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Evaluating Health and Disease in Sub-Saharan Africa: Minimally Invasive Collection of Plasma in the Malawi Longitudinal Study of Families and Health (MLSFH)

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Abstract

Background: The collection of biomarker-based indicators of adult health and fitness is an important addition to socioeconomic surveys since these indicators provide valuable insights into the biological functions, and the complex causal pathways between socioeconomic environments and health of adult individuals. Other than select Demographic and Health Surveys (DHS), there are almost no population-based sources of biomarker-based indicators of adult health in sub-Saharan Africa (SSA), where most population-based biologic data are focused on HIV, other STDs, malaria, or nutritional status. While infectious diseases—such as HIV and malaria—attract the majority of research and NGOs attention in sub-Saharan Africa, there is an important need to understand the general determinants of adult health in SSA since the region will rapidly age in the next decades in ways that are significantly different from the aging patterns in other developing regions due to the AIDS epidemic, and chronic diseases will increasingly become relevant for understanding the health of sub-Saharan populations. **Methods and Design:** We document our protocol for the collection of biomarker-based health indicators as a pilot project within the Malawi Longitudinal Study of Families and Health (MLSFH), and we provide basic descriptive information about the study population and the collected biomarker-based indicators of adult health obtained from respondents in rural Malawi. LabAnywhere kits were used to obtain blood plasma from 980 adult men and women living in Balaka, the southern-most region in rural Malawi. The procedure allows for the non-invasive collection of blood plasma, but has not been previously used in the context of a developing country. We collected biomarkers for inflammation and immunity, lipids, organ function, and metabolic processes. We specifically collected wide-range CRP, total cholesterol, LDL, HDL, total protein, urea, albumin, blood urea nitrogen, creatinine, random blood glucose and HbA1c assays. Overall, the mean values of the biomarkers are below the lower limits of clinical guidelines for adult populations in the U.S. and other developed countries, and only small proportions of the sample are above the upper limits of the normal clinical ranges as defined by U.S. standards. The correlational patterns of the collected biomarkers are consistent with observations from developed countries, and the comparison with other low-income populations such as the Tsimane in Bolivia or the Yakuts in Siberia show remarkably similar age-specific patterns of the biomarkers despite differences in the mode of blood sampling. **Discussion:** The MLSFH biomarker sample makes a potentially important contribution to understanding the health of the adult populations in low income environments. The present study confirms that the collection of such biomarkers using the LabAnywhere system is feasible in rural sub-Saharan contexts: the refusal rate was very low in the MLSFH and following the procedures described above, only a small fraction of the biomarker samples could not be analyzed by LabAnywhere. The system therefore provides an attractive alternative to the collection of dried blood spots (DBS) and venous blood samples, providing a broader range of potential biomarkers than DBS and being logistically easier than the collection of venous blood.

Keywords

Biomarkers, Field methods, Health, Malawi

Disciplines

Demography, Population, and Ecology | Social and Behavioral Sciences | Sociology

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Abstract

Background: The collection of biomarker-based indicators of adult health and fitness is an important addition to socioeconomic surveys since these indicators provide valuable insights into the biological functions, and the complex causal pathways between socioeconomic environments and health of adult individuals. Other than select Demographic and Health Surveys (DHS), there are almost no population-based sources of biomarker-based indicators of adult health in sub-Saharan Africa (SSA), where most population-based biologic data are focused on HIV, other STDs, malaria, or nutritional status. While infectious diseases—such as HIV and malaria—attract the majority of research and NGOs attention in sub-Saharan Africa, there is an important need to understand the general determinants of adult health in SSA since the region will rapidly age in the next decades in ways that are significantly different from the aging patterns in other developing regions due to the AIDS epidemic, and chronic diseases will increasingly become relevant for understanding the health of sub-Saharan populations.

Methods and Design: We document our protocol for the collection of biomarker-based health indicators as a pilot project within the Malawi Longitudinal Study of Families and Health (MLSFH), and we provide basic descriptive information about the study population and the collected biomarker-based indicators of adult health obtained from respondents in rural Malawi. LabAnywhere kits were used to obtain blood plasma from 980 adult men and women living in Balaka, the southern-most region in rural Malawi. The procedure allows for the non-invasive collection of blood plasma, but has not been previously used in the context of a developing country. We collected biomarkers for inflammation and immunity, lipids, organ function, and metabolic processes. We specifically collected wide-range CRP, total cholesterol, LDL, HDL, total protein, urea, albumin, blood urea nitrogen, creatinine, random blood glucose and HbA1c assays. Overall, the mean values of the biomarkers are below the lower limits of clinical guidelines for adult populations in the U.S. and other developed countries, and only small proportions of the sample are above the upper limits of the normal clinical ranges as defined by U.S. standards. The correlational patterns of the collected biomarkers are consistent with observations from developed countries, and the comparison with other low-income populations such as the Tsimane in Bolivia or the Yakuts in Siberia show remarkably similar age-specific patterns of the biomarkers despite differences in the mode of blood sampling.

Discussion: The MLSFH biomarker sample makes a potentially important contribution to understanding the health of the adult populations in low income environments. The present study confirms that the collection of such biomarkers using the LabAnywhere system is feasible in rural sub-Saharan contexts: the refusal rate was very low in the MLSFH and following the procedures described above, only a small fraction of the biomarker samples could not be analyzed by LabAnywhere. The system therefore provides an attractive alternative to the collection of dried blood spots (DBS) and venous blood samples, providing a broader range of potential biomarkers than DBS and being logistically easier than the collection of venous blood.

