Technical Note 60

Young Lives

Collecting Hair Samples to Measure Cortisol in the Young Lives Study: Protocols and Fieldwork Results

Marta Favara, Andrea Tartakowsky, Juliana Quigua,
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About Young Lives

Young Lives is an international study of poverty and inequality, following the lives of 12,000 children in four countries (Ethiopia, India, Peru and Vietnam) since 2001. **www.younglives.org.uk**

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Contents

The authors

Andrea Tartakowsky (Blavatnik School of Government, University of Oxford) and Juliana Quigua (Young Lives, University of Oxford) led the preparation of this technical note. All the other co-authors contributed through critical review, comments and revisions. Marta Favara (Young Lives, University of Oxford) had oversight and leadership responsibility. Together with Alan Sánchez (Young Lives, University of Oxford), she conceptualised the note and provided guidance throughout the drafting process. The note uses material related to cortisol collection from the fieldwork manual and training materials used for the Round 7 data collection, which were authored by Sofia Carrera (Northwestern University), Katherine Curi-Quinto (Instituto de Investigación Nutricional) and Anthony I. Aquino (Instituto de Investigación Nutricional). Additional conceptual information was provided by Sofia Carrera. All the authors reviewed and approved the final version for publication.

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Summary

In Round 7, Young Lives collected hair samples from study participants for the first time, with the aim of measuring cortisol levels, a correlate of chronic stress. This technical note summarises the scientific basis for using hair cortisol as an indicator of chronic stress, outlines the sample collection protocols, and describes how Young Lives country teams in Ethiopia, India and Peru implemented these protocols in practice. It offers a useful tool to assess Young Lives' process of hair sample collection, makes recommendations for future rounds, and provides other researchers with valuable information to produce large-scale cortisol data in international cohort studies.

1 Introduction

Mental health is a fundamental component of human development and well-being. While a growing body of literature links mental health and climate change, most studies originate from high-income countries, with results that are not generalisable to lower- and middle-income countries (LMICs) (Hayes et al. 2018; Hwong et al. 2022). This gap is particularly concerning given the high burden of mental health issues (Jacob et al. 2007), increasing pressure of climate change (Bathiany et al. 2018) and severe shortage of financial and human resources allocated to mental health issues in LMICs (World Health Organization 2021). In its Round 6 survey, Young Lives collected self-reported information about the mental health of its study participants as young adults, then aged 19–20 and 26–27 (Porter et al. 2021). In Round 7, these measurements were repeated and, for the first time, a self-reported Perceived Stress Scale (PSS-10) was administered. Young Lives also launched its cortisol study in Round 7 and collected hair samples with the aim of measuring participants' cortisol levels.

Cortisol is produced by the body's stress-response system and chronically elevated levels have been associated with poor mental and physical health. Moreover, hair cortisol is considered an objective and reliable non-invasive measure of chronic stress over the past several months. Once analysed, the newly collected information, along with the bank of data collected from infancy to adulthood, will enable understanding of the patterns of long-term stress and to what extent growing up in poverty and being exposed to stressful circumstances over the life course, including circumstances linked to climate change, affect mental (and physical) health in adulthood.

Although hair cortisol has been collected in other studies, most of these have small sample sizes and have been conducted in developed countries. It is uncommon to find longitudinal, large-sample studies of hair cortisol for LMICs. To the best of our knowledge, the Young Lives cortisol study is the largest study in the Global South to date examining cortisol levels from hair samples. Between 2023 and 2024, Young Lives collected 5,230 hair samples: 1,935 in Peru, 2,290 in India and 1,005 in Ethiopia. Methodologically, this study advances our understanding of how to collect non-invasive biomarker samples in developing countries. Empirically, it contributes to the understanding of the relationship between self-reported mental health – including perceived stress, depression and anxiety – and an objective measure of the physiological stress response obtained from hair samples.

The rest of this note is organised as follows. Section 2 presents the theoretical basis of cortisol assessment as a measure of stress, along with the advantages and disadvantages of using hair cortisol compared to other available methods. Section 3 describes the ethical aspects involved in the collection of hair samples and the protocols used to collect and handle the samples in the field. Section 4 outlines the logistics of hair sample collection in Ethiopia, India and Peru, including the challenges encountered when applying the protocols. Section 5 covers the logistics involved in transporting samples to a laboratory abroad for analysis, and Section 6 concludes.

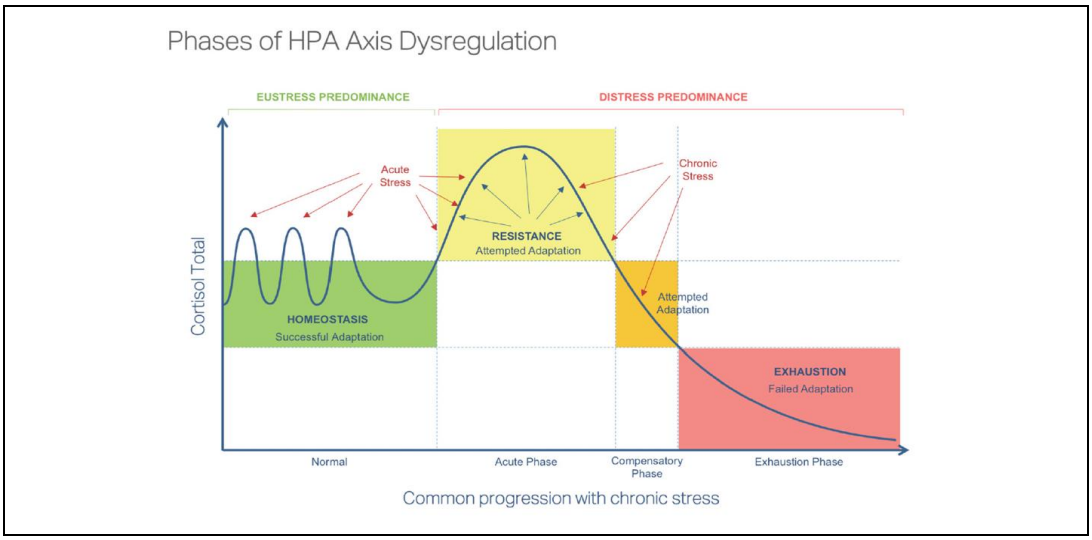
2 Hair cortisol to measure stress

2.1 Cortisol as a measure of stress

Cortisol is a vital hormone produced by the hypothalamic-pituitary-adrenal (HPA) axis that regulates various physiological processes in the body, including metabolism and energy utilisation. In addition to these functions, cortisol is considered a measure of stress, as human bodies release cortisol in response to acute psychosocial stressors, such as job interviews (Sapolsky, Romero and Munck 2000; Goodman, Janson and Wolf 2017; Helminen et al. 2019). Furthermore, cortisol serves as an important indicator of chronic stress-related conditions, such as major depressive disorders and severe melancholia, because of the excessive secretion of this hormone (Yehuda et al. 1996; Dedovic and Ngiam 2015; Powers et al. 2016; Gunnar and Quevedo 2007; Yehuda and Seckl 2011). In contrast, for other mental health issues like post-traumatic stress disorder (PTSD), chronic pain and fatigue disorders, there is a reduced secretion of cortisol (Gunnar and Quevedo 2007; Yehuda and Seckl 2011).

