Impaired Mineral Metabolism in Cushing's Syndrome: Parathyroid Function, Vitamin D Metabolites and Osteopenia.

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Abstract

To clarify the mechanism for the impaired mineral metabolism in Cushing's syndrome, the clinical features, biochemical parameters before and after oral calcium load, and vitamin D metabolism were compared between two groups of patients of endogenous Cushing's syndrome (17 cases) with and without osteopenia. The patients with osteopenia [OP (+): 7 cases, all female] were older (42.7 ± 8.3 y. o.) and had a longer duration (117 ± 75 M) of the syndrome than those without osteopenia [OP (-): 33.8 ± 8.9 y. o., 36 ± 25 M]. OP (-) showed a blunted hypercalciuria after oral calcium load (63.7 ± 20.4 to 90.9 ± 36.1 mg/g·Cr), while OP (+) had higher levels of urinary excretion of calcium (fasting: 120.4 ± 37.5 , and after oral calcium load: 235.6 ± 72.6 mg/g·Cr), of cyclic AMP (7.6 ± 1.1 nmol/dl·GF), and of plasma $1,25(\text{OH})_2\text{D}$ (76.6 ± 34.0 pg/ml) than OP (-) (u-cAMP: 3.2 ± 2.0 nmol/dl·GF, $1,25(\text{OH})_2\text{D}$: 27.9 ± 16.3 pg/ml). These results indicate that 1) elderly female patients with Cushing's syndrome of long duration are susceptible to OP, 2) during the early phases of the syndrome, reduced intestinal calcium absorption with sustained calciuria (probably through the inhibition of calcium reabsorptive effect of PTH by glucocorticoid) induces negative calcium balance, leading to 3) a development of secondary hyperparathyroidism which stimulates $1,25(\text{OH})_2\text{D}$ synthesis. Thus, the mechanism involving bone resorption stimulated by excess PTH along with the direct inhibition of bone formation by glucocorticoid seems to play an important role in a progressive development of OP in Cushing's syndrome.

Patients with Cushing's syndrome suffer from an impairment of mineral metabolism, such as osteopenia and urolithiasis (Howland *et al.*, 1958; Ross *et al.*, 1966) as one of the major complications. Chronic administration of pharmacologic doses of glucocorticoid inhibits the intestinal calcium

absorption with normal or increased urinary excretion (Hahn *et al.*, 1979; Klein *et al.*, 1977; Lukert and Adams., 1976) leading to a persistent negative calcium balance which results in a progressive loss of bone mass (Hahn *et al.* 1976). However, the mechanisms, involved in these impairments of mineral homeostasis, especially in relation to the function of calcium regulating

hormones, are still controversial. Some studies have demonstrated a secondary increase in parathyroid hormone (PTH) secretion (Findlings *et al.*, 1982; Fucik *et al.*, 1975; Hahn *et al.*, 1979; Lukert and Adams., 1976; Suzuki *et al.*, 1983), while others have shown no difference in PTH secretion during hyper- and euglucocorticoid states (Seeman *et al.*, 1980; Slovik *et al.*, 1980). The previous reports on vitamin D metabolism indicated that glucocorticoids decrease (Klein *et al.*, 1977; Lindgren *et al.*, 1982; Suzuki *et al.*, 1983), increase (Findlings *et al.*, 1983; Hahn *et al.*, 1981), or do not change (Hahn *et al.*, 1977; Seeman *et al.*, 1980; Slovik *et al.*, 1980) plasma 25OHD or 1,25(OH)₂D. The heterogeneity of the subjects such as the presence or absence and clinical stage of underlying disease, dose and duration of glucocorticoid excess, and the degree of impairment of mineral metabolism might make some differences in these results.

To clarify the mechanism involved in the development of impaired mineral metabolism in Cushing's syndrome, a clinical study was carried out among the patients with endogenous Cushing's syndrome, and the clinical and biochemical features were compared between two groups of patients with and without osteopenia.

Subject and Method

Subjects

Seventeen patients with Cushing's syndrome (eight cases with Cushing's disease, eight cases with adrenal adenoma, and one case with ectopic ACTH syndrome, age range 18–52 y. o.) were studied. The diagnosis of Cushing's syndrome was established by the standard dexamethasone suppression test, adrenal scintigram, and plasma ACTH level.

