

Adrenocortical incidentalomas and bone: from molecular insights to clinical perspectives.

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Adrenocortical incidentalomas and bone: from molecular insights to clinical perspectives.

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1 **Abstract**

2 Adrenal incidentalomas constitute a common clinical problem with an overall prevalence of around 2-
3 3%, but are more common with advancing age being present in 10% of those aged 70y. The majority
4 of these lesions are benign adrenocortical adenomas (80%), characterized in 10-40% of the cases by
5 autonomous cortisol hypersecretion, and in 1-10% by aldosterone hypersecretion. Several
6 observational studies have shown that autonomous cortisol and aldosterone hypersecretion are more
7 prevalent than expected in patients with osteopenia and osteoporosis: these patients have accelerated
8 bone loss and an increased incidence of vertebral fractures. In contrast to glucocorticoid action, the
9 effects of aldosterone on bone are less well understood. Recent data, demonstrating a concomitant co-
10 secretion of glucocorticoid metabolites in patients with primary aldosteronism, could explain some of
11 the metabolic abnormalities seen in patients with aldosterone hypersecretion.

12 In clinical practice, patients with unexplained osteoporosis, particularly when associated with other
13 features such as impaired glucose tolerance or hypertension, should be investigated for the possible
14 presence of autonomous cortisol or aldosterone secretion due to an adrenal adenoma. Randomized
15 intervention studies are needed, however, to investigate the optimum interventions for osteoporosis
16 and other co-morbidities in these patients.

17
18 **Keywords:** adrenal, incidentaloma, autonomous cortisol hypersecretion, primary aldosteronism, bone,
19 osteoporosis.

20

21 Introduction

22 The term adrenal incidentaloma refers to any clinically unsuspected adrenal lesion that is detected
23 incidentally during imaging for other indications [1,2]. With widespread use of imaging techniques,
24 adrenocortical incidentalomas constitute a common clinical problem with a prevalence of more than
25 10% in people 70 years or more [1-6]. Adrenal incidentalomas can be benign or malignant,
26 functioning or nonfunctioning, unilateral or bilateral. The vast majority are benign adrenocortical
27 adenomas (ACA, 80%) [1,2] with the most frequent endocrine dysfunction being ‘autonomous cortisol
28 hypersecretion’, previously termed ‘subclinical Cushing’s syndrome’ [1,2,7-10], while primary
29 aldosteronism (PA) seems to be the most frequent hormonal secretion in Korean population with
30 adrenal incidentaloma [11]. Depending on definitions used, the prevalence of excess cortisol secretion
31 amongst these adrenocortical lesions ranges from 10-40%. In contrast, the frequency of aldosterone
32 hypersecretion varies from 1% to 10% according to various tests used [1,2,6,12,13]. Recent data,
33 however, indicate that excess cortisol secretion is also seen in PA, and that this may account for some
34 of the metabolic abnormalities seen in these patients [14]. Furthermore, the cut-off used to define
35 whether an adrenocortical incidentaloma is ‘functioning’ or ‘non-functioning’ is important, since
36 patients with apparently non-functioning adrenal incidentalomas, as defined by a serum cortisol
37 post dexamethasone of <1.8 ug/dL, still have excess risk of what may be reasonably considered to be
38 cortisol-dependent co-morbidities [15].

39 The estimated cost of fragility fracture in the UK was £2.3 billion in 2011, but with this rising to a
40 predicted cost of £6 billion by 2036, mostly due to the cost of hip fracture [16]. Bone loss and
41 osteoporosis are well-established complications of glucocorticoid excess, be it from endogenous
42 Cushing’s syndrome or exogenous sources [17]. Given the wide prevalence of adrenal incidentaloma
43 with low-grade cortisol-excess (1-4% of the ageing population), it is important to understand what
44 effect there may be on bone health, as this may have a very significant impact at the population level.
45 In light of this, many studies over the past two decades have sought to investigate the effect of
46 subclinical hypercortisolism on bone health in patients with adrenal incidentalomas.

