CORRESPONDENCE

Hypertension and ascorbic acid

Sir—We are surprised that Stephen Duffy and colleagues (Dec 11, p 2048) failed to mention the results of previous trials of vitamin C and blood pressure, which we summarised in a systematic review published in a Medline-listed peer-review journal in 1997. We have tabulated the methods and results of their trial with those of the other three trials of vitamin C and the difference in blood pressure in treatment and control groups (table). Most of the patients in the previous trials had hypertension. All four trials were of similar duration and used comparable doses of vitamin C.

We were not able to formally combine the results of these trials because none reported the standard error of the between-group difference and only two reported either CIs or p values that would allow the standard error to be calculated. Nevertheless, these trials suggest that if vitamin C does have any effect on blood pressure at all, the true effect is likely to be much smaller than that reported by Duffy and colleagues.

All the trials reported so far, including that by Duffy and colleagues, are too small to provide conclusive evidence of an effect of vitamin C on blood pressure, especially if (as the current trial evidence suggests) the true effect is not large. Furthermore, systematic reviews based on small trials are also susceptible to publication bias. Any future trials should be larger and should report CIs for estimated treatment effects.

*Andy Ness, Jonathan Sterne
Department of Social Medicine, University of Bristol, Bristol BS8 2PR, UK


Sir—A very attractive hypothesis to support the study by Stephen Duffy and colleagues is that ascorbic acid may reduce blood pressure through a nitric-oxide (NO)-mediated mechanism. Vitamin C has been shown to improve endothelium-dependent vasodilation in essential hypertension1 and in patients with hypercholesterolaemia, and to restore NO-mediated flow-dependent dilation in patients with heart failure. Because plasma cyclic guanosine monophosphate (cGMP) did not change in their patients after treatment with ascorbic acid, the investigators could not show an effect of ascorbic acid on NO activity. However, plasma cGMP may not be sensitive enough to reflect a change in NO bioactivity, as the investigators admit. By measuring endogenous NO in the exhaled air, Schilling and colleagues found that hypertensive patients exhaled significantly less NO than healthy volunteers.

We assessed the effect of oral intake of ascorbic acid (1 g/day for 2 weeks, after 2 weeks of placebo) on the respiratory production of NO in a single-blind sequential study. Eight healthy volunteers (aged 49–9 [SD 14] years, four men) who were not smokers and who had uncomplicated hypertension (untreated diastolic pressure >90 mm Hg in four, a history of taking antihypertensive drugs in four, taking diuretics in three, and β-blocking drugs in one) and eight control participants (age 46–9 [14] years, four men) were studied. Exhaled NO concentration was measured by chemiluminescence method with an NO analyser (Sievers Instruments, Boulder, CO, USA). Participants inhaled NO-free air to total lung capacity and then they were asked to exhale at a constant flow (45 mL/s) against a high resistance (20 mm Hg, to exclude nasal air contamination) into mylar balloons. NO concentration was analysed within 1 h.

After taking vitamin C, exhaled NO increased significantly in patients with hypertension from 11·1 [1·1] ppb after placebo to 13·5 [0·9] ppb (p<0·001), while mean blood pressure decreased significantly from 112·6 [3·3] mm Hg after placebo to 107·9 [3·7] mm Hg (p<0·01). The decrease of mean blood pressure was a result of a significant decrease of systolic blood pressure (from 150·6 [8·6] mm Hg to 141·2 [7·4] mm Hg, after placebo and vitamin C, respectively; p<0·001).

Hypertension and ascorbic acid

<table>
<thead>
<tr>
<th>Study</th>
<th>Patient number</th>
<th>Design</th>
<th>Duration (weeks)</th>
<th>Intervention</th>
<th>Blood pressure (mm Hg)</th>
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<tbody>
<tr>
<td>Osilesi O, et al, 1991</td>
<td>20</td>
<td>Cross over 6 weeks</td>
<td>1000 mg per day</td>
<td>Systolic: –6·3 (p&lt;0·05)</td>
<td>Diastolic: 0·6 NS</td>
</tr>
<tr>
<td>Lovat LB, et al, 1993</td>
<td>27</td>
<td>Cross over 4 weeks</td>
<td>400 mg per day</td>
<td>–0·2 to –5·3</td>
<td>0·2 to –1·9 NS</td>
</tr>
<tr>
<td>Ghosh SK, et al, 1994</td>
<td>48</td>
<td>Controlled 6 weeks</td>
<td>500 mg per day</td>
<td>–2·5 NS</td>
<td>1·2 NS</td>
</tr>
<tr>
<td>Duffy SJ, et al, 1995</td>
<td>39</td>
<td>Controlled 4 weeks</td>
<td>2000 g bolus then 500 mg per day</td>
<td>–11 (p&lt;0·03)</td>
<td>–6 (p&lt;0·24)</td>
</tr>
</tbody>
</table>

*Duration of treatment period or periods, this does not include run in or washout periods. †Between group mean difference at follow-up. ‡The reported effects varied according to whether the blood pressure was measured lying or standing and whether the participant received vitamin C or placebo first. These values are the range of differences reported and not the CI of the difference. NS=not significant.

Trials of vitamin C supplementation and blood pressure

THE LANCET • Vol 355 • April 8, 2000 1271
In control participants no significant change was observed in exhaled NO and in blood pressure after taking vitamin C from 127 to [1-2] ppb to 12 [1-5] ppb and from 91-4 [4-3] mm Hg to 91 [4-2] mm Hg, respectively. Our results suggest that patients with hypertension have decreased NO availability in the respiratory tract, probably as a result of excessive oxidative burden. There is cumulating evidence that ascorbic acid increases NO bioactivity through its antioxidant effects. We postulate that in patients with hypertension the increase in exhaled NO after vitamin C mirrors a systemic increase in NO bioactivity, which might explain the observed hypotensive effect.