High cortisol levels are also associated with long-term stressors, such as declines in food availability, environmental shocks, socioeconomic stressors, and others (Dowd, Simanek and Aiello 2009; Chemin, de Laat and Haushofer 2013; Vaghri et al. 2013; Bermúdez-Millán et al. 2019; Howells et al. 2022). There is evidence to suggest that early-life experiences may dysregulate the HPA axis, affecting cortisol levels in adulthood (Gunnar and Quevedo 2007). This dysregulation can manifest in different ways, such as hyper- or hypo-responsiveness (Rovnaghi et al. 2021). For instance, the baseline level of cortisol may rise, the peak response to a stressor may diminish, and/or the recovery time from peak to baseline may lengthen. Notably, there is growing evidence that early-life and/or chronic stress ultimately leads to lower cortisol production (Figure 1), contrary to the typical belief that more stress always equates to an increase in cortisol. Individuals who have experienced early-life chronic stress show mixed responses, exhibiting either higher cortisol levels (the 'resistance' phase) or lower levels (the 'exhaustion' phase), both of which can be detrimental to overall health.

Figure 1. *Cortisol levels versus time*



Source: Rovnaghi et al. (2021).

2.2 Advantages and disadvantages of hair cortisol versus alternative measures of cortisol

2.2.1 Advantages

Hair collection has been adopted as a non-invasive, cost-effective and objective medium for measuring cortisol levels, offering several advantages over other media, such as saliva, blood and urine (Toly, Fiala and Shi 2022). One key benefit is that hair cortisol reflects cumulative cortisol secretion over several months. Given that human hair grows approximately 1cm per month, analysing hair samples provides a retrospective measure of long-term cortisol levels (Stalder and Kirschbaum 2012), unlike blood or saliva samples, that only capture cortisol levels at the time of collection (Turpeinen and Hämäläinen 2013) and may be influenced by the collection process (e.g. response to needles). Additionally, hair cortisol measures are relatively stable over time when repeated samples are collected from the vertex posterior of the head. This makes hair collection particularly valuable for detecting the effects of long-term exposure to harmful stressors, in contrast to measures of acute stress that have fewer enduring consequences and are more sensitive to the timing of sample collection.[1]

Furthermore, hair samples are easier to collect, store and transport compared to other bio samples. While blood, urine and saliva samples have been widely used to measure cortisol levels (Hellhammer, Wüst and Kudielka 2009; Bozovic, Racic and Ivkovic 2013; El-Farhan, Rees and Evans 2017), the collection of these samples is complex (and expensive) and in some cases may need to be undertaken by a specialist or in a laboratory. Moreover, these types of samples require immediate refrigeration or freezing and prompt analysis to prevent sample degradation. Improper chain-of-custody management or delays in processing can compromise sample integrity, potentially leading to inaccurate data interpretation or the need to discard samples. In contrast, hair collection does not need to be performed in a laboratory or by a health specialist; it can be done during a regular in-house visit by a trained fieldworker. After being collected, the hair needs to be protected from light and humidity but can otherwise be easily stored at room temperature and analysis can be postponed for years if needed (Russell et al. 2012). These features largely facilitate data collection and lower costs in the field.

Finally, hair collection provides an objective measure of stress that is not prone to survey effects, such as social desirability bias, which is an important advantage for measuring individuals' levels of stress in large-scale studies. In this sense, it can be analysed alongside other subjective measures of stress to provide a holistic picture of participants' mental health. This is relevant to research in LMICs as empirical evidence suggests that the relationship between perceived stress and cortisol is not always consistent across different studies and populations (Faresjö et al. 2013; García-León et al. 2018).

2.2.2 Disadvantages

The collection of hair samples to measure cortisol levels in large-scale studies also poses some challenges and difficulties. First, additional ethical approvals from government agencies

[1] Cortisol levels have been shown to vary over the course of a day, with slight increases in the morning and decreases in the evening (Weitzman et al. 1971).

and participants are needed on top of those related to self-reporting of mental health.[2] Second, training for fieldworkers is more complex and time-consuming, as hair collection protocols must be thoroughly explained and hands-on workshops with volunteers are required to ensure proper practice and adherence to these protocols. Third, participants may have cultural or religious reasons for refusing to provide a hair sample. This can create discomfort with the request and as a result, may lead to a low success rate in hair collection. Even when participants agree to take part in the study, there are challenges in collecting samples that meet the necessary standards for analysis. For example, participants may be bald or have very short hair. Additionally, they may have undergone hair treatments (such as bleaching, colouring, perming or chemical straightening) or may have patterns of continuous exposure to direct sunlight and a high frequency of hair washing, which can also influence the results. However, this evidence is not consistent across studies (Greff et al. 2019).

3 Cortisol study design and protocols for Young Lives Round 7

Round 7 survey activities took place in Ethiopia, India and Peru only.[3] This section provides an overview of the activities involved in pre-fieldwork preparation, including obtaining ethical approvals and preparing materials; the protocol followed by enumerators to inquire consent and identify eligibility, and the instruction during and after hair collection.

3.1 Pre-fieldwork preparation

3.1.1 Ethical approval

The first step for fieldwork preparation is to produce the necessary information to apply for ethical approval. Since the coordination of Young Lives is based at the University of Oxford, ethical clearance had to be obtained first from the university and later from each of the study countries. In Ethiopia, approval was granted by Addis Ababa University, the College of Education Ethical Review Committee and the National Research Ethical Review Committee of the Ministry of Education; in India, from the Institute of Genetics and Hospital for Genetic Diseases and Centre for Economic and Social Studies (CESS) Ethics Committee; and in Peru, from the Research Ethics Committee at the Instituto de Investigación Nutricional (IIN).

3.1.2 Fieldwork manual

A fieldwork manual, including the protocol for hair sample collection, was first prepared in English and later, along with the questionnaire, professionally translated into all the languages spoken by participants in the study countries ahead of the fieldwork training. It contained detailed, step-by-step explanations of the procedure for collecting a hair sample, how to store

[2] Despite this challenge, securing ethical approvals for collecting blood or saliva would likely have been more difficult.

[3] Data was not collected in Vietnam due to a change in government procedures for the international transfer of personal data.

it safely, and how and when to deliver it to a fieldwork supervisor. To provide more clarity, images of all these processes were included in the manual.

3.1.3 Training activities

Supervisors and enumerators from each study country received training from specialists, which included both theoretical and practical sessions. In addition to the fieldwork manual, the training materials comprised presentation slides on cortisol and its importance as well as videos demonstrating how to collect hair samples. With the assistance of local volunteers, each country's team created at least two videos outlining the correct techniques for collecting samples from participants with either long or short hair.

The first training activities in Peru took place in March and April 2023 at the IIN in Lima. Before the training, the research team adapted the translated fieldwork manual to the Peruvian context, considering the specific characteristics of hair samples (types), available materials and potential field constraints. The first training session was held for supervisors, followed by training sessions for enumerators. Feedback from both sessions was incorporated into the fieldwork manual. Since Peru was the first country to run training sessions for fieldworkers, feedback was solicited to refine the protocols, which would later be used in all the other study countries. The training material had three main purposes: explaining what cortisol is and the importance of measuring it in Round 7; the associated questions in the Young Lives questionnaire; and how to correctly collect and store hair samples. Apart from attending presentations, enumerators were also required to practice hair-sample collection with the help of volunteers. During these practical sessions, each enumerator had the opportunity to collect at least two hair samples and benefited from sharing tips with each other.

Training activities in India took place in May 2023 at the CESS in Hyderabad. The training materials were adapted from those originally created for Peru, incorporating lessons learnt from the training activities and pilot conducted there. The supervisors' training session lasted half a day and was followed by a practical hair collection activity, allowing supervisors to apply what they had learnt. Later in May, cortisol training activities for enumerators were organised, spanning several days to ensure comprehensive instruction and practice. The enumerators filmed two example videos for collecting short and long hair samples.

Training activities in Ethiopia took place during August and September 2023 at the Policy Studies Institute (PSI) in Addis Ababa. The training content was essentially the same as used in India; however, some new elements were introduced due to distinctive cultural factors that make hair collection more challenging in Ethiopia. For instance, in some areas there is the belief that hairlocks could be used for satanism or sorcery.

