Q J Med 1998; **91**:627–633

Improvement of factor VII clotting activity following long-term NCPAP treatment in obstructive sleep apnoea syndrome

K. CHIN, H. KITA, T. NOGUCHI, N. OTSUKA, T. TSUBOI, T. NAKAMURA, K. SHIMIZU, M. MISHIMA and M. OHI

From the Department of Clinical Physiology, Chest Disease Research Institute, Kyoto University, Kyoto, Japan

Received 16 February 1998 and in revised form 23 June 1998

Summary

Obstructive sleep apnoea syndrome (OSAS) is a very common disorder. Patients with OSAS are at an increased risk for cardiovascular events. It has also been reported that a 25% rise in factor VII clotting activity (FVIIc) is associated with a 55% increase in ischaemic heart disease death during the first 5 years. We examined the effects of nasal continuous positive airway pressure (NCPAP) treatment on FVIIc in patients with OSAS. FVIIc was investigated prospectively in 15 patients with OSAS before (mean ± SEM apnoea and hypopnoea index (AHI) 61.5 ± 4.2 and after (AHI 3.0 ± 0.9) NCPAP treatment for immediate relief, at 1 month after treatment and at over 6 months. FVIIc levels gradually decreased after NCPAP treatment. After 6 months of NCPAP treatment, FVIIc levels had decreased significantly (before 141.1 ± 11.7% vs. after 6 months 110.7 ± 6.2%; $p < 0.01$). Six of the seven patients whose FVIIc levels were over 140% before the NCPAP treatment had FVIIc levels below 130% after 6 months or 1 year of NCPAP treatment. This decrease in FVIIc after long-term NCPAP treatment could improve mortality in OSAS patients. If patients, especially obese ones, present with high FVIIc of unknown origin, it would be prudent to check for OSAS.

Introduction

Obstructive sleep apnoea syndrome (OSAS) is a very common disorder, and recent studies suggest that it affects up to 4–5% of the general population.[1,2] Mortality is increased in untreated OSAS patients, and they are also at increased risk for cardiovascular disease.[3,4] The blood coagulation system is one of the most important physiological systems, and is part of the causation of cardiovascular disease.[5–8] Fibrinogen and factor VII clotting activity (FVIIc) are independent risk factors for ischaemic heart disease.[9] In particular, FVIIc strongly predicts fatal episodes.[9,10] FVIIc is also correlated with the serum cholesterol concentration,[9] the serum triglyceride level,[11] and the body mass index.[12]

The rate of mortality in OSAS patients is improved by nasal continuous positive airway pressure (NCPAP) treatment.[3] Therefore, we hypothesized that the hypoxaemia, changes in sympathetic activity including elevated blood pressure,[13] intrapleural pressure oscillations and several other stresses[14] caused by repetitive obstructive sleep apnoea might influence FVIIc, and that NCPAP treatment might also have an effect on the FVIIc levels. We prospectively investigated the immediate, short (one month) and long-term (over 6 months) effects of NCPAP treatment on FVIIc levels in 15 patients with OSAS.

Methods

Patients

Fifteen male patients with OSAS (apnoea and hypopnoea index (AHI) >20) who had undergone NCPAP therapy were studied. Polysomnography was

Address correspondence to Dr K. Chin, Department of Clinical Physiology, Chest Disease Research Institute, Kyoto University, 53 Shogoin-kawaharacho, Sakyo-Ku, Kyoto 606-01, Japan. e-mail: chink@kuhp.kyoto-u.ac.jp

performed before the NCPAP therapy, and again on the first night of the NCPAP therapy. The diagnosis of OSAS and the selection of the NCPAP candidates was as previously described.[15] Another eight patients with OSAS, who were also candidates for NCPAP treatment, served as control subjects. This study was approved by the local Institute Committee, and all patients gave their informed consent prior to the study.