Since the distribution of the osteopenia in Cushing's syndrome appears to involve predominantly the axial skeleton (vertebrae and rib) which is rich in trabecular bone (Hahn *et al.*, 1974), the routine X-ray films of chest and abdomen were reviewed by radiologists for

the evaluation of osteopenia (i. e. marked demineralization and vertebral compression fracture). According to the radiological reports, the patients were divided into two groups, one with marked changes of osteopenia and the other without such changes.

Study Protocol

After the patients were hospitalized and maintained on a diet which contains approximately 600 mg of calcium and 10 g of sodium chloride for at least 3 days, the basal plasma samples were obtained for the determination of immunoreactive PTH (iPTH) and vitamin D metabolites. The oral calcium load tests (Pak *et al.*, 1975) were performed in ten patients.

Analyses

Serum iPTH was determined by a radioimmunoassay (RIA) kit (Eiken Immunochemical Laboratory, Ohji, Tokyo, Japan) (Koide *et al.*, 1981). Vitamin D metabolites were measured by competitive protein binding assay [25OHD and 24,25(OH)₂D] or radioreceptor assay [1,25(OH)₂D] after the extraction of metabolites from plasma by Sephadex LH-20 and high pressure liquid chromatography (Imawari *et al.*, 1982). Urinary cyclic AMP was determined by an RIA kit (Yamasa Shoyu, Choshi, Chiba, Japan). Serum and urinary calcium, phosphorus, and creatinine were measured by a multichannel autoanalyzer. The serum calcium level was corrected for protein binding (Payne *et al.*, 1973). Statistical analyses were performed using Student's *t* test.

Results

Clinical features (Table 1)

Seven patients, all female, showed marked changes of osteopenia. The age at the diagnosis of Cushing's syndrome was slightly older in patients with osteopenia than in those without it. The estimated duration with the clinical symptoms of the syndrome was significantly longer in patients with osteopenia than in those without it. The degree of hypercortisolism expressed by the serum cortisol level and urinary excretion of 17-OHCS was not different between two groups.

Table 1. Clinical features of Cushing's syndrome in patients with and without marked osteopenia

Clinical features	OP (+) n=7	OP (-) n=10	P
Sex	(M: O, F: 7)	(M: 3, F: 7)	
Age (y. o.)	42.7 ± 8.3	33.8 ± 8.9	<0.1
Duration (m)	117 ± 75	36 ± 25	<0.01
Plasma cortisol (μg/dl)	22.8 ± 5.8	20.7 ± 4.6	NS
Urinary 17-OHCS (mg/g·Cr)	16.2 ± 5.6	15.0 ± 3.4	NS

OP: Osteopenia. Values are the mean ± SD.

Fig. 1. Serum calcium corrected for protein (s-Ca, ●) and phosphorus (s-P, ○), urinary calcium (u-Ca) and cyclic AMP (u-cAMP) during fasting and after calcium load (indicated by arrows) in patients with marked osteopenia [OP (+)], in those without it [OP (-)], and in normal subjects (N: n=18). Values are the mean and SD and normal range for serum measures (■: Ca, □: P). Open bars represent urinary excretion per gram of creatinine (g·Cr) and dotted bars represent that per 100 ml of glomerular filtration rate (dl·GF). Values which are different from fasting value (paired data) or between two groups [OP (+) vs. OP (-)] are indicated (+, $P < 0.1$; *, $P < 0.05$; **, $P < 0.01$).

Mineral metabolism

Serum calcium corrected for protein and serum phosphorus were within the normal range and not different between two groups (Fig. 1, upper panels). In the patients without osteopenia, serum calcium and phosphorus did not change after oral calcium load. Patients with osteopenia showed a slight but significant increase in serum calcium and phosphorus levels after oral calcium load. In the patients without osteopenia, the mean urinary excretion of calcium during fasting was normal and did not increase significantly after oral calcium load (Fig. 1, right in the middle panels). In the patients with osteopenia, the mean urinary excretion of calcium during fasting was not in the hypercalciuric range but significantly higher than that of the patients without osteopenia. After oral calcium load, the mean urinary excretion of calcium increased by a degree compatible with that of normal subjects (Fig. 1, left in the middle panels).