3.1.4 Pilots

Apart from the small-scale practical sessions that followed the cortisol training activities, each country tested the cortisol module on a much larger scale during the pilots for the Round 7 collection. In Peru, the cortisol section of the questionnaire and the protocols for the collection of hair samples were assessed in late May 2023 in a pilot implemented in Cañete (a province south of Lima). Throughout the pilot, the hair sampling process was closely monitored and support provided for sample collection, along with a review of key concepts related to the cortisol module.

The Ethiopian team also tested participants' willingness to hypothetically provide a hair sample (i.e. if they were asked to) in a pre-pilot in ten sites in Tigray, Amhara, Oromia and Addis Ababa in May 2023, following their training activities. Several difficulties related to cultural beliefs regarding hair collection were detected and addressed before the training and the wider study. India was the last country to carry out trials of the cortisol module in a pilot that took place in July 2023.

3.2 Consent and eligibility

All Young Lives participants were invited to provide a hair sample and requested to sign a consent form. The form included information about the nature, purpose and risk of the study and it was separate from the general Round 7 data collection consent form. Therefore, participants could take part in the Round 7 survey without participating in the hair collection. Upon reading the consent form, enumerators registered whether the participant agreed to provide a small lock of hair. When asking them to provide their consent, fieldworkers were required to show participants a picture of an example hair sample that they carried with them to provide a clear illustration of how the sample would be collected in practice and how much hair would be taken. If participants hesitated or were not inclined to provide a sample, enumerators were instructed to show them a pre-recorded video of the hair sample collection process. To provide participants with a realistic view of what the process would entail, videos were pre-recorded for each country, featuring both women and men.

Before proceeding with the collection of a hair sample, fieldworkers were required to check the participants' eligibility to take part in the cortisol study. There were two eligibility criteria:

1. Length of the hair: fieldworkers were instructed to determine whether their hair length was at least 3cm by simple observation, if possible, or by using a comb and a ruler (see Figure 2). Participants were eligible for hair collection only if their hair, when pulled straight, measured at least 3cm. For individuals with coiled or kinky hair, the eligibility criterion was a minimum length of 2cm.
2. Health conditions: participants were asked whether they had ever received a diagnosis of hypo- or hypercortisolism, such as Addison's disease or Cushing's syndrome. Both diseases cause abnormal levels of cortisol; therefore, participants who reported having these issues were not eligible for the cortisol study.

After checking for the two eligibility criteria, fieldworkers asked participants to reconfirm their consent to participate in the cortisol collection. If participants decided to withdraw, they were encouraged to provide an explanation for their decision. The enumerators then provided a detailed explanation of the study's nature, objectives and the intended use of the data to address any potential concerns. If participants still chose not to participate after this clarification, fieldworkers respected their decision and moved on to the next section of the Young Lives questionnaire. Fieldworkers recorded the reasons (e.g. cultural) why a participant declined to participate.

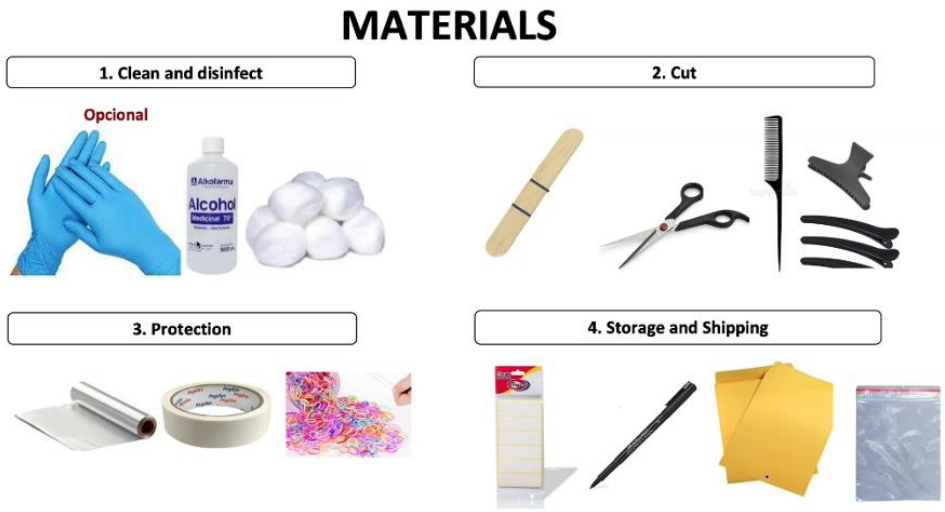
In some instances, participants may have agreed to provide a lock of hair but were unable to do so for practical reasons, such as having very short hair or products in their hair. As a result, it became necessary to arrange follow-up visits for sample collection. In these cases, the country teams recommended contacting participants by phone first to confirm their availability for a revisit. This also allowed them to verify that participants still consented to provide a sample and to ensure that their hair was of the appropriate length or condition for

collection. Costs and logistics (for example, if the team was still in the cluster or in a nearby area) were also considered to decide whether it was worth revisiting the participant.

3.3 Protocol for enumerators' hair sample collection

Figure 2. *Materials required for hair sample collection*

The collection of hair samples was fully integrated into the protocol and plan for Round 7 data collection. Procedures were performed just before the self-administered questionnaire, for which enumerators needed to find a quiet space where the respondents had some privacy (e.g. away from other household members and external interference). This section outlines the step-by-step procedure followed by fieldworkers.



Source: Training material for Round 7 data collection.

Step 1. Check the material needed for the hair sample collection. Before starting the procedure, fieldworkers were required to verify they had all the necessary equipment in place (see Figure 2).

Step 2. Prepare the working area. Fieldworkers instructed the respondents to sit comfortably, preferably in a chair, and aimed to create a relaxed atmosphere, ideally in a private space. Next, they sanitised the comb, scissors, hair clippers and their hands. Hair elastics, aluminium foil, tape, envelopes (either paper or plastic) and a marker were then placed on a surface, ready for use.

Step 3. Put on gloves (optional). If gloves make it difficult to collect the sample, particularly in cases of short hair, the fieldworker may decide not to use them. In such cases, they were required to wash and sanitise their hands before and after collecting the sample.

Step 4. Prepare what is needed to store the hair sample. Fieldworkers prepared a piece of aluminium foil and a paper or plastic envelope (depending on the country). The envelope was labelled with the participant ID, date and time. In Peru, the team also included location and collector ID details. If the hair sample was short, the enumerator labelled it without using a rubber band. The pre-filled label to be attached to the hair sample once collected was double-checked and any missing or incorrect information was updated.