Protocol

For antecubital venous blood sampling, 21-gauge needles and siliconized vacutainers were used, and the minimal stasis technique within 1 min was used. The first 10 ml was collected into tubes without any additives, and was used for lipid analysis or discarded. The next 2×2 ml was collected into citrated tubes, and was used for the FVIIc and fibrinogen analyses. The blood samples were drawn at 3:30 pm following 3 h fasting before the polysomnography, and again at 8:30 am after fasting and post-polysomnography. Total cholesterol and triglyceride levels, high-density lipoprotein (HDL) cholesterol and FVIIc were also measured 6 months after the NCPAP treatment from fasting samples. Some of the patients also had fasting blood samples for their FVIIc taken at 1 month and at over 1 year after the NCPAP treatment. Low-density lipoprotein (LDL) cholesterol was calculated by the Friedwald formula.[16] During the study, no changes were made to the patients' medications.

The blood samples used for the plasma fibrinogen and FVIIc analyses were centrifuged immediately at 3000 rpm for 10 minutes at 4 °C, and the separated plasma was frozen at −20 °C. Plasma fibrinogen samples were analysed as previously reported.[15] The FVIIc samples taken at 3:30 p.m. before the polysomnography and at 8:30 a.m. after the polysomnography were frozen at −20 °C, transported to another laboratory, and were then frozen at −60 °C. The FVIIc samples were all analysed within 1 week of transportation. FVIIc levels were measured by a photometric method.[17]

The OSAS patients in our hospital had to wait for almost 2 months for polysomnography, without or with the NCPAP therapy. Therefore, the control subjects also had their fasting blood samples analysed twice over the 2 months.

Data analysis

The data are presented as means ± SEM. The FVIIc data were tested by non-parametric methods, the other parameters by parametric methods. The FVIIc data and other parameters could be obtained from 15 patients before and after 6 months NCPAP treatment. The FVIIc data from some of the patients at 1 month, 1 year, and 18 months after the NCPAP treatment could not be obtained. Therefore, we first compared the FVIIc and other data at before vs. 6 months after NCPAP treatment, using Wilcoxon's rank-test, or a two-tailed paired *t*-test. The FVIIc data obtained at 1 month ($n=11$), 1 year ($n=8$) and 18 months ($n=4$) post-NCPAP were tested by Friedman's test, including the before and after 6 months NCPAP therapy data. If a significant difference ($p<0.05$) was found by Friedman's test, then the differences before vs. after treatment were retested by Wilcoxon's rank-test.

Results

All of the 15 NCPAP patients and eight control patients were male. Their age, body mass index (BMI), FVIIc and several other parameters are shown in Table 1. The effects of the NCPAP therapy are shown in Table 2. Patients undergoing NCPAP treatment generally visit the hospital every 1 or 2 months. We obtained the NCPAP compliance data by a questionnaire, because we could not measure the exact NCPAP run time. Twelve of the patients used NCPAP every day for more than 5 h per night, and three patients used the NCPAP 6 days per week for more than 5 h per night.

As previously reported,[15] the plasma fibrinogen levels were significantly different in the morning vs. in the afternoon, and with vs. without NCPAP. In contrast, the FVIIc levels in the morning vs. in the afternoon and with vs. without NCPAP were not significantly different (Table 2). However, FVIIc levels gradually decreased. After 6 months (204 ± 10 days) of NCPAP therapy, fasting FVIIc levels as well as blood pressure had decreased significantly, compared to before NCPAP therapy ($p<0.01$) (Table 3 and Figure 1). In contrast, the other parameters, some of which have been reported to influence the FVIIc levels, did not change significantly (Table 3). There was no seasonal bias in the blood samples. In all of the patients except for two, the FVIIc dropped to below 130% after NCPAP treatment for 6 months. Six of the 7 patients whose FVIIc levels were over 140% before the NCPAP treatment had FVIIc levels below 130% after 1 year of NCPAP treatment (Figure 1). The FVIIc remained below 130% not only after 12 months of NCPAP treatment (Figure 1), but also at 18 months after the treatment ($n=4$) (Figures 1 and 2). Patient 2 stopped the NCPAP treatment for 3 months because he could not buy or rent the NCPAP equipment at that time, and his FVIIc rose again. However, his FVIIc levels decreased again after the resumption of NCPAP therapy (Figure 2). There was no significant correlation between FVIIc

