Residual Adrenal Function in Autoimmune Addison's Disease: Improvement After Tetracosactide ($ACTH_{1–24}$) Treatment

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Context: Despite lifelong steroid hormone replacement, there is excess morbidity and mortality associated with autoimmune Addison's disease. In health, adrenocortical cells undergo continuous self-renewal from a population of subcapsular progenitor cells, under the influence of ACTH, suggesting a therapeutic possibility.

Objective: We aimed to determine whether tetracosactide (synthetic $ACTH_{1–24}$) could revive adrenal steroidogenic function in autoimmune Addison's disease.

Design, Setting, and Patients: Thirteen patients (aged 16–65 y) with established autoimmune Addison's disease for more than 1 year were recruited at the Newcastle University Clinical Research Facility.

Intervention: The intervention included a 20-week study of regular sc tetracosactide ($ACTH_{1–24}$) therapy.

Main Outcome Measures: Serum and urine corticosteroids were measured during medication withdrawal at baseline and every 5 weeks during the study.

Results: Serum cortisol levels remained less than 100 nmol/L in 11 of 13 participants throughout the study. However, two women achieved peak serum cortisol concentrations greater than 400 nmol/L after 10 and 29 weeks of tetracosactide therapy, respectively, allowing withdrawal of corticosteroid replacement. Concurrently, urine glucocorticoid and mineralocorticoid metabolite excretion increased from subnormal to above the median of healthy controls. One of these responders remains well with improving peak serum cortisol (672 nmol/L) 28 months after stopping all treatments. The other responder showed a gradual reduction in serum cortisol and aldosterone over time, and steroid therapy was recommenced after a 28-week period without glucocorticoid replacement.

Conclusion: This is the first study to demonstrate that established autoimmune Addison's disease is amenable to a regenerative medicine therapy approach. **(*J Clin Endocrinol Metab* 99: 111–118, 2014)**

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Abbreviation: DHEAS, dehydroepiandrosterone sulfate.

Autoimmune Addison's disease is caused by destruction of the adrenal cortex with failure of adrenal steroid production owing to an autoimmune response directed against steroid 21-hydroxylase and other adrenal steroidogenic enzymes (1, 2). A diagnosis of autoimmune Addison's disease necessitates lifelong corticosteroid therapy, most commonly hydrocortisone taken two or three times daily and fludrocortisone taken once daily. Cessation of this medication, or inappropriate dose adjustment during intercurrent illness, leads to a life-threatening adrenal crisis, coma, and ultimately death. Such adrenal crisis occurs in about 8% of primary adrenal failure patients per year and is unpredictable, (3, 4), which limits the activities of many patients with autoimmune Addison's disease. In addition, two studies have shown decreased life expectancy of individuals with Addison's disease (5, 6), and there is also an increased risk of low bone mineral density and excess of hip fractures (7, 8). Furthermore, patients with autoimmune Addison's disease suffer from worse quality of life and have lower rates of employment and higher rates of disability than sex- and age-matched healthy individuals (9). For most autoimmune Addison's patients, therefore, daily steroid replacement is adequate to control symptoms but is not a perfect solution to restore them to full health and normal life expectancy. With this in mind, our group and others have taken several different approaches to try to improve health and well-being in autoimmune Addison's disease patients over recent years (10–14).

It is well established that adrenocortical tissue has intrinsic plasticity, (15, 16) with transient adrenal atrophy and functional adrenal failure during exogenous glucocorticoid administration, owing to suppression of pituitary ACTH secretion. Similarly, there is adrenal hypertrophy and hyperfunction during the chronic ACTH stimulation of Cushing's disease. This adrenal plasticity is believed to be based on the presence of adrenocortical progenitor or stem cells, which continuously repopulate the adrenal cortex in an ACTH-responsive fashion. We hypothesized that the immune response in autoimmune Addison's disease may spare these adrenocortical progenitor cells because they are not believed to express the steroidogenic enzymes that are the target of the immune response (17, 18). Thus, adrenal plasticity could be preserved in autoimmune Addison's patients, and this has the potential to be used to improve or restore adrenal steroidogenesis in autoimmune Addison's disease. Therefore, we performed a 20-week trial of regular sc tetracosactide ($ACTH_{1–24}$) administration in patients with established autoimmune Addison's disease to try to ameliorate adrenal failure.