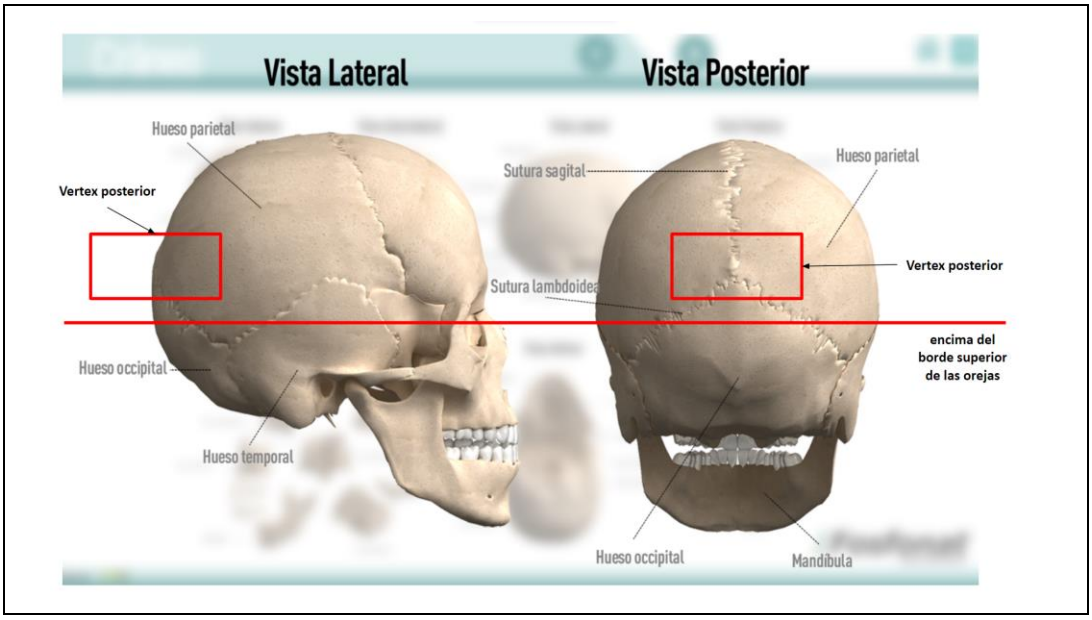
Step 5. Check consent and pre-existing health condition eligibility criteria. Fieldworkers were required to double-check that the participant had consented to be part of the hair sample study and that they did not have a prior diagnosis of hypo- or hypercortisolism.

Step 6. **Explain the procedure to the participant**.

Step 7. **Ask the participant to sit up straight in a chair, keep still and face forward, with their chin parallel to the floor**.

Step 8. Identify the vertex posterior region. The enumerator located the vertex posterior at the back of the respondent's head (see Figures 3 and 4) by placing their fingers on the tops of the respondent's ears and creating a horizontal 'line' with their hands. The thumbs were brought together at the centre of the respondent's head, allowing the enumerator to identify the vertex posterior as a circular area approximately 5cm in diameter just above this point. The hair sample could then be collected from any location within this circle. Finally, the enumerator verified that the respondent's hair met the required length.

Figure 3. *Vertex posterior 1.*



Source: Fieldwork manual for Round 7 data collection.

Step 9. Prepare the lock of hair. First, the enumerator sectioned off a 1cm x 1cm area using a comb. If necessary, the enumerator used hair clips to pin back the surrounding hair (see Figure 5). If the hair sample was shorter than 5cm, an elastic band was placed near the root before cutting the hair. Only one lock of hair was required, with an approximate thickness similar to a rubber band of 1cm in diameter, which should weigh about 20mg (see Figure 6). When possible, the enumerator should select a spot in the vertex posterior region that would be naturally covered by the rest of the participant's hair after collection.

Step 10. Collect the hair sample. To cut a lock of hair, the enumerator positioned the scissors as close to the roots as possible while keeping the blades flat against the scalp. For long hair, the sample should not be cut to shorten it; the lock of hair must retain the same length as the participant's hair.

Step 11. Attach the hair sample to aluminium foil. All hair samples had to be protected from light and contamination by using a piece of aluminium foil. The procedure to attach the hair sample depended on the length of the lock of hair:

- For a hair lock longer than 5cm: The sample had to be attached to the foil using tape only, ensuring that the tape does not cover the 3cm of interest. The tape was placed at the 5cm mark to keep the sample aligned. Additionally, an arrow was drawn on the tape with a permanent pen or marker, pointing towards the hair roots (see Figures 7 and 8). The remainder of the hair was rolled and then carefully wrapped in foil.
- For a hair lock shorter than 5cm but longer than 3cm: No tape was used. Instead of tape, an elastic was used to tie the hair lock near the roots, trying to keep the sample aligned (see Figures 7 and 8). Then, the tied hair sample was placed on the aluminium foil and securely packed.
- For a hair lock shorter than 3cm: No hair elastic or tape was used, to prevent damaging the small sample. The hair lock was wrapped in foil, folding the pack very tightly to keep the sample secure and protected from light.

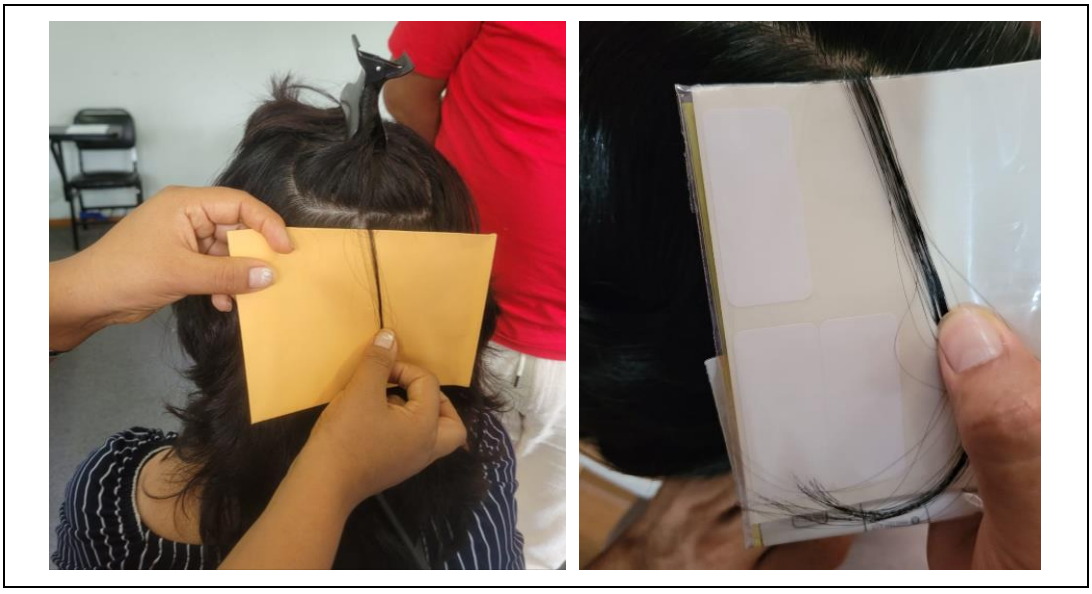
Figure 4. *Vertex posterior 2*

Source: Fieldwork manual for Round 7 data collection.

Figure 5. *Vertex posterior 3*

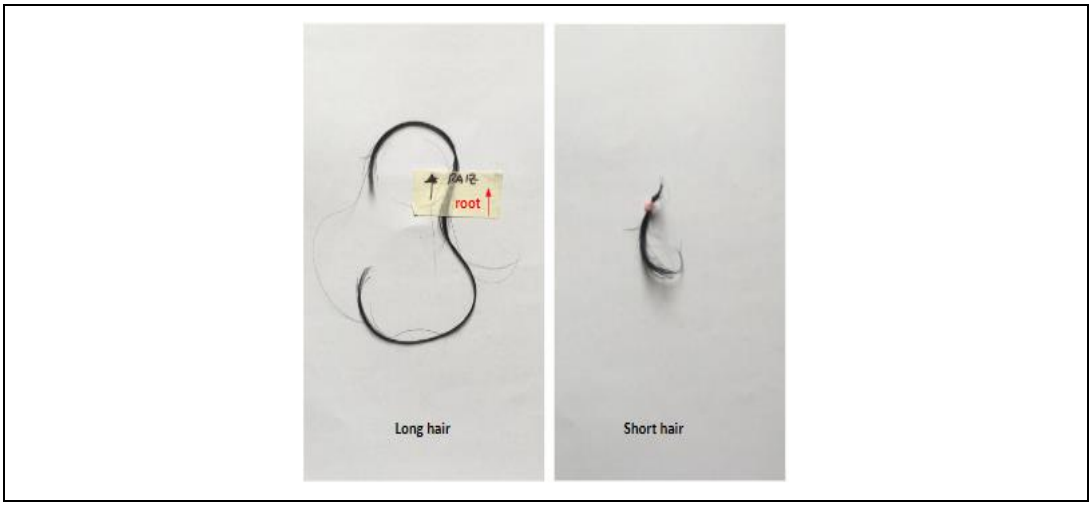
Source: Fieldwork manual for Round 7 data collection.