Table 1 Characteristics of OSAS patients with and without (control) NCPAP

Variable (NCPAP)	NCPAP ($n=15$)	Control ($n=8$)	p
Age (years)	45.2(3.1)	52(3.1)	NS
Height (m)	1.70(0.02)	1.68(0.01)	NS
Weight (kg)	90.3(4.9)	77.4(2.9)	NS
Body mass index (kg/m^2)	31.1(1.3)	27.3(0.9)	NS
PaO_2 (mmHg)	79.2(2.3)	81.7(1.7)	NS
$PaCO_2$ (mmHg)	41.6(0.8)	41.5(1.3)	NS
pH	7.41(0.01)	7.41(0.01)	NS
$FEV_{1.0}$/FVC	79.5(1.2)	76.8(1.8)	NS
Apnoea-hypopnoea index	61.5(4.2)	50.2(5.5)	NS
Lowest nocturnal SaO_2 (%)	57.5(3.4)	61.1(3.2)	NS
Total-cholesterol (mmol/l)	5.91(0.27)	5.28(0.19)	NS
Triglyceride (mmol/l)	2.13(0.27)	2.72(0.41)	NS
FVIIc (%)	141.1(6.2)	134.8(5.9)	NS
Systolic-BP (mmHg)	141.3(4.7)	140.3(5.0)	NS
Diastolic-BP (mmHg)	89.9(3.2)	90.3(3.5)	NS

NCPAP, nasal continuous positive airway pressure; $FEV_{1.0}$, forced expiratory volumes at 1.0 s; FVC, forced vital capacity; SaO_2, arterial oxygen saturation; FVIIc, factor VII clotting activity; BP, blood pressure; NS, not significant. Values are means (SEM).

Table 2 Effects of NCPAP on OSAS

	Pre-NCPAP	During NCPAP	p
Apnoa-hypopnoea index	61.5(4.2)	3.0(0.9)	<0.0001
Mean nocturnal SaO_2 (%)	89.5(1.1)	94.7(0.3)	<0.0001
Lowest nocturnal SaO_2 (%)	57.5(3.4)	87.7(1.2)	<0.0001
D-Fibrinogen ($n=13$) (g/l)	0.18(0.03)	0.04(0.04)	0.0056
D-FVIIc ($n=12$) (%)	6.8(5.5)	2.4(2.5)	NS

NCPAP, nasal continuous positive airway pressure; SaO_2, arterial oxygen saturation; D-Fibrinogen, the difference between the plasma fibrinogen levels in the morning versus in the previous afternoon; D-FVIIc, the difference between factor VII clotting activity in the morning versus in the previous afternoon. Values ($n=15$) are means (SEM).

Table 3 Patient characteristics

	Pre	6 months	p
Body weight (kg)	90.1(4.8)	88.4(4.9)	NS
Cholesterol (mmol/l)	5.91(0.27)	5.60(0.23)	NS
HDL cholesterol (mmol/l)	1.07(0.06)	1.12(0.09)	NS
LDL cholesterol (mmol/l)	3.90(0.22)	3.63(0.23)	NS
Triglyceride (mmol/l)	2.06(0.20)	1.83(0.24)	NS
Fibrinogen (g/l)	2.99(0.16)	2.92(0.17)	NS
FVIIc (%)	141.1(11.7)	110.7(6.2)	0.0054
Systolic BP (mmHg)	141.3(4.7)	135.5(3.5)	0.0419
Diastolic BP (mmHg)	89.9(3.2)	80.7(2.3)	0.0001

Pre, before NCPAP treatment; 6 months, after 6 months NCPAP treatment; other abbreviations as Table 1. Values ($n=15$) are means (SEM).

and the AHI ($r=0.12$, not significant) or the lowest SaO_2 during sleep ($r=0.013$, not significant).