1
2 47 The aim of this article is to outline the known effects of cortisol and aldosterone on bone and
3
4 48 summarize the main studies that have assessed bone health in patients with adrenal incidentalomas.
5

6 49 7 50 **Methods**

8 51 A literature search was conducted in PubMed in English language, in order to identify publications on
9
10 52 adrenal incidentalomas and bone **until the end of June 2018**. We collected, analyzed and qualitatively
11
12 53 resynthesized data regarding the effects cortisol and aldosterone on bone metabolism, as well as
13
14 54 studies that have assessed bone health in patients with adrenal incidentalomas. We present in turn
15
16 55 updated information regarding the mechanisms of action of cortisol and aldosterone on bone and
17
18 56 clinical evidence from patients with adrenal incidentalomas with autonomous cortisol hypersecretion
19
20 57 or hyperaldosteronism or both. We also discuss clinical implications and provide recommendations on
21
22 58 appropriate management.
23
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28

29 60 **Cortisol hypersecretion and bone.**

30 61 *Effects of cortisol on bone metabolism: mechanisms of action.*

31 62 Glucocorticoids are important for bone development by affecting osteoblast differentiation [18,19], but
32
33 63 excessive quantities seem to have a negative impact on bone health [20] and this impact will be
34
35 64 analyzed here. In patients with adrenal incidentalomas with increased secretion of glucocorticoid to
36
37 65 levels insufficient to cause classic Cushing's syndrome, the 'sub-clinical' levels may still be sufficient
38
39 66 to **increase the risk of vertebral fractures due to a decrease of bone mineral density (BMD) and bone**
40
41
42 67 **quality [20,21].**

43
44 68 **Evidence showing the effect of glucocorticoid on bone deriving primarily from *in vitro* and *in vivo***
45
46 69 **models of mouse treated with glucocorticoid. Osteoporosis induced by glucocorticoid excess is due**
47
48 70 **mainly to a direct effect on cells involved in bone remodelling (osteoblast, osteocytes, osteoclast and**
49
50 71 **their precursors) [20], which express the glucocorticoid receptors (GRs) that mediated the main action**
51
52 72 **of cortisol [22]. The principal effect of the cortisol excess is a reduction of bone formation through a**
53
54 73 **suppression of osteoblast activity mediated by an upregulation of peroxisome proliferator-activated**
55
56 74 **receptor (PPAR)- γ [23] and an inhibition of the wingless (wnt)/ β -catenin signaling pathway (Fig. 1)**
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75 [24-26]. These mechanisms favor the differentiation of mesenchymal progenitors to adipocytes instead
76 of osteoblasts, resulting in a decreased number of osteoblasts and in an increasing of osteoblast
77 apoptosis [27,28]. Cortisol excess stimulates the expression in osteocytes of sclerostin which seems to
78 be a key role in the inhibition of the wnt pathway in osteoblast (Fig.1) [29,30]. In mouse models of
79 glucocorticoid-induced osteoporosis it has been showed that the treatment with anti-sclerostin
80 antibody prevented the reduction of bone mass and strength in comparison to placebo [30]. Moreover,
81 the treatment with these antibodies prevented osteocytes from apoptosis in rodents [31]. The
82 suppression of osteoblasts differentiation associated with an increased osteoblasts and osteocytes
83 apoptosis causes a reduction of bone formation (Fig.1).

84 Cortisol excess favors also bone resorption through an alteration of the receptor activator for NF- κ B
85 ligand (RANKL)/osteoprotegerin (OPG) ratio produced by osteoblasts and osteocytes (Fig.1) [32-34].
86 RANKL is a regulator of recruitment, activation and survival of osteoclasts, whereas, OPG acts as a
87 decoy receptor for RANKL preventing its interaction with RANK and causing the inhibition of
88 osteoclastogenesis [35]. An *in vivo* mouse model demonstrated that glucocorticoids treatment
89 decreased secretion of OPG rather than elevating RANKL expression in osteocyte cells [34]. The
90 modified RANKL/OPG ratio by cortisol increases the RANKL activity and promotes the bone
91 resorption (Fig.1) [32-34]. Moreover, glucocorticoids stimulate the production of the macrophage
92 colony-stimulating factor (M-CSF) that stimulates osteoclastogenesis together with RANKL [36].
93 However, this effect of bone resorption is only transient and usually decreases over time due to a
94 suppression of osteoblasts and osteocytes activity [37]. Therefore, the decrease in bone formation
95 rather than increase in bone resorption plays a key role in osteoporosis induced by cortisol excess
96 [28,19].

