Low Circulating Estradiol and Adrenal Androgens Concentrations in Men on Glucocorticoids: A Potential Contributory Factor in Steroid-Induced Osteoporosis

G. Hampson, N. Bhargava, J. Cheung, S. Vaja, P.T. Seed, and I. Fogelman

Reductions in circulating estradiol concentrations could be implicated in the pathogenesis of steroid-induced osteoporosis (SIOP) in men. We assessed serum estradiol and adrenal androgens (dehydroepiandrosterone sulfate [DHEAS] and androstenedione) in 77 men (group A: idiopathic osteoporosis [IOP], n = 38, aged [mean ± SD] 57.7 ± 12.1 years; group B: SIOP, n = 39, aged 55.3 ± 13.1 years). We also studied the relationship between bone mineral density (BMD) and serum estradiol in the group of men with SIOP. In group B, we observed a higher prevalence of low serum testosterone concentrations (<9.0 nmol/L) (_P_ = .0052), which was significantly correlated with steroid dosage (_r_ = -0.42, _P_ = .0089) and estradiol concentrations (_r_ = 0.42, _P_ = .012). There was a significant positive association between BMD at the lumbar spine and serum estradiol (_P_ = .004) in the men with SIOP (group B). A high proportion of subjects had low serum estradiol concentrations (<48 pmol/L) in both groups (group A: 44.7 %, group B: 36 %). Serum adrenal androgens concentrations were also significantly suppressed in group B (serum androstenedione— group A: 4.99 ± 1.8; Group B: 2.1 ± 1.6 nmol/L; _P_ = .0001). Serum DHEAS was undetectable in 59% of patients in group B versus 6% in group A (_P_ = .001). Reductions in androstenedione also correlated with steroid dosage (_r_ = -0.35, _P_ = .01). In conclusion, the data show that adrenal androgens synthesis is markedly suppressed in men with SIOP. The clinical relevance of this finding remains to be determined. This study also shows a positive association between serum estradiol and BMD and a high prevalence of low serum estradiol in men with SIOP. Low serum estradiol may contribute to bone loss in men with SIOP.

OSTEOPOROSIS IN MEN is an important, although underestimated, public health problem. Up to 30% of hip fracture and 20% of vertebral fractures occur in men.[1] The major causes are primary or idiopathic (IOP) or glucocorticoid-induced osteoporosis (SIOP).

It is now becoming increasingly clear that estrogens play a central role in the development of the male skeleton. This was first demonstrated in males with inherited aromatase deficiency[2] and mutations in the estrogen receptor (ER-α) gene.[3] Several studies have also shown a positive association between serum estradiol concentrations and bone mineral density (BMD)[4,5] and the biochemical markers of bone turnover in men.[4] Estrogen in men is derived from testosterone and the adrenal androgens, dehydroepiandrosterone (DHEA) and androstenedione, by the actions of the microsomal P450 enzyme aromatase.[6]

The relationship between estrogen and skeletal integrity in men with SIOP remains unknown. Although the pathogenesis of SIOP is multifactorial, changes in gonadal and adrenal steroids have been implicated.[7,8] Glucocorticoid therapy has been shown to lead to a dose-dependent reduction in testosterone.[9] In postmenopausal women on glucocorticoid, adrenal suppression results in a reduction in androstenedione, testosterone, and estrone.[10] However, data on the extent of adrenal suppression in men with SIOP on long-term glucocorticoid are lacking.

We hypothesize that as estrogen has beneficial effects on the

male skeleton, it is also likely to play a major role in the pathogenesis of SIOP in men. The aim of this study was to assess circulating estrogen concentration and its relationship to BMD in men with SIOP. We also measured serum concentrations of testosterone and adrenal androgens as these hormones have a major role, through aromatization, in the maintenance of estrogen level.

MATERIALS AND METHODS

Subjects

We studied 77 men who were recruited consecutively from the metabolic bone clinic at Guy's Hospital. The local research ethics committee had approved the study and all the patients gave informed consent. All patients were asked to complete a questionnaire, which included questions about smoking, alcohol consumption, family history, medication, level of exercise, past medical history, and history of fractures.