Figure 6. *Minimum hair sample collection*



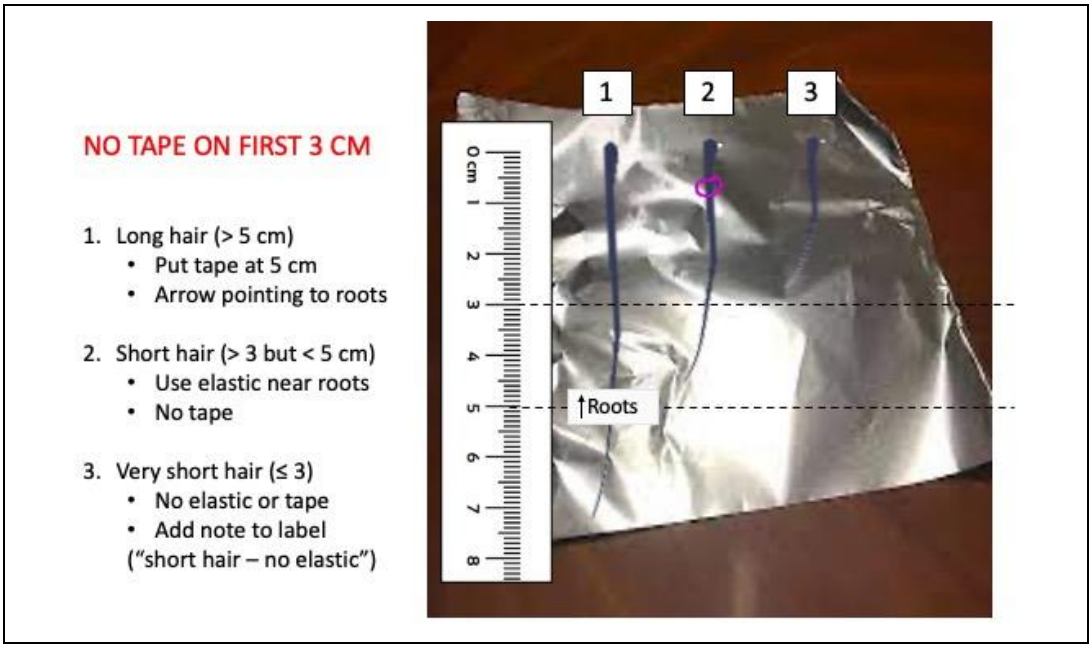
Source: Fieldwork manual for Round 7 data collection.

Figure 7. *Examples of hair samples*



Source: Fieldwork manual for Round 7 data collection.

Figure 8. *Types of hair samples*



Source: Fieldwork manual for Round 7 data collection.

Step 12. Label and pack the hair sample. A label containing the participant ID, date, time, location and collector ID was affixed to the foil package. Alternatively, this information could be written directly on the foil. If the hair sample was short and not secured to the foil with tape or tied together with an elastic band, a note stating 'short hair – no elastic' was included. Next, the foil package containing the hair sample was placed into either a paper envelope or a small zip-lock bag. If it had not yet been labelled, the paper envelope or small zip-lock bag was labelled with exactly the same information written on the foil package.

3.4 Post-cortisol collection survey questions

Directly after obtaining either the hair sample or rejection from participants, enumerators registered whether the hair sample was successfully obtained. If the sample was taken, they recorded the date and time of collection, along with the ID of the fieldworker who took the sample. If the sample could not be obtained, the enumerator provided the reasons for this, from the following options:

- Participant refused at the last minute
- Participant moved too much
- Hair was not long enough
- Equipment did not work
- Equipment was missing
- Other, to be specified.

Some additional data was recorded for participants who provided a hair sample, which is important to consider when analysing the cortisol data. First, participants were asked about their natural hair colour and how often they wash their hair. Second, they reported whether their hair is frequently exposed to high heat (due to the use of hair dryers, hair straighteners, hair curlers, etc.) and how frequently this exposure occurs. Third, the questionnaire enquired whether participants have had any hair treatment over the past three months, such as bleach, colour, perm, chemical straightening or relaxer, and if so, how long ago that treatment took place was also recorded. Fourth, participants were asked how many hours a day their hair is exposed to direct sunlight and whether they regularly wear a hat, scarf or any other garment to protect their hair from the sun. Fifth, participants reported whether they take over-the-counter medications for any scalp conditions and whether they have taken steroids (such as inhaled medications, steroid injections in a joint, or other oral steroids such as prednisone, dexamethasone or aldosterone) in the last three months. If participants reported having taken steroids, they were asked to specify how recently they had used these medications.

3.5 Protocols for country teams' storage and transportation of hair samples

Since the processes of storing and transporting hair samples involved several stages performed by different country team members, protocols were defined for each of them. The materials recommended in these protocols are summarised in Figure 9.

3.5.1 Protocol for enumerators

While enumerators were still collecting data and hair samples within a specific cluster, they kept the hair samples with them. During this stage of fieldwork, it was important to place all paper envelopes containing hair samples into a canvas bag (Figure 9, No. 1) and store the bag in a dark location. This provided additional protection from sunlight for the samples. When enumerators arrived at the base of their designated cluster, they gave all the hair samples to their fieldwork supervisors.

Figure 9. *Materials used for storage of collected samples*

(1) Dark canvas bag; (2) sealed plastic box; (3) opaque locked box or drawer cabinet; (4) large zip lock bags (optional); (5) silica gel sachets (optional).

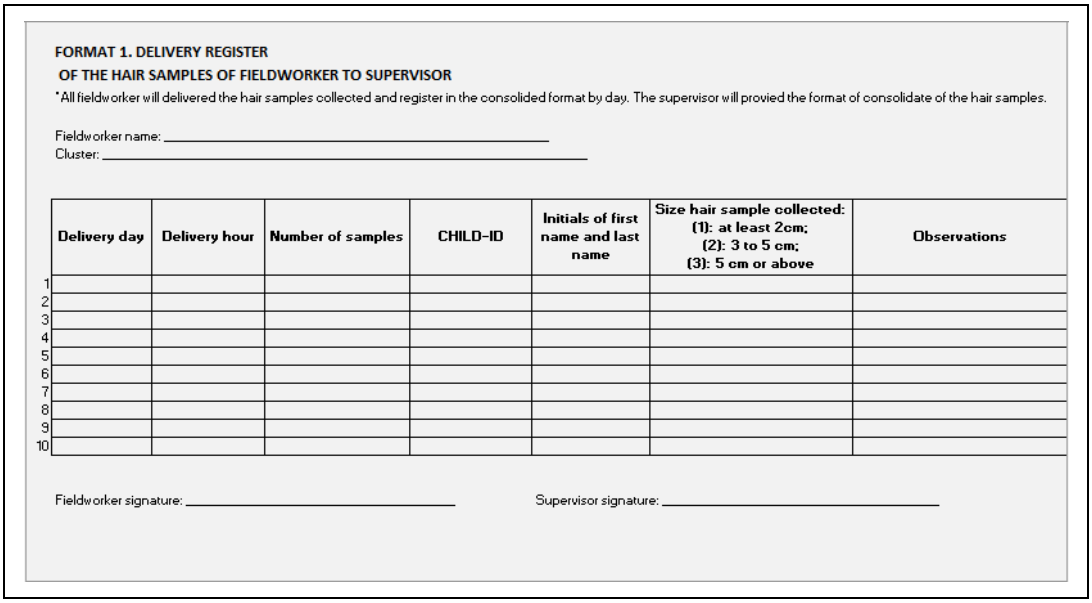
(1) (2) (3) (4) (5)

3.5.2 Protocols for fieldwork supervisors

Once supervisors received the hair samples from enumerators, they stored them at room temperature in one or more sealed plastic boxes (Figure 9, No. 2) and registered the date and time at which the supervisor received each of the samples. The format for this registration must contain: name of the fieldworker who collected the sample, name of the supervisor who received the sample, date and time of the sample delivery, the correlative number of samples per fieldworker per day, identification information of each sample (participant ID and initials of their names, as they appear on the label of each sample), and the approximate size of the collected hair sample. Figure 10 illustrates the format for the registry of collected samples.