*Giovanni Rolla, Luisa Brussino, Renata Carra, Erika Garbella, Caterina Bucci*

Dipartimento di Scienze Biomediche e Oncologia Umana, Università di Torino, via Genova 3, 10126 Torino, Italy (e-mail: rolla@molinette.unito.it)


Sir—After reading the research letter by Stephen Duffy and colleagues we examined the effect of vitamin C supplementation on blood pressure in the Arterial Disease Multiple Intervention Trial (ADMIT). ADMIT was a prospective double-blind placebo controlled clinical trial in patients with peripheral arterial disease who were randomly assigned in a factorial design antioxidant vitamins versus placebo (vitamin C 1000 mg a day, vitamin E 800 IU per day, β-carotene 24 mg per day, or placebo), warfarin versus aspirin, and niacin versus placebo. There was no difference in systolic or diastolic blood pressure between patients randomly assigned antioxidant vitamins (n=181) compared with those randomly assigned placebo (n=182) by repeated measures analysis of variance.

Analysis limited to people with high blood pressure (higher than 140/90 mm Hg; n=177) showed no effect of vitamin supplementation on blood pressure when compared with placebo (table). There was no interaction between antioxidant vitamins and niacin or warfarin with respect to systolic or diastolic blood pressure. These data from the prospective double-blind placebo controlled ADMIT clinical trial, in conjunction with most published reports,1,2,3 suggest that the effect of ascorbic acid on blood pressure is probably non-existent or negligible.

*John B Kostis, Alan C Wilson, Clifton R Lacy*

UMDNJ-Robert Wood Johnson Medical School, New Brunswick, NJ 08903–0019, USA


Sir—Stephen Duffy and colleagues report that daily supplementation of 500 mg of ascorbic acid for 1 month has a beneficial effect on blood pressure in patients with hypertension. Because ascorbic acid supplementation in their study did not reduce indices of oxidative stress (F2 isoprostanes), they suggest that the effect of the ascorbic acid supplementation does not seem to be linked to the antioxidant property of the substance and conclude that the mechanism of blood pressure reduction by vitamin C remains unclear.

We have three queries. In 1991 we reported a possible antihypertensive effect of ascorbic acid.2 Evidence that the same effect was reproduced by each of two other antioxidants—ie, thipronine and glutathione—strongly supported the hypothesis that the antihypertensive effect of ascorbic acid was due to its antioxidant activity. In their study, Duffy and colleagues do not report possible dietary changes of the study participants. Because eating more or less fruit and vegetables for as short a period as 1 week is enough to alter antioxidant status, we believe that such information could be made available before drawing any conclusions.

An acute effect of ascorbic acid on blood pressure was not found in the study by Duffy and colleagues. An acute vasodilatory effect of ascorbic acid has been shown in several studies3,4 and may well be dose-related. Intravenous administration of 1 g ascorbic acid has been reported to reduce blood pressure in hypertensive patients but not in normotensive individuals.5 To confirm or exclude the acute hypotensive effect of oral ascorbic acid, a comparison of the effects of different doses—eg, 2 g versus 500 mg—might identify an oral dose of ascorbic acid effective in rapidly lowering blood pressure.

In a study by Levine and colleagues6 acute administration of ascorbic acid, 2 g orally, versus placebo, produced an improvement in endothelial dysfunction in patients with coronary artery disease. In these individuals, about 50% of whom had hypertension, blood pressure values after treatment were similar in the vitamin C and placebo groups, while acute variations in blood pressure as a consequence of ascorbic acid administration were not illustrated. If such data, particularly those regarding the subset of participants with hypertension could be collected, they could help clarify the point.

From a clinical point of view there are at least two concerns about the use of ascorbic acid: the possibility of increased risk of urinary oxalate stones during therapy with ascorbic acid and the increased production of advanced glyated endproducts induced by vitamin C in patients with diabetes. Moreover, while nearly all studies confirm that blood-pressure lowering reduces mortality from cardiovascular disease, no
intervention study based on vitamin C tablet administration has ever shown the same effect.

*Antonio Ceriello, Enrico Motz, Dario Giugliano
*Department of Pathology and Medicine, University of Udine, 33100 Udine, Italy; and Department of Geriatric Medicine and Metabolic Disease, II University of Naples, Naples (e-mail: Antonio.Ceriello@Dipmsc.uniiud.it)


Sir—Stephen Duffy and colleagues1 did not find any changes in the concentrations of nitric oxide, prostacyclin, or lipid peroxides to explain the blood-pressure lowering action of ascorbic acid. I have suggested that some of the beneficial actions of ascorbic acid could be due to their ability to enhance prostaglandin E1 (PGE1) production.2

In vitro, ascorbic acid (range 10–100 µg/mL) caused a dose-dependent and significant enhancement of conversion of dihomo-γ-linolenic acid (DGLA) to PGE1, and to thromboxane B1 (TXB1) by human platelets, but did not have any effect on the conversion of arachidonic acid (AA) to PGE1, and TXB2.2 This action of ascorbic acid on prostaglandin metabolism may explain its platelet antiaggregatory and antihypertensive action since PGE1, is a potent platelet antiaggregatory and vasodilator. Studies done by McCarron and colleagues3 showed that intake of vitamins A and C was low in the group of participants who had hypertension compared with controls. It is also known that a dietary intake of 300–450 mg per day ascorbic acid reduces blood cholesterol concentrations by at least 15–70 mg%.3 Ascorbic acid can also lower plasma glucose concentrations in patients with type II diabetes.4

Duffy and colleagues did not measure PGE1 concentrations in the plasma or in the platelets. I suggest that the beneficial action of ascorbic acid observed in their study is a result of enhanced formation and release of PGE1 by platelets, endothelial cells, or leucocytes. Since the interaction between various nutrients, the precursors of eicosanoids and eicosanoids, free radicals, nitric oxide, and endothelium appears complex, and may be relevant to the pathobiology of essential hypertension, it is important to look at all these factors in a comprehensive manner.