97 It should be noted that the severity of the skeletal effect of hypercortisolism could due to individual
98 sensitivity to cortisol that may modify the overall phenotype observed. Some of the variability in
99 sensitivity in different tissues in the same individual may be mediated by the repertoire of co-
100 activators and co-repressors that are present in a given tissue. Moreover, evidence suggests that at least
101 some of the variable sensitivity to glucocorticoids in bone is conferred by polymorphisms of the GR
102 [38]. Moreover, local regeneration of cortisol by 11-beta hydroxysteroid dehydrogenase type 1

103 (11 β HSD1) may contribute further to these effects [39].

104 **Hypercortisolism influences mineral and bone metabolism also through indirect effects mediated by**
105 **calcium (Ca⁺⁺) and parathyroid hormone (PTH) [20]. Cortisol reduces intestinal Ca⁺⁺ absorption and**
106 **increases renal Ca⁺⁺ excretion, with a final Ca⁺⁺ negative balance that may deteriorate bone**
107 **mineralization. Opposing data are reported regarding PTH levels, a marker of bone resorption, in**
108 **patients with adrenal incidentaloma and. Two studies by the group of Chiodini showed that in**
109 **female patients with adrenal incidentaloma autonomous cortisol hypersecretion and**
110 **autonomous cortisol hypersecretion had higher PTH levels in comparison to patients with**
111 **inactive adrenal masses [40,41]. Higher levels of PTH in these patients were not shown by**
112 **studies from other groups [42,43]. However, regardless of an increase plasma levels, PTH**
113 **correlated inversely with femoral BMD [40,42,43]. More consistently observed are lower**
114 **blood osteocalcin levels, a marker of bone formation, in patients with autonomous cortisol**
115 **hypersecretion in comparison to patients with inactive adrenal incidentaloma or healthy**
116 **controls [41,40,42,44]. The decrease of osteocalcin levels is due to the inhibition of**
117 **osteoblastic activity and increase of osteoblastic apoptosis caused by cortisol excess [20].**
118 **However, this finding was not confirmed by other studies [43,45]. It is important to note that**
119 **the discordance observed between studies on PTH and osteocalcin levels is likely due to the**
120 **small sample size of the studies and the different criteria used for the definition of**
121 **autonomous cortisol hypersecretion [21].**

122 Taken together, it is clear that the overall level of cortisol secretion needed to have deleterious effects
123 differs by tissue and by individual, but that over time even subtle increases of endogenous cortisol
124 secretion has a net effect favoring bone loss.

125
126 *Clinical evidence from patients with adrenal incidentaloma and autonomous cortisol*
127 *hypersecretion.*

128 Evidence for the effect of cortisol hypersecretion on bone health also comes from clinical studies too.
129 Although glucocorticoids impair bone turnover with inhibition of osteoblastic activity [42,46,40], in

130 patients with adrenal incidentalomas initial BMD studies did not find significant differences between
131 those deemed to have autonomous cortisol secretion and controls [40,47,48]. Two studies assessing
132 more homogeneous populations with adrenal incidentalomas, one including eugonadal males [45] and
133 one including post-menopausal women [43], demonstrated significantly decreased BMD in patients
134 with autonomous cortisol hypersecretion compared to those without. This decrease was however,
135 mainly within the limits of osteopenia and not sufficient to be classed as osteoporosis [43].

136 Several observational studies from one Italian center provide data that autonomous cortisol
137 hypersecretion in patients with ACA is associated not only with accelerated bone loss but also with
138 increased incidence of vertebral fractures [49-51]. Chiodini et al. included only women (70 patients
139 and 84 controls) to avoid gender-related effects on bone and divided participants according to
140 premenopausal and postmenopausal status. Subclinical hypercortisolism was associated with higher
141 prevalence of fractures and reduced volumetric bone mass at the lumbar spine, independent of gonadal
142 status. BMD, however, was mainly affected by menopausal status [49]. Another retrospective study
143 including 287 patients with adrenal incidentalomas and 194 controls, showed that BMD was
144 significantly lower in lumbar spine and femoral neck in patients with autonomous cortisol
145 hypersecretion than nonfunctioning adenoma and controls. Fracture prevalence and spinal deformity
146 index were also significantly higher in those with subclinical hypercortisolism regardless of age,
147 gender, menopausal status and BMD [50]. In a prospective study by the same group, 103 consecutive
148 patients with adrenal incidentalomas were followed-up in order to evaluate the fracture risk over time.
149 It was shown that the group of patients with autonomous cortisol hypersecretion had a higher rate of
150 vertebral fractures (82%) compared to baseline (56%), regardless of age, gender, body mass index
151 (BMI), BMD and menopause, and this incidence was higher than that seen in patients with
152 nonfunctioning adrenal incidentalomas [51]. It is likely that the fractures reported in these studies are
153 being disclosed by very sensitive methodologies, since in routine clinical practice such a high rate of
154 clinically significant fractures is not usually seen.