Subjects and Methods

Study participants

Thirteen patients between the ages of 16 and 65 years who had autoimmune Addison's disease for more than 1 year were recruited either from the endocrine clinics of the Newcastle upon Tyne National Health Service hospitals (n = 12) or self-referred after an ethics committee agreed advertisement in the national Addison's disease self-help group quarterly newsletter (n = 1). Eligibility criteria included biochemical evidence of severe primary adrenocortical failure, as judged by a basal morning serum cortisol of less than 100 nmol/L or subnormal serum cortisol response to 250 μg tetracosactide (Synacthen, Alliance Pharma Plc), with peak cortisol levels of less than 300 nmol/L, which was established at least 1 year prior to the study and persisted at baseline assessments. Patients with active infectious diseases, asthma, acute psychosis, significant cardiopulmonary illnesses, chronic renal or liver disease, or pregnancy or other conditions that would preclude tetracosactide treatment were excluded (a full list of eligibility criteria is given in the Supplemental Information, published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org). We identified 16 patients for possible inclusion in the study (see Figure 1). Three did not meet the inclusion criteria, with two not having autoimmune primary adrenal failure (pituitary disease) and one excluded due to asthma. The study was registered as NCT01371526 at clinicaltrials.gov. Ethical approval was granted by the Northeast Research Ethics Committee (reference 10/H0903/14).

Study design and treatment regimen

We conducted an open-label trial of synthetic tetracosactide ($ACTH_{1–24}$; Synacthen depot) to stimulate adrenocortical function in adults with established autoimmune Addison's disease. Participants were recruited between September 2010 and April 2012. Eleven of the participants were taking hydrocortisone as glucocorticoid replacement, whereas two patients who had not felt well during hydrocortisone therapy were taking prednisolone (Table 1). All patients took fludrocortisone. The study consisted of two sequential treatment periods, both aimed to maximally stimulate adrenal steroidogenesis, each lasting 10 weeks. During the first period, participants self-administered 1 mg Synacthen depot sc on alternate days. After the 10-week assessment, the eight participants who did not respond in the first period were randomly allocated to either a continuous 24-hour sc infusion of Synacthen (10 μg/h) or 12-hour Synacthen administration with 30-minute 10-μg boluses each hour, from midnight to midday, using sequentially opened, preordered sealed envelopes that contained randomly generated allocations. Infusion pumps (OmniPoD; Ypsomed UK) were programmed by investigators, and participants replenished the reservoir with tetracosactide every third day. For participants who responded during the first period, Synacthen depot injections were continued until the peak stimulated cortisol was greater than 400 nmol/L. Then steroid replacement therapy was judiciously titrated down, with regular monitoring of well-being, blood pressure, and serum electrolytes. Tetracosactide treatment was also weaned off over 8–10 weeks once glucocorticoid replacement was stopped with regular review of participant clinical status.