U N Das
EFA Sciences LLC, Norwood, MA 02062, USA

Sir—We thank the correspondents for their interest in our work. We were therefore interested in endothelium-dependent dilation, but did not cite the comment about PGE1 by UN Das. However, all but one of those studies showed improvement in endothelium-dependent dilation, but no change in blood pressure. Ceriello and colleagues suggest that we should examine the effect of a higher dose of ascorbic acid, but apparently missed the observation that placebo treatment was associated with a 7–10 mm Hg reduction in blood pressure suggests that some unmeasured factors may have confounded the results, or that the blood-pressure measurements were inaccurate.

Antonio Ceriello and colleagues previously reported1 a decrease in blood pressure after high-dose intravenous ascorbic acid, but in their study this effect lasted only 20 min, and thus has limited relevance to the question of long-term treatment of hypertension. They cite several other studies, including work by us, showing an acute vasodilatory effect of ascorbic acid. However, all but one of those studies showed improvement in endothelium-dependent dilation, but no change in blood pressure. Ceriello and colleagues suggest that we should examine the effect of a higher dose of ascorbic acid, but apparently missed the fact that we reported that a 2 g dose had no effect on blood pressure at our 2-h timepoint. We agree that dietary antioxidants could influence blood pressure, but argue that it is unlikely that a change in diet explains our positive results since we employed a randomised study design, and no patients reported any change in dietary habits during the 1 month study period.

Our study did not provide a mechanistic explanation for the blood pressure lowering effect of ascorbic acid. We were therefore interested in the comment about PGE1 by U N Das. However, it is our understanding that, in general, PGE1, is not thought to be an important regulator of vascular tone in human beings. As stated in our study, we strongly agree that there is a need for a...
carefully-conducted, large-scale study of the long-term effects of ascorbic acid on blood pressure in patients with hypertension. Such a study should be done before this treatment can be recommended.

*Stephen J Duffy, Joseph A Vita
Evans Department of Medicine and Whitaker Cardiovascular Institute, Boston University School of Medicine, Boston, MA 02118, USA (e-mail: jvita@bu.edu)


### Antenatal corticosteroids: is more better?

Sir—The Jan 22 commentary by G N Smith and colleagues1 is timely because although the use of antenatal maternal steroids to reduce death and disability in pre-term infants is well established, a culture of blame is developing around obstetricians who fail to prescribe steroids to women at risk in the 10 days or so preceding delivery. These potentially dangerous drugs have been widely used and their use is increasing, possibly with a relative diminution of benefit. The question of adverse effects on development should not be addressed in premature survivors, in whom there are many confounding variables, but in infants born at term after single or multiple doses of steroids have been given. There are many such infants aged up to about 6 years. While awaiting the results of large multicentre studies, which are aiming to answer the question, a quick case-control study may, or may not, provide a rapid resolution of major fears. Our own attempt to set up such a study 2 years ago foundered on poor record linkage between pharmacy and maternity data systems at a time when the latter were being upgraded. Other units with better linkage systems might undertake such a trial.

*Robert B Fraser, Peter Stewart
Department of Obstetrics and Gynaecology, Northern General Hospital NHS Trust, Sheffield S5 7AU, UK


### Increased body-mass index in patients with narcolepsy

Sir—Seiji Nishino and colleagues (Jan 1, p 39)1 showed that patients with narcolepsy have reduced concentrations of hypocretin (orexin) in their cerebrospinal fluid. Because hypocretins stimulate food intake in animal models, Siegel hypothesised2 that patients with narcolepsy should be lean, rather than obese. However, in an earlier report Honda and colleagues3 suggest that in patients with narcolepsy the frequency of obesity and of non-insulin dependent diabetes is increased compared with a non-healthy control group of psychiatric patients.

To gather more reliable information, we retrospectively identified all patients of 14–70 years who had narcolepsy, and who were treated in the Max-Planck-Institute of Psychiatry, Munich, Germany, between 1988 and 1999 (n=45). We selected all patients with: excessive daytime sleepiness; cataplectic attacks; multiple sleep-onset REM (rapid eye movement) periods (SOREMs); and who were HLA-DR2 positive. In these 35 patients (24 men, 11 women) we recorded the body-mass index (BMI). At the time of height and weight measurements 18 patients (mean age 36·3 [SD 17·3] years) had never been treated pharmacologically for the disease. 17 patients (mean age 48·5 [12·6] years) had previously received tricyclic antidepressants, psychostimulants, or combinations of these drugs before.

The BMIs were plotted into BMI-percentiles representative of the German male and female population, respectively (figure). The distribution of the BMIs differed significantly from the rectangular distribution on the 0–100 interval (Kolmogorov-Smirnov test). This was apparent both in male (mean BMI percentile 75·6 [23·0]) and female (61·2 [32·5]) patients. The mean BMI-percentile of those patients who had never been treated for narcolepsy (74·8 [25·0]) was in the same range.