155 Interestingly, fracture risk was not directly predicted by BMD, as 40% of fractures occurred in patients
156 with normal or only slightly reduced BMD [50,51]. Therefore, it is possible that both bone mass and
157 bone quality may be disordered. In further study from the same group, bone microarchitecture was

158 assessed by measurement of the trabecular bone score (TBS) in patients with adrenal incidentalomas
159 and concluded that bone quality in autonomous cortisol hypersecretion is altered [52]. Furthermore, it
160 was shown that a combination of low TBS and low BMD was highly predictive for fractures, whilst
161 the converse was true for those with a normal TBS plus high BMD, in whom a lower rate of fractures
162 was observed [52]. A very recent study provided evidence that patients with mild autonomous cortisol
163 secretion presented significantly decreased trabecular bone score (TBS), but not BMD when compared
164 with patients with non-secreting incidentalomas [53]. TBS may be proved as a promising, non-
165 invasive, inexpensive tool for the routine assessment of these patients in clinical practice.
166 A meta-analysis including six relevant studies has shown that patients with bilateral ACA had a higher
167 prevalence of autonomous cortisol hypersecretion compared to patients with unilateral incidentalomas
168 of the same size as the largest of the bilateral adenomas [54]. Only one study from this analysis
169 investigated bone parameters in patients with unilateral vs. bilateral adrenal incidentalomas and
170 reported a higher prevalence of fractures in those patients with bilateral adenomas. Interestingly, this
171 higher prevalence remained significant even after adjusting for subclinical hypercortisolism, BMI, age
172 and lumbar spine BMD [55].

173 When managing patients with adrenal incidentaloma in clinical practice, it would be very useful to
174 know which biochemical parameter of cortisol hypersecretion is the most reliable for predicting
175 increased fracture risk. However, this is difficult as the diagnosis of autonomous cortisol secretion
176 itself is still a matter of debate [56]. It is worth noting that the Italian group with the most studies on
177 the topic is based on the presence of two out of the following three alterations for the diagnosis of
178 subclinical hypercortisolism: 1) increased urinary free cortisol (UFC) levels (>193.1 nmol/24 h) 2)
179 unsuppressed serum cortisol levels after 1-mg overnight dexamethasone (Dex) suppression test (serum
180 cortisol after Dex > 82.8 nmol/liter), and 3) low ACTH levels (<2.2 pmol/liter) [45,43,49-52].

181 A recent study from Italy found that serum cortisol levels after 1 mg dexamethasone-suppression test
182 greater than 2.0 mg/dL (55 nmol/L) are independently associated with both prevalent and incident of
183 vertebral fracture as well as with an increased risk of new vertebral fractures at diagnosis and during
184 follow-up [57]. This association between the degree of biochemical cortisol hypersecretion and the
185 risk for vertebral fracture was expected and is in accordance with previous studies, most of which

186 **come from** a single Italian group [45,43,49-52]. Interestingly, this association was independent of
187 BMD and supports the notion that reduced bone quality is the most significant parameter leading to
188 skeletal fractures as a consequence of cortisol excess [52]. 24-h urinary free cortisol and plasma
189 adrenocorticotrophic hormone (ACTH) levels were shown to be not statistically associated with fracture
190 risk. A potential explanation for plasma ACTH not being a useful marker is the differing sensitivity of
191 various tissues to glucocorticoids: bone tissue may be affected even before suppression of
192 hypothalamic-pituitary-adrenal axis is evident [57].
193 Surgical treatment of ACA in small groups of patients with autonomous cortisol hypersecretion has
194 been associated with improvement of various parameters, including weight, blood pressure, glucose
195 and lipid metabolism [58,59]. However, here the data are still too limited, and some studies report no
196 benefit. The European guidelines on the management of adrenal incidentaloma recommend
197 adrenalectomy only in the minority of cases, and based on careful individualised treatment decisions.
198 Recent data showed a 30% reduction of vertebral fracture risk after adrenalectomy in selected patients
199 [60]; this finding is potentially important and underlines the pathophysiological association between
200 cortisol hypersecretion, reduced bone quality and fractures in patients with ACA. However, it is
201 important to note that the majority of studies on bone in patients with adrenal incidentaloma come
202 from one group [49,50,58,45,57,51,55] **and** before making wide-ranging treatment recommendations it
203 is crucial **to have larger studies in various populations.**

