Residual adrenal function in autoimmune Addison's disease: Improvement following tetracosactide (ACTH₁-₂₄) treatment.

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**Full Eligibility criteria**

*Inclusion criteria:*

- Age 16-65
- Biochemical evidence of adrenal failure (basal cortisol <100nmol/L or peak cortisol after 250µg ACTH₁₋₂₄ <300nmol/L)
- Confirmation of primary origin of adrenal failure (basal ACTH level >55ng/L)
- Established adrenal failure for 12 months or more
- Normal or atrophic adrenals on cross-sectional imaging (if performed): compatible with autoimmune Addison’s disease
- If female, willing to take secure contraceptive measures for duration of study
- Willing to travel to research unit for study
- Willing to attend steroid replacement education session

*Exclusion criteria*

- Pregnant, breastfeeding or plan for pregnancy within 12 months
- Significant heart, chest, renal or hepatic disease
- Cancer (excluding non-melanoma skin cancer)
- Asthma
- Currently symptomatic or untreated peptic ulcer disease
- Acute psychosis
- Known non-immune cause for adrenal failure eg. -linked adrenal leukodystrophy or inborn error of steroidogenesis or adrenal development
- Known allergy to synacthen (ACTH₁₋₂₄)
**Supplemental Table 1. Steroid replacement regimens of participants at study entry**

<table>
<thead>
<tr>
<th>Participant</th>
<th>Glucocorticoid (daily dosage, mg)</th>
<th>Fludrocortisone (daily dosage, µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Hydrocortisone (25)</td>
<td>100</td>
</tr>
<tr>
<td>02</td>
<td>Prednisolone (5)</td>
<td>200</td>
</tr>
<tr>
<td>03</td>
<td>Hydrocortisone (15)</td>
<td>100</td>
</tr>
<tr>
<td>04</td>
<td>Hydrocortisone (17.5)</td>
<td>100</td>
</tr>
<tr>
<td>05</td>
<td>Hydrocortisone (17.5)</td>
<td>150</td>
</tr>
<tr>
<td>06</td>
<td>Prednisolone (6)</td>
<td>150</td>
</tr>
<tr>
<td>07</td>
<td>Hydrocortisone (15)</td>
<td>100/200 alternate days</td>
</tr>
<tr>
<td>08</td>
<td>Hydrocortisone (20)</td>
<td>100</td>
</tr>
<tr>
<td>09</td>
<td>Hydrocortisone (17.5)</td>
<td>150</td>
</tr>
<tr>
<td>10</td>
<td>Hydrocortisone (17.5)</td>
<td>150/100 alternate days</td>
</tr>
<tr>
<td>11</td>
<td>Hydrocortisone (20)</td>
<td>100</td>
</tr>
<tr>
<td>12</td>
<td>Hydrocortisone (20)</td>
<td>100</td>
</tr>
<tr>
<td>13</td>
<td>Hydrocortisone (25)</td>
<td>150</td>
</tr>
</tbody>
</table>
Supplemental Table 2. Longitudinal course of basal plasma ACTH and serum cortisol levels in participant 02 & 06, during and after tetracosactide (ACTH₁₋₂₄) therapy.