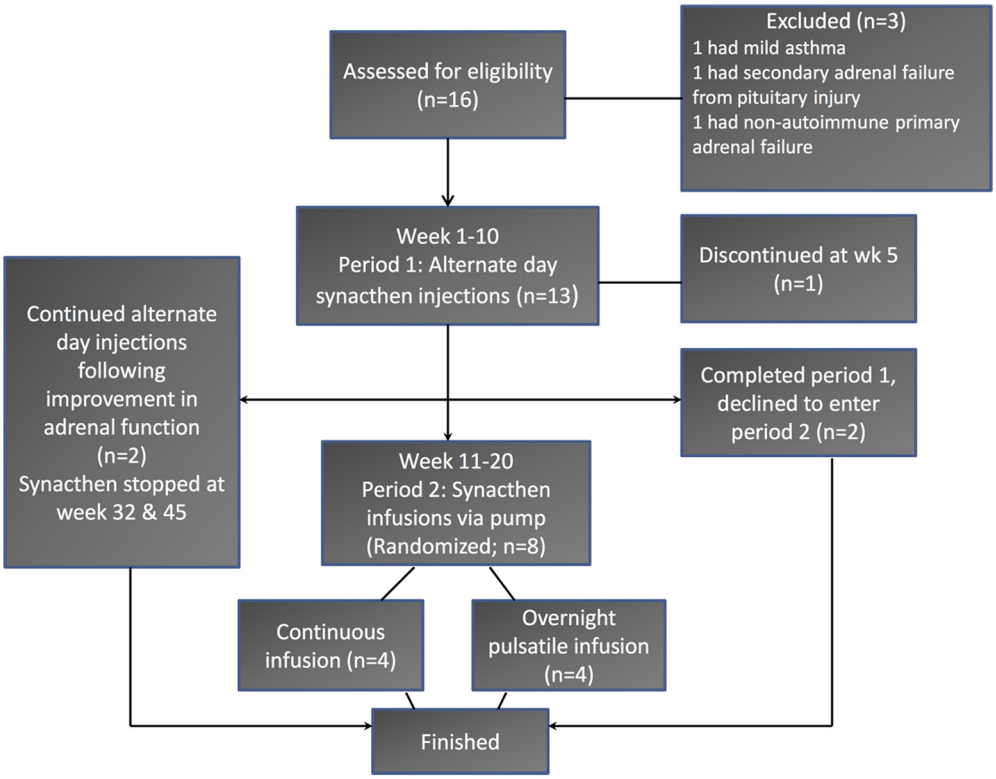

Figure 1. Participant flow chart (Consolidated Standards of Reporting Trials).

Outcome measures and assessments

After baseline testing, outcome measurements were made at weeks 5, 10, 15, and 20. All of these assessments were made after 36–42 hours of withdrawal from regular steroid replacement therapy, during which participants were admitted to the Clinical Research Facility. These measurements included baseline plasma ACTH, plasma renin activity, aldosterone, cortisol, dehydroepiandrosterone sulfate (DHEAS), androstenedione, and 21-hydroxylase antibodies. Serum cortisol was measured using a competitive chemiluminescent immunoassay on a Centaur platform (Siemens) with a limit of detection of 25 nmol/L. Serum aldosterone was measured with a solid-phase RIA (Coat-a-Count kit; Diagnostic Products Corp), with a limit of detection of 70 pmol/L. Serum androstenedione and DHEAS measurements were made by solid-phase competitive chemiluminescent immunoassay (Immulite; Siemens), with limits of detection of 1.05 nmol/L and 0.1 μmol/L, respectively. Plasma ACTH was measured by a solid-phase, two-site sequential chemiluminescent as-

Table 1. Patient Baseline Characteristics

Patient Identification	Sex; Age, y	Time Since Diagnosis of AAD, y	Peak Serum Cortisol, nmol/L[a]	Plasma ACTH, ng/L[b]	21-Hydroxylase Antibody, U/mL[c]	Thyroid Peroxidase Antibody, U/mL[d]	Associated Autoimmunity	Oral Steroid Regimen	Previous Adrenal Crisis
01	F; 65	12	<24	828	3.1	63	HT	HC, FC	Yes
02	F; 43	8	219	225	6.3	191	HT	PD, FC	No
03	F; 44	6	<24	>1250	170	253	HT	HC, FC	Yes
04	M; 46	19	<24	417	2.3	16	Nil	HC, FC	Yes
05	F; 23	8	<24	752	0.3[e]	51	HT	HC, FC	No
06	F; 36	4	184	174	27.7	11	HT	PD, FC	No
07	F; 16	2	<24	1080	5.1	8	Nil	HC, FC	No
08	M; 45	3	<24	610	12.3	12	T1DM	HC, FC	No
09	F; 54	1.5	30	>1250	617	9	Nil	HC, FC	No
10	F; 24	6	<24	805	3073	<5	T1DM	HC, FC	No
11	F; 48	6	32	645	1607	>600	HT	HC, FC	No
12	F; 35	1	<24	550	73	<5	Nil	HC, FC	No
13	F; 27	1	<24	1000	101	105	Nil	HC, FC	Yes

Abbreviations: F, female; FC, fludrocortisone; HC, hydrocortisone; HT, hypothyroidism; M, male; PD, prednisolone; T1DM, type 1 diabetes mellitus.

[a] Reference range for serum cortisol response after 250 μg $ACTH_{1-24}$ is greater than 550 nmol/L. To convert from nanomoles per liter to micrograms per deciliter, divide by 27.6.

[b] Reference range for morning plasma ACTH is 10–55 ng/L.

[c] Reference range for serum 21-hydroxylase antibodies is less than 1.0 U/mL.