1. INTRODUCTION

The collection of biomarker-based indicators of health as part of population-based samples has only recently become available for large-scale survey research, and it represents a potentially important addition to socioeconomic surveys as these biomarker-based indicators provide valuable insights into the biological function and the complex causal pathways between socioeconomic environments and health [1–8]. Numerous studies have established the relevance of biomarker collection for measuring the efficacy of immune system function [9–11], malnutrition [12–14], and diabetes [15] on morbidity and mortality [4]. Increasingly panel studies in industrialized countries (such as the Health and Retirement Study (HRS) in the U.S. and the English Longitudinal Study of Aging (ELSA) in the UK) are among the larger studies collecting biomarker-based health indicators [4, 7, 16, 17]. However, very few population-based studies in the developing world have followed suit, despite the considerable relevance of biomarker-based assessments of health in the context of low income countries. Exceptions include several extant isolated populations that are under intensive study—including the Cebu in the Philippines [18] and the Tsimane in Bolivia [19, 20]—to investigate the evolution of human immune response to various physiologic challenges and its relevance for human health [21]. There are almost no population-based sources of general biomarker-based health indicators in sub-Saharan Africa (SSA). Most population-based biomarker collections in the region—for instance those conducted as part of Demographic and Health Surveys (DHS) [22, 23]—have focused on HIV, other STDs, malaria and nutrition status (e.g. anemia). While infectious diseases—such as HIV and malaria—often attract the majority of research and NGOs attention in sub-Saharan Africa [24], there is an important need to collect and analyze biomarkers of general health. Over the next decades the adult populations in SSA will age rapidly in ways that will likely be distinct from other developing countries due to the exposure to the AIDS epidemic [25–28]. Chronic diseases will increasingly become relevant for understanding the health of sub-Saharan populations. In this context, biomarker-based health indicators can provide important information on normal physiological processes and signal pathogenic processes and/or diseases [4, 17]. They can clarify how social and behavioral factors influence health [4], and can help to identify pathways that are important for predicting morbidity and mortality trends [29]. Moreover, because biomarker-based health information is not subject to the same measurement errors as self-reported health indicators, biomarker-based measures of adult health may be particularly useful in surveys in developing countries where the measurement of health is problematic and little is known about disease incidence, duration, and prevalence or cause-specific mortality among adult and elderly individuals [1].

The Malawi Longitudinal Study of Families and Health (MLSFH), a longitudinal panel study in rural Malawi that provides a rare record of more than a decade’s duration of demographic, socioeconomic and health conditions in one of the world’s poorest countries, has augmented its collection of socioeconomic data with biomarker-based indicators of adult health in 2008–09. The MLSFH biomarker sample is a subset of about 900 MLSFH respondents and it provides 12 biomarker-based indicators of respondents’

general health, focusing specifically on inflammation, cardiovascular risks, metabolic processes and organ function. The aim of this paper is to document the protocol for the biomarker data collection conducted for this sub-sample of MLSFH, and to provide basic descriptive information about the study population and the collected biomarker-based indicators of health among adult individuals in a low income sub-Saharan population. These MLSFH biomarkers provide new opportunities to study aspects of health and disease in a context characterized by a mature HIV/AIDS epidemic, high levels of poverty and high levels morbidity and mortality.

2. BACKGROUND

Malawi is an opportune environment for studying epidemiologic and demographic transitions in real time. It is one of the poorest countries in the world, ranked 153 of 169 in terms of the human development index [30], with about 15% of its population considered “ultra-poor”, i.e., with an estimated food consumption below the minimum level of dietary energy requirement (the proportion ultra-poor in Malawi has declined from 24% in 1998) [31]. Life expectancy at birth is estimated to be 47 years (44 years for males/47 years for females) in 2009, with healthy life expectancy at birth estimated at 44 years in 2007 [32]. While the Malawian per capita income is below the sub-Saharan average, Malawi is similar to other SSA countries and countries in the World Bank low income group in terms of life expectancy, infant mortality, child malnutrition, access to clean water, literacy and educational enrollment [32, 33]. In rural areas, such as the study sites of the MLSFH, the majority of individuals engage in home production of crops, primarily maize, squash, tomatoes, potatoes, nuts, dark green leafy vegetables, and fruit, complemented by some market activities. The local diet is characterized by high intakes of vegetables and grains (especially maize) and low consumption of protein-dense food, especially animal-based proteins. Tuberculosis [34, 35], malaria [36, 37], and endemic parasites (e.g., soil-transmitted helminths (STH) and schistosomiasis) also have a relatively high prevalence [38–40]. Nearly 80% of all deaths in Malawi are caused by infectious diseases including HIV/AIDS, malaria, TB, schistosomiasis, and nutritional deficits [41]. Chronic conditions, on the other hand, continue to be relatively rare; in 2002, for example, cerebrovascular and cardiovascular diseases accounted for only 6.0% of all deaths. This combination of low rates of chronic disease mortality but high levels of communicable disease mortality are emblematic of the early stage of the epidemiologic transition, i.e., the transformation of disease patterns that is (or has been) experienced by all societies over time [42]. Sub-Saharan African countries, including Malawi, however are distinct in their epidemiological transition due to the presence of the HIV/AIDS epidemic. The effects of the HIV/AIDS epidemic on the epidemiological transition is only poorly understood [43–45]. Moreover, while HIV/AIDS is widespread, the vast majority of the population—more than 85% of adults aged 15–49—is HIV negative [46], and HIV-negative individuals also confront a high disease-risk environment characterized by high levels of poverty, malaria, TB, endemic parasites, poor sanitation, limited access to health care facilities, and, episodic malnutrition. Studies collecting biomarker-based health indicators that represent both HIV+ and HIV– individuals, as is the case of the

MLSFH biomarker data collection described below, are therefore important for evaluating the health conditions of sub-Saharan populations, and the effects that population aging and the HIV/AIDS epidemic have for population health in this region.

3. METHODS

3.1 *Malawi Longitudinal Study of Families and Health (MLSFH)*

The Malawi Longitudinal Study of Families and Health (MLSFH; formerly, Malawi Diffusion and Ideational Change Project, MDICP) is a longitudinal panel data collection with survey waves in 1998, 2001, 2004, 2006, 2008 and 2010 that is currently focused on studying the mechanisms that individuals, families, households, and communities develop and use in a poor rural setting to cope with the impacts of high morbidity and mortality in their immediate living environment [47–58]. The MLSFH is implemented in three sites in rural Malawi: Rumphi (in the northern region), Mchinji (in the central region), and Balaka (in the southern region). These rural regions are similar in terms of their overall economic context that is based on subsistence agriculture; however, the study regions also reflect interesting heterogeneity in terms of marriage patterns [59], religious affiliations [60], schooling [53, 61], patrilineal vs. matrilineal inheritance and land-ownership, and HIV prevalence, thus reflecting a range of socioeconomic/health/demographic conditions and permitting the evaluation of contextual effects, including risk environments, on estimated relations pertaining to health and its behavioral and socioeconomic determinants.