These results suggest that patients with narcolepsy have a higher BMI...
than population controls. This could result from altered eating behaviour or energy homeostasis. Because no obvious differences between medicated and drug-naive patients were apparent, we suspect that the higher BMI is linked to the pathophysiology of the disease. This association could arise from a direct pathogenic link that involves hypocretins. Alternatively, it may be a consequence of disease-related behaviour—eg, reduced locomotor activity, increased amounts of sleep, or other aspects of behaviour resulting in a lower energy expenditure. Because it remains unclear whether and how an increased BMI in narcolepsy is associated with decreased hypocretin concentrations,1 BMI and hypocretin concentrations should be studied in parallel in patients with narcolepsy. Moreover, the association between BMI and hypocretin concentrations should also be explored in patients with other disorders of excessive sleepiness as well as in healthy and obese controls. Andreas Schuld, Johannes Hebebrand, Frank Giller, *Thomas Polimächer

*Max Planck Institute of Psychiatry, D-80804 Munich, Germany; and Department of Child and Adolescent Psychiatry and Institute for Medical Biometry and Epidemiology, Philipps-Universität Marburg, Marburg, Germany (e-mail: topo@mpipsykl.mpg.de)


### Comparison of health indicators between Matlab (ICDDR:B intervention area) and Bangladesh

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Matlab (ICDDR:B intervention area)</th>
<th>Bangladesh</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contraceptive prevalence rate (%)</td>
<td>70</td>
<td>49</td>
</tr>
<tr>
<td>Crude death rate (per 1000)</td>
<td>6-6</td>
<td>8-0</td>
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<tr>
<td>Infant mortality rate (per 1000 livebirths)</td>
<td>50</td>
<td>67</td>
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<tr>
<td>Child death rate (1–4 years)</td>
<td>4-5</td>
<td>11-0</td>
</tr>
<tr>
<td>Life expectancy at birth</td>
<td>67-7</td>
<td>59-8</td>
</tr>
</tbody>
</table>

**Authors’ reply**

Sir—We have some concerns regarding the article on management of reproductive-tract infections in Bangladesh, by Sarah Hawkes and colleagues (Nov 20, p 1776).1 The investigators worked with highly selected women served by an intensive family-planning programme. The women were also married and regular attendants of maternal and child health/family-planning clinics. Further, participants appeared to be older, of greater parity, and more likely to be users of contraceptives compared with those that declined involvement. These concerns can be confirmed by reanalysis of the original data available from the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR:B). These results, available to the investigators, should have been presented in the paper submitted to The Lancet. In addition, ICDDR:B reported that the risk assessment component—without which syndromic management can not be fully evaluated—was not explored as recommended. In a previous study,2 Wasserheit and colleagues articulately described why extrapolation of conclusions associated with reproductive health behaviour and services in this population subgroup was not possible. Extrapolation of such data from this study to Bangladesh and the rest of Asia is poor science and a grave mistake.

ICDDR:B is a state-of-the-art research institute. Researchers from the institute have been working in the vicinity of Matlab for over 25 years. Because of several interventions in this area, in particular the intensive family planning home delivery service, there is a significant difference between Matlab and other parts of the country.

We have reviewed over 60 reports on diagnosis of sexually-transmitted diseases (STD) and management experience from the non-governmental-organisation and research communities in Bangladesh. The conclusions and policy decisions taken differed substantively3 from those of Hawkes and colleagues. Nevertheless, persistence in presenting and publishing these interpretations have had a strong influence on donors’ willingness to fund STD services. The subject is technical and some donor representatives had difficulties grasping the issues. Extensive time and energy has gone into resolving the confusion created. Scientists, consultants, and representatives of ICDDR:B, the Government of Bangladesh, the Bangladesh Rural Advancement Committee, Bangladesh Women’s Health Coalition, WHO, Marie Stopes, the Association for Voluntary Surgical Contraception, the Population Council, USAID, and the World Bank became involved. The study by Hawkes and colleagues was reviewed and rejected by the international and national scientists involved locally. STD management in the 5th Health and Population plan remains based on the syndromic approach.

Fawzia Rasheed, Enamul Karim
Ave Louis Yung-St, 1290 Versoix, Switzerland, and Institute of Health Sector Development, London, UK (e-mail: rasheedf@bluewin.ch)

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and highlighted by Rasheed and Karim, we know of no evidence that high quality family planning services alone (especially those where condom use is low, as in this population) have any impact on STI prevalence.

Rasheed and Karim seem to believe that we are reporting results from our population surveys done as part of the same project. The results of our paper were based on women self-reporting to maternal and child health (MCH) family-planning clinics with symptoms of possible reproductive-tract infections. The results pertain to clinical settings only, and, no women declined to participate in this clinic-based survey. We did not extrapolate from the clinics to the general population in Matlab or beyond. The fact that younger and non-exploring girls might not attend family-planning services is acknowledged in the paper. This non-attendance is typical of many situations and not limited to Matlab. Therefore, no matter how good the intervention tool in MCH-family-planning clinics, it may still miss these girls and women at potential risk. Even the presence of services dedicated to STI management are acknowledged to have a very limited impact on STI rates in the absence of interventions to promote appropriate health seeking and possible community screening.