204 205 **Primary aldosteronism and bone.**

206 *Effects of aldosterone on bone metabolism: mechanisms of action.*

207 Contrary to the well-studied mechanisms, which underline the link between autonomous cortisol
208 secretion and bone, less is known regarding the link between hyperaldosteronism and osteoporosis.

209 Over the last two decades, several small studies have demonstrated that aldosterone excess is likely to
210 affect bone turnover through a direct effect on bone cells and through indirect mechanisms via **PTH**
211 and oxidative stress [61-66] (Fig. 2).

212 The direct effect of aldosterone on bone metabolism is still poorly understood. Mineralocorticoid
213 receptors (MRs) are expressed in human and rat osteoclasts, osteocytes and osteoblasts [67,68],

214 suggesting a direct effect of aldosterone on bone turnover. MRs are present also in normal and
1
2 215 adenomatous parathyroid tissue [69,70]. Furthermore, a positive association between the
3
4 216 aldosterone/renin ratio and serum PTH concentration has been demonstrated in normal individuals
5
6 217 [71], suggesting that aldosterone may directly regulate PTH synthesis and secretion (Fig. 2).
7
8 218 Moreover, *in vivo* and observational human studies suggest that MR antagonists (MRA) have a
9
10 219 beneficial effect on bone metabolism. In rat models, treated with aldosterone and salt for 4-6 weeks,
11
12 220 bone loss was attenuated after administration of the MRA spironolactone [72,73,61]. Similarly,
13
14 221 patients with PA treated with spironolactone showed decreased urinary calcium loss and improved
15
16 222 BMD [74-76]. However, a recent single-center, double-blind, randomized, placebo-controlled trial
17
18 223 demonstrated no effects of eplerenone on bone turnover markers in patients with primary
19
20 224 hyperparathyroidism, suggesting that MR antagonism may not be relevant in primary
21
22 225 hyperparathyroidism, but could have efficacy in condition of hyperparathyroidism secondary to
23
24 226 hyperaldosteronism [77].
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27
28 227 The interaction between MR and bone has been further examined in animal models. In rats treated
29
30 228 with aldosterone and salt, there was a significant increase in urinary and fecal excretion of Ca^{++} and
31
32 229 magnesium (Mg^{++}), with a consequent progressive reduction of plasma ionized Ca^{++} and Mg^{++} levels
33
34 230 [61]. Urinary losses of Ca^{++} and Mg^{++} were the result of expanded extravascular fluid volume resulting
35
36 231 in decreased resorption of sodium (Na^+), Ca^{++} and Mg^{++} in the proximal tubule of the nephron with a
37
38 232 consequent increase of their excretion in the distal tubule. Because aldosterone stimulates Na^+
39
40 233 resorption, but not that of Ca^{++} and Mg^{++} at the distal tubule, this causes a marked increase of Ca^{++} and
41
42 234 Mg^{++} excretion [78,79], with the lowering of Ca^{++} and Mg^{++} leading to secondary
43
44 235 hyperparathyroidism, stimulating bone resorption and a significant reduction of BMD and cortical
45
46 236 bone strength (Fig. 2) [61,80].
47
48
49 237 In the same rat model, a significant reduction of plasma $\alpha 1$ -antiprotease activity and an increase of
50
51 238 lymphocyte hydrogen peroxide production was reported after aldosterone-sodium treatment for 1-6
52
53 239 weeks in comparison to control group [61,80,81]. The authors hypothesized that aldosterone promotes
54
55 240 a systemic condition of oxidative stress and inflammation that could result in increased osteoblast and
56
57 241 osteocyte apoptosis, and reduced bone formation (Fig. 2) [82,83].
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242 In conclusion, hyperaldosteronism affects bone turnover through several direct and indirect
1 mechanisms, most of which act through an increase of serum PTH levels.
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4 244