The MLSFH started in 1998 with a sample of 1,541 ever-married women aged 15–49 and 1,065 of their spouses. In 2001, respondents were re-interviewed, along with any new spouses since 1998. In 2004, the study added two new components to the data-collection: a new additional sample of approximately 1,500 adolescents, and free HIV testing and a voluntary counseling on the HIV test results for all respondents. The MLSFH returned for a fourth wave of survey data collection and a second round of HIV testing in 2006, and it followed-up in 2008 and 2010 with two additional rounds of wide-ranging survey data for about 4,000 respondents aged between 21 (10th percentile) and 67 (90th percentile). Detailed descriptions of the MLSFH sample selection, data collection, and data quality are provided on the project website <http://www.malawi.pop.upenn.edu>, in a Special Collection of the online journal *Demographic Research* that is devoted to the MLSFH [62], and in a recent follow-up publication that incorporates the 2004 and 2006 MLSFH data [48]. Comparisons with the Malawi Demographic and Health Survey (DHS) showed that the MLSFH sample population is reasonably representative of the rural Malawi population [48, 63].

Some core features of the MLSFH survey data that are particularly relevant for analyses in connection with biomarker-based health indicators include: (a) measured weight and height of respondents in 2008 to calculate the body mass index (BMI) as a simple, reliable, albeit crude indicator of body fat measures [64, 65]; repeated measures of mental and physical health since 2006 using the SF12-scale; (b) repeated HIV tests (2004, 2006 and 2008) [58, 66] identifying 345 HIV+ individuals, and detailed measures of HIV risk perceptions since 1998 [51, 52, 67]; (c) detailed demographic and socioeconomic data

of respondents, including marital history and sexual-partnership histories; (d) comprehensive measures of economic and social shocks experienced by respondents and their households, including mortality of family members, crop failures/income losses, changes in health of individuals and their family members (since 1998); and (e) extensive information on social networks and social capital, including participation in social groups (since 1998) and unique information about family networks, intergenerational transfers and resource sharing in extended family networks (since 2006) [54, 57, 68, 69].

3.2 MLSFH Biomarker Collection: Minimally Invasive Collection of Plasma in the Field

We use the term “biomarker” as defined by Crimmins and colleagues [70], that is, as an objectively measured trait that prior research has shown to be a reliable indicator of normal biologic or pathologic processes that are common to human aging. The MLSFH biomarker collection focused on blood serum indicators as these indicators are pertinent to the study of health and aging in the sub-Saharan African context. Collecting such biomarkers in surveys inevitably involves balancing the ease and cost with specimen stability and assay reliability. These concerns are exacerbated in developing countries with poor infrastructures for health care and transportation. Overcoming such obstacles is necessary if we are to improve our understanding of population health in resource-poor countries that are transitioning from an acute disease regime to one increasingly dominated by chronic conditions [71, 72].

To date, the primary method for obtaining biomarkers in developing countries has been dried blood spots (DBS). McDade pioneered these techniques for collecting and storing small blood samples and developing assays for important biomarkers, such as hsCRP, a marker of inflammation [29, 73, 74]. The infrastructure for processing DBS assays has not kept pace with the large volume of DBS obtained in large, nationally representative surveys. Moreover, the biomarkers that can be obtained from DBS remain restricted. To avoid the complications associated with DBS, the MLSFH has tested a new approach for collecting measures of population health and their adaptability to extreme conditions in tropical zones. Our results indicate the reproducibility of biomarkers obtained from the LabAnywhere (previously Demecal) system (LabAnywhere, Haarlem, The Netherlands) [75], a new system for the collection of blood plasma that has been used in other large-scale biomarker collections in developed countries [76–78]. This biomarker collection as part of the MLSFH was approved by the IRB at the University of Pennsylvania (May 9th, 2008) and by the Malawi National Health Sciences Research Council (NHSRC) (December 8th, 2008).

The LabAnywhere kits used for the MLSFH biomarker collection require only a few drops of blood harvested from a lancet puncture of a sanitized fingertip. A sponge device is used for absorbing the drop of blood. After the sponge turns completely red, it is dropped into a container with buffer fluid. A gentle swinging motion for 40 seconds is necessary to release the dilution buffer. A filter is used to separate the red blood cells from the plasma. The distinctive feature of this system is that the blood is pressed through a patented filter that separates out plasma. Unlike a clinic based procedure for obtaining blood plasma, the LabAnywhere system does not require the use of a centrifuge. The

reliability, sensitivity, and specificity of the test kits have been demonstrated by LabAny-where in the Netherlands, and the applications of test specific recovery factors yielded a good correlation with results of venous blood samples [75]. In general, LabAnywhere plasma samples are stable for 4 days at 4°C, 2–3 days at room temperature and 1 day at 37°C.

An important advantage of using the LabAnywhere kits for blood sampling is the minor discomfort it causes to study participants. The kits offer an ideal combination providing viable blood samples for analysis and a non-invasive means of collection. As such, the LabAnywhere method offers several advantages over the other common means of collecting blood samples such as dried blood spots (DBS) or venipuncture [17]. Blood sample collection through DBS has several disadvantages including potential damage and loss of viability caused by the drying of blood, and insufficient blood for testing provided on the filter paper. While intravenous blood collection avoids the problems of DBS, it involves a much more invasive procedure and requires collection by trained phlebotomists. The LabAnywhere kit allows for the preparation of plasma from just one single drop of blood at any location, at any time (e.g., at the homes of MLSFH respondents). Up to 16 assays can be done with this plasma. Because the plasma has been diluted, the Lab-Anywhere analyser technology will measure very small amounts premitting this quantity of assays. The disadvantages of the LabAnywhere method to collect and analyze blood plasma compared to other approach are summarized and discussed in more details by McDade [17].

There are numerous biomarkers of population health that could potentially inform our understanding of adult health in rural Malawi. Those that we chose have demonstrated analytic utility in studies of both, in developed countries [4]—notably in the U.S. [79–83] and Japan [84]—and in developing countries, including resource-poor countries such as Nigeria [85, 86], Amazonian Bolivia [20, 87], Mexico [88], the Philippines [89], South Africa and other sub-Saharan populations [90, 91], Aboriginal Australia [92], and indigenous Africans [93, 94].