In the control group without NCPAP treatment, FVIIc levels did not show any significant changes during almost the entire 2 months (47.9 ± 4.4 days) (Figure 3). Neither the serum cholesterol concentration nor the triglyceride levels in the control group changed significantly during this period (cholesterol:

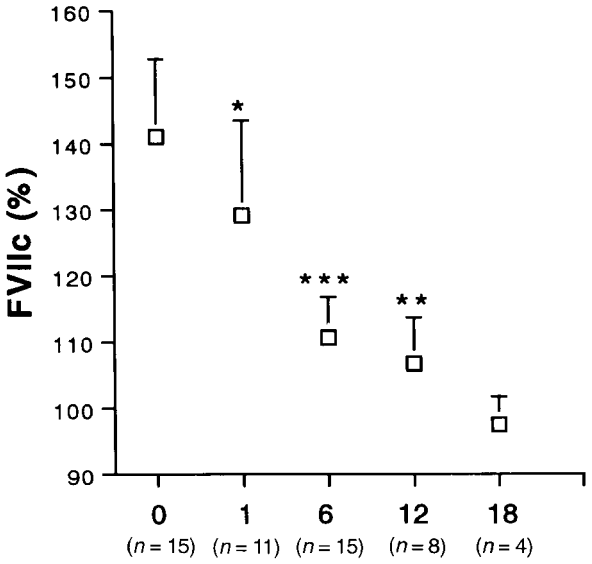

Figure 1. Changes in FVIIc in patients with OSAS after NCPAP treatment. Bars indicate SEM. *$p=0.018$; **$p=0.018$; ***$p=0.0054$; all compared to FVIIc value before NCPAP treatment.

from 5.28 ± 0.19 to 4.89 ± 0.16 mmol/l; triglyceride: from 2.72 ± 0.41 to 2.25 ± 0.27 mmol/l).

Discussion

In this study, the FVIIc in patients with severe OSAS decreased significantly, but neither cholesterol levels, triglyceride levels nor body mass index changed significantly during 6 months of NCPAP treatment. It has been reported that there are seasonal variations in FVIIc,[18] but there was no seasonal bias detected in this study. In the Northwick Park Heart Study,[9] a one standard deviation rise in the FVIIc (25.1% standard) was associated with a 55% increase in ischaemic heart disease death during the first 5 years. Therefore, a decrease in the FVIIc of more than 30% during the 6 months in this study, namely from 141.1% to 110.7%, is clinically noteworthy.

FVIIc and NCPAP treatment

High FVIIc levels are correlated with serum cholesterol,[9] serum triglyceride level[11] and body mass index.[12] In this study, these factors did not change significantly during NCPAP treatment (Table 3). However, NCPAP treatment[19] did improve the repetitive OSAS with severe negative pressure, hypoxaemia, microarousals and associated changes in blood pressure and/or sympathetic activity.[13,20,21] Therefore, OSAS associated with hypoxaemia, microarousals and changes in blood pressure and/or sympathetic activity might have an effect on the FVIIc. However, there was no significant relationship between disease severity, expressed as the AHI or the lowest SaO_2, and FVIIc. Recently, polymorphisms in the coagulation factor VII gene have been reported.[22] Therefore, the FVIIc levels might have a genetic predisposition, and OSAS might exacerbate the FVIIc levels. It has been reported that occupational stress has significant effects on the FVIIc levels,[23] and it has also been reported that the levels of stress protein (heat shock protein 72) during sleep in patients with OSAS show significant changes with and without NCPAP treatment.[14] Therefore, several indirect stresses caused by the repetitive apnoeas might also have significant effects on the FVIIc levels. In addition to the changes mentioned above, there is also the possibility that the severe negative pressure experienced during obstructive sleep might have an effect on the blood vessels in the thorax (contraction of the vessels, changes in blood flow etc.), thus activating FVIIc. However, neither the exact mechanism underlying this phenomenon, nor the other effects of NCPAP treatment[24] are known.