After completing data collection in a cluster, which usually takes around 15 days, samples must be packaged in a sealed box containing approximately 100 samples – this corresponds to the typical number of participants in each cluster. These boxes were then sent to the headquarters in each country using specialised courier services. For remote areas, the boxes were shipped by air. A similar procedure was followed for Young Lives participants who have migrated and now live far from their original clusters.

Figure 10. *Delivery register of the hair samples collected by fieldworker to supervisor (example from Peru)*



FORMAT 1. DELIVERY REGISTER
OF THE HAIR SAMPLES OF FIELDWORKER TO SUPERVISOR

*All fieldworker will delivered the hair samples collected and register in the consolided format by day. The supervisor will provied the format of consolidate of the hair samples.

Fieldworker name: _____
Cluster: _____

	Delivery day	Delivery hour	Number of samples	CHILD-ID	Initials of first name and last name	Size hair sample collected: (1): at least 2cm; (2): 3 to 5 cm; (3): 5 cm or above	Observations
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							

Fieldworker signature: _____ Supervisor signature: _____

3.5.3 Protocol for countries' headquarters

Once received at each country's headquarters, plastic boxes containing hair samples were stored at room temperature in an opaque locked box or drawer cabinet (Figure 9, No. 3) until the data collection in that country had been completed (from four to six months, depending on the country). The date of arrival of all hair samples at the headquarters was recorded in a delivery register similar to that used by fieldwork supervisors (Figure 10). During the period that hair samples stayed at countries' headquarters waiting to be sent to a laboratory for analysis, it was recommended that the box or cabinet that contained them be kept fully closed and locked in order to avoid exposure to sunlight.

In cases of high humidity at the country's headquarters, paper envelopes or small bags containing individual hair samples were placed in large plastic zip-lock bags (hermetic) along with silica gel sachets, prior to storing them in a locked box or drawer cabinet. While zip-lock bags prevented humidity from penetrating the samples, the silica gel sachets absorbed any excess humidity inside the plastic bags. In Peru, the sachets were replaced every three months due to humidity.

4 Organisation of fieldwork and hair-sample collection results

4.1 Dates of data collection

Peru was the first country to start Round 7 data collection, on 20 June 2023. Although the main part of the fieldwork in Peru was completed by early December 2023, it was extended until 31 January 2023 due to the need to search for migrants. Hair samples and cortisol-associated data were collected over all that period. As some participants from Lima were unable to provide hair samples on the day of their interview because their hair was not long enough, the country team decided to revisit them later to collect the samples. Out of 44 participants who qualified for a second attempt at hair collection, the team successfully obtained 11 samples by the end of the fieldwork.

Fieldwork in India started on 2 August 2023. Similar to Peru, the main stage of data collection concluded on 1 November 2023, but the search for participants who had recently migrated within the country extended the fieldwork activities, including the collection of hair samples, until 25 January 2024. During fieldwork, the country team found 163 respondents who gave their consent to give a hair sample, but several practical reasons – including lack of time and short hair – prevented them from doing so on the interview day. In response, the country team coordinated revisits via phone with those participants and successfully collected hair samples in 103 cases.

In Ethiopia, fieldwork first commenced in Addis Ababa, the Southern Nations, Nationalities, and Peoples' (SNNP) region and Oromia on 13 October 2023 and was then extended to Tigray and Amhara during the same month. The main part of the fieldwork, which included hair sample collection, concluded on 5 January 2024. Due to the ongoing conflict in certain areas and the risks for fieldworkers, it was necessary to conduct a phone survey instead of an in-person survey in two Amhara cities. The phone survey started on 2 April 2024 and finished on 30 April 2024, but no hair samples were collected at this stage. Like Peru and India, Ethiopia also organised revisits, not only to recover hair samples that could not be collected on the first visit due to short hair but also to amend data-entry errors in the cortisol section of the questionnaire. This was the case for 65 participants.

4.2 Hair-sample collection results and success rates

Table 1 summarises the total sample collection in the three study countries of Round 7: Ethiopia, India and Peru. The study collected 5,230 hair samples, corresponding to an overall success rate of 75% of cortisol collection (total number of hair samples out of total number of Round 7 interviews). Most participants (85%) consented to participate in the study but not all were eligible due to various reasons.[4] However, almost all eligible participants provided a hair sample. From the total hair samples, 3,847 were from Younger Cohort participants (aged 21)

[4] Eligible participants were those who provided informed consent, had sufficiently long hair and did not have a diagnosis of hypo- or hypercortisolism.

and 1,383 from Older Cohort participants (aged 29). The higher collection among the Younger Cohort was attributed not only to the larger sample size but also to higher consent, eligibility and success rates. Women had a lower consent rate than men but their success rate was higher, which resulted in a higher number of samples from this group. This was mainly due to the larger proportion of men who were ineligible due to having short hair.

Table 1. *Number of hair samples collected and success rates for Young Lives Round 7*

Sample size	Younger Cohort			Older Cohort			Both cohorts		
	Men	Women	Total	Men	Women	Total	Men	Women	Total
Round 7 interviews	2,535	2,399	4,934	1,014	982	1,996	3,549	3,381	6,930
Consenting participants	2,281	2,006	4,287	862	758	1,620	3,143	2,764	5,907
(% total interviews)	**90%**	**84%**	**87%**	**85%**	**77%**	**81%**	**89%**	**82%**	**85%**
Eligible participants	1,919	1,952	3,871	649	741	1,390	2,568	2,693	5,261
(% total interviews)	**76%**	**81%**	**78%**	**64%**	**75%**	**70%**	**72%**	**80%**	**76%**
Hair samples collected	1,902	1,945	3,847	645	738	1,383	2,547	2,683	5,230
(% total interviews)	**75%**	**81%**	**78%**	**64%**	**75%**	**69%**	**72%**	**79%**	**75%**

Table 2 presents the same information but at the country level. India and Peru exhibited high success rates of more than 85% for participants from both cohorts. This is mainly due to these countries' high consent and eligibility rates. Ethiopia had considerably lower success rates, close to 49% overall. The main reason for this was the high number of participants with short hair in the vertex posterior, which made them ineligible. Table 2 also reveals that the differences in success rates by both participants' cohort and gender detected for the whole Round 7 sample hold for Ethiopia and Peru. Section 5.2 provides more in-depth analysis of the plausible reasons for these differences across cohorts and countries.