The clinical characteristics of all patients are summarized in Table 1. They were divided into 2 groups: group A (n = 38) with IOP and group B (n = 39) with SIOP. Subjects in group A were invited to participate in the study if they met the following criteria: (1) a T score (BMD values expressed as number of SDs above or below the young adult mean value) of less than -2.5 at the hip or spine and/or the presence of one or more low trauma fractures. The patients in group B were included in the study if they had a history of oral glucocorticoid intake and were receiving treatment for prevention of bone loss and fractures according to the consensus guidelines[8]; a T score of less than -1.5 at the spine and/or hip, or a history of previous low trauma fractures. Seventeen patients in group A and 9 in group B had previously sustained a low trauma fracture. This was determined from the clinical history and spine x-rays performed at first presentation to the metabolic bone clinic.

The mean (SD) age of the patients in groups A and B was 57.7 (12.1) and 55.3 (13.1) years, respectively. There was no significant difference in their body mass index (BMI): group A, 24.8 (3.4); and group B, 25.4 (4.4); or in BMD at the lumbar spine and hip (Table 1).

Group B patients had been on oral steroids for 9.6 (8.1) years and the mean current prednisolone dosage was 14.3 (11.3) mg. They were on steroids for a variety of clinical conditions, including asthma (n = 14), post–renal transplant (n = 7), myasthenia gravis (n = 4), sarcoidosis

Table 1. Summary of Clinical Characteristics of the Study Population

	Group A (n = 38)	Group B (n = 39)
Age (yrs)	57.7 ± 12.1	55.3 ± 13.1
BMI (kg/m^2)	24.8 ± 3.4	25.4 ± 4.4
BMD, spine (g/cm^2)	0.823 ± 0.118	0.847 ± 0.154
BMD, total hip (g/cm^2)	0.828 ± 0.123	0.817 ± 0.116
Prevalence of fractures	44.7%*	13%
Current and exsmoker	34.2%	20.5%
Alcohol intake (>20 U) per week	12.7%	10.5%

NOTE. Values are mean ± SD unless indicated otherwise.
*P = .01.

(n = 3), myo/neuropathies (n = 3), and chronic inflammatory bowel disease (n = 1). At the time of entry in the study, they were all stable on their current maintenance dose of steroids and were managed as outpatients. All group B patients were being treated for SIOP and were taking bisphosphonates and calcichew D3 (2 tablets daily, equivalent to 1 g of calcium and 800 IU of cholecalciferol) (n = 21), bisphosphonates only (n = 9), and calcichew D3 only (same dosage as above) (n = 9). There had been no significant change in their BMD over the previous 12 months (as defined by a percent annual change of > 1%). They denied any clinical history suggestive of new fractures over the last year such as acute onset of sharp back pain. However, vertebral x-rays to assess "silent" vertebral deformities were not examined.

Patients in group A had no obvious secondary causes for osteoporosis such as corticosteroid use, hypogonadism, alcoholism, gastrointestinal disorders, endocrine disorders, or vitamin D deficiency. On clinical examination they had no evidence of hypogonadism. None of them had excessive alcohol intake. Renal function was normal. Serum calcium, phosphate, parathyroid hormone (PTH), vitamin D, and testosterone were all within normal limits. Thirteen (34%) had a positive family history of osteoporosis. Twenty were on bisphosphonates and calcichew D3, 11 were on bisphosphonates only, and 7 were taking calcichew D3 only.

Methods

Bone mineral density. BMD was measured by dual-energy absorptiometry (DXA) on a Hologic (Waltham, MA) QDR-2000. BMD was measured at the lumbar spine and hip. Baseline BMD values, prior to institution of treatment, as well as values during attendance at clinic for follow-up visits, were obtained on all subjects.

Biochemical tests. Serum electrolytes, urea, creatinine, albumin, calcium, and phosphate was measured on all patients at recruitment by standard laboratory procedures on the Beckman LX 20 analyzer (Beckman Coulter, Fullerton, CA). Serum PTH was measured on the Nicholas Advantage (Nichols Institute, Diagnostics, Middlesex, UK) by a chemiluminometric assay. The reference range of the assay is 10 to 65 ng/L. Serum 25(OH) vitamin D was determined by radioimmunoassay using a commercial kit following a rapid extraction procedure (DiaSorin, Stillwater, MN). The assay reference range is 40 to 195 nmol/L.

