Sir—Kaare Christensen and colleagues'<sup>1</sup> analyses and figures are convincing. The relation between sex, age, social status, and loss of teeth in Denmark and other industrialised countries is a well documented fact.<sup>24</sup> We are, however, not convinced that the alleged cause-effect relation between number of children and loss of teeth is substantiated by this study. There is no evidence of a biological mechanism that links pregnancy with an increased risk of dental disease.

We are aware of the difficulties in identification of a cause and establishing a causal association in epidemiological studies.⁵ The statistical association shown in Christensen's study might be indirectly causal, because an important cofactor, or co-player, has been forgotten in their reasoning. This co-player is the dentist. Teeth are very seldom lost; teeth are extracted by dentists. These women were childbearing many years ago and their dentists' attitudes might have played an important part in the decision to extract or not to extract. Dentists at that time might have internalised the common proverb "A child, a tooth". In other words, the association between number of children and number of teeth could be explained sociologically in a particular segment of the health service rather than as a biological fact.

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## Authors' reply

Sir—Among Danish female twins born in the first part of the twentieth century, we saw a negative association between number of children and number of teeth. A similar study among women from the developing world would be an excellent opportunity to further test this finding, because the association was strongest

among women of lower socioeconomic status. Alexander Walker summarises such a study from South Africa, which did not corroborate our finding. The African women studied were much vounger than the Danish women, the sample size was considerably smaller, and groups of one to three and five or more children were used. It was not clear which group women with none or four children belonged to in Walker's study. All these factors will reduce the power of the study to detect an association. However, it is likely that different mechanisms are responsible in the two settings that are very different in terms of nutrition, life style, and dental health care system.

Flemming Scheutz and Sven Poulsen speculate that it is the dental health care system that influences the negative association between number of children and number of teeth. They believe that "many dentists at that time may have internalised the common proverb 'A child, a tooth'". We cannot rule this out, but we believe that it is unlikely that dentists have asked female patients about their reproductive history to decide on whether to extract teeth or not. Dentists might have extracted more teeth among women with more children because these women had poorer teeth and not because they had more children.

Our data refer to Danish women born 80 years ago and it is not clear whether the results are generally valid. However, two smaller Swedish studies report findings that are similar.<sup>1,2</sup> The proverb "a tooth per child" is wellknown in Scandinavia, Germany, Russia, and Japan: this suggests that an association between childbearing and loss of teeth has been common in countries with probably manv different dental practice. More studies populations of present from and developing industrialised countries are needed to test whether a negative association between number of children and number of teeth exists, and if so, what the mechanisms are.

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## Packed-cell volume in athletes

Sir—J Marx and P Vergouwen (Aug 8, p 451)<sup>1</sup> report that subjectively healthy people (athletes and non-athletes) who do not take recombinant human erythropoietin (EPO) may be excluded from cycling competitions because their basal packed-cell volume is over 0.50 (rule of the Union Cycliste Internationale).

Since doping is widespread, one should examine the scientific basis of the measurement of the analytes and the interpretation of the results. However, it seems that the effects of preanalytical factors are neglected.

Preanalytical factors are factors that affect the specimen before its final analysis. They can be divided into invivo (or biological) and in-vitro factors.<sup>2</sup> The in-vivo factors occur in a person before and during the specimen collection. Some may be controllable (eg, diet, physical activity, posture, use of tourniquet, site of specimen collection, time of the day) and some are not (eg, genetic factors, sex, age). The in-vitro factors include equipment used in the collection and the processing of the specimen (transport, storage, preparation for the analysis).

With the packed-cell volume, preanalytical factors such as posture, physical activity, and use of tourniquet are important. For instance, in some individuals a change in posture may lead to a 20% increase in the packedcell volume.<sup>3</sup> Other factors such as fasting/food intake and time of the day have a slight but significant effect<sup>4</sup> although sometimes great variation between individuals has been observed.

We found that ingestion of breakfast in 51 subjectively healthy volunteers led to a significant average increase of only  $+1\cdot3\%$ ; however, the extreme changes were  $-7\cdot6\%$  and  $+8\cdot5\%$ .<sup>4</sup> The results obtained from specimens taken at 11.30 h are  $0\cdot7\%$  (extremes  $-2\cdot8$ ;  $+5\cdot3$ ), higher than those taken at 08.00 h (preprandial).<sup>4</sup>

To give reliable results and to treat the athletes as fairly as possible, the specimen collection should be standardised and monitored. Regulation of fluid intake should also be considered. We suggest that all specimens should be collected by an experienced laboratory technician and after at least 15 min of sitting before venepuncture with no tourniquet. This procedure has proved to buffer the effect of various preanalytical factors.<sup>2</sup> Documentation concerning procedure should be provided. Specimens with visible haemolysis should be discarded.