As we clearly state in the conclusion, syndromic management has been well tested as a management tool in several settings (specifically for symptomatic men, and people with genital ulcers). The use of this tool in women complaining of vaginal discharge is, however, recognised to be difficult. Based on WHO guidelines (which propose that risk factors for STIs should be locally determined), 4 we calculated the cost and effectiveness of using a tool which was being widely discussed in Bangladesh at the time of the study. We are not aware of any report by ICDDR:B that “risk assessment . . . was not explored as recommended” 5 and we note that Rasheed and Karim do not cite a reference for this claim. Like others in Bangladesh, we have found that this tool does not work well to treat STIs 6 in women with vaginal discharge, but is more effective in managing the more common vaginal infections in these women.

The importance of these findings lies in the ability of policy makers to recognise that there is epidemiological diversity of infection of the reproductive tract, and that women, in particular, will suffer a clinical and social disservice if programmes set up to tackle STI do not recognise this.

Sarah Hawkes, Linda Morison, David Mabey, Rosanna Peeling, Susie Foster

Clinical Research Unit, London School of Hygiene & Tropical Medicine, London WC1E 7HT, UK; and Laboratory Centre for Disease Control, Winnipeg, Canada


Sir—Sarah Hawkes and colleagues’ study (Nov 20, p 1776) 1 was originally designed to describe the prevalence of sexually transmitted infections (STIs) in various subgroups of a population in Matlab, but it focuses on women sampled from a community subgroup in a long-term (20 years) family-planning intervention. This project established contraceptive support for all eligible women through community health workers (CHWs), who were living in the same neighbourhood and therefore, were part of the community. These CHWs visited the women on a fortnightly basis. Such frequent contact is unlikely to be achieved in a government programme. I question whether this study can be used to draw conclusions, generalise findings, or consider altering policy recommendations.

First the Matlab women have been regularly encouraged for over two decades to attend the health centre if they detected any alteration in vaginal discharge or menstrual patterns. Hawkes’ study further encouraged this practice. Therefore, that women without infection attended the clinics is not surprising. This pattern surely led to a dilution of potential cases with infections and to arguments pointing towards wastage of resources. The logic that calls for limiting uninfect ed women’s access to clinics as a way to reduce such wastage is fundamentally flawed.

Second, confidentiality was likely to be a consideration. Treatment for STIs might have been sought in private pharmacies, especially since CHWs’ record and report the treatment provided. If this were so, the true prevalence of STIs might have been underestimated. Third, estimation of STI symptoms or signs in partners of women reporting through this system probably further distorted interpretation of data.

These factors are likely to result in underestimation of the true prevalence of STIs in this population and the positive predictive value of STD syndromic management, and to reduce the representativeness of the aetiological spectrum of vaginal discharge in relation to other similar populations. The system of reporting of partners symptoms may have contributed to an increased treatment of false-positive cases and to raise the costs of the strategy. None of these factors could have been dealt with through statistical analyses or examination of cost-effectiveness.

Attempts to assess syndromic management within Bangladesh should be based on experience within the prevailing health-care system if policy conclusions are to be drawn. In Bangladesh, the government system at best makes contact with clients every 3 months, and is presently moving away from household visits to a system of an essential package of services (to include STI management) at cluster point clinics. It would, therefore, be detrimental to argue to reduce investment in health care or non-high-risk females on the basis of this study. Furthermore, research and non-governmental-organisation experience with STD syndromic management give results and conclusions 2 contrary to those given by Hawkes and colleagues.

Efficacy studies should concentrate on evaluating changes of vaginal discharge in relation to reproductive events, as described in earlier work in the same population. 3 Public-health initiatives clearly need to be validated within specific contexts before they are implemented. 4

Andres de Francisco
Global Forum for Health Research, World Health Organization, 1211 Geneva 27, Switzerland

4 Trollope-Kumar K. Symptoms of reproductive-tract infection—not all that they seem to be. Lancet 1999; 354: 1745–46.
**Association studies of genetic polymorphisms and complex disease**

Sir—Giovanni Gambaro and colleagues [Jan 22, p 308] made an appeal for more resources to be made available for research into functionally important gene variants. We believe that it is even more important to discover the functional significance of gene variants, especially since modifying the genome is an increasingly unattractive therapeutic option.

The insertion/deletion (I/D) polymorphism of the angiotensin-converting enzyme (ACE) gene, cited as a rare triumph for the blunderbuss approach to studying the association of genetic polymorphisms and disease, is a case in point. This polymorphism affects plasma ACE activity and has been linked to myocardial infarction and other cardiovascular diseases. We and others have reported a decline in the frequency of the DD genotype with increasing age,6 which may be consistent with decreased survival of those with this genotype. Although the link between the deleted sequence and increased expression of the ACE gene has been elucidated, the association between ACE activity and disease in human beings has been neglected.

Increased angiotensin II is believed by many to be the pathogenetic mechanism. However, the biggest surprise in cardiology at the turn of the century was the unexpected effectiveness of an ACE inhibitor (ACEI) in preventing cardiovascular disease in the Heart Outcomes Prevention Evaluation (HOPE) and MICRO-HOPE studies and the equally unexpected failure of an ACEI in hypertensive patients with age.7 Thus, while the molecular mechanism. However, the biggest surprise in cardiology at the turn of the century was the unexpected effectiveness of an ACE inhibitor (ACEI) in preventing cardiovascular disease in the Heart Outcomes Prevention Evaluation (HOPE) and MICRO-HOPE studies and the equally unexpected failure of an ACEI in hypertensive patients with age.

