A 33-year-old woman presented with amenorrhea and weight gain of 27.2–31.8 kg, despite diet and exercise, as well as progressively worsening acne. Symptoms began subsequent to a spontaneous abortion 5 years earlier and had become especially concerning during the past year. There was no notable family medical history. The patient did not report taking any prescription or over-the-counter medication and denied tobacco, alcohol, or illicit drug use. She had a blood pressure of 144/86 mmHg, heart rate of 88 beats/min, temperature of 37.1 °C, and was 1.8 m tall and weighed 105.7 kg (body mass index, 33.4). The results of a physical examination were otherwise normal.

Initial laboratory evaluation results included a negative point-of-care urine human chorionic gonadotropin test and concentrations within reference intervals for thyroid-stimulating hormone (TSH), prolactin, leutinizing hormone (LH), and follicle-stimulating hormone (FSH) (Table 1). Further testing revealed a low estradiol concentration (20 pg/mL, reference interval, 24 –706 pg/mL), as well as increased total testosterone (96 ng/dL (DPC, Siemens, Malvern PA), reference interval, 10 – 80 ng/dL) and dehydroepiandrosterone-S (DHEA-S) (594 /H9262 9262 /H11021 340 /H9262 g/dL, reference interval, 340 /H9262 g/dL) (Table 1). She was diagnosed with polycystic ovary syndrome (PCOS) and treated with metformin, which she did not tolerate, and local creams for her acne were ineffective.

Approximately 1 year later, the patient presented with continuing amenorrhea and complained of mood swings and depression as well as easy bruising and hirsutism. She was referred to an endocrinologist for further evaluation. Laboratory testing at this time included a basic metabolic panel. All measurements were within reference intervals, as were concentrations of TSH, LH, and FSH (Table 1). A random 17-hydroxyprogesterone (17-OHP) was within reference intervals, and estradiol was at the lower limit of the reference interval (28 pg/mL). Prothrombin time and partial thromboplastin time were both within reference intervals. Total testosterone (132 ng/dL) and DHEA-S (812 /H9262 mg/dL) remained increased.

**DISCUSSION**

**OVERVIEW OF SECONDARY AMENORRHEA**

Amenorrhea is classified as either primary (failure to achieve menarche) or secondary, which is the cessation of menses for 3 months or more. Secondary amenorrhea is not, in itself, a cause for concern; however, it can be a symptom of other pathological states. Secondary amenorrhea will affect approximately 5% of women of reproductive age and those who are affected often will not demonstrate an obvious etiology for their symptoms. Therefore, a systematic evaluation is required to establish a definitive diagnosis (1).

The most common underlying cause of secondary amenorrhea is pregnancy. Once pregnancy is ruled out, TSH and prolactin can be measured to investigate other causes such as hypothyroidism and hyperprolactinemia. In the absence of hypothyroidism and hyperprolactinemia, secondary amenorrhea is likely due to either outflow tract obstruction or hypogonadism. The low estrogen concentrations observed in this case narrowed the differential to hypoestrogenism. Hypo-
gonadism is further classified as either a normo-, hyper-, or hypogonadotropic state, which is differentiated on the basis of laboratory measurement of LH and FSH. In this patient, both LH and FSH were within reference intervals. Normogonadotropic hypogonadism is most frequently associated with hyperandrogenism, as demonstrated by increased testosterone concentrations in this case. Secondary amenorrhea in the background of hyperandrogenic hypogonadism can be due to PCOS, nonclassical (late-onset) congenital adrenal hyperplasia (CAH), or Cushing syndrome.

PCOS is by far the most common of these 3 causes, affecting approximately 6% of women of reproductive age. The presentation may be characterized by amenorrhea, infertility, hirsutism, and metabolic disturbance often manifesting as type 2 diabetes mellitus, insulin resistance, or metabolic syndrome accompanied by obesity (2). However, before a diagnosis of PCOS is made, both CAH and Cushing syndrome should be ruled out since they will manifest similarly (3).

Nonclassical CAH results most commonly from a deficiency of 21-hydroxylase that leads to accumulation of 17-OHP, which is then shunted into androgen synthesis (4). The condition is characterized by masculinization of adolescent and adult female patients, distinguishing it from classical CAH, which presents in infancy and early childhood (4). In cases in which PCOS is suspected, a random 17-OHP measurement is usually sufficient to rule out CAH before a definitive diagnosis of PCOS is made (3–4).