[d] Reference range for thyroid peroxidase antibodies is less than 35 U/ml

[e] Participant 5 had positive adrenal cell autoantibodies as measured by immunofluorescence at diagnosis.

say on an Immulite platform (Siemens), with a limit of detection of 5 ng/L. There was no cross-reactivity for tetracosactide in this assay. In addition, an overnight (12-hour) urine collection was assessed for steroid metabolite excretion by gas chromatography/steroid mass spectrometry as previously described (19). A short tetracosactide test (with cortisol measured prior to and 30 and 60 min after 250 μg im tetracosactide injection) was then performed starting between 8:30 and 9:00 AM. Measurements of serum steroid 21-hydroxylase antibodies were made in duplicate by immunoprecipitation of radiolabeled recombinant 21-hydroxylase expressed in yeast (*Saccharomyces cerevisiae*) (RSR Ltd) (20).

Results

Patient baseline characteristics

Thirteen subjects (11 women and two men), with a median age of 43 years (range 16–65 y) were enrolled in the study. All participants showed elevation of plasma ACTH and impaired serum cortisol response to tetracosactide at baseline (Table 1). Twelve participants completed the first treatment period of the study (to 10 wk), with two participants responding to tetracosactide depot. Eight participants went on to the second treatment period (tetracosactide infusion), completing the full 20-week protocol. One participant withdrew after the week 5 assessment owing to pain at injection sites (Figure 1).

Adrenal steroidogenic function

During the screening visit, 9 of the 13 participants had undetectable tetracosactide-stimulated peak serum cortisol concentration. We observed detectable cortisol levels (≥25 nmol/L) in four of them including two participants (subjects 02 and 06) with tetracosactide-stimulated peak cortisol concentrations of 219 and 184 nmol/L, respectively (Table 1).

After 10 and 20 weeks of treatment with tetracosactide depot, 10 of the 12 participants had a peak serum cortisol concentration that remained below 100 nmol/L (Figure 2A). Similarly, the same group of participants had serum aldosterone levels below the threshold for detection (Figure 2B). In contrast, we observed an increase in serum cortisol concentration in participants 02 and 06 by week 5. Nevertheless, their corresponding serum aldosterone levels started to rise above the threshold for detection only at weeks 20 and 40, respectively.

At week 10, participant 02 had a peak serum cortisol concentration of 462 nmol/L, so her oral steroid replacement was judiciously weaned down, with close monitoring to ensure continued well-being and patient safety. She ceased oral prednisolone at week 13, fludrocortisone at week 23, and tetracosactide injections at week 32 without any deleterious consequences. Since stopping medication, she has experienced a few episodes of cold-like symptoms and one significant episode of viral gastroenteritis with diarrhea but has neither needed to take steroid medication nor hospital treatment during these illnesses. Both her peak serum cortisol and aldosterone concentrations were normal, at 672 nmol/L and 273 pmol/L, 72 weeks after weaning off all therapies other than levothyroxine for autoimmune hypothyroidism. At the current time, she has remained well for 28 months without steroid replacement.

At week 29, participant 06 had a peak serum cortisol concentration of 441 nmol/L and her oral steroid replacement therapy was then progressively reduced, ceasing oral prednisolone at week 36, tetracosactide injections at week 45, and fludrocortisone at week 57. She remained well with the staged reduction of steroid and Synacthen therapy, but her peak serum cortisol concentrations decreased progressively. Oral steroid replacement therapy was restarted when she became symptomatic at week 64. Details of the presenting biochemical features of these responding patients are shown in Table 2.

Serum androstenedione was detectable in six participants at baseline (including the two men) and increased progressively in patients 02 and 06 (Figure 2C). At baseline serum DHEAS was detectable only in the two male participants but was slow to change, rising into the reference range (>1.0 μmol/L) only in female participant 02, 100 weeks after the start of the study (Figure 2D). Urinary excretion of glucocorticoid precursors and active glucocorticoid metabolites gradually increased in patients 02 and 06 from below the fifth centile to above the median of healthy female controls at 10 and 40 weeks, respectively (Supplemental Figure 1). This was paralleled by increases in urinary mineralocorticoid metabolite excretion; however, androgen precursor and active androgen metabolite excretion was slower to increase.

21-Hydroxylase antibodies

All patients except participant 05 had a positive 21-hydroxylase antibody concentration (>1.0 U/mL) at baseline. None of the patients demonstrated significant changes in the 21-hydroxylase antibody level at either 10 or 20 weeks of the study ($P > .05$, paired t test) (Supplemental Figure 2). Similarly, participants 02 and 06, who were followed up for 65 and 80 weeks, respectively, did not show any significant increase in serum antibody concentrations.

