

## SWAN Repository Dataset Documentation

**Study:** App 046, “Adrenocorticotrophic hormone (ACTH)”, Lasley

**Datasets:** SWANRep046\_ACTH

**Cohort:** SWAN

Summary, Adrenocorticotrophic hormone (ACTH), also known as corticotropin data, Repository Protocol #46

### 1. Who is included in the dataset?

A subset of 176 participants who had existing previously-measured circulating adrenal androgen measurements. Each participant provides data from up to 3 SWAN visits (00, 01, 02). The dataset includes 248 measurements from these 176 collections/participants.

### 2. Language and translation

There were no issues regarding language and translation.

### 3. Included variables

Variable name	Description of the variable	Codes
ARCHID	Encrypted SWAN subject ID	
VISIT	SWAN annual visit	00, 01, 02
PLATE	Microtiter plate assignment	1 through 7
MEANRESULT	Average of duplicate measurements of ACTH	

### 4. Background, description of ACTH assay

Based on positive and statistically significant associations of DHEAS with symptoms, which were attenuated upon adjustment for other participant characteristics, we hypothesized that a related adrenal steroid that was not being measured could be supporting these intriguing associations. We selected androstenediol as the candidate analyte to measure, due to its well-characterized biological activity.

The purpose of this investigation was to determine if higher adrenal steroids were associated with a pattern of shift in ACTH which would indicate either: 1) a requirement for the hypothalamus/pituitary (source of ACTH) to increase to compensate for the redirection of cortisol precursors at the level of pregnenolone; 2) or if an increase in adrenal androgens was associated with a decline in ACTH indicating that the cause for the increase in androgens also increased cortisol and this feedback then decreased the need for the hypothalamus/pituitary to produce ACTH.

The determination of ACTH was performed in duplicate on 400 µL EDTA plasma (200 µL per determination). The ACTH immunoassay used (ALPCO Diagnostics, Salem, HN) is a two-site ELISA [Enzyme Linked ImmunoSorbent Assay] for the measurement of the biologically active 39 amino acid chain of ACTH. A goat polyclonal antibody to human ACTH, purified by affinity chromatography, and a

mouse monoclonal antibody to human ACTH are specific for well-defined regions on the ACTH molecule. One antibody is prepared to bind only the C-terminal ACTH 34-39 and this antibody is biotinylated. The other antibody is prepared to bind only the mid-region and N-terminal ACTH 1-24 and this antibody is labeled with horseradish peroxidase [HRP] for detection. In this assay, calibrators, controls, or patient samples are simultaneously incubated with the enzyme labeled antibody and a biotin coupled antibody in a streptavidin-coated microplate well. At the end of the assay incubation, the microwell is washed to remove unbound components and the enzyme bound to the solid phase is incubated with the substrate, tetramethylbenzidine (TMB). An acidic stop solution is then added to stop the reaction and convert the color to yellow. The intensity of the yellow color is directly proportional to the concentration of ACTH in the sample. A dose response curve of absorbance unit vs. concentration is generated using results obtained from the calibrators. Concentrations of ACTH present in the controls and patient samples are determined directly from this curve. ACTH levels were measured in one hundred and thirty-four (134) apparently normal individuals in the U.S. with the ACTH ELISA. The values obtained ranged from 7.0 to 63 pg/mL. Based on statistical tests on skewness and kurtosis, the population, when transformed logarithmically, follows the normal or Gaussian distribution. The geometric mean + 2 standard deviations of the mean were calculated to be 6.17 to 58.2 pg/mL. The assay range (Standard curve) is 0.0- 165 pg/mL. The assay measures analyte concentrations to 165 pg/mL with a minimum reportable concentration (lowest standard) of 5 pg/mL, and a LLD (sensitivity) of 0.22 pg/mL. The inter assay coefficient of variation reported by the manufacturer is 7.1% at 42.3 pg/mL and 6.9% at 287.8 pg/mL. The intra assay coefficient of variation reported by the manufacturer is 6.7% at 42.2 pg/mL and 2.3% at 269.9 pg/mL.