Sir—Giovanni Gambaro and colleagues blame the rather modest results of association studies of genetic polymorphisms and complex diseases almost entirely on the incompetence of clinical researchers and their lack of understanding of basic genetic principles. I believe that this paints a rather lopsided picture of this issue.

Most geneticists have generally been trained in classical genetics involving family-based linkage studies, and association and case-control studies are the bread-and-butter work of the clinical epidemiologist. Clinical researchers are, in fact, aware of the potential for recruitment bias, concerned about the sensitivity and specificity of methods for patient characterisation (now popularly referred to as phenotyping), and familiar with the statistical aspects of analysing such data. The geneticist is generally the least qualified person to appreciate the complexity of the clinical syndrome at hand. Thus, while the molecular geneticist is obsessed (and rightly so) with technical aspects of genotyping or data analyses, a clinician-researcher trained in patient-oriented research is more likely to appreciate and deal with the task of recruiting and phenotyping patients.

Few geneticists will recognise that even the measurement of a simple routine parameter—such as blood pressure—raises the simple questions of when, how, and by whom the parameter was measured. Molecular biologists often falsely assume that data gathered during a routine work up of a patient constitute the result of a scientific experiment. In reality, routine clinical data are probably the most unreliable source of scientific information one could possibly imagine. Few of the published association studies would have made it to print had they crossed the desk of a referee trained in clinical epidemiology rather than that of a molecular geneticist.

The call should therefore not be for less but rather for more involvement of the clinical researcher, who must be appropriated with the necessary funds to actually carry out the phenotyping that will allow reliable and meaningful analyses of the genetic material. Wasting laboratory resources on studying ill-defined DNA, cellular, and tissue samples derived from routine clinical practice, rather than from expensive dedicated recruitment and phenotyping programmes, is clearly something that must be stopped. In contrast to the appeal by Gambaro and colleagues for more studies on cells and tissues, I quote a Nature Medicine editorial: “A sizeable part of the biochemical research community is recognising that the popular practice of reductionist biological experimentation does not hold up beyond the simplest questions . . . Instead there is a move to recognise that only an interrogation of the entire complex system will allow one to fully understand the system.”8 I have nothing to add.

Ayä M Sharma
Universitätsklinikum Benjamin Franklin, Freie Universität Berlin, 12200 Berlin, Germany


Sir—In their excellent Viewpoint, Giovanni Gambaro and colleagues express several pitfalls in genetic case-control association studies, with the angiotensin converting enzyme (ACE) gene polymorphism as a case in point. The major failings of such studies are lack of large numbers of patients with a condition of interest, lack of an adequate control group, and ethnic heterogeneity. Erroneous results may occur as a result of several confounding factors, including population stratification (founder effect), multiple hypothesis testing, and sub-group analysis. Gambaro and colleagues do not mention that, in addition, publication bias may be a major problem, whereby positive results are...
easy to publish in major journals, whilst more robust, better designed studies with negative results may not be published. These factors will give an overestimate of the significance of positive associations.

The imbalance of published data is why the study by the International Studies of Infarct Survival (ISIS) collaborators is so welcome. The original finding of a positive association of the D allele of the ACE gene with myocardial infarction was reported by Cambien and colleagues, and subsequently questioned by us. As Gambaro and colleagues state, huge numbers of subsequent studies have shown conflicting results. The ISIS collaborators have done an almost perfect association study, with large number of well phenotyped cases and controls. The study is powerful enough to refute all previous positive associations, and should be the last word on the ACE gene polymorphism and cardiovascular disease.

Investigators from both studies fail to point out that any positive association should be reproduced in larger cohorts, and be tested for linkage in family based studies. A powerful method of showing both association and linkage is to use the transmission disequilibrium test, which requires DNA from an affected patient, and their parents, and examines the transmission of alleles from a heterozygous parent to the affected offspring. Significant variance from the expected Mendelian ratio of 50:50 would suggest that the allele has a role in the susceptibility to the disease in question. A second method is to use the affected sib-pair approach, which has been used successfully in research into type-1 diabetes. These methods require multicentre collection of families and large co-operative groups, but they are the way forward in unravelling the complex genetics of polygenic disease. Case-control studies still have a role in hypothesis testing, but they must involve large numbers to provide meaningful results.

Tahseen A Chowdhury
Jeffrey Keilson Diabetic Centre, Central Middlesex Hospital, London NW10 7NS, UK


Sir—Giovanni Gambaro and colleagues illustrate the pitfalls of the current population association studies of genetic polymorphisms of complex diseases. However, they do not explain how the results might be used to change our strategy for prevention and treatment at a population level.

The strength of the association between a candidate gene polymorphism (allele) and a complex disease (measured as odds ratios in case-control studies) is used to try and gauge the importance of an underlying aetiology. However, to do this the allele must be causally associated, and not in linkage disequilibrium, with another gene allele implicated in the disease. This odds ratio is often used inappropriately to try and predict how important that particular polymorphism may be in a population.

The likely impact at a population level of a particular factor is predicted by the population attributable risk (PAR), a measure that indicates how much of the disease would be removed from the population if we were able to remove (or specifically target) the effect of the risk factor. For a causative allele variant, the population attributable risk depends both on the genetic relative risk and the frequency of the allele within the population under study. The possible implications at the population level can be illustrated by the T594M polymorphism (of the β sub-unit of the epithelial sodium channel) and the C825T polymorphism (of the G-protein β3 subunit) that appear to be associated with blood pressure in black people. Both polymorphisms encode for proteins that could affect the ability of the kidney to handle sodium and, in principle, be associated with an increase in blood pressure. Both associations show large odds ratios (2·0–4·0). If we assume that these two polymorphisms are causally associated with the development of high blood pressure, how would this affect our strategy for preventing and treating high blood pressure in black people?