Sex hormones and adrenal androgens assays. Serum testosterone, sex-hormone binding globulin (SHBG), and DHEAS were measured on the Centaur (Bayer plc, Newbury, UK) and Immulite (Immulite, Glyn Rhonwy, UK) analyzers, respectively, by chemiluminometric assays. The interassay coefficient of variation (CV) for total testosterone was 4.4% and 4.7% at concentrations of 3.3 nmol/L and 12.7 nmol/L, respectively. The interassay CV for SHBG was 9.2% and 8% at SHBG concentrations of 12 nmol/L and 35 nmol/L, respectively. The between-assay precision for DHEAS was less than 10%. The lower limit of detection of the DHEAS assay is 0.8 μmol/L (concentration of the

lowest standard). Androstenedione was assayed by radioimmunoassay using a commercial kit (Ortho-Clinical Diagnostics, Amersham, UK). The interassay CV was 6.3% and 4.8% at concentrations of 2.2 and 7.1 nmol/L. The lower limit of detection of the assay was 0.34 nmol/L. The reference ranges for serum testosterone, DHEAS (age-related), and androstenedione had been derived by the local laboratory and is used in routine clinical practice. Estradiol was measured by radioimmunoassay using the ESTR-US-CT kit (CIS Bio International, Gif-Sur-Yvette, France). The reference range in men as quoted by the manufacturer is 23 to 266 pmol/L with a mean of 117 pmol/L.

Statistics

Statistical analyses were performed using the STATA 5 statistical software (STATA Corp, College Station, TX). Data were analyzed using multiple regression analysis with robust standard errors. Differences in prevalence between the 2 groups were analyzed using Pearson's chi-square test. Correlation was determined using Spearman's rank correlation in cases of nonparametric distribution (Fig 1). Data were age-adjusted.

RESULTS

Calcium Metabolism

There was no significant difference in the mean (SD) serum calcium (normal range, 2.2 to 2.6 mmol/L), phosphate (normal range, 0.8 to 1.5 mmol/L), PTH (normal range, 10 to 65 ng/L), and 25(OH) vitamin D (normal range, 40 to 195 nmol/L) between the 2 groups after exclusion of the renal transplant
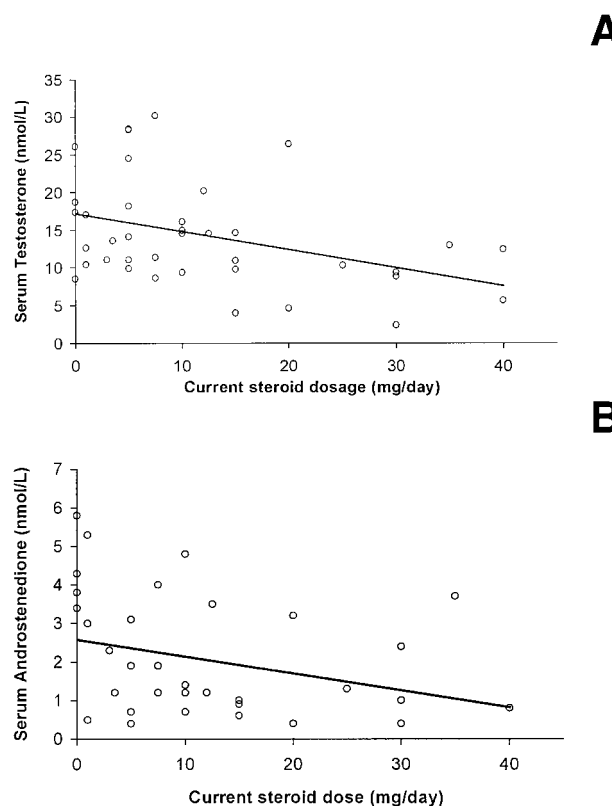

Fig 1. Correlation between current steroid dosage and (A) serum testosterone (r = -0.42, P = .009), and (B) serum androstenedione (r = −0.35, P = .01) in group B subjects.