5
6 245 *Clinical evidence from patients with adrenal incidentaloma and hyperaldosteronism.*
7

8 246 Evidence regarding the link between hyperaldosteronism and bone metabolism is also derived from
9
10 several observational studies. Aldosterone hypersecretion can be detected in 1-10% of patients with
11 247
12 adrenal incidentalomas [1,2]. Together with bilateral adrenal hyperplasia (BAH), the aldosterone-
13 248
14 producing adenomas (APA, also termed ‘Conn adenoma’) represent more than 90% of cases of PA;
15 249
16 the remaining cases of PA are due to unilateral adrenal hyperplasia and aldosterone-producing
17 250
18 carcinoma [84]. Several observational studies showed significantly higher PTH levels, lower serum
19 251
20 Ca⁺⁺ levels and higher urinary Ca⁺⁺ excretion in patients with PA in comparison to those with essential
21 252
22 hypertension (EH) [63,64,85,74,86,87], and a higher prevalence of osteoporosis [63,64,66,65,88,74].
23 253
24

25 254 Salcuni et al. reported the first association between hyperaldosteronism and osteoporosis in patients
26
27 with APA [63]. In 11 patients with APA there was decreased BMD at the lumbar spine, total and
28 255
29 femoral neck (13%, 8% and 11%, respectively), an increased prevalence of osteoporosis (73 vs. 20%)
30 256
31 and a higher incidence of vertebral fractures (46 vs. 13%), in comparison to 15 patients with
32 257
33 nonfunctioning incidentalomas. Moreover, the increased urinary Ca⁺⁺ excretion and elevated PTH
34 258
35 levels found in APA patients were reversed after adrenalectomy or spironolactone treatment [63].
36 259
37

38 260 The reversibility of secondary hyperparathyroidism in PA patients after surgical or medical treatment
39
40 was supported by two other observational studies [85,86]. Ceccoli et al. compared PA patients (46
41 261
42 with APA and 70 with BAH) with 110 EH patients, finding significant increases in PTH levels and
43 262
44 urinary Ca⁺⁺ excretion, and decreased serum Ca⁺⁺ levels (with comparable vitamin D concentrations)
45 263
46 [85]. Interesting, PTH levels were higher in patients with APA than in those with BAH [85]. Similarly
47 264
48 Pilz et al. showed higher PTH levels in a small group of patients with PA (5 APA and 5 BAH)
49 265
50 compared to 182 with EH; moreover, they observed that the normalization of PTH levels was more
51 266
52 pronounced in patients operated for APA than those treated with MRA for BAH [86]. It is important to
53 267
54 note that in both studies the PA group had significantly higher blood pressure than the EH group, and
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56 that arterial hypertension itself can increase urinary Ca⁺⁺ excretion with consequent secondary
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270 hyperparathyroidism [89]. Nevertheless, a larger observational study demonstrated higher urinary Ca^{++}
271 excretion, lower serum Ca^{++} levels and higher PTH levels in 73 patients with PA in comparison to 73
272 patients with EH and 40 healthy controls [64], without differences in blood pressure between PA and
273 EH groups, suggesting that aldosterone itself may be involved in the stimulation of PTH secretion in
274 PA. No differences were seen in anthropometric and biochemical characteristics between patients with
275 APA and BAH [64].

276 Another observational study comparing 105 consecutive patients with hypertension, of whom 44 with
277 APA and 61 with EH, showed that in the APA group there were significantly higher plasma PTH
278 levels compared to the EH group ($P < 0.001$), despite similar urinary Ca^{++} excretion and vitamin D
279 levels [87]. Similar to previous studies, PTH levels were normalized in patients with APA after
280 adrenalectomy. Moreover, the authors demonstrated the expression of the PTH receptor, at mRNA and
281 protein levels, in APA tissues and speculated that PTH, by acting on these receptors, may contribute to
282 hyperaldosteronism despite the suppression of the angiotensin-renin system [87].