Assuming causality and a relative risk of 2·0, the presence of the 594M allele (frequency 2·4%) would be responsible for a small proportion of hypertension in the black population (PAR 2·3%). A high-risk strategy would seem appropriate because it would be possible to genotype all black people to identify the few people at risk and target them for intervention. For instance, it is possible that those with the 594M variant may be particularly sensitive to amiloride. By contrast, assuming a similar odds ratio, the population attributable risk for the 825T allele is much higher (PAR 44%) because a large proportion of the population carries the variant (79%). Assuming causality, the knowledge of the presence or absence of the allele would be of little practical value: a population-based strategy would seem more appropriate—eg, a reduction in salt intake.

We share the enthusiasm for identification of new genetic variants for complex diseases, but it is easy to underestimate the importance of causality and the implications for prevention and treatment at a population level. Bernard Keavney and colleagues say that there is a need for studies of candidate genes to involve large populations, but they do not propose how the results could be applied to population prevention and treatment. So far, a large effort in population-based molecular studies has produced rather modest results. There is a need for caution in investing disproportionate resources into large-scale genetic epidemiology, when a moderate investment in known modifiable environmental causes of complex diseases could lead to a substantial reduction of disease in the population—eg, cardiovascular disease.

*Francesco P Cappuccio, Giuseppe A Sagnella, Graham A MacGregor
Blood Pressure Unit, Department of Medicine, St George’s Hospital Medical School, London SW17 0RE, UK (e-mail: f.cappuccio@sgms.ac.uk)

Reforming the borderline personality diagnosis

Sir—In his Dec 18/25 commentary1 Peter Tyrer says that the borderline personality disorder is a diagnostic categorisation that has probably “run its course” and he proposes reclassification of this disorder. His suggestion is sustained, in part, by his interpretation of four reports published in the American Journal of Psychiatry. However, three of these four reports can be held up as evidence that the diagnostic validity of borderline personality disorder categorisation is valid. To begin with, Tyrer highlights a point from the paper by Herpertz and colleagues2 that the affective psychological responses of these four reports can be held up as evidence that the diagnostic validity of borderline personality disorder categorisation is valid. To begin with, Tyrer highlights a point from the paper by Herpertz and colleagues2 that the affective psychological responses of the patients. But in the article, the investigators say that the same patients had unexpectedly low endodermal responses to different stimulus categories, which indicated physiological underarousal. From the article by Hodey and colleagues3 Tyrer gathers that a high degree of expressed emotion to the behaviour of patients with borderline personality disorder by their relatives was not predictive of outcome. However, the article shows that a good clinical outcome is strongly associated with high levels of emotional reaction by the family towards the patient’s disturbances. Investigators of the third article cited by Tyrer do not imply that there is any need of reform in diagnosis of borderline personality disorder because they state that psychoanalytically oriented treatment programmes are effective in these patients. But in the fourth article cited by Tyrer the investigators highlight a diagnosis discrepancy: 0.4% of patients were diagnosed as having borderline personality disorder in clinical settings whereas this percentage rose to 14% after standard research-oriented structured interview. This discrepancy shows that we are dealing with an unpredictable and spurious diagnosis. However, the investigators of this article do not contend the diagnostic validity of borderline personality disorder but contend the deficient methodology for proper diagnosis. Therefore, the investigators of these four articles have theoretical positions that are at odds with the interpretations advanced by Tyrer.

Also, what about other insights into patients with borderline personality disorder, such as autonomic episodic memory deficiencies,1 higher density of the first-cycle of rapid-eye-movements, or the naltrexone responsiveness of the borderline personality disorder dissociative symptoms and abnormalities in opioidergic brain systems? Tyrer’s opinion about the looseness (and perhaps ugliness) of the term “borderline” is probably right, but reclassification perhaps is premature. We should wait until we know more about the core neurophysiological markers of this disease (or diseases) to be able to change this diagnostic categorisation properly.

Salvador Vale
Unidad de Investigaciones Clínicas, Antiguo Hospital Concepción Beástegui, Regina 7, CP 06080, México D F, México (e-mail: svale@dfl.telmex.net.mx)


Swedish cancer register: corrected data

Sir—Because of an erroneously updated variable that indicated year of death in the Swedish cancer register, the results reported in our research letter (Sept 25, p 1093)1 need correction. The error meant that we underestimated the annual mortality and hence overestimated the prevalence of cancer. The corrected numbers show a stronger overall prevalence trend as well as a stronger relative effect of improved survival compared with the original data.

The number of people who developed cancer during the preceding 5 years increased from 20 937 to 52 176 (149%) for men and from 29 925 to 57 964 (94%) for women. The trends partitioned into population growth, ageing, survival, and incidence are shown in the figure. Improved survival accounts for 40% of the change for both sexes. The relative shares of the change (men, women, respectively) on the log scale were as follows: population growth 13%, 21%; population ageing 20%, 22%; survival 41%, 38%; incidence 26%, 18%.

The corrected results underscore our previous conclusions. The steep increase in prevalence of cancer is explained mainly by good forces such as improved cancer survival and increased general life expectancy. Hence, as concluded earlier, the increase in prevalence of cancer is a greater burden for the healthcare system than it is for public health because it requires more spending on health care irrespective of improvements in public health.