Parathyroid function

Since only three cases with osteopenia had a detectable concentration of iPTH in plasma (0.44 ± 0.12 ng/ml, detection limit of 0.2 ng/ml), the parathyroid function was estimated from the urinary excretion of cyclic AMP. In the patients without osteopenia, the mean urinary excretion of cyclic AMP was normal and not suppressed after oral calcium load (Fig. 1, right in lower panels). In the patients with osteopenia, the mean

urinary excretion of cyclic AMP was above the upper limit of the normal range and significantly higher than that of the patients without osteopenia. The mean urinary excretion of cyclic AMP was decreased significantly after oral calcium load in the patients with osteopenia (Fig. 1, left in the lower panels).

Vitamin D metabolites (Table 2)

Plasma levels of 25OHD and 24,25(OH)₂D were in the lower range of those for normal subjects, and the mean levels of these metabolites were not different between two groups.

The plasma levels of 1,25(OH)₂D in the patients without osteopenia were in the lower range of those for normal subjects and those in the patients with osteopenia were in the upper range of those for normal subjects. The mean plasma level of the metabolite was significantly higher in the patients with osteopenia than that in the patients without it.

Discussion

In the present study, the analyses of the clinical features revealed that the patients with Cushing's syndrome with marked osteopenia were all female and had a longer duration of the syndrome than those without osteopenia. The clinical study on the mineral metabolism demonstrated that the cases without osteopenia showed a

Table 2. Vitamin D metabolites in patients with and without marked osteopenia

Metabolites	Normal	OP (+) n=4	OP (-) n=7	P
250 HD (ng/ml)	19 ± 5 (10-31)	13.88 ± 3.71 (9-18)	16.29 ± 4.75 (10-23)	NS
24,25 (OH) ₂ D (ng/ml)	2.3 ± 1.4 (0.4-4.8)	0.30 ± 0.28 (0.1 - 0.5)	0.67 ± 0.32 (0.2 - 1.1)	NS
1,25 (OH) ₂ D (pg/ml)	37 ± 11 (26-62)	76.60 ± 34.00 (40-120.9)	27.90 ± 16.31 (7.5-49.5)	<0.01

OP: Osteopenia. Values are the mean+SD and range in parentheses.

blunted hypercalciuric response after oral calcium load, while the cases with marked osteopenia had higher levels of urinary excretion of calcium (fasting and after oral calcium load), of cyclic AMP, and of plasma $1,25(\text{OH})_2\text{D}$ than those without osteopenia.

Since the patients with marked osteopenia were all female and older than those without osteopenia, the elderly female patients with Cushing's syndrome seem to be more susceptible to osteopenia. A similar finding has been reported in steroid induced osteopenia among patients with rheumatoid arthritis, and interpreted as a reflection of the reduced bone mass already present in elderly female patients before initiation of glucocorticoid administration (Saville and Kharmosh, 1967).

The long duration of Cushing's syndrome seems to play an important role in the development of osteopenia. Since the close correlation between the development of osteopenia and the duration of glucocorticoid administration has been demonstrated (Hahn *et al.*, 1981), our findings indicate that in endogenous Cushing's syndrome, the duration of hypercortisolism also contributes to the development of osteopenia.

The blunted hypercalciuric response after oral calcium load seen in the cases without marked osteopenia reflects an impairment of intestinal calcium absorption as reported previously in glucocorticoid excess (Gallaher *et al.*, 1973; Hahn *et al.* 1979; Kimberg *et al.*, 1971; Lukert *et al.*, 1973). The mechanism involved in this impairment has been attributed to either the derangement of hepatic production of 25OHD (Avioli *et al.*, 1968), the low level of $1,25(\text{OH})_2\text{D}$ in plasma (Lindgren *et al.*, 1968) under glucocorticoid excess, or direct inhibition of calcium absorption by glucocorticoid under normal vitamin D metabolism (Kinberg *et al.*, 1971; Seeman *et al.*, 1980). In our patients without marked osteopenia who also

showed impaired calcium absorption, the plasma levels of 25OHD and $1,25(\text{OH})_2\text{D}$ were distributed in the lower range of those for normal subjects. These findings seem to favor the mechanism of impaired calcium absorption involving abnormal vitamin D metabolism rather than the direct effects of glucocorticoid excess.