Limitations

One major limitation was that the time between the two FVIIc samplings in the controls was almost 2 months, because they had to wait 2 months for polysomnography. It would have been better to increase the duration of the intervention in the controls to >6 months, or to take those patients who had already received NCPAP treatment off their NCPAP for several months to observe whether a rise in the FVIIc levels would occur. However, there are ethical problems with such a protocol, because we would have knowingly exposed these patients to what we honestly believe is harmful (severe OSAS with high FVIIc levels).[25] In addition to the control data, based on the results from patients 1 and 2 (both had high FVIIc levels before the NCPAP treatment which decreased significantly with NCPAP treatment for over 2 or 3 years), the significant decrease in the FVIIc levels associated with long-term NCPAP treatment is likely to be valid. Furthermore, when patient 2 stopped the NCPAP treatment for 3 months, his FVIIc levels rose. However, his FVIIc levels decreased again after the resumption of NCPAP (Figure 2). Recently, there have been some objections raised to the relationship between OSAS and the increased mortality due to cardiovascular disease.[26] However, there is extensive experimental and epidemiological evidence which suggests that enhanced blood coagulation is a risk factor for atherogenesis and thrombogenesis,[5–8] and it is likely that the best measure of coagulable potential is still FVIIc.[27] FVIIc, in addition to the fibrinogen levels, is an independent risk factor for ischaemic heart disease.[9] Furthermore, FVIIc strongly