283 Very recently, Salcuni et al. observed a higher prevalence of PA in a group of 322 consecutive
284 subjects screened for osteoporosis who were not taking drugs affecting bone and mineral metabolism
285 and who had no prior diagnosis of secondary osteoporosis, compared to a non-osteoporotic control
286 group (5.2% vs 0.9%, $P = 0.066$). The prevalence of PA was higher still in those who also had
287 osteoporosis and hypertension (13.9%), fracture and hypertension (14.8%), fracture and hypercalciuria
288 (11.1%), and osteoporosis, hypertension and hypercalciuria (26.1%), emphasizing the potential
289 interplay between PA and bone [88]. In this study, osteoporosis was associated with PA (OR=10.42;
290 95% CI 1.21-90.91), as well as age (OR=1.06; 95% CI 1.03-1.09) and BMI (OR=1.11; 95% CI 1.05-
291 1.17), but not with EH (OR=1.23; 95% CI 0.72-2.1) [88].

292 Another recent study including 56 PA patients, 16 of whom had APA, and 56 matched healthy
293 controls identified PA as a risk factor for vertebral fractures independently of blood pressure, glycated
294 hemoglobin and lipid levels [65]. There were no differences in the vertebral fracture rate in patients
295 with APA in comparison to those with BAH, despite higher aldosterone plasma levels in patients with
296 APA. Contrary to previous observational studies [63,64,88], there were no significant differences in
297 PTH levels and BMD in PA patients compared to controls [65]. This discrepancy could be due to the

298 design of the study, which focused on vertebral and not cortical bone [65]. A large population-based
299 study suggested that PA was associated with higher risk of bone fracture; however, a reduced risk of
300 fracture in women with both APA or BAH after MRA treatment was not observed, a result which
301 might reflect the duration of disease [66].

302 However, similar to what is observed in autonomous cortisol secretion, the majority of data regarding
303 PA and bone metabolism came from observational studies of a small cohort of patients evaluated in a
304 single center. Multicenter observational studies and randomized interventional studies, which
305 investigate the efficacy of MRA or adrenalectomy for the prevention of osteoporosis, are urgently
306 needed.

307 Very recent data suggest the potential role of co-secretion of mild glucocorticoid excess in the
308 development of comorbidities in patients with PA. Using mass spectrometry-based analysis of the 24-h
309 urinary steroid metabolic profiling a concomitant presence of mild glucocorticoid metabolite excess
310 was demonstrated in a large proportion of patients with PA (provocatively termed by the authors as
311 ‘Connshing’s’ syndrome) [14]. Interesting, in the group of patients with co-secretion of aldosterone
312 and cortisol, metabolic parameters such as increased BMI, insulin resistance, diastolic blood pressure,
313 waist circumference and high-density lipoprotein were associated with cortisol levels and not with
314 aldosterone levels [14]. Arlt et al. suggested that the co-secretion of cortisol in patients with PA may
315 contribute in the pathogenesis of co-morbidities observed in these patients, including osteoporosis
316 [90,14]. However, prospective randomized studies are needed to confirm this result, and to assess
317 whether those patients with PA identified as having the glucocorticoid-rich metabolic profile but who
318 do not undergo surgery, need glucocorticoid antagonist in addition to MRA to counteract the adverse
319 metabolic risk [14].

320

321 **Conclusion and implications for management.**

322 Adrenocortical incidentalomas constitute a common clinical problem with a prevalence of up to 10%
323 in elderly [1-4], mostly being represented by benign ACA and often being associated with
324 corticosteroid excess [1,2,7-9].