Safety and serological studies

All participants experienced transient redness, itch, and swelling at the tetracosactide injection sites. Areas of erythema and swelling ranged up to a maximum of 4 cm in

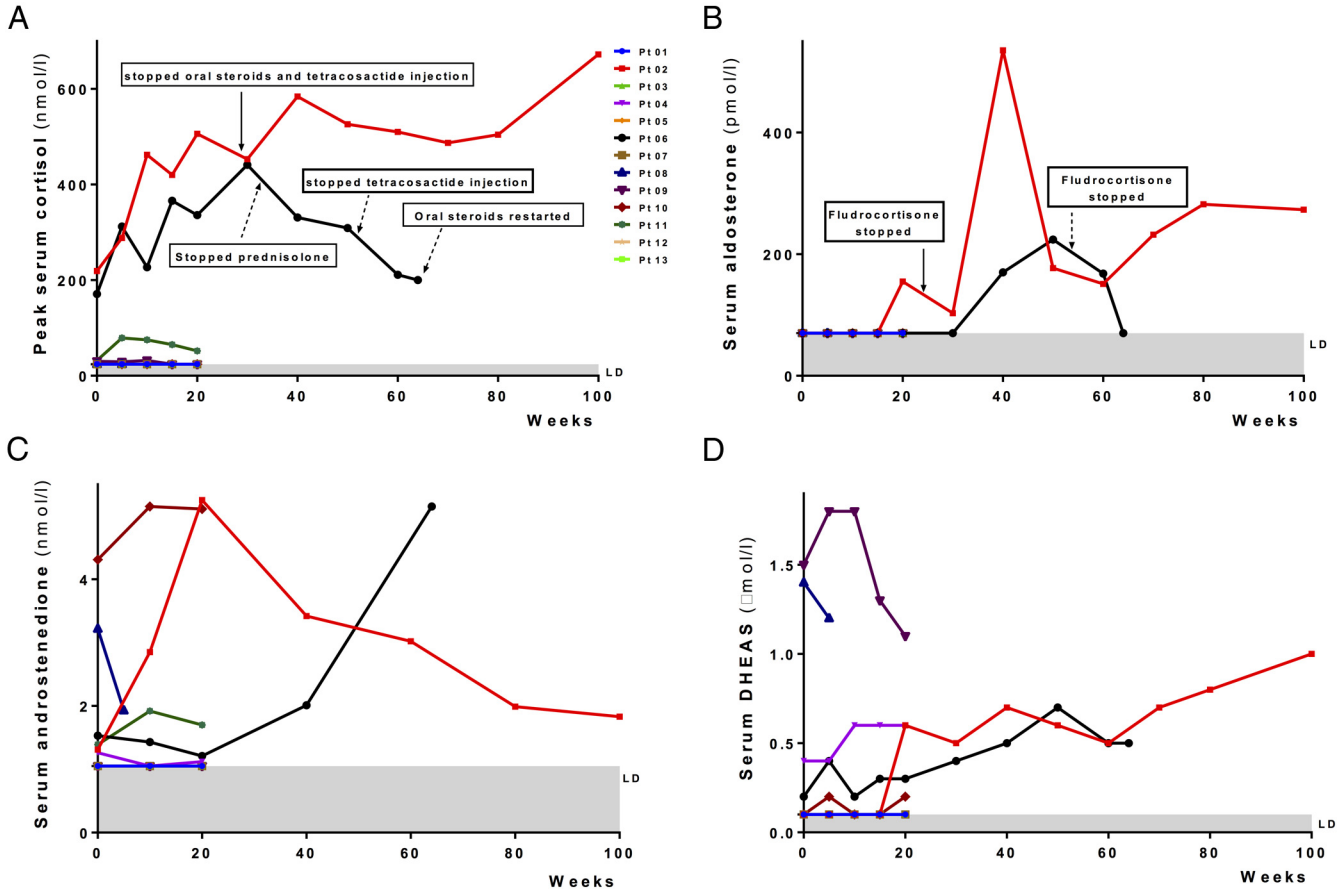

Figure 2. Longitudinal course of serum steroid hormones in autoimmune Addison's disease patients during and after tetracosactide ($ACTH_{1–24}$) therapy. A, Serum cortisol and all other steroid hormone measurements were made after 36–42 hours of withdrawal from regular glucocorticoid and fludrocortisone replacement therapy. For each time point, we recorded the highest serum cortisol observed during a short tetracosactide test (250 μg im, with blood sampling at baseline and 30 and 60 min after injection). The normal response to this stimulus is a peak cortisol concentration greater than 550 nmol/L. The assay limit of detection (LD) was 25 nmol/L, shown as the shaded horizontal area. To convert serum cortisol from nanomoles per liter to micrograms per deciliter, divide by 27.6. B, Serum aldosterone concentrations. The reference range for recumbent serum aldosterone is 100–450 pmol/L, with a limit of detection (LD) of 70 pmol/L, shown as the shaded horizontal area. Several patients' data are superimposed on this limit of detection line. To convert serum aldosterone from picomoles per liter, to nanograms per deciliter, divide by 27.7. C, Serum androstenedione concentrations. The limit of detection (LD) was 1.05 nmol/L, shown as the shaded horizontal area. Several patients' data are superimposed on this limit of detection line. The female reference range is 1.2–14.3 nmol/L and the male reference range is 1.4–9.1 nmol/L. To convert serum androstenedione from nanomoles per liter to nanograms per deciliter, divide by 0.0349. D, Serum DHEAS concentrations. The limit of detection (LD) was 0.1 μmol/L (shown as the shaded horizontal area). Several patients' data are superimposed on this limit of detection line. The female reference interval is 1.0–9.2 μmol/L and the male reference interval is 2.4–11.6 μmol/L. To convert serum DHEAS from micromoles per liter to micrograms per deciliter, divide by 0.0271.

diameter and resolved over 48 hours after each sc injection.

Four of nine premenopausal female participants developed menstrual disturbance, with early menstruation and occasional lower abdominal cramps during the study. In addition, participant 02 felt unwell during the short tetracosactide testing at week 25, with generalized weakness, nausea, and abdominopelvic cramps, similar to severe menstrual pain. Participant 11 also experienced lower abdominal cramps and vomiting during the short tetracosactide tests at weeks 15 and 20. There was complete resolution of symptoms within 120 minutes of the tetracosactide injection, except for patient 11, who developed intermenstrual bleeding for 24 hours after the 20-week test.