Table 2. Serum Sex Hormones and Adrenal Androgens Concentration in the Study Subjects (groups A and B)

	Group A	Group B	Normal Range
Serum testosterone	16.86 ± 5.4	14.15 ± 6.98	9-30 nmol/L
Serum free testosterone	347.82 ± 91.6	331.21 ± 121.23	134-844 pmol/L
Serum estradiol	55.13 ± 24.26	66.56 ± 35.33	26-266 pmol/L
Serum androstenedione	4.99 ± 1.77*	2.02 ± 1.55	4.4-10.6 nmol/L
Serum DHEAS, median (range)	2.9 (0.8-11.8)†	0.8 (0.8-9.9)	(40-60yr) 0.8-6.9μmol/L

NOTE. Values are mean ± SD unless indicated otherwise.
*$P = .0001$.
†$P = .001$.

patients (calcium—group A: 2.37 [0.09]; group B: 2.41 [0.09]; phosphate—group A: 1.14 [0.22]; group B: 1.13 [0.21]; 25(OH) vitamin D—group A: 49.5 [21.3]; group B: 35.4 [18.2]; PTH—group A: 30.8 [13.6]; group B: 33.7 [11.6]). The renal transplant patients (n = 7) had higher serum PTH concentrations: 94.4 (54.4) ng/L. Their mean (SD) serum calcium and phosphate were 2.47 (0.19) and 1.0 (0.23) mmol/L, respectively. Analysis of the data excluding the renal transplant patients did not lead to any significant changes. Data from these patients were therefore included in subsequent analyses of the group with SIOP (detailed below).

Sex Steroids

There was a significant negative correlation between serum total testosterone and current steroid dosage ($r = -0.42$, $P = .0089$) (Fig 1A). Serum total testosterone was lower in the patients on steroids (Table 2), although the results just failed to reach significance ($t = -1.9$, $P = .0618$). However, a significantly higher proportion of patients in group B had serum testosterone below the normal range (< 9 nmol/L) (n = 8 in group B v n =0 in group A, $P = .0052$).

There was no significant difference in serum estradiol concentrations between the 2 groups. Seventeen subjects in group A (44.7%) and 14 in group B (36%) had serum estradiol values below 48 pmol/L (≈1 SD below the mean). There was a significant positive association between serum estradiol levels and BMD at the lumbar spine in group B ($t = 3.096$, $P = .004$) (Fig 2). The significant positive association was also present when using baseline BMD data instead of follow-up values.
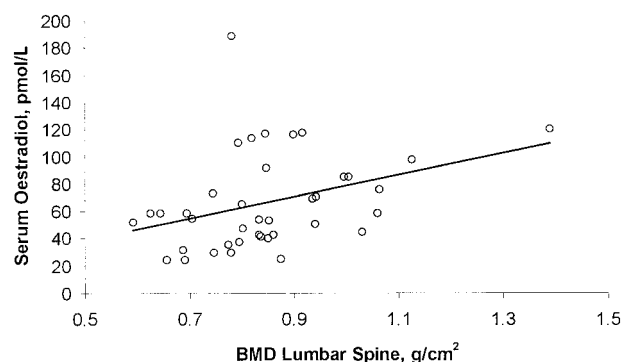
There was no significant reduction in BMD on follow-up, after institution of treatment. BMD values remained steady.

There was a significant correlation between log-transformed serum testosterone concentrations and estradiol in the steroid-treated group (group B) ($r = 0.4$, $P = .012$). However, we observed no correlation between log-transformed serum testosterone and estradiol levels in group A ($r = 0.14$, $P = .42$) (Fig 3).

A

B

Fig 2. Association between serum estradiol and BMD at the lumbar spine in group B ($P = .004$).
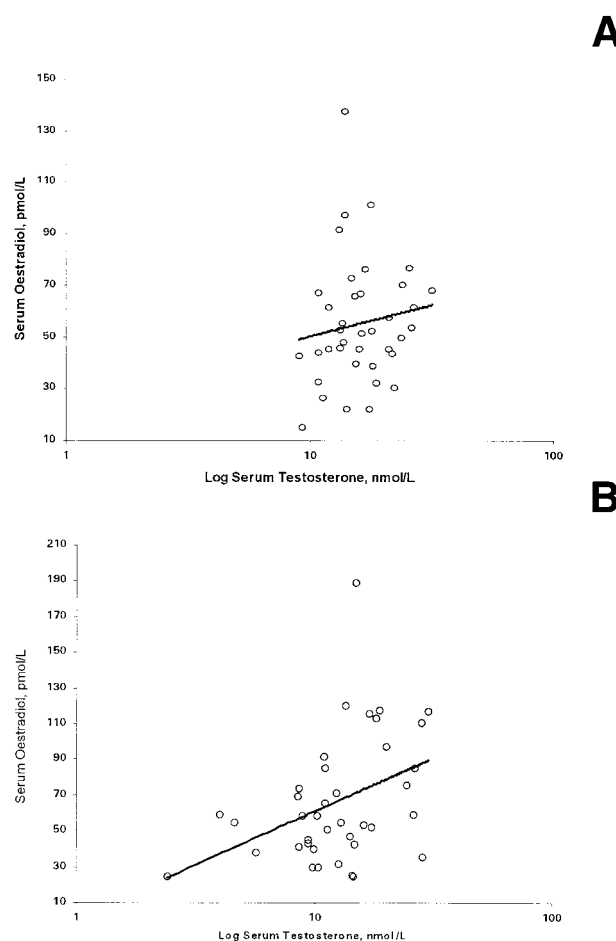
Fig 3. Correlation between serum estradiol and testosterone in (A) group A ($r = 0.14$, $P = .42$) and (B) group B ($r = 0.4$, $P = .012$).

