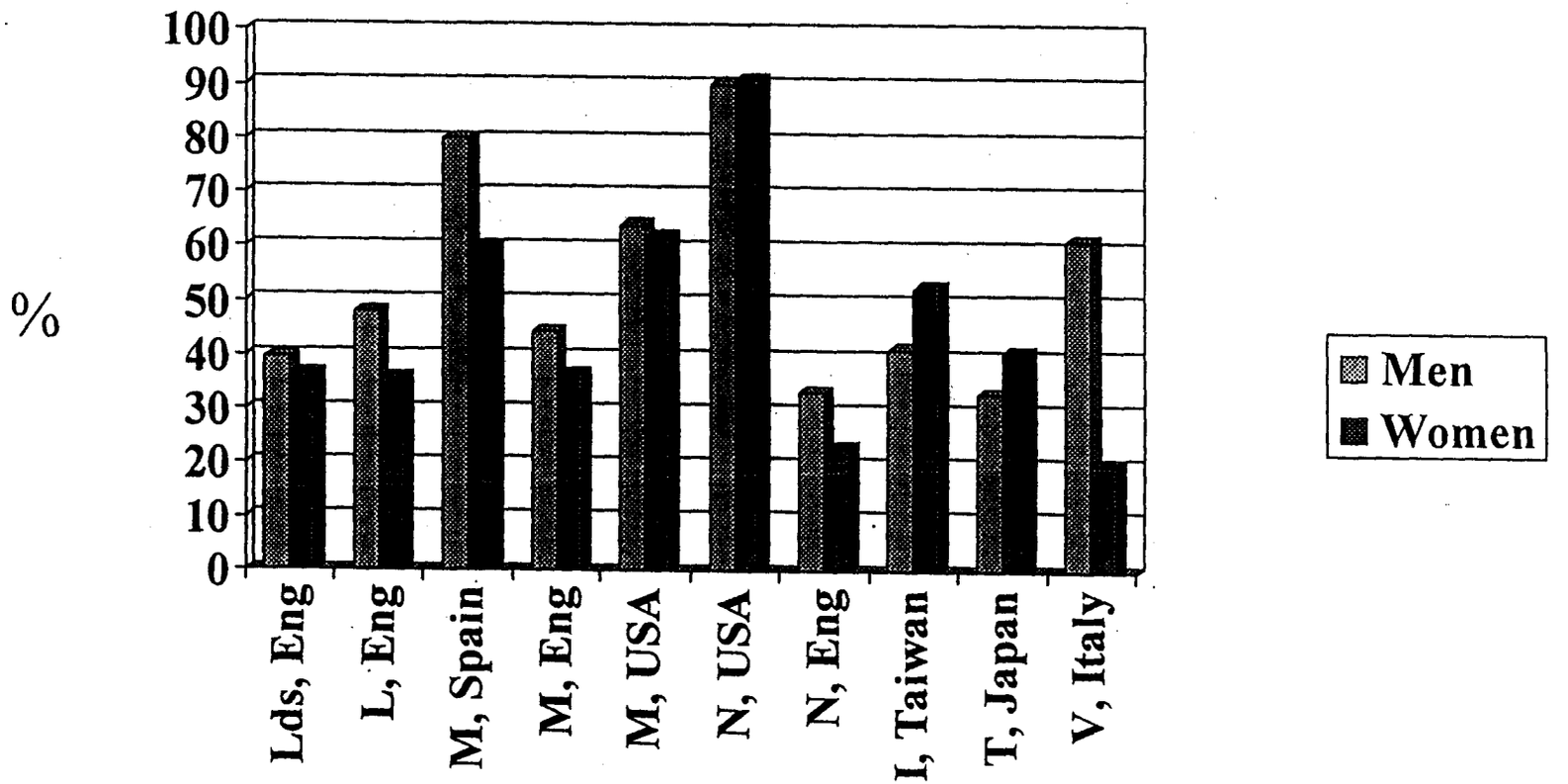

Figure 4 Percentage of first ever minor amputations associated with diabetes