Magnus Stenbeck
Centre for Epidemiology, National Board of Health and Welfare, 106 30 Stockholm, Sweden (e-mail: magnus.stenbeck@ssos.se)


Thiomersal in vaccines

SIR—Thiomersal is an organic mercurial compound that has been used for over 60 years as an antimicrobial agent in vaccines to prevent contamination. It is present in commonly used vaccines such as diphtheria-tetanus-pertussis (DTP) vaccine and tetanus toxoid (TT) as well as certain brands of hepatitis B (HB) and Haemophilus influenzae type b (HiB) vaccines, but not in live bacterial or viral vaccines. The use of thiomersal has probably prevented death or illness in countless infants by reducing the risk of contamination of for example, opened multidose vials.

There is a need to minimise exposure to mercury from all sources such as food (especially certain fish), pharmaceuticals, and biological products. In July, 1999, the US Public Health Service (USPHS) and American Academy of Pediatrics (AAP) issued a joint statement concerning thiomersal in vaccines,1 which prompted international public debate about preservatives and their safety. At doses much higher than those used in vaccines,
that drug-induced exposure to methyl mercury in the diet. provide recommendations for safe the US Food and Drug Administration (EPA), the US Agency for Toxic Environmental Protection Agency Organisations such as WHO, the US approaches a level that is of concern. mercury have been used to determine thiomersal remains uncertain. from low concentrations of exposure to vaccines that, while not obviously concentrations, it seems that some about the implications of various sources should be minimised, has led to a paradigm shift in the perception of risk from thiomersal. The public’s overall tolerance for risk in the absence of obvious benefit to the individual has greatly diminished, particularly when the source of risk is perceived as man-made and potentially avoidable.1

Removing thiomersal (and with it the risk from mercury) from vaccines is not a simple task. If the condemnation of thiomersal were to be too strong, many vital vaccines might be withdrawn from production, resulting in a global supply crisis as well as a loss of public confidence in vaccines. The risk from contamination of multidose vials would increase and lives would be put at risk from, for instance, toxic-shock syndrome.

**Mercury exposure from thiomersal in typical immunisation schedules**

<table>
<thead>
<tr>
<th>Age</th>
<th>Vaccines</th>
<th>Hepatitis B (HB) vaccine</th>
<th>Mercury dose (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Scheme A</td>
<td>Scheme B</td>
<td>Scheme A</td>
</tr>
<tr>
<td>Birth</td>
<td>BCG, OPV 0</td>
<td>HB 1</td>
<td>12-5</td>
</tr>
<tr>
<td>6 weeks</td>
<td>DTP 1, OPV 1, Hib 1</td>
<td>HB 2</td>
<td>62-5</td>
</tr>
<tr>
<td>10 weeks</td>
<td>DTP 2, OPV 2, Hib 2</td>
<td>HB 2</td>
<td>62-5</td>
</tr>
<tr>
<td>14 weeks</td>
<td>DTP 3, OPV 3, Hib 3</td>
<td>HB 3</td>
<td>62-5</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>187-5</td>
</tr>
</tbody>
</table>

**Hazards of translation of non-English medical publications in Medline**

Sir—To our astonishment an article appeared in Medline as “Diagnosis of systemic fungal infections in haematology”. This referred to a publication, by the Working Group on Infections in Haematology and Oncology of the German Association for Haematology and Oncology, on standard recommendations in diagnosis of systemic fungal infections in haematology. The paper was published in a German medical journal *Deutsche Medizinische Wochenzeitschrift*. In the words of Gertrude Stein “A rose is a rose is a rose is a rose” and a mushroom is a fungus but not all fungi are mushrooms. Mushroom poisoning or mycetismus is defined as distress resulting from the consumption of a fungal organism. The investigators were not referring to the consumption of mushrooms and it should have been obvious to the translators that “systemic mushroom poisoning in haematology” cannot possibly be the correct translation. English is today’s international Esperanto, and the number of medical journals published in English are on the increase. However, each country has a certain number of journals published in the mother tongue and the need for translation arises the moment this information has to be made internationally accessible, as for example, in Medline. Translation in itself is a potentially rich source of errors and one has to bear that in mind when using international sources of data such as Medline. Even though, one would not expect a mistake to be quite as gross as it was in this instance, resulting in a complete distortion of the original meaning.

*Orhan Sezer, Angelika Böhme
*Universitätsklinikum Charité, Humboldt-Universität, 10098 Berlin, Germany; and Klinikum der Johann Wolfgang Goethe Universität, Frankfurt (e-mail: sezer@charite.de)

**DEPARTMENT OF ERROR**

Results at 1 year of outpatient multidisciplinary pulmonary rehabilitation: a randomised controlled trial—In this Article by T L Griffiths and colleagues (Jan 29, p 362), the name of the tenth author should be A A Jones.

Lung cancer—In this Seminar by P C Hoffman and colleagues (Feb 5, p 479), the second sentence of the second paragraph under the heading “Treatment of small-cell lung cancer” on page 483 should be, “A major randomised study showed that the poor survival group had a median survival of 15 months . . . “

Implications of discontinuation of doxazosin arm of ALLHAT—In this Commentary (March 11, p 863), by F H Messerli, the end of paragraph 3 should have read: “Instead, the board found that low-dose diuretic therapy conferred more overall cardiovascular benefits than did doxazosin. Although the ALLHAT showed no difference in diastolic pressure, there was a 3 mm Hg difference in systolic pressure, which would not account for the congestive heart failure but could explain why users of doxazosin had 25% more cardiovascular events than did users of chlorthalidone. The minor difference in blood pressure indicates that drug-induced changes in insulin resistance . . .”