325 Several observational studies have shown that ACA with autonomous cortisol hypersecretion is more
1 prevalent than expected in patients with osteopenia and osteoporosis and that the autonomous cortisol
2 326 hypersecretion is associated with accelerated bone loss and increased incidence of vertebral fractures
3
4 327 [49-51]. Similarly, hyperaldosteronism is associated with a higher prevalence of osteoporosis
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6 328 [63,64,66,65,88,74]. Contrary to what is known about glucocorticoid action, the effects of aldosterone
7
8 329 on bone metabolism are less well understood and seem mostly due to an indirect effect through the
9
10 330 increase of urinary Ca^{++} excretion, leading to compensatory secondary hyperparathyroidism
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12 331 [85,64,63,61]. However, recent data using urinary steroid metabolic profiling, has shown a mild
13
14 332 cortisol co-secretion in a subgroup of patients with APA and that it may account for some of the
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16 333 metabolic abnormalities seen in these patients, including osteoporosis [14,90].
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23 335 The recent European Society of Endocrinology (ESE) / European Network for the Study of Adrenal
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25 336 Tumors (ENSAT) guidelines suggest screening of patients with ACA and autonomous cortisol
26
27 337 secretion for vertebral fractures at least once at the time of diagnosis (by re-evaluation of CT images or
28
29 338 by X-ray), while no consensus was reached by the experts concerning the assessment of BMD with
30
31 339 dual-energy x-ray absorptiometry (DXA) [1]. The data summarized above suggest that BMD may not
32
33 340 be accurate for fracture risk assessment in patients with ACA and autonomous cortisol secretion, and
34
35 341 that TBS may be more useful, or at the very least used in combination.
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38 342 In everyday clinical practice, patients with unexplained osteoporosis, particularly when associated
39
40 343 with other metabolic symptoms (impaired glucose tolerance, hypertension or hypercalciuria), should
41
42 344 be investigated for the possible presence of adrenal incidentaloma associated with autonomous cortisol
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44 345 secretion or aldosterone hypersecretion. Thus, patients with ACA and osteopenia, osteoporosis or
45
46 346 vertebral fractures might benefit from therapeutic adrenalectomy or when it is not possible from
47
48 347 specific medical treatment, such as glucocorticoid antagonist therapy or MRA, to mitigate against the
49
50 348 comorbidities due to hormone excess [1,12,14]. Furthermore, it is possible that patients with PA, who
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52 349 do not undergo surgery, might need also glucocorticoid antagonist in addition to MRA if they have a
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54 350 glucocorticoid metabolite profile [14]. All these suggestions are derived from observational studies;
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56 351 more data, especially from prospective, randomized, controlled intervention trials, are needed to
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352 investigate further the optimum surgical or medical interventions to ameliorate osteoporosis and other
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2 353 co-morbidities due to ACA associated with autonomous cortisol secretion or hyperaldosteronism.
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6 355 **Compliance with Ethical Standards**

8 356 **Conflict of interest**

10
11 357 The authors declare that they have no conflict of interest.
12

13 358 **Ethical approval**

14
15 359 This article does not contain any studies with human participants or animals performed by any of the
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17 360 authors.
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676 **Figure legends**

677 **Fig. 1 Direct effects of cortisol excess on bone metabolism**

678 Endogenous glucocorticoid excess negatively affect osteoblast, osteocytes and osteoclast, which
679 expressed glucocorticoid receptors (GRs). These action include an upregulation of peroxisome
680 proliferator-activated receptor (PPAR)- γ [23] and an inhibition of the wingless (wnt)/ β -catenin
681 signaling pathway [24-26], leading to mesenchymal progenitor cells differentiating preferentially into
682 adipocyte that results in a decreased number of osteoblasts and in an increasing of osteoblast apoptosis
683 and a consequent reduction of bone formation [28]. This mechanism is also stimulated by sclerostin
684 produced by osteocytes [30]. Another key mechanism is the increase of the receptor activator for NF-
685 κ B ligand (RANKL)/osteoprotegerin (OPG) ratio produced by osteoblasts and osteocytes [32-34] that,
686 together with the increased macrophage colony-stimulating factor (M-CSF) [36], stimulates
687 osteoclastogenesis and bone resorption.

689 **Fig. 2 Mechanisms of action of aldosterone on bone metabolism**

690 Aldosterone excess could affect bone turnover directly by binding mineralcorticoid receptors (MRs)
691 expressed in osteoclasts, osteocytes and osteoblasts [67]. Furthermore, aldosterone regulate PTH
692 synthesis and secretion through the MRs expressed in cells of parathyroid glands [69,70]. Indirectly,
693 aldosterone excess regulates bone metabolism through parathyroid hormone (PTH) and oxidative
694 stress. Hyperaldosteronism expands the extravascular fluid volume that causes a marked increase of
695 urinary excretion of calcium (Ca^{++}) and magnesium (Mg^{++}) in the distal tubule of the nephron, with a
696 progressive reduction of serum Ca^{++} and Mg^{++} levels. The resulting hypocalcemia and
697 hypomagnesemia stimulate the secretion of PTH, with a consequent secondary hyperparathyroidism,
698 which induces bone resorption and a reduction of the bone mineral density (BMD) [61]. Moreover,
699 aldosterone excess reduces plasma α 1-antiprotease activity and increases lymphocyte hydrogen
700 peroxide production, promoting a condition of oxidative stress resulting in increased osteoblast and
701 osteocyte apoptosis, and reduction of bone formation [61,81].