The impairment of calcium absorption induced by long term glucocorticoid would probably induce secondary hyperparathyroidism. Most studies have demonstrated an increase in PTH secretion during hypercortisolism (Findlings *et al.*, 1982; Fucik *et al.*, 1975; Hahn *et al.*, 1979; Lukert and Adams, 1976; Suzuki *et al.*, 1983), despite a few reports indicating no change in the plasma iPTH level during hyper- and euglucocorticoid states (Seeman *et al.*, 1980; Slovik *et al.*, 1980). In the present study, plasma iPTH concentrations were measurable only in a few cases with marked osteopenia. These controversy might be derived from the heterogeneity of the subjects, such as the degree of bone involvement or the duration of glucocorticoid excess. The methodological differences in RIA of PTH such as sensitivity and specificity with heterogeneous PTH fragments in plasma might also contribute to producing controversial results. Therefore, in the present study the parathyroid function was estimated using urinary excretion of cyclic AMP as an index. In the patients with marked osteopenia, the mean urinary excretion of cyclic AMP was elevated and suppressible by oral calcium load, suggesting the presence of secondary hyperparathyroidism. The elevation of serum phosphorus after oral calcium load probably reflects the suppression of phosphaturia induced by secondary hyperparathyroidism. While the excess PTH should increase tubular calcium reabsorption, the urinary excretion of calcium remained normal in patients with marked osteopenia. This finding may reflect the impaired

calcium conservation by the kidney due to the interference with renal action of PTH by glucocorticoid. Similar findings have demonstrated (Laake, 1960; Suzuki *et al.*, 1983) and regarded as an important factor in the development of secondary hyperparathyroidism under glucocorticoid excess (Suzuki *et al.*, 1983).

Regarding vitamin D metabolism in the hyperglucocorticoid state, plasma 25OHD or 1,25(OH)₂D values have been reported to be decreased (Klein *et al.*, 1977; Lindgren *et al.*, 1982; Suzuki *et al.*, 1983), increased (Findlings *et al.*, 1983, Hahn *et al.*, 1981), or unchanged (Hahn *et al.*, 1977; Seeman *et al.*, 1980; Slovik *et al.*, 1980). In our series, plasma 25OHD and 24,25(OH)₂D values were in the lower range of those for normal subjects either in the presence or in the absence of marked osteopenia. The mean plasma 1,25(OH)₂D value for the entire subjects studied was within the range for normal subjects. However, the mean plasma 1,25(OH)₂D level was significantly higher in the cases with marked osteopenia than in those without it, suggesting that glucocorticoid excess lasting long enough to cause osteopenia results in an increase in the plasma level of this metabolite. Since the plasma level of 25OHD was low and the serum phosphorus level was normal, the plasma 1,25(OH)₂D level was elevated probably through parathyroid hormone excess.

The mechanism involved in these changes in parathyroid function and vitamin D metabolism are summarized as follows. During the early phase, hypercortisolism reduces intestinal calcium absorption probably by impairing vitamin D metabolism with sustained calciuria probably by inhibiting calcium reabsorption. This state of negative calcium balance for longer duration induces a secondary increase in PTH secretion to maintain a normal calcium balance. Excess PTH stimulates 1,25(OH)₂D synthesis to normalize impaired intestinal

calcium absorption. The urinary loss of calcium, however, continues leading to a persistent secondary hyperparathyroidism. Excess PTH also stimulates bone resorption resulting in osteopenia along with the direct inhibitory effects of glucocorticoid on bone formation (Jewsey and Riggs, 1970; Jee *et al.*, 1970). A similarity in the distribution and histological changes of bone loss in glucocorticoid induced osteopenia and primary hyperparathyroidism (Hahn *et al.*, 1974; Jee *et al.*, 1980), and abolishment by parathyroidectomy of osteoclastic response to steroid administration in animals (Jee *et al.*, 1970) indicate the importance of excess PTH in the development of the steroid induced osteopenia.

Although the single primary cause is difficult to define in advanced osteopenia in Cushing's syndrome, the functional states of calcium regulating hormones are somewhat different in the early stage from those in longstanding Cushing's syndrome. The understanding of these mechanisms will help in devising specific methods for treatment and prevention of the impairment of calcium metabolism under glucocorticoid excess based on the functional states of calcium regulating hormones.

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