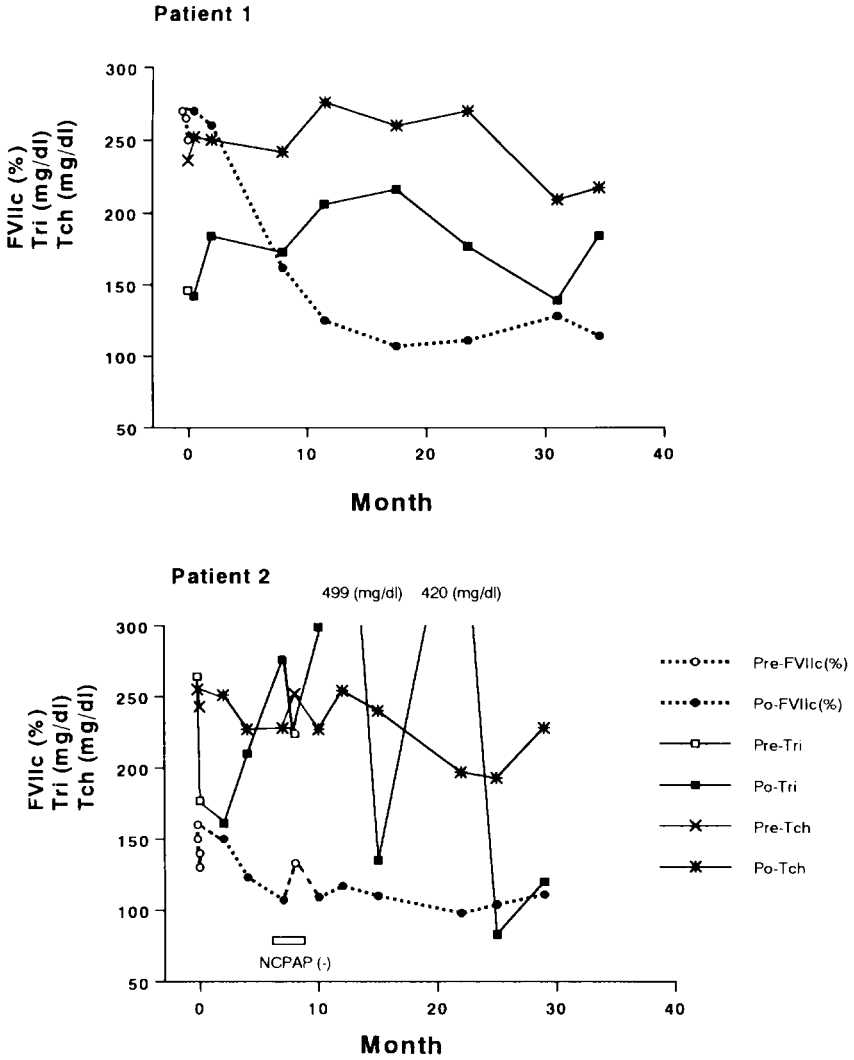

Figure 2. Changes in FVIIc levels in two patients with OSAS after NCPAP treatment. Patient 2 stopped the NCPAP treatment for 3 months because he could not buy or rent NCPAP equipment at that time, and his FVIIc levels rose again. However, his FVIIc levels decreased after the resumption of NCPAP therapy. Tri, triglyceride; Tch, total cholesterol; Pre, before NCPAP treatment; Po, after NCPAP treatment. Conversion to SI units: triglyceride 1 mg/dl ≈ 0.01129 mmol/l, total cholesterol 1 mg/dl ≈ 0.02586 mmol/l.

predicts fatal episodes.[9,10] Therefore, the decrease in FVIIc levels, as well as the morning decrease in plasma fibrinogen levels, associated with long-term NCPAP treatment should attenuate the mortality of OSAS patients (Figure 1, Table 2).[15]

In our laboratory, we could not measure the activated factor VII (FVIIa) levels. This measurement may also be important because of the variable sensitivity among one-stage assays to the presence of previously-activated factor VII.[10,28] Although we found a significant decrease in the FVIIc of OSAS patients following long-term NCPAP treatment, and we are confident that the effects of NCPAP therapy on FVIIc are of genuine clinical significance, further examinations should be performed to evaluate possible differences in methodology.

In a recent study, the prevalence of OSAS in middle-age was demonstrated to be surprisingly high.[1,2] In general, obese subjects ordinarily have high serum cholesterol and/or triglyceride levels. Obese subjects are also more prone to develop OSAS. Therefore, the previous reports[9,11,12] probably included a considerable number of undiagnosed OSAS patients, and thus the results (i.e. the relationship between FVIIc and serum cholesterol or triglyceride levels) might have been affected by these subjects with OSAS. It is probably important to check for OSAS in obese patients when obesity-associated diseases are investigated.

We did not measure the exact NCPAP run time,[29] and the small number of subjects in our trial and some methodological difficulties all contribute to the limitations of this study. Nevertheless, the attenuation of FVIIc levels with long-term NCPAP therapy, as

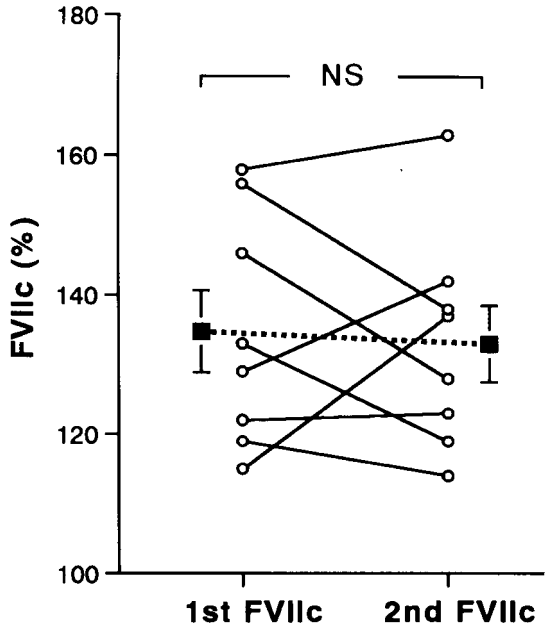

Figure 3. Changes in the FVIIc levels of control patients with OSAS. Values from the same patients are connected. Means are indicated ■; bars indicate SEM. The period between the first and second FVIIc measurements was 47.9±4.4 days. 1st-FVIIc, FVIIc level at the first measurement; 2nd-FVIIc, FVIIc level at the second measurement; NS, not significant.