Table 2. *Number of hair samples collected and success rates for Young Lives Round 7 by country*

Ethiopia									
Sample size	**Younger Cohort**			**Older Cohort**			**Both cohorts**		
	Men	**Women**	**Total**	**Men**	**Women**	**Total**	**Men**	**Women**	**Total**
Round 7 interviews	722	684	1,406	333	299	632	1,055	983	2,038
Consenting participants	585	436	1,021	250	163	413	835	599	1,434
(% total interviews)	**81%**	**64%**	**73%**	**75%**	**55%**	**65%**	**79%**	**61%**	**70%**
Eligible participants	352	392	744	120	147	267	472	539	1,011
(% total interviews)	**49%**	**57%**	**53%**	**36%**	**49%**	**42%**	**45%**	**55%**	**50%**
Hair samples collected	352	387	739	122	144	266	474	531	1,005
(% total interviews)	**49%**	**57%**	**53%**	**37%**	**48%**	**42%**	**45%**	**54%**	**49%**

Table 2. *Number of hair samples collected and success rates for Young Lives Round 7 by country continued*

India									
Sample size	**Younger Cohort**			**Older Cohort**			**Both cohorts**		
	Men	**Women**	**Total**	**Men**	**Women**	**Total**	**Men**	**Women**	**Total**
Round 7 interviews	987	839	1,826	410	437	847	1,397	1,276	2,673
Consenting participants	914	740	1,654	361	368	729	1,275	1,108	2,383
(% total interviews)	**93%**	**88%**	**91%**	**88%**	**84%**	**86%**	**91%**	**87%**	**89%**
Eligible participants	873	735	1,608	331	368	699	1,204	1,103	2,307
(% total interviews)	**88%**	**88%**	**88%**	**81%**	**84%**	**83%**	**86%**	**86%**	**86%**
Hair samples collected	861	733	1,594	328	368	696	1,189	1,101	2,290
(% total interviews)	**87%**	**87%**	**87%**	**80%**	**84%**	**82%**	**85%**	**86%**	**86%**

Peru									
Sample size	**Younger Cohort**			**Older Cohort**			**Both cohorts**		
	Men	**Women**	**Total**	**Men**	**Women**	**Total**	**Men**	**Women**	**Total**
Round 7 interviews	826	876	1,702	271	246	517	1,097	1,122	2,219
Consenting participants	782	830	1,612	251	227	478	1,033	1,057	2,090
(% total interviews)	**95%**	**95%**	**95%**	**93%**	**92%**	**92%**	**94%**	**94%**	**94%**
Eligible participants	694	825	1,519	198	226	424	892	1,051	1,943
(% total interviews)	**84%**	**94%**	**89%**	**73%**	**92%**	**82%**	**81%**	**94%**	**88%**
Hair samples collected	689	825	1,514	195	226	421	884	1,051	1,935
(% total interviews)	**83%**	**94%**	**89%**	**72%**	**92%**	**81%**	**81%**	**94%**	**87%**

5 Assessment of protocols for hair sample collection in Young Lives Round 7

The Round 7 questionnaire provided enumerators with the opportunity to include open comments in the cortisol module for each participant, which were recorded in the databases for further analysis. Additionally, all enumerators had the opportunity to provide feedback about the whole process and to assess each section of the study by completing the enumerators' survey. Each country team also organised an in-person feedback session to bring enumerators and fieldworkers together to reflect on the successes and setbacks of the fieldwork.

Apart from the enumerators' accounts, interviews with countries' project coordinators, field coordinators and researchers, as well as with the experts who defined the protocols for the Young Lives cortisol-data collection, were conducted with the purpose of better understanding the implementation of cortisol protocols in each country and the main strengths, difficulties and challenges of the cortisol collection process. This section presents the main results of all these assessments.

5.1 Enumerators' survey results

Enumerators from Peru positively assessed the hair sample collection protocol and related questions, describing them as easy to follow. This part of the Round 7 survey was generally positively received, with almost 87% of enumerators indicating that it was good or very good. Moreover, the proportion of negative assessments (measured as enumerators who assessed the section as 'bad' or 'very bad') was around 3%, with ten other sections of the survey receiving worse assessments. Despite this, the hair sample procedure was the section that received the most rejections from participants in Peru, compared to other sections such as the reading comprehension test and self-administered questionnaire. Therefore, the Peru team suggested including a variable inquiring about reasons for rejection (no consent) in future rounds of data collection.

In India, the proportion of enumerators who evaluated this section of the survey as 'good' or 'very good' was very high (93%). When combining the percentages of enumerators who rated the cortisol section of Young Lives as 'bad' (3%) or 'very bad' (3%) and considering their average overall assessment, this section received the second-worst evaluation in India.

Among the three countries studied, Ethiopia had the lowest evaluation of this section in Round 7. This was largely due to the significant challenges that enumerators faced in explaining the module's purpose to participants and in obtaining their consent. Only 50% of enumerators rated the module as 'good' or 'very good', while nearly 20% considered it 'bad' or 'very bad'. These results indicate that it was the worst-rated section of the Young Lives study in Ethiopia.

5.2 Country teams' assessment of hair sample collection

Analysis of all the information provided by the three country teams reveals that the fieldwork manual, the clear protocols, the training sessions (both theoretical and practical) and the experience acquired during the pre-pilots and pilots of the study were the main facilitating factors that enabled the success of the cortisol collection process.

In particular, country teams emphasised that the complementarity among the various elements of the fieldwork preparation was crucial in refining the protocols prior to data collection. For instance, the Peru team described how the comments and feedback provided by enumerators (especially those who have been part of several rounds of the Young Lives study) during both the theoretical and practical training sessions led the authors of the training material to introduce several changes to the fieldwork manuals. The Ethiopia country team agreed that the pre-pilot was crucial in anticipating certain difficulties with collecting hair samples that had not been observed in the other two countries and in preparing fieldworkers to respond to them. For example, cultural and religious objections to providing hair samples were identified, as well as the common practice of using butter in hair and braiding, which meant additional challenges to hair sample collection. In response, recommendations for addressing these situations were added to both the fieldwork manual and the training sessions.

In terms of the practical application of protocols, the most common challenges, obstacles and difficulties that country teams identified that they encountered during the cortisol collection process are set out below.

5.2.1 Obtaining participants' consent

All country teams found it both difficult and time-consuming to explain to participants the objectives of the hair collection and the destination of the samples. In India, enumerators perceived some distrust from participants regarding the intended use of hair samples. Despite this, the India team achieved high success rates, which they attributed to participants' familiarity with fieldworkers, as many had worked with them in several previous rounds of the Young Lives study.

In Peru, where success rates were also high, the country team emphasised the importance of preparing adequate communication material about the sample collection procedure to be presented to participants along with the informed consent form. In particular, according to the Peru team, the use of laminated hair samples, which showed the exact amount of hair needed for cortisol analysis (see Figure 11), helped to obtain consent, as participants could observe that the amount of hair they were being asked to provide was very small.

Figure 11. *Example of laminated hair sample used to facilitate informed consent in Peru*

Ethiopia was the country that faced the highest proportion of rejections. Fieldworkers reported that they had spent considerable time and effort attempting to obtain consent, which extended the total interview time. This was particularly problematic in some areas due to beliefs that hair locks could be used for satanism or sorcery. The Ethiopia team also observed that a very important factor in obtaining consent is that close family members are not against hair collection. For instance, during the data collection process in SNNP, some enumerators had to return hair samples to participants the day after collection, due to the disapproval of relatives, who insisted on getting their hair back. The issue of relatives trying to convince participants not to give hair samples was also observed in India, although no samples had to be returned as a consequence. The Ethiopia team proposed that, in future rounds of the cortisol study, enumerators should encourage participants to seek approval from their close family members before providing a hair sample, to avoid initial consent later being denied after participants discuss it with their spouses or parents.

Participants expressed several cultural and religious reasons for their reluctance to provide hair samples, as well as concerns about distrust. For instance, some participants in India indicated that they had offered their hair to a deity, while some Peruvian participants had made a promise not to cut their hair until after their child was born. Additionally, Muslim women in India and Ethiopia mentioned that they are not permitted to show their hair in public. There were also aesthetic concerns; fieldworkers noted that some participants feared that the absence of a hair lock would be noticeable.