The biomarkers that were collected as part of the MLSFH include: wide-range CRP (wrCRP) as a measure of inflammation and the immune function [95]; a lipids panel consisting of cholesterol, LDL, HDL, and triglycerides, as measures for risk factors for cardiovascular disease; markers of renal function and clearance (total protein, uric acid, albumin, urea/blood urea nitrogen (BUN), and creatinine); and circulating blood glucose and HbA1c (only in cases when the blood glucose was above the normal range) as markers of the metabolic function. Few, if any, biomarkers are free-standing reliable diagnostic tools, and neither are the ones listed above. Many of the MLSFH biomarkers are standard blood work commonly ordered for mid-life adults. These are important measures for comparative purposes, but the environment in which participants live can amend their interpretation. In the context of Malawi, low levels of total protein are more likely to signal inadequate nutrition, rather than renal disease. Enteric infections in Malawi, rare in the U.S., can impair intestinal transport of nutrients regardless of diet or anemia in Malawi. Low levels of circulating glucose may be an important adaptation to unpredictable energy inputs rather than a symptom of pathology, per se.

Although the biomarkers collected as part of the MLSFH are well-known, we briefly discuss our reasons for their selection, the critical levels used for obtaining indicators of health risks, mostly for the U.S. and similar developed contexts, and the anticipated relations of each biomarker to others we measure.

3.2.1 Lipids

Total cholesterol (TC), high-density lipoprotein (HDL), low density lipoprotein (LDL), and triglycerides (TG) All lipids are fats that store energy for quick release, and to varying degrees, all lipids are recognized risk factors for cardiovascular disease in the developed world. For example, in the absence of other risk factors, the American Heart Association considers a total cholesterol reading of less than 200mg/dl desirable, 200-230 mg/dl borderline, and in excess of 240mg/dl as conveying a high risk for cardiovascular disease. The optimal level of HDL in a U.S. context is 50 mg/dl, but not less than 35 mg/dl, and less than 100mg/dl is recommended for LDL. Normal fasting triglyceride levels in the U.S. are below 150 mg/dl; 150-199 mg/dl is considered borderline high, 200-499 mg/dl high, and 500 mg/dl and greater very high [96]. However, because participants from the Balaka region in Malawi live in rural environments, with food being produced at relatively high energy expenditures but yielding few protein-dense calories, we expected that the distribution of lipids among Malawi participants will be more concentrated at the lower end of distributions than observed in European or U.S. populations.

3.2.2 Metabolic processes

Glucose and HbA1c: Random blood glucose, also known as a non-fasting blood sugar, is a biomarker for the efficiency of the metabolic system. Glucose is the main source of energy for the body. Insulin, the hormone that cells use to metabolize the glucose, is produced in the pancreas. It is released into the blood in response to levels of circulating glucose. A random blood glucose (RBG) test has two advantages: it does not require respondent fasting and it is less expensive. But because fasting is not a prerequisite for the test, the RBG measure is less precise. The normal range for a random blood sugar test is 70–100 mg/dl. At its most advanced stage, high blood sugar, if untreated, increases the odds of renal and liver failure, as well as neuropathy, a malfunction of the central nervous system, for which we did not collect a biomarker. HbA1c measures the percentage of hemoglobin—a protein in red blood cells that carries oxygen—is coated with sugar (glycated). Rather than measuring blood sugar levels at one point of time, the HbA1c test shows an average blood sugar level for the past two to three months. HbA1c below 5 percent is seen as normal level and a target, although it can range from 4.5 to 6 percent. People with diabetes are characterized by elevated HbA1c levels and for them a level of about 7 percent is a target. In the MSFH biomarker data collection, HbA1c was not measured for the entire sample, but only for those study participants who showed elevated blood glucose levels (i.e., 12 study participants with a mean value of HbA1c of 5.53 and 0.71 std. dev.).

3.2.3 Biomarkers of organ function

Creatinine: A major function of the kidneys is to filter out waste and water from the blood. Creatinine is one of the waste products in the blood created by the normal breakdown of muscles; circulating levels of creatinine are fairly reliable indicator of the efficacy of kidney. Regardless of the etiology of the problem, as the kidneys become impaired, creatinine levels in the blood rise because of poor clearance. Normal levels of creatinine in the blood are approximately 0.6 to 1.2 mg/dl in adult males and 0.5 to 1.1 mg/dl in adult females. In malnourished persons or those who experience weight loss or wasting, such as common in persons with HIV/AIDS or cancer, creatinine levels may be surpassed. Crimmins and colleagues also note that serum creatinine is generally less reliable than urinary creatinine [70]. Any condition that impairs the function of the kidneys will increase creatinine level in the blood.

Albumin: Like creatinine, serum albumin is used to assess renal and liver function. Albumin is the protein of highest concentration in the blood and maintains oncotic pressure of blood to prevent its leakage into tissue. The normal (U.S.) range for of albumin is 3.5 to 5.5 mg/dl. A low albumin level is correlated with inflammation and malnutrition while high levels signal dehydration. Low concentrations of albumin, even within the normal range, have been positively related to coronary disease [97].

Total protein: Unlike fats and carbohydrates, proteins are not stored in the body. They are continuously broken down (metabolized) into amino acids that are used as building blocks for other proteins. The LabAnywhere test is a rough measure of all the proteins found in the plasma, principally albumin and globulin. The normal range of the test is 6.0 to 8.3mg/dl.

Uric Acid: Uric acid is produced in the body from purine metabolism and excreted by the kidneys. Elevations and decreases in uric acid are associated with human disease and conditions; for instance, elevated uric acid is associated with gout, starvation, metabolic syndrome or kidney stones, and decreased uric acid is associated with multiple sclerosis. Normal values of uric acid range between 3.5 and 7.2 mg/dl.

Urea/Blood Urea Nitrogen (BUN): Blood carries proteins for use by cells throughout the body. After the cells use the protein, the remaining waste products are returned to the blood as urea, a compound containing nitrogen. Healthy kidneys take urea out of the blood and send it to the bladder for excretion. If kidneys are not working well, the urea stays in the blood. Normal blood contains 7 to 20 milligrams of urea per deciliter of blood. A BUN result of more than 20 mg/dl indicates that kidneys are not functioning normally. Other possible causes of an elevated BUN include dehydration, gastrointestinal bleeding or heart failure.