well as the attenuation of the morning increases in the plasma fibrinogen concentration,[15] may contribute to an overall improvement in the mortality due to cardiovascular events in patients with OSAS. If patients, especially obese ones, present with high FVIIc levels of unknown origin, it would be prudent to determine whether these patients have OSAS, because the prevalence of OSAS is so high.[1,2]

Acknowledgements

The authors thank the staff of the Department of Laboratory Medicine, Chest Disease Research Institute, Kyoto University, for their kind assistance in measuring several blood chemical indices and measures of pulmonary function.

Supported in part by grants from No. 09670608 from the Japanese Ministry of Education, Science and Culture, the Japanese Ministry of Health and Welfare, and France Bed Medical Home Care Research Subsidy Foundation.

References

1. Young T, Palta M, Dempsey J, Skatrud J, Weber S, Badr S. The occurrence of sleep-disordered breathing among middle-aged adults. *N Engl J Med* 1993; **328**:1230–5.
2. Davies RJ, Stradling JR. The epidemiology of sleep apnoea. *Thorax* 1996; **51**(Suppl 2):S65–S70.
3. He J, Kryger MH, Zorick FJ, Conway W, Roth T. Mortality and apnoea index in obstructive sleep apnea. *Chest* 1988; **94**:9–14.
4. Partinen M, Jamieson A, Guilleminault C. Long-term outcome for obstructive sleep apnea syndrome patients. Mortality. *Chest* 1988; **94**:1200–4.
5. Wilhelmsen L, Svardsudd K, Korsan-Bengsten K, Larsson B, Welin L, Tibblin G. Fibrinogen as a risk factor for stroke and myocardial infarction. *N Engl J Med* 1984; **311**:501–5.
6. Tofler GH, Brezinski D, Schafer AI, Czeisler CA, Rutherford JD, Willich SN, Gleason RE, Williams GH, Muller JE. Concurrent morning increase in platelet aggregability and risk of myocardial infarction and sudden cardiac death. *N Engl J Med* 1987; **316**:1514–18.
7. Kannel WB, Wolf PA, Castelli WP, D'Agostino RB. Fibrinogen and risk of cardiovascular disease. *JAMA* 1987; **258**:1183–6.
8. Qizibash N, Jones L, Warlow C, Mann J. Fibrinogen and lipid concentrations as risk factors for transient ischaemic attacks and minor ischaemic strokes. *Br Med J* 1991; **303**:605–9.
9. Meade TW, Mellows S, Brozovic M, Miller GJ, Chaknabarti RR, North WR, Haines AP, Stirling Y, Imeson JD, Thompson SG. Haemostatic function and ischaemic heart disease: principal results of the Northwick Park Heart Study. *Lancet* 1986; **ii**:533–7.
10. Heinrich J, Balleisen L, Schulte H, Assmann G, van de Loo J. Fibrinogen and factor VII in the prediction of coronary risk. Results from the PROCAM study in healthy men. *Arterioscler Thromb* 1994; **14**:54–9.
11. Iso H, Folsom AR, Wu KK, Finch A, Munger RG, Sato S, Shimamoto T, Terao A, Komachi Y. Hemostatic variables in Japanese and Caucasian men. *Am J Epidemiol* 1989; **130**:925–34.
12. Meade TW, Imeson J, Stirling Y. Effects of changes in smoking and other characteristics on clotting factors and the risk of ischaemic heart disease. *Lancet* 1987; **ii**:986–8.
13. Carlson J, Davies R, Ehlenz K, Grunstein R, Hedner J, Podszus T, Sinoway L, Stradling J, Telakivi T, Zwillich C. Obstructive sleep apnea and blood pressure elevation: what is the relationship? *Blood Pressure* 1993; **2**:166–82.
14. Noguchi T, Chin K, Ohi M, Kita H, Otsuka N, Tsuboi T, Satoh M, Nakai A, Kuno K, Nagata K. Heat shock protein 72 level decreases during sleep in patients with obstructive sleep apnea syndrome. *Am J Respir Crit Care Med* 1997; **155**:1316–22.
15. Chin K, Ohi M, Kita H, Noguchi T, Otsuka N, Tsuboi T, Mishima M, Kuno K. Effects of NCPAP therapy on fibrinogen levels in obstructive sleep apnea syndrome. *Am J Respir Crit Care Med* 1996; **153**:1972–8.
16. Friedwald WT, Levy RI, Frederickson DS. Estimation of the concentration of low density lipoprotein cholesterol in plasma, without use of preparative ultracentrifugation. *Clin Chem* 1972; **18**:499–502.
17. Dati F, Barthels M, Conard J, Fluckiger J, Girolami A, Hanseler E, Huber J, Keller F, Kolde HJ, Muller-Berghaus G, Samama M, Thiel W. Multicenter evaluation of a chromogenic substrate methods for photometric determination of prothrombin time. *Thromb Haemost* 1987; **58**:856–65.
18. Woodhouse PR, Khaw KT, Plummer M, Foley A, Meade TW. Seasonal variations of plasma fibrinogen and factor VII

