# Hair Cortisol Measurement and Relationships with Growth Among Amazonian Shuar Children

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## Introduction

Cortisol is a hormone secreted by the adrenal gland in response to stress. It is a widely-used biomarker of stress. Cortisol can be analyzed from saliva, blood, urine, and hair. However, collecting saliva, urine, and blood only provide "point samples" (reflecting acute/short-term cortisol level), making it challenging to examine chronic physiological stress

Advantages of hair collection:

- ❑ Non-invasive
- ❑ Hair grows at a rate of 1 cm/month, allowing assessment of cumulative concentrations of cortisol, and therefore stress, over a period of months corresponding to the length of hair used

Figure 1: Hair sample collected from a Shuar child. The arrow indicates the hair near the scalp. A 3 cm segment of hair was measured from the scalp, weighed, and then processed to determine the average cortisol concentration for the three months prior to collection

## Research Objectives

- ❑ Optimize hair cortisol extraction protocol
- ❑ Establish reference levels of hair cortisol in Shuar children
- ❑ Examine relationships between cortisol and growth parameters

## Methods

Protocol Optimization

- ❑ Compared how well we could mince or grind up the hair using:
  - Surgical scissors
  - A Wiley Mill

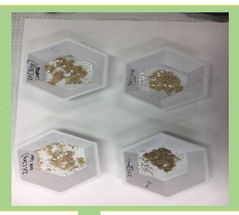
  - Bead homogenizer (BeadRuptor 12)

- ❑ Processing the samples with surgical scissors took an excessive amount of time and did not result in a powder alike consistency
- ❑ Wiley mill failed to grind hair samples finely
- ❑ Lastly, we used BeadRuptor12 and found that processing hair samples in 7-ml reinforced tubes with ~16 2.8mm ceramic beads for two cycles on high for 30 seconds each yielded the most pulverized hair

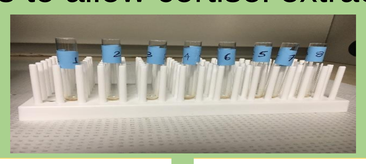
- ❑ Cortisol was then extracted from pulverized hair samples using a modified version of the method of Meyer et al. (2014) and assayed in duplicate using high sensitivity salivary cortisol enzyme immunoassay kits manufactured by Salimetrics

Wash hair samples with isopropanol to remove exogenous cortisol and air dry for 3 days

Pulverize samples



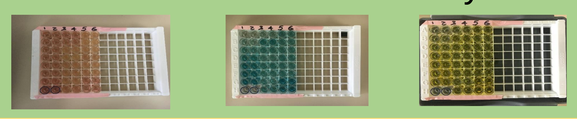
Add methanol to the samples and rotate for 24 hours to allow cortisol extraction



Evaporate methanol using nitrogen gas and heat


Reconstitute cortisol extract in enzyme-linked immunosorbent assay (ELISA) buffer

Run competitive enzyme-linked immunosorbent assay



Modifications to the Meyer et al. (2014) protocol

- ❑ Ceramic beads were used to pulverize the hair samples
- ❑ Hair samples were processed in 7-mL tubes rather than 1.5 mL tubes
- ❑ Methanol (used to extract the cortisol from the pulverized hair sample) was evaporated using nitrogen gas and heat (60° C) for ~ 25 minutes
- ❑ Samples were reconstituted in using 75 ul of enzyme-linked immunosorbent assay diluent rather than 200 ul as specified in Meyer et al.

Application to Shuar population:

- ❑ Optimized protocol was applied to hair samples collected from Shuar participants (3-19 years old) in Amazonian Ecuador

## Results

- ❑ This research describes refinements of an existing method and helps make this technique available to other researchers
- ❑ Thirty-two of 51 Shuar samples (62.7%) had extremely low levels of cortisol (below the limit of detection of the assay). These results are consistent with the very low levels of cortisol found in saliva samples from Shuar
- ❑ For those hair samples with measurable cortisol levels, the mean cortisol concentration was 4.22 pg/mg, while the range was 0.13 – 21.73 pg/mg. The standard deviation was 4.75
- ❑ Nineteen of 32 Shuar samples (59.4%) with extremely low levels of cortisol were individuals taller than the average height (~123 cm)

## Implications

- ❑ Cortisol level in saliva is widely used as a biomarker of stress. Hair is typically easier to collect and store than saliva collection
- ❑ By measuring hair cortisol, it is possible to evaluate overall stress levels over longer periods of time (1-3 months based on length of hair assessed) than possible with saliva samples
- ❑ The Global Health Biomarker Lab will soon offer fee-for-service testing of hair cortisol to researchers

## Important Considerations When Extracting Cortisol from Hair

- ❑ To avoid contamination (external cortisol that might be present from sweat), hair samples should be washed in isopropanol
- ❑ Ensure hair sample is sufficiently ground to maximize cortisol extraction

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