3.2.4 Biomarkers of the Immune System

C-reactive protein (CRP): CRP is the most commonly used marker of inflammation and infection. As an acute-phase response protein, CRP can increase as much as 1000-fold in 24 hours. At elevated levels CPR indicates systemic infection or tissue damage, and levels above 3.0 mg/l are generally considered as indicating a high risk for cardiovascular disease. We assayed only this one biomarker of immune function. Were it not for budget

constraint of this project, we would have preferred to include IL-6 that up-regulates CRP, and an IGG panel that can be used to detect antibodies to specific diseases. Among the commercially available CRP tests, we used the wide-range CRP (wrCRP) assay because it detects levels of CRP in the range of 0.012–16.0 mg/l, and thus is sensitive to and measures both very low and very high levels.

There are few studies of CRP in low-resource countries against which to compare the distribution of CRP. In Malawi, we would expect median and mean levels of CRP to be classified as high-risk range (≥ 3.0 mg/l) for cardiovascular disease and related events, such as myocardial infarction, or more relevantly, advancing HIV infection [98].

3.3 Health survey and anthropometric measurements

The biomarker data collection was accompanied by a brief personal questionnaire to gather information on the respondent's living environment, including sources of drinking water, type of sanitation, prior malaria exposure, and regular use of insecticide-treated bednets. We also asked participants when they last ate, what they had (particularly, protein and carbohydrates), about how much they consumed, if they have been recently infected with malaria and/or other infectious diseases that dominate morbidity patterns in Malawi. If the respondent was female, we also asked if she was currently pregnant.

3.4 Study population for MLSFH biomarker collection

For the MLSFH biomarker collection, we first identified one of the three MLSFH field-work sites to conduct this biomarker collection (logistical and monetary considerations precluded conducting this data collection in all three sites). Due to our particular interest in the interaction between HIV and the biomarker measures, we chose the site with the highest HIV prevalence, Balaka (in the southern region). Next, we drew our sample in two stages. First, all respondents who were found HIV positive in a previous round of the study were included in the sample. Next, in addition to the HIV positive respondents, we drew a random sample of approximately 1500 respondents (aged ≥ 20 years) from the 2500 total respondents in the MLSFH Balaka sample. Because of weather obstacles we were able to re-contact 1,031 individuals. Of these, 49 respondents (4.7%) refused to participate, and we collected biomarker specimens for 982 cases. Of the 982 cases, approximately 60 cases had previously tested positive for HIV. Like the MLSFH study population, the MLSFH biomarker sample is longitudinally linked across MLSFH survey waves and therefore providing socioeconomic and health data prior and after the biomarker collection; it is also linked between husbands and wives, and the linkages between parents and children are in process.

3.5 Field procedures, training and data management

The LabAnywhere test kits were delivered directly to Malawi in September 2008. A test-run of all field procedures for about 70 cases was completed just prior to the full-scale data collection in early January 2009, including the freezing protocol in Malawi, the transport protocol, and the assays themselves. In this trial we were interested in how well the integrity of the plasma samples was maintained and the reliability of the assays

themselves. The actual field work commenced in mid January and was completed by early February, 2009.

Because the administration of the LabAnywhere system in a developing country context such as Malawi is ideally done using personnel trained in biomarker specimen collection, the MLSFH biomarker collectors team consisted of 25 individuals who had previously been trained by the Malawian Government in finger prick blood collection as part of HIV voluntary counseling and testing. They underwent one-week of training in the use of the kits prior to beginning biomarker collection in Balaka. Training included the specific procedures for collecting samples using the LabAnywhere kit and for the proper storage and transportation of the samples to the fieldwork headquarters; training also included the ethical considerations of biomarker collection in the field, informed consent, and the requirements for protecting respondents privacy and the confidentiality of the collected data. All biomarker collectors participating in the training were required to collect test samples that were sent to LabAnywhere for assessment. Only biomarker collectors who produced usable samples from their test kits during the training were certified by LabAnywhere and hired for the MLSFH biomarker collection on the main sample.

Using village guides who knew the respondents, the MLSFH biomarker collectors visited the home of the assigned respondent, obtained informed consent (either by signature or thumbprint from illiterate study participants) for the biomarker collection, and then collected the plasma using the LabAnywhere kit. The informed consent process involved a detailed discussion of the data collection process, the use of the biomarkers for research purposes, the risk of study participation and the limited value of the collected biomarkers for identifying any specific diseases the respondent might have. To protect the respondent's confidentiality, the specimen were marked with a special identification number known only to the MLSFH Biomarker Coordinator, and only the coordinator was able to link the plasma samples with the respondents and their personal information. The MLSFH biomarker collectors placed the label with this identification number on the biomarker sample. After successfully collecting and labeling the plasma sample, the specimen collector returned it to the biomarker coordinator, who stored all plasma samples in a cooler.

Upon returning from the field each day, the biomarker coordinator checked all samples to verify that they were collected and labeled properly; all plasma samples were then stored in a -20°C freezer until they were shipped to LabAnywhere. At the end of each week, all biomarker samples were cross-checked with field records, and sent via DHL from Malawi to the LabAnywhere laboratory in the Netherlands for testing. For the ground and air transport to the Netherlands, the samples were packed in a special cooler with ice packs provided by LabAnywhere, which were designed specifically for transporting the frozen blood samples. Minimum/maximum thermometers were packed in each transport cooler. LabAnywhere was able to analyze 92.7%, or 910 of the 982, samples they received. None were discarded because of inadequate temperature control. Each shipment also included a list of identification numbers, so that the entry of test results by LabAnywhere preserved confidentiality of participants. After the biomarker

processing was completed by LabAnywhere (less than one week after arriving in Amsterdam), LabAnywhere sent the results to MLSFH. LabAnywhere also prepared a database with the assayed values and individual IDs were mapped to the extant database maintained by the MLSFH. After eliminating outliers that are likely to be erroneous measurements (see below), the MLSFH biomarker sample includes 906 respondents with at least one valid biomarker measurement.

Upon receiving the test results, MLSFH convened an information session in all participating villages during which potential health concerns identified by the tests were discussed. Individual respondents were given the option to discuss privately their results with a health care counselor. The MLSFH also worked with local health clinics to follow up on any potential health issues that were identified by the biomarker tests. However, except for referrals to local health clinics, no specific treatments were provided as part of the MLSFH biomarker study.