Adrenal Androgens

Androstenedione was significantly reduced in the subjects in group B (Table 2). Thirty-four patients (87%) had levels below the normal range (<4.4 nmol/L).

DHEAS was also significantly reduced in group B. Serum concentrations were undetectable (<0.8 μmol/L) in 23 (59%) patients in group B as compared to 2 (6%) in group A (*P* = .001). The median and range of DHEAS in the 2 patient groups are given in Table 2, as the distribution of values was nonparametric. This was accounted for in the statistical analysis (the data for DHEAS were log-transformed).

A significant negative correlation was also observed between serum androstendione and current steroid dosage (Fig 1B) (*r* = -0.35 *P* = .01).

DISCUSSION

This study was undertaken to evaluate serum estradiol and its association with BMD in men with SIOP. The secondary aim was to assess the serum adrenal androgens and testosterone concentrations in the study population as estradiol is derived from these hormones.

Our study confirms the previously documented[11] high prevalence of low serum estradiol concentrations (<48 pmol/L) in men with IOP. Indeed, it has recently been shown that elderly men with bioavailable estradiol levels of less than 40 pmol/L had significantly higher rates of bone loss and levels of bone resorption markers than men with bioavailable estradiol levels above 40 pmol/L, further demonstrating the importance of estradiol on bone metabolism in men.[12] The cause of low serum estradiol in men with IOP is still unclear, although a reduction in aromatase activity causing reduced conversion of testosterone to estrogen has been postulated.[11] This could also explain our findings of a lack of correlation between serum testosterone and estradiol in our patients with IOP. As previously reported in men with IOP,[4,5] we showed for the first time a positive association between serum estradiol concentrations and BMD in men with SIOP. The association was still significant when baseline BMD prior to institution of treatment was used in the analysis. Moreover, BMD values remained steady on follow-up, following treatment. This association was significant at the lumbar spine, emphasizing the importance of estrogen on trabecular bone. We also demonstrated a similar prevalence of low serum estradiol in the group with SIOP. Several factors could contribute to low serum estradiol concentrations in men with SIOP. First is the reduction in serum testosterone concentrations in men on glucocorticoids. This is further supported by our finding of a positive correlation between serum testosterone

and estradiol in the patients on glucocorticoids. We, like others,[9] observed a reduction in serum testosterone that correlated significantly with the steroid dosage in the subjects with SIOP, implying that the major contributory factor to the reduction in serum testosterone in these patients is glucocorticoid therapy. Moreover, although the patients with SIOP were on glucocorticoids for a variety of clinical conditions, they were all stable clinically on their current steroid dose. Second, low serum estrogen concentrations in men with SIOP could be due to suppression of adrenal androgen synthesis. Our results show a profound decrease in DHEAS and androstenedione in these patients as previously documented in women.[10] Indeed, as with serum testosterone, we observed a significant negative correlation between serum androstenedione and steroid dosage. However, the magnitude of suppression was larger than that observed for testosterone.