5.2.2 Securing a hair sample after consent

In the three countries, the main obstacle to obtaining a hair sample from participants who had already agreed to provide one was too short hair or head shaving. The fact that these two situations are more prevalent in men than in women explains why the success rates for hair collection were considerably higher for women than for men. Another factor that made it difficult to get hair samples from some was the lack of time to perform the procedure, as the interviews were already lengthy. The country team in India noted that this issue primarily affected participants from urban areas who had long working hours. This is consistent with the finding that success rates were higher for 21-year-olds than for 29-year-olds, since the latter are more likely to have full-time jobs outside the home. Country teams dealt with these difficulties by organising revisits for hair collection at a later date (see Section 4.1), typically by the end of fieldwork. The coordination of these revisits via phone calls provided participants with the opportunity to choose a more convenient time to provide a hair sample, as well as to have longer hair compared to the first attempt. Apart from those two obstacles, 'participant refusing at the last minute' and 'participant moving too much during the procedure to obtain a hair sample' were also mentioned as difficulties in obtaining hair samples by enumerators in Ethiopia.

5.2.3 Using and carrying hair-collection material

In general, all the country teams positively assessed the usefulness of the material for collecting and storing hair samples. However, many fieldworkers mentioned the inconvenience of carrying all these implements, besides the anthropometrics material, when conducting interviews in remote rural areas, when going to distant areas looking for migrants or when attempting cortisol revisits, due to their large volume and heavy weight. Therefore, they recommended assessing the total weight of materials enumerators need to carry before acquiring them for future rounds of the study.

Although the protocol recommended using gloves for sanitary reasons, fieldworkers found it very difficult to cut a lock of hair while wearing them. As a result, they often chose not to wear gloves and instead carefully sanitised their hands before and after collecting hair samples. Because of this, the India team suggested that if gloves are to be used in future study rounds, thinner and tighter gloves should be provided. The country teams also noted that if glove use is not recommended in future rounds, it would be worth evaluating whether the questionnaire should identify specific health conditions prior to sample collection and whether additional hygiene and safety protocols should be included when participants have any of those conditions.

5.2.4 Storing and preserving hair samples

As described in Section 3.5, light and humidity are the main threats to the preservation of hair samples, so the protocols are intended to describe the optimal conditions for their maintenance before being sent to a laboratory for analysis. Although the three country teams followed these, there was some cross-country variation in their application. For instance, regarding the storage of samples, the Peru country team used large zip-lock bags with silica gel sachets to store groups of paper envelopes containing individual hair samples (previously packed in aluminium foil). The India team did not use paper envelopes but rather small zip-lock bags to store each of the aluminium foil packages containing the hair samples. The

Ethiopia team only used paper envelopes to store individual hair samples already wrapped in aluminium foil and did not use zip-lock bags.

The decisions on storage materials depended on humidity levels, storage duration and the availability and cost of materials locally. For example, the Peru team's choices reflected the country's high humidity levels – over 80% year-round – and the fact that it was the first country to start fieldwork, requiring longer storage of samples. The Peru team also carefully monitored humidity and replaced silica gel sachets once they absorbed enough water. Although Addis Ababa in Ethiopia also experiences relatively high humidity – up to 80% in August and an annual average near 60% – zip-lock bags were hard to find and silica gel sachets were costly, so they were not used. In India, with a lower average humidity of about 45%, the team decided that small zip-lock bags were enough to protect the samples.

A retrospective analysis of the strategies employed by country teams suggested that the use of silica gel should be included in protocols as mandatory and systematic, rather than optional only in countries with high humidity levels during fieldwork months. As seen, although only the Peru team used silica gel sachets, the levels of humidity in Ethiopia can also be high, which could potentially reduce the quality of the hair samples collected there.

Regarding the possibility that excess light or high temperatures could damage the samples, fieldworkers from Ethiopia suggested that the protocols should include more precise instructions on how to protect samples after collection and before handing them over to their supervisors. This is especially important during periods when fieldworkers are constantly on the move and often staying in rented accommodation. In particular, some fieldworkers reported uncertainty about whether certain places where they stayed and kept the hair samples were adequate enough to meet the protocol of 'storing samples in a dark room at room temperature'.

5.2.5 Transporting hair samples to country teams' headquarters

With respect to the transportation of samples to country teams' headquarters, the three teams also proceeded in a slightly different way. Fieldwork supervisors in Peru opted to pack the plastic boxes containing hair samples in cardboard boxes and send them to Lima by land transportation once the fieldwork had been completed in each cluster. In contrast, the India team instructed supervisors to hand over all the hair samples collected at their sentinel sites in person once a month to its headquarters in Hyderabad. In the case of Ethiopia, hair samples were transported to the country team's headquarters in two phases. In the first phase, which occurred in the middle of the fieldwork process, supervisors from Oromia and Addis Ababa were asked to bring the hair samples to Addis Ababa in person, and the samples from SNNP and Tigray were personally collected by the country's project coordinator and field coordinator, respectively. In the second phase, all supervisors were required to bring the remaining hair samples to the headquarters in Addis Ababa, once the fieldwork had been completed.

Regardless of their chosen means of transportation, all the country teams evaluated this phase of the process positively and reported no major difficulties. The Peru team also emphasised that, at some point, they evaluated the possibility of using air transportation to send the samples to its headquarters in Lima; in the end, they opted for a land-based postal service because airlines have stricter protocols for carrying biological samples, which would have made the transportation process more complicated.

6 Conclusions and looking forward

As part of the its research agenda centred on mental health and well-being during Round 7, Young Lives provided informed consent and requested a hair sample from all study participants in Ethiopia, India and Peru (no Round 7 data collection took place in Vietnam). Despite the challenges noted by the country teams in the field, the operation as a whole was successful. Overall, Young Lives obtained hair samples from 75% of the study participants – 49% in Ethiopia, 86% in India and 87% in Peru. Most participants consented to participate in the study (85%), although not all were eligible, primarily due to having short hair. The steps taken to anticipate challenges and consider cultural factors significantly contributed to the success of this data collection. In particular, the previous preparation, including the adaptation of sample collection based on cultural and contextual factors, as well as the explanations provided during the informed consent process, along with training and pilot procedures, were crucial.

These samples will be used to measure cortisol levels (hair cortisol) with the aim of identifying patterns of long-term stress between and within country samples, the impact of shocks and crises, as well as the interrelation between stress and other dimensions of human development, thereby improving our understanding of the causes and consequences of long-term stress.

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Young Lives

A Longitudinal Study of Poverty & Inequality

About Young Lives

Young Lives is an international study of poverty and inequality, following the lives of 12,000 children in four countries (Ethiopia, India, Peru and Vietnam). Young Lives is a collaborative research programme led by a team in the Department of International Development at the University of Oxford in association with research and policy partners in the four study countries.

Through researching different aspects of children's lives across time, we seek to improve policies and programmes for children and young people.

Young Lives Research and Policy Partners

Ethiopia

- *Policy Studies Institute*
- *Pankhurst Development Research and Consulting plc*

India (Andhra Pradesh and Telangana)

- *Young Lives India*
- *Centre for Economic and Social Studies, Hyderabad (CESS)*

Peru

- *Grupo de Análisis para el Desarollo (GRADE)*
- *Instituto de Investigación Nutricional (IIN)*

Vietnam (from Round 1–6)

- *Centre for Analysis and Forecast, Viet Nam Academy of Social Sciences (CAF-VASS)*
- *General Statistics Office of Viet Nam (GSO)*

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