3.6 Transformation and cleaning of biomarker data

Some of the biomarker measures obtained by the lab fell outside the plausible range, possibly as a result of measurement problems or instabilities of the blood samples. We identified outliers as values that were 2 interquartile ranges below the 25th or about the 75th percentile, and replaced these with missing values. In addition, CRP values of zero were replaced with missing. In total, the following outliers were identified: 3 for total cholesterol (TC), 16 for HDL, 10 for LDL, 4 for triglycerides (TG), 1 for glucose (RBG), 6 for creatinine, 23 for albumin, 14 for total protein (TP), 2 for uric acid, 5 for urea (BUN), 48 for CRP (for CRP, all of which were zero values). Log transformations of all biomarkers are calculated since log transformations are preferable for some of the very skewed distributions—such as for CRP—to reduce the influence of extreme values on the results.

4. RESULTS AND DISCUSSION

Table 1 provides summary statistics for the MLSFH biomarker study population. For 906 respondents, all living in the Balaka (southern) region, at least one valid biomarker indicator is available. The mean age is about 42–43 years, with about 50% of the study population older than 40. The MLSFH biomarker study population is therefore relatively old, especially when compared to the median age of the Malawi population that is around 17 years. This overrepresentation of elderly individuals in the MLSFH, which is an attractive aspect for analyses using the MLSFH biomarkers, is the result of the aging of the MLSFH panel population recruited in 1998 and the addition of a MLSFH *parent sample* in 2008 that includes all respondents' living parents. About 80% of the study population is married, and because the original MLSFH sample included women and their current husbands, slightly more men than women are married in the study population. As expected for the Balaka region, about 70% of respondents are Muslim, with the remainder mostly belonging to various Christian dominations and only a small fraction reporting no religion. More than 1/2 of women in the study population, and close to 1/3 of men in the study population, have not attended school, and only a very small fraction has attended

Table 1: Summary statistics for the study population

| | Females mean (sd) | Males mean (sd) | Total mean (sd) |
|---|--------------------------------|------------------------------|------------------------------|
| # of observations | 571 | 335 | 906 |
| Age (in 2008) | 42.17 (17.75) | 43.54 (16.87) | 42.68 (17.43) |
| Age Group | | | |
| < 30 | 0.296 | 0.307 | 0.300 |
| 30–39 | 0.205 | 0.131 | 0.178 |
| 40–49 | 0.186 | 0.152 | 0.173 |
| 50–59 | 0.144 | 0.209 | 0.168 |
| 60–69 | 0.0823 | 0.140 | 0.104 |
| 70+ | 0.0876 | 0.0597 | 0.0773 |
| Married (in 2008) | 0.762 | 0.892 | 0.809 |
| Muslim (vs Christian/other/none) | 0.691 | 0.706 | 0.696 |
| Schooling attainment | | | |
| <i>No school</i> | 0.575 | 0.320 | 0.483 |
| <i>Primary level</i> | 0.399 | 0.618 | 0.478 |
| <i>Secondary level</i> | 0.0256 | 0.0615 | 0.0386 |
| Body Mass Index (BMI) (2008) | | | |
| <i>Underweight (BMI < 18.5)</i> | 0.143 | 0.118 | 0.135 |
| <i>Normal (18.5 ≤ BMI < 25)</i> | 0.750 | 0.837 | 0.777 |
| <i>Overweight (25 ≤ BMI < 30)</i> | 0.0915 | 0.0345 | 0.0737 |
| <i>Obese (BMI ≥ 30)</i> | 0.0156 | 0.00985 | 0.0138 |
| BMI unknown | 0.215 | 0.396 | 0.282 |
| HIV positive | 0.0835 | 0.0496 | 0.0715 |
| Subjective health | | | |
| <i>Fair/Poor</i> | 0.158 | 0.100 | 0.136 |
| <i>Good</i> | 0.307 | 0.195 | 0.265 |
| <i>Very good</i> | 0.279 | 0.298 | 0.286 |
| <i>Excellent</i> | 0.256 | 0.407 | 0.312 |
| Resp.'s household has | | | |
| <i>access to potable water</i> | 0.843 | 0.880 | 0.857 |
| <i>metal roof on house</i> | 0.144 | 0.159 | 0.149 |
| <i>pit latrine</i> | 0.782 | 0.838 | 0.802 |
| <i>mosquito nets</i> | 0.816 | 0.828 | 0.821 |
| <i>mosquito nets treated with insecticide</i> | 0.652 | 0.662 | 0.655 |

Notes: Descriptive statistics are calculated for respondents with at least one valid biomarker measure.

secondary school.

About 12–14% of the study population is underweight with a BMI below 18.5; and the vast majority has a normal BMI between 18.5 and 25. A small fraction of respondents, somewhat more for females than for males, are somewhat overweight with a BMI between 25 and 30, and virtually none of the MLSFH biomarker respondents are obese with a BMI ≥ 30 . Height and/or weight, and therefore BMI, is missing for about 20% of female and 40% of male respondents.

Among women, 8% are HIV positive (based on at least one HIV positive test during 2004–2008 among respondents with at least one test in this period), and about 5% of males are HIV positive [58, 66]. Despite the relatively high levels of morbidity and mortality experienced by rural Malawians, about 50% of female and 70% of male MLSFH biomarker respondents described their subjective health as either “very good” or “excellent”, a pattern that is consistent with other low income populations [99–104].

More than 80% of MLSFH respondents have access to potable water—mostly through a covered well or bore hole—and about 80% of respondents live in households with a pit latrine. About 15% of respondents’ house has a metal roof, which is an indicator of relative wealth. While the majority of respondent’s households has at least one mosquito net, only around 65% of respondent’s household have nets that have been treated with insecticide.

Table 2 documents the means, std. deviations and percentiles of the biomarker-based health indicators collected as part of the MLSFH. Some noteworthy patterns of the biomarker distributions in Table 2 include:

Total Cholesterol (TC): The overall mean of total cholesterol is 110 mg/dl (median = 108), with an interquartile range of 88.8–131. Only the four largest observations in our sample are considered as having an elevated risk for heart disease by U.S. standards (ie, TC > 200 mg/dl). *High-density lipoprotein (HDL)*: The overall mean of HDL is 32 mg/dl (median = 31), with an interquartile range of 23–39. Only about 5% of women or men have optimal levels HDL levels, defined as HDL ≥ 50 mg/dl, and the majority of the sample has levels of HDL that are too low based on U.S. clinical standards. *Low-density lipoprotein (LDL)*: The overall mean LDL in the overall sample is 59 mg/dl (median = 58), with an interquartile range of 42–73. By U.S. standards, only the top 5% of sampled individuals had elevated levels of LDL (LDL > 100 mg/dl). *Triglycerides (TG)*: The median level of triglycerides in the MLSFH biomarker sample is 53 (mean = 59.5), with an interquartile range of 35–71. The vast majority of respondents have TG levels well within the normal range, and only the top 1.4% of respondents women had triglycerides above 150 mg/dl.