activity in the elderly: winter infections and death from cardiovascular disease. *Lancet* 1994; **343**:435–9.

19. Sullivan CE, Issa FG, Berthon-Jones M, Eves L. Reversal of obstructive sleep apnea by continuous positive airway pressure applied through the nose. *Lancet* 1981; **i**:862–5.
20. Somers VK, Dyken ME, Clart MP, Abboud FM. Sympathetic neural mechanism in obstructive sleep apnea. *J Clin Invest* 1995; **96**:1897–904.
21. Otsuka N, Ohi M, Chin K, Kita H, Noguchi T, Hata T, Nohara R, Hosokawa R, Fujita M, Kuno K. Assessment of cardiac sympathetic function with iosine-123-MIBG imaging in obstructive sleep apnea syndrome. *J Nucl Med* 1997; **38**:567–72.
22. Iacoviello L, DiCastelnuovo A, Knijff PD, D'Orazio A, Amore C, Arboretti R, Kluft C, Donati MB. Polymorphisms in the coagulation factor VII gene and risk of myocardial infarction. *N Engl J Med* 1998; **338**:79–85.
23. Frimerman A, Miller HI, Laniado S, Keren G. Changes in hemostatic function at times of cyclic variation in occupational stress. *Am J Cardiol* 1997; **79**:72–5.
24. Engleman HM, Martin SE, Deary IJ, Douglas NJ. Effect of continuous positive airway pressure treatment on daytime function in sleep apnoea/hypopnoea syndrome. *Lancet* 1994; **343**:572–5.
25. Stradling J. Sleep apnoea and the misuse of evidence-based medicine. *Lancet* 1997; **349**:201–2.
26. Wright J, Johns R, Watt I, Melville A, Sheldon T. Health effects of obstructive sleep apnoea and the effectiveness of continuous positive airways pressure: a systemic review of the research evidence. *Br Med J* 1997; **314**:851–61.
27. Meade TW. Hypercoagulability and ischaemic heart disease. *Blood Rev* 1987; **1**:2–8.
28. Miller GJ, Stirling Y, Esnouf MP, Heinrich J, van de Loo J, Kienast J, Wu KK, Morrissey JH, Meade TW, Martin JC, Imeson JD, Cooper JA, Finch A. Factor VII-deficient substrate plasma depleted of protein C raises the sensitivity of the factor VII bio-assay to activated factor VII in test samples: results of an international collaborative study. *Thromb Haemost* 1994; **71**:38–48.
29. Engleman HM, Martin SE, Douglas NJ. Compliance with CPAP therapy in patients with the sleep apnoea/hypopnoea syndrome. *Thorax* 1994; **49**:263–6.