Two participants developed wheals at the previous abdominal tetracosactide infusion sites within 10 minutes of the administration of im tetracosactide during retesting of adrenal function (at wk 15 and 20). They both also reported a synchronous itchiness and redness over palms and soles. They remained hemodynamically stable throughout the events, which resolved after an hour.

Discussion

Autoimmune Addison's disease is a chronic condition that is currently considered incurable and available replacement therapy is imperfect in failing to mimic physiological corticosteroid secretion. We sought to find out whether

Table 2. Biochemical and Clinical Characteristics for Participants 02 and 06 at Diagnosis

Biochemical Tests (Reference Range)	Participant 02	Participant 06
Sodium (133–146 mmol/L)	131	130
Potassium (3.5–5.3 mmol/L)	5.7	4.7
Urea (2.5–7.8 mmol/L)	9.4	5.7
Creatinine (55–95 μmol/L)	112	92
Baseline cortisol	195	273
Peak cortisol after stimulation with 250 μg $ACTH_{1-24}$ (>550 nmol/L)	212	279
ACTH (10–55 ng/L)	498	55
Renin (1.0–5.5 pmol/L/h)	8.7	7.5
Aldosterone (100–450 pmol/L)	72	<70

patients with established autoimmune Addison's disease could have salvageable adrenal steroidogenic function that could be stimulated using the synthetic analog of ACTH, tetracosactide ($ACTH_{1-24}$, Synacthen). Four of the 13 participants had detectable serum cortisol at baseline testing, and two of these had progressive improvement in adrenal steroidogenic function during treatment with Synacthen. In both of these patients, we were able to withdraw daily steroid replacement medication with maintenance of good health. In one participant this was transient, with a progressive decrease in stimulated serum cortisol once tetracosactide injections were stopped, which required reinstatement of glucocorticoid replacement after 28 weeks. In the other participant, the treatment has led to a sustained improvement in adrenal steroidogenesis, such that she has remained well and without steroid replacement medication for 28 months at the time of writing this paper. Nevertheless, despite a peak cortisol level of 672 nmol/L during the stimulation testing, she does not currently have normal adrenal function, with residual elevation of plasma ACTH (159 ng/L; reference range 10–55) and renin (14 pmol/L·h; reference range 1.0–5.5).