The marked reductions in the adrenal androgen concentrations could have important clinical implications. First, these androgens are aromatized to estrogens.[6] Indeed, in intervention trials in men, DHEA replacement has been shown to lead to significant increases in circulating estrogens.[13] In addition, DHEA has been shown to have direct anabolic properties and could therefore counteract some of the catabolic properties of glucocorticoids. DHEA has been demonstrated to stimulate both osteoblast proliferation and differentiation in vitro.[6] In a relatively recent study, DHEA replacement for 12 months has been shown to increase BMD by stimulating bone formation as indicated by increases in serum osteocalcin, a marker of bone formation, in postmenopausal women aged 60 to 70 years.[14] Trials in men have been relatively of short duration (6 months), but have shown beneficial effects on body composition with increases in muscle mass.[15] No significant side effects were reported in men.[13,15] Controlled intervention trials are indicated in order to assess fully the effects of DHEA replacement in patients with glucocorticoid-induced osteoporosis.

In summary, this cross-sectional study shows for the first time a positive association between serum estradiol and BMD and a high prevalence of low serum estradiol in men with SIOP. Low serum estradiol could contribute to bone loss in men with SIOP, although the cross-sectional nature of this study precludes a definitive conclusion. Further longitudinal studies are needed. Moreover, we have also demonstrated that adrenal androgens synthesis is profoundly suppressed in this group. The clinical relevance of this finding remains to be studied further in controlled trials to assess the potential benefits of DHEA replacement therapy in men with SIOP.

REFERENCES

1. Eastell R, Boyle IT, Compston J, et al: Management of male osteoporosis: Report of the UK Consensus Group. Q J Med 91:71-72 , 1998

2. Morishima A, Grumbach MM, Simpson ER, et al: Aromatase deficiency in male and female siblings caused by a novel mutation and the physiological role of estrogens. J Clin Endocrinol Metab 80:3689-3698, 1995

3. Smith EP, Boyd J, Frank GR, et al: Estrogen resistance caused by a mutation in the estrogen receptor gene in a man. N Engl J Med 339:599-603, 1998

4. Khosla A, Melton LJ, Atkinson EJ, et al: Relationship of serum sex steroid levels and bone turnover markers with bone mineral density in men and women: A key role for bioavailable estrogen. J Clin Endocrinol Metabo 83:2266-2274, 1998

5. Van Den Beld AW, De Jong FH, Grobbee DE, et al: Measures of bioavailable serum testosterone and estradiol and their relationship with muscle strength, bone density and body composition in elderly men. J Clin Endocrinol Metab 85:3276-3282, 2000

6. Hofbauer LC, Khosla S: Androgen effects on bone metabolism: Recent progress and controversies. Eur J Endocrinol 140:271-286, 1999

7. Pearce G, Tabensky DA, Delmas PD, et al: Corticosteroid-induced bone loss in men. J Clin Endocrinol Metab 83:801-806, 1998

8. Eastell R, Reid DM, Compston J, et al: A UK consensus group on management of glucocorticoid-induced osteoporosis: An update. J Intern Med 244:271-292, 1998

9. Reid IR, Ibbertson HK, France JT, et al: Plasma testosterone concentrations in asthmatic men treated with glucocorticoids. Br Med J 291:574-577, 1985

10. Crilly RG, Cawood M, Marshall DH, et al: Hormonal status in normal, osteoporotic and corticosteroid-treated post menopausal women. J R Soc Med 71:733-736, 1978

11. Carlsen CG, Soerensen TH, Eriksen EF: Prevalence of low serum estradiol levels in male osteoporosis. Osteoporosis Int 11:697-701, 2000

12. Khosla S, Melton LJ, Atkinson EJ, et al: Relationship of serum sex steroid levels to longitudinal changes in bone density in young versus elderly men. J Clin Endocrinol Metab 86:3555-3561, 2001

13. Arlt W, Haas J, Callies F, et al: Biotransformation of oral dehydroepiandrosterone in elderly men: Significant increase in circulating estrogens. J Clin Endocrinol Metab 84:2170-2176, 1999

14. Labrie F, Diamond P, Cusan L, et al: Effect of 12-month dehydroepiandrosterone replacement therapy on bone, vagina and endometrium in post menopausal women. J Clin Endocrinol Metab 82:3498-3505, 1997

15. Morales AJ, Hanbrich RH, Hwang JY, et al: The effect of six months treatment with a 100 mg daily dose of dehydroepiandrosterone (DHEA) on circulating sex steroids, body composition and muscle strength in age-advanced men and women. Clin Endocrinol 49:421-432, 1998