<table>
<thead>
<tr>
<th>Study weeks (participant 02)</th>
<th>0</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>24</th>
<th>32</th>
<th>34</th>
<th>51</th>
<th>64</th>
<th>74</th>
<th>82</th>
<th>104</th>
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</thead>
<tbody>
<tr>
<td>Cortisol &lt;sub&gt;basal&lt;/sub&gt; (nmol/L)</td>
<td>192</td>
<td>224</td>
<td>409</td>
<td>336</td>
<td>506</td>
<td>498</td>
<td>394</td>
<td>339</td>
<td>452</td>
<td>502</td>
<td>421</td>
<td>447</td>
<td>606</td>
</tr>
<tr>
<td>Cortisol &lt;sub&gt;30 mins&lt;/sub&gt; (nmol/L)</td>
<td>214</td>
<td>267</td>
<td>451</td>
<td>396</td>
<td>472</td>
<td>539</td>
<td>434</td>
<td>388</td>
<td>489</td>
<td>486</td>
<td>466</td>
<td>473</td>
<td>659</td>
</tr>
<tr>
<td>Cortisol &lt;sub&gt;60 mins&lt;/sub&gt; (nmol/L)</td>
<td>219</td>
<td>288</td>
<td>462</td>
<td>420</td>
<td>475</td>
<td>509</td>
<td>453</td>
<td>413</td>
<td>526</td>
<td>504</td>
<td>487</td>
<td>504</td>
<td>672</td>
</tr>
<tr>
<td>ACTH &lt;sub&gt;basal&lt;/sub&gt; (ng/L)</td>
<td>225</td>
<td>162</td>
<td>143</td>
<td>71</td>
<td>2236</td>
<td>1179</td>
<td>1192</td>
<td>2728</td>
<td>341</td>
<td>286</td>
<td>228</td>
<td>190</td>
<td>159</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Study weeks (participant 06)</th>
<th>0</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>27</th>
<th>35</th>
<th>39</th>
<th>42</th>
<th>46</th>
<th>64</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortisol &lt;sub&gt;basal&lt;/sub&gt; (nmol/L)</td>
<td>184</td>
<td>257</td>
<td>176</td>
<td>315</td>
<td>255</td>
<td>381</td>
<td>374</td>
<td>324</td>
<td>265</td>
<td>371</td>
<td>211</td>
</tr>
<tr>
<td>Cortisol &lt;sub&gt;30 mins&lt;/sub&gt; (nmol/L)</td>
<td>171</td>
<td>284</td>
<td>201</td>
<td>366</td>
<td>311</td>
<td>428</td>
<td>393</td>
<td>331</td>
<td>269</td>
<td>377</td>
<td>203</td>
</tr>
<tr>
<td>Cortisol &lt;sub&gt;60 mins&lt;/sub&gt; (nmol/L)</td>
<td>184</td>
<td>312</td>
<td>227</td>
<td>364</td>
<td>336</td>
<td>441</td>
<td>397</td>
<td>327</td>
<td>269</td>
<td>363</td>
<td>200</td>
</tr>
<tr>
<td>ACTH &lt;sub&gt;basal&lt;/sub&gt; (ng/L)</td>
<td>174</td>
<td>36</td>
<td>272</td>
<td>599</td>
<td>587</td>
<td>867</td>
<td>1126</td>
<td>3135</td>
<td>2127</td>
<td>1852</td>
<td>1985</td>
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</tbody>
</table>
Figure legends

Supplemental Figure 1A. Longitudinal course of urinary steroid metabolite excretion in autoimmune Addison’s disease patients during and after tetracosactide (ACTH1-24) therapy

Urinary steroid metabolite excretion was measured by gas chromatography/mass spectrometry (see methods section). Data from the 11 female participants are presented in comparison to results obtained in 55 healthy women with the median of the normal cohort indicated as a dotted line and the shaded area representing the 5th-95th centile range. Results observed in the two male participants are shown in the supplementary figure S1b.

Panel A: The sum of the metabolites of the glucocorticoid precursors 17-hydroxyprogesterone (17-hydroxy-pregnanolone, pregnanetriol) and 11-deoxycortisol (tetrahydro-11-deoxycortisol) are plotted against time (reference range median 493 [5th-95th centile 167-821] µg/24h).

Panel B: The sum of the active glucocorticoid metabolites (cortisol, tetrahydrocortisol, 5α-tetrahydrocortisol, α-cortol, β-cortol, cortisone, tetrahydrocortisone, α-cortolone, and β-cortolone) are plotted against time (reference range 5867 [2935-9632] µg/24h).