Glucose (RBG): The overall mean RBG in the overall sample is 75 mg/dl (median = 68.5), with an interquartile range of 61–85. Only about 10% of respondents participants had RBG above the 100mg/dl cut-off.

Creatinine: The median level of creatinine in the MLSFH biomarker sample was .71 (mean = .73), with an interquartile range of .60–.83. All but the lowest quartile of the distribution is within the normal range by U.S. standards, and thus we did not find evidence of elevated creatinine levels in the MLSFH biomarker sample.

Table 2: Summary statistics for the biomarker-based health indicators

| | N | Mean | std. | Percentiles | | | | |
|--|-------|-------|------|-------------|------|-------|-------|-------|
| | | | | 5th | 25th | 50th | 75th | 95th |
| Total cholesterol (TC) (mg/dL) | 904.0 | 110.4 | 29.6 | 65.6 | 88.8 | 108.1 | 131.3 | 162.2 |
| High-density cholesterol (HDL) (mg/dL) | 891.0 | 32.0 | 10.8 | 15.4 | 23.2 | 30.9 | 38.6 | 50.2 |
| Low-density cholesterol (LDL) (mg/dL) | 897.0 | 59.0 | 22.3 | 27.0 | 42.5 | 57.9 | 73.4 | 96.5 |
| Triglycerides (TG) (mg/dL) | 902.0 | 59.5 | 29.6 | 26.5 | 35.4 | 53.1 | 70.8 | 115.0 |
| Glucose (RBG) (mg/dL) | 904.0 | 75.0 | 19.5 | 52.3 | 61.3 | 68.5 | 84.7 | 113.5 |
| Creatinine (mg/dL) | 901.0 | 0.73 | 0.19 | 0.45 | 0.60 | 0.71 | 0.83 | 1.06 |
| Albumin (ALB) (g/DL) | 884.0 | 3.64 | 0.44 | 2.90 | 3.36 | 3.63 | 3.92 | 4.34 |
| Total protein (TP) (mg/DL) | 893.0 | 6.89 | 0.83 | 5.52 | 6.36 | 6.86 | 7.40 | 8.28 |
| Uric Acid (mg/dL) | 905.0 | 4.45 | 1.18 | 2.69 | 3.70 | 4.37 | 5.21 | 6.56 |
| Urea (BUN) (mg/dL) | 902.0 | 10.7 | 3.13 | 6.16 | 8.68 | 10.4 | 12.3 | 16.5 |
| C-reactive protein (CRP) (mg/L) | 845.0 | 4.50 | 11.8 | 0.10 | 0.20 | 0.70 | 2.80 | 25.0 |

Albumin (ALB): The mean and median of albumin is 3.6g/dl, with an interquartile range of 3.4–3.9. About 38% of the sample have below normal levels of albumin. *Total protein (TP)*: The mean for total protein is 6.89g/dl, with a median of 6.86 and an interquartile range of 6.36–7.40. All but the bottom 12% and the top 4% of the MLSFH biomarker respondents are within the normal range for TP. *Uric Acid*: The median level of uric acid in the MLSFH biomarker sample is 4.37 (mean = 4.45), with an interquartile range of 3.70–5.21. The normal range for uric acid varies by laboratory standards, but typically is in the range of 3.6 mg/dl and 8.3 mg/dl. *Urea/Blood Urea Nitrogen (BUN)*: The overall mean of blood urea nitrogen in the MLSFH biomarker sample is 10.7 mg/dl (median = 10.4), with an interquartile range of 6.2–12.3. None of the MLSFH respondents has an elevated level above 20mg/dl, which would suggest kidney problems based on U.S. standards.

C-reactive protein (CRP): The distribution of CRP is very skewed, with the mean level of 4.5 exceeding the median level of .70 by a factor of 6.4. The interquartile range of CRP is .20–2.80 mg/l. Contrary to our expectation of finding widespread elevated level of CRP in the study population, 21% of the MLSFH biomarker study population have CRP levels that are considered normal by the U.S. standards of 3.0 mg/l.

Table 3 describes the correlation coefficients between the different biomarkers. The correlation pattern of the MLSFH biomarkers is consistent with observations from developed countries indicating that there are significant and positive correlations among all lipid measures (TC, HDL, LDL, TG), except for TG and HDL that are negatively correlated. Glucose is positively correlated with all the lipids, except HDL, and all indicators of renal and liver function. Albumin and creatinine are positively correlated, although only albumin is inversely correlated with CRP. This is as expected because albumin is a negative acute-phase protein, and the albumin concentration falls approximately 20 percent during the inflammatory process. CRP is negatively correlated with the lipids TC, HDL and LDL, and it is positively correlated with TG. It has a weak correlation with the other biomarkers, except for albumin, with which it is slightly negatively correlated. The generally weak correlation of the biomarkers with each other, except for lipids that are relatively highly correlated with each other, indicates that these biomarkers are indicators of different biological processes and reflect different dimensions of the respondent's health.

Very few of the MLSFH biomarkers fall in the high risk categories as defined by U.S. standards, and this is particularly the case of biomarkers associated with cardiovascular disease (CVD) such as total cholesterol, LDL, triglycerides, albumin and CRP. HDL is the exception, where a sizable proportion of the MLSFH sample is in the high risk group. To evaluate these findings in a comparative context, Table 4 therefore compares both the median levels of total cholesterol, HDL, LDL, triglycerides, albumin and CRP in the MLSFH biomarker sample, and the percentage in the high risk category for each of these biomarkers, with the Tsimane of Bolivia, an extensively studied low income population [19, 20, 87, 105]. The top part of the table compares the median levels, for each age group, between these two populations, and the bottom part compares the percentage in the high risk categories. Despite differences in the collection of blood samples and in their