Similarly to PCOS and CAH, Cushing syndrome can be an underlying cause of secondary amenorrhea and will present as hyperandrogenic hypogonadism. Cushing syndrome is characterized by a prolonged increase of cortisol concentrations. The most common causes of Cushing syndrome are iatrogenic, specifically the use of glucocorticoids as antiinflammatory or immunosuppressive therapies. However, Cushing syndrome can also be endogenous due to cortisol overproduction. In this case the underlying pathophysiology is further subdivided into adrenocorticotropic hormone (ACTH) dependent or ACTH independent. Cushing disease (Cushing syndrome caused by pituitary ACTH overproduction) is the cause of 80% of endogenous ACTH-dependent Cushing syndrome cases typically due to an ACTH-producing pituitary adenoma (5). The remaining 20% of ACTH-dependent cases are due to extrapituitary (ectopic) tumors (5). ACTH-independent Cushing syndrome is caused by adrenocortical hyperplasia or tumors (5).

Although all cases of Cushing syndrome result from prolonged exposure to cortisol, the clinical manifestation varies widely (6). The most common presenting symptoms include truncal obesity and skin changes, including acne, purple striae, and thinning of the skin, which results in a propensity to bruising (7). Other symptoms include menstrual irregularities, hirsutism, impaired glucose metabolism and diabetes, hypertension, proximal muscle weakness and atrophy, fatigue, and neuropsychological symptoms such as depression and mood instability (5). Many patients will have only isolated symptoms, making the clinical picture unclear. Furthermore, these symptoms are very common in the general population and can be caused by many etiologies, most of which are much more common than Cushing syndrome. The consequence is that patients with Cushing syndrome are frequently misdiagnosed and treated for other conditions, often for many years, before the correct diagnosis is achieved (6).

The most common screening tests for suspected Cushing syndrome are the low-dose dexamethasone suppression test and a 24-h urinary free cortisol (7). Late-night salivary cortisol measurements are also used. Cortisol production is usually suppressed at night, but not in Cushing syndrome, and salivary cortisol concentrations reflect the free plasma concentration (7). However, although saliva is easy to collect, it is not a routine matrix in most clinical laboratories and confounding factors (such as sex and age) have not been well characterized. Guidelines for the diagnosis of Cushing syndrome published by the Endocrine Society recommend using any of these 3 tests to screen patients (7). If the initial result is positive, another of the 3 screening tests should be used as confirmation.

### Table 1. Laboratory values on the initial and return visits.

<table>
<thead>
<tr>
<th></th>
<th>TSH, mIU/mL</th>
<th>Prolactin, ng/mL</th>
<th>LH, mIU/mL</th>
<th>FSH, mIU/mL</th>
<th>Estradiol, pg/mL</th>
<th>Testosterone, ng/dL</th>
<th>DHEA-S, µg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference Interval</td>
<td>0.34–5.60</td>
<td>1–24</td>
<td>1–105</td>
<td>4–22</td>
<td>24–706</td>
<td>10–80</td>
<td>&lt;340</td>
</tr>
<tr>
<td>Initial Visit</td>
<td>4.23</td>
<td>15</td>
<td>7</td>
<td>7</td>
<td>20**</td>
<td>96*</td>
<td>594*</td>
</tr>
<tr>
<td>Return Visit</td>
<td>3.91</td>
<td>25*</td>
<td>6</td>
<td>7</td>
<td>28</td>
<td>132*</td>
<td>812*</td>
</tr>
</tbody>
</table>

* Indicates values that exceed the reference interval.
In this case, a 24-h urine free cortisol was increased (157.1 μg/dL, reference interval, ≤45 μg/dL). A low-dose (1-mg) dexamethasone suppression test was performed. Baseline cortisol was 25 μg/dL and decreased to 8 μg/dL after dexamethasone administration; however, the recommended cutoff for suppression was <5 μg/dL. Therefore, the result was considered positive and established a diagnosis of Cushing syndrome.

Subsequent to the initial diagnosis, Cushing syndrome must be further subdivided into ACTH-dependent or ACTH-independent types. Lastly, to differentiate between a pituitary and extrapituitary source of ACTH, the corticotropin-releasing hormone (CRH) stimulation test is used (8). The gold standard CRH test is performed using bilateral inferior petrosal sinus sampling (9). Exaggerated increases in ACTH concentrations after CRH administration are indicative of Cushing disease.