Panel C: The sum of the mineralocorticoid metabolites (3α,5β-tetrahydroaldosterone, tetrahydrocorticosterone, 5α-tetrahydrocorticosterone, tetrahydrodeoxycorticosterone, 5α-tetrahydrodeoxycorticosterone, tetrahydro-11-dehydrocorticosterone, and 5α-tetrahydro-11-dehydrocorticosterone) are plotted against time (reference range 432 [167-821] µg/24h).

Panel D: The sum of the androgen precursor metabolites dehydroepiandrosterone, 16α-dehydroepiandrosterone, 5-pregnanediol and 5-pregnanetriol are plotted against time (reference range 1003 [177-3897] µg/24h).

Panel E: The sum of the major active androgen metabolites androsterone and etiocholanolone are plotted against time (reference range 2139 [484-4790] µg/24h).

Supplemental Figure 1B.

Longitudinal measurements of 24-h urinary steroid excretion (µg/24h) in the two male participants (patients 4 and 8) at study baseline, and following tetracosactide treatment. Normal reference ranges derived from healthy male controls (n=27; 20-60 years) are
represented by the median (indicated by the dotted line) and the 5th to 95th centile range (represented by the shaded areas).

**Panel A:** The sum of the metabolites of the glucocorticoid precursors 17-hydroxyprogesterone (17-hydroxy-pregnanolone, pregnanetriol) and 11-deoxycortisol (tetrahydro-11-deoxycortisol) are plotted against time (normal range median 1007 [5th-95th centile 405-2196] µg/24h).

**Panel B:** The sum of the active glucocorticoid metabolites (cortisol, tetrahydrocortisol, 5α-tetrahydrocortisol, α-cortol, β-cortol, cortisone, tetrahydrocortisone, α-cortolone, β-cortolone) are plotted against time (normal range 10329 [4447-18496] µg/24h).

**Panel C:** The sum of mineralocorticoid metabolites (3α,5β-tetrahydroaldosterone, tetrahydrocorticosterone, 5α-tetrahydrocorticosterone, tetrahydrodeoxyxorticosterone, 5α-tetrahydrodeoxyxorticosterone, tetrahydro-11-dehydrocorticosterone, 5α-tetrahydro-11-dehydrocorticosterone) are plotted against time (normal range 628 [279-1631] µg/24h).

**Panel D:** The sum of the androgen precursor metabolites, dehydroepiandrosterone, 16α-dehydroepiandrosterone, 5-pregnanediol and 5-pregnanetriol are plotted against time (normal range 2262 [237-10548] µg/24h).

**Panel E:** The sum of the major androgen metabolites, androsterone and etiocholanolone, are plotted against time (normal range 4067 [1660-12017] µg/24h).

**Supplemental Figure 2. Serum 21-hydroxylase autoantibodies**

The concentration of serum 21-hydroxylase autoantibodies over time is shown (note logarithmic Y-axis). The reference range is <1·0 U/ml. Samples from participants 02 and 06 were run in two separate batches (weeks 1-40 and weeks 40 onwards) and measurements corrected using duplicate samples run in both assays.
Supplemental Figure 1A

Graphs showing changes in glucocorticoid precursor metabolites, glucocorticoid metabolites, mineralocorticoid metabolites, and androgen precursor metabolites over weeks.
Supplemental Figure 1B

A

Glucocorticoid metabolites (µg/24h)

B

Mineralocorticoid metabolites (µg/24h)

C

Androgen metabolites (µg/24h)

D

Androgen precursor metabolites (µg/24h)

E

Androgen metabolites (µg/24h)
Supplemental Figure 2

Serum 21-hydroxylase antibody (U/ml)

Weeks

Pt 01
Pt 02
Pt 03
Pt 04
Pt 05
Pt 06
Pt 07
Pt 08
Pt 09
Pt 10
Pt 11
Pt 12
Pt 13