Our study also demonstrates that autoimmune Addison's disease is a heterogeneous condition, with 2 of 13 participants having useful residual adrenal steroidogenic function. We also observed a small rise in serum cortisol in a third individual (participant 11). Contrary to expectation, the patients with residual adrenal function were not those with the most recently diagnosed disease. Our two patients with useful recovery had been treated for autoimmune Addison's disease 4 and 8 years prior to participating in this study. In contrast, four participants who were diagnosed with autoimmune Addison's disease within 2 years before the baseline visit had no residual steroidogenesis that we could demonstrate. This suggests that certain autoimmune Addison's disease patients may have residual steroidogenic function that is durable over long periods of time. One previous study of autoimmune Addison's disease patients showed increases in serum cortisol in 10 of 27 patients during tetracosactide testing, despite prior dexamethasone administration (0.5 mg twice) (21). Along with our own data, this suggests that low-level residual steroidogenic function may be present in up to 30% of autoimmune Addison's disease patients. There are only two individuals reported in the literature with spontaneous recovery from established Addison's disease (22, 23). In light of our findings, it appears probable that those two patients also had residual steroidogenic function that improved owing to endogenous ACTH stimulation once the patients had decided to reduce or stop their steroid medication.

Residual islet β-cell function (marked biochemically by insulin C-peptide positivity) is well described in about 20% of patients with autoimmune type 1 diabetes, and our findings could represent a parallel state of partial destruction of the target organ in an autoimmune disease process. In C-peptide positive type 1 diabetes patients, the residual insulin secretion is significant and has been correlated with improved glycemic control and fewer microvascular complications (24–26). Approximately 40% of autoimmune Addison's disease patients are reported to have never been hospitalized with an adrenal crisis (3, 4), and this was also the case for our two participants who exhibited responsiveness to tetracosactide therapy. It is possible that long-term freedom from adrenal crises could be owing to low-level residual adrenal function in certain autoimmune Addison's disease patients.

Regular, high-dose tetracosactide therapy was not without adverse effects, the most significant of which was menstruation-like lower abdominal cramps during repeat tetracosactide stimulation testing for the assessment of adrenal function in two of nine premenopausal women and menstrual disturbance in four of nine women. Menstrual disturbance is a well-described side effect of chronic ACTH administration (27); however, little is known about the role of ACTH or melanocortin receptors in uterine function and this clearly warrants further investigation. An additional two participants had allergic cutaneous reactions during repeat tetracosactide testing.

By demonstrating that certain autoimmune Addison's disease patients have residual steroidogenic function several years after diagnosis, we provide new and valuable information about the heterogeneity of the natural history of the disease. We also show that such residual adrenal function is clinically important because it has been exploited to ameliorate the condition in this study by administration of exogenous trophic ACTH stimulation. An important ramification of our study is that, at the time of first treatment of autoimmune Addison's disease using

standard replacement therapy, it is unknown whether the exogenous glucocorticoid and mineralocorticoid treatment that decreases trophic ACTH drive will compound the adrenal failure by producing an additional degree of steroid-induced adrenal suppression/atrophy. It will be important to identify additional markers of residual adrenal function to enable this patient group to be distinguished in the future.

Despite a concern over the potential for recrudescence of the autoimmune response, there has been no worsening of humoral antiadrenal autoimmunity over time in patients 02 and 06, as judged by serum 21-hydroxylase antibody concentrations. This is consistent with the hypothesis that adrenocortical steroidogenesis may protect the adrenal against immune attack because the high local glucocorticoid concentrations impair the function of antigen-presenting dendritic cells and several other immune cells (28, 29). Nevertheless, T lymphocyte reactivity against adrenal antigens was not studied, and an ongoing or renewed T cell attack could have contributed to the early decline in steroidogenic function in patient 06. Our study is unique in showing that tetracosactide stimulation can lead to the recovery of adrenal function after long-term adrenal insufficiency in autoimmune Addison's disease. The approach we describe needs further exploration; however, it is likely that a proportion of autoimmune Addison's disease patients could have similar long-term benefits from such therapy.

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