RESOLUTION OF THE CASE
An increased ACTH (92 pg/mL, reference interval, 5–27 pg/mL) confirmed that the Cushing syndrome was ACTH dependent. Furthermore, a subsequent CRH-stimulation test revealed exaggerated increases in both cortisol and ACTH in peripheral blood (Fig. 1), confirming a diagnosis of Cushing disease. In Cushing disease, at least a 50% rise in ACTH and a 20% rise in cortisol 30 min after CRH administration compared to baseline have been described as criteria providing 91% sensitivity and 95% specificity for pituitary Cushing (10). In adrenal Cushing, the low ACTH and high cortisol concentrations at baseline are not affected by CRH injection. In ectopic Cushing, the high ACTH and high cortisol concentrations at baseline are usually not altered by the CRH administration. An MRI was performed and revealed a pituitary tumor measuring 0.4 cm, which was removed by transsphenoidal adenectomy. Subsequently, both ACTH and cortisol concentrations returned to reference intervals. The patient’s Cushing disease is currently in remission and her amenorrhea is resolving, with the additional symptoms of hyperandrogenic hypogonadism improving over time.

SUMMARY
Secondary amenorrhea is a symptom that can be indicative of a more serious underlying condition. Therefore, it is critical to correctly diagnose the cause of secondary amenorrhea so that the patient can be effectively treated. Appropriate treatment of this patient for Cushing disease was delayed due to misdiagnosis, which is frequent in patients with this fairly rare condition. It is imperative that clinicians be aware of conditions that present similarly and the relevant tests to correctly differentiate between those conditions (1, 3, 7). Definitive diagnosis relies on proper laboratory evaluation, and this case illustrates that a defined, sequential approach to laboratory testing should be used to arrive at the correct diagnosis quickly and efficiently.

Fig. 1. Results of the CRH stimulation test.
In this patient, ACTH concentrations increased 2-fold and cortisol concentrations increased by 50% subsequent to CRH stimulation. These results were consistent with Cushing disease. Open circles, cortisol; filled squares, ACTH. Dotted lines, reference interval for cortisol; dashed lines, reference interval for ACTH.

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POUNDS TO REMEMBER

- The most common causes of secondary amenorrhea include pregnancy, hypothyroidism, and hyperprolactinemia.
- Laboratory evaluation of secondary amenorrhea should be done in a step-wise manner to correctly elucidate the underlying cause and should include human chorionic gonadotropin, TSH, prolactin, FSH, and LH.
- Both CAH and Cushing syndrome must be ruled out as the underlying cause of secondary amenorrhea in a background of hyperandrogenic hypogonadism before PCOS can be definitively diagnosed.
- Low-dose dexamethasone suppression dynamic testing, 24-h urinary free cortisol, and late-night salivary cortisol concentration are the screening tests for Cushing syndrome recommended by the Endocrine Society (7).
- Further dynamic testing of individuals with increased ACTH is useful to differentiate Cushing disease from ectopic ACTH production.

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Commentary

Lynn D. Loriaux*

This woman presented with weight gain, hirsutism, and secondary amenorrhea. Common causes of secondary amenorrhea (pregnancy, menopause, prolactinoma, over exercise, and significant weight loss) were excluded on the first visit. She was diagnosed with PCOS, a sign, not a disease. She was actually treated for metabolic syndrome, weight-related insulin resistance, but there was no evidence for that. One year later she also had depression and easy bruising, which can be caused by disordered clotting, vasculitis, and capillary fragility. The clotting cascade was within reference intervals, and the purpura was presumably nonpalpable, excluding vasculitis. Capillary fragility secondary to glucocorticoid excess should be suspected. She had 1 of 2 sensitive signs of Cushing syndrome, weight gain (90% prevalence) and acanthosis nigricans secondary to insulin resistance (88%). Specific findings are hypokalemia (96%), osteopenia (97%), and proximal muscle weakness (93%). Hypokalemia was presumably absent, but the other 2 were not mentioned. The case for Cushing syndrome rests on easy bruising and weight gain. The indicated confirmatory test is 24-h urinary free cortisol, which was 3 times the reference interval for this woman. Dexamethasone suppression was performed, but in this scenario this test has a positive pre-