Table 3: Correlation coefficients for biomarker measure

| | TC | HDL | LDL | TG | RBG | Creat. | ALB | TP | Uric A. | BUN | CRP |
|--|--------|--------|--------|-------|-------|--------|--------|-------|---------|-------|-------|
| Total cholesterol (TC) (mg/dL) | 1.00* | | | | | | | | | | |
| High-density cholesterol (HDL) (mg/dL) | 0.58* | 1.00* | | | | | | | | | |
| Low-density cholesterol (LDL) (mg/dL) | 0.89* | 0.30* | 1.00* | | | | | | | | |
| Triglycerides (TG) (mg/dL) | 0.24* | -0.14* | 0.20* | 1.00* | | | | | | | |
| Glucose (RBG) (mg/dL) | 0.11* | 0.01 | 0.11* | 0.10* | 1.00* | | | | | | |
| Creatinine (mg/dL) | 0.09* | -0.09* | 0.07* | 0.10* | 0.26* | 1.00* | | | | | |
| Albumin (ALB) (g/DL) | 0.26* | 0.23* | 0.18* | 0.00 | 0.19* | 0.26* | 1.00* | | | | |
| Total protein (TP) (mg/DL) | 0.17* | 0.03 | 0.18* | 0.17* | 0.15* | 0.22* | 0.61* | 1.00* | | | |
| Uric Acid (mg/dL) | -0.01 | -0.15* | 0.03 | 0.15* | 0.18* | 0.47* | 0.11* | 0.24* | 1.00* | | |
| Urea (BUN) (mg/dL) | 0.02 | -0.09* | -0.01 | 0.06 | 0.23* | 0.47* | 0.12* | 0.10* | 0.34* | 1.00* | |
| C-reactive protein (CRP) (mg/L) | -0.17* | -0.24* | -0.12* | 0.18* | 0.00 | 0.02 | -0.13* | 0.04 | 0.07* | 0.04 | 1.00* |

Note: * p -value < 0.05

processing (venous blood for the Tsimane, and LabAnywhere kits for the MLSFH), the age-specific patterns of the biomarkers shown above are remarkably similar. This finding lends credibility to those obtained using the LabAnywhere system rather than collecting venous blood as was done in the Tsimane study. The majority in either population fell into the U.S. high-risk range only with respect to high density lipoprotein (HDL), the so-called good cholesterol, with most respondents in both populations having too low HDL levels.

For CRP, we can also compare the MLSFH biomarker sample to another low-CRP population of “ultramarathon runners”. In this population, CRP can be markedly suppressed, independent of adiposity, with median CRP levels being less than half of the control median [106]. The Yakut, a subsistence population in Siberia, have also been shown to have low CRP levels [107]. In the Yakut, the median CRP is 0.76, compared to a median CRP of .70 in the MLSFH biomarker sample. Comparing the MLSFH biomarker sample CRP levels to a range of countries from very modern such as the US and UK to the Yakut and Brazil [108], the MLSFH median CRP level of .70 is clearly the lowest, except for Japan with a median of 0.16 mg/L for men and 0.09 among women.

5. CONCLUSIONS

The MLSFH biomarker sample makes a potentially important contribution to the different populations in low income countries for which biomarker-based health indicators are available. The present study confirms that the collection of such biomarkers using the LabAnywhere system is feasible in rural sub-Saharan contexts: refusal rates to the biomarker collection was very low in the MLSFH, and following the procedures described above, only a small fraction of the biomarker samples could not be analyzed by LabAnywhere. The system therefore provides an attractive alternative to the collection of dried blood spots (DBS) and venous blood samples, providing a broader range of potential biomarkers than DBS and being logistically easier than the collection of venous blood.

Several important questions arise from the MLSFH biomarker collection and analyses that need to be more carefully addressed in future research. For example, the generally very low proportion of respondents that are classified as “high risk” based on any of the collected biomarkers is very low. The implications of this finding are currently not well understood. Do, for instance, these inter-country differences in CRP indicate that Malawians are in better health than the Yakut, or Aborigines in the remote Northern Territories of Australia [92], or in worse health than the Japanese populations [109]? More likely, the results suggest that the conventional critical values, which are mostly validated for developed countries, are not applicable for low income SSA contexts. For example, very low HDL has been observed with acute or chronic inflammatory response, as indicated by elevated levels of mean hsCRP and reduced albumin [110]. Changes in lipoproteins are noted to occur during the acute-phase reaction to inflammation. Similarly, inflammation and acute phase proteins may alter/reverse cholesterol transport by HDL [111]. Increasingly, HDL is also thought of as a component of the innate immune system because of its capacity to efflux cholesterol during the acute phase response [112].

Table 4: CVD risk factors

| | <i>Malawi</i> | | | | | <i>Tsimane</i> | | | | | |
|---|---------------------------|-------|-------|-------|-------|---------------------------|-----------|-------|-------|------|-----|
| | <i>Mean/median levels</i> | | | | | <i>Mean/median levels</i> | | | | | |
| | Age Group | | | | Total | Age Group | | | | N | |
| | 40–49 | 50–59 | 60–69 | 70+ | Total | N | 40–49 | 50–59 | 60–69 | 70+ | N |
| <i>Mean values by age:</i> | | | | | | | | | | | |
| Body Mass Index (BMI) | 21.7 | 21.5 | 21.2 | 20.3 | 21.3 | 381 | 23.9 | 24.4 | 23.2 | 22.1 | 477 |
| Total cholesterol (TC) (mg/dL) | 111.7 | 116.7 | 123.8 | 123.1 | 117.4 | 472 | 144 | 144 | 136 | 134 | 203 |
| High-density cholesterol (HDL) (mg/dL) | 32.8 | 32.3 | 33.9 | 30.5 | 32.5 | 465 | 37 | 37 | 37 | 35 | 172 |
| Low-density cholesterol (LDL) (mg/dL) | 58.5 | 63.5 | 66.0 | 68.6 | 63.1 | 471 | 80 | 79 | 76 | 72 | 170 |
| Triglycerides (TG) (mg/dL) | 56.8 | 63.5 | 66.6 | 70.3 | 62.9 | 471 | 137 | 142 | 116 | 121 | 203 |
| Albumin (ALB) (g/DL) | 3.60 | 3.60 | 3.60 | 3.46 | 3.58 | 460 | – | – | – | – | – |
| C-reactive protein (CRP) (mg/L) | 4.52 | 5.57 | 4.93 | 6.06 | 5.17 | 448 | 9.9 | 6.8 | 7.2 | 15.1 | 205 |
| <i>Median values by age:</i> | | | | | | | | | | | |
| C-reactive protein (CRP) (mg/L) | 0.75 | 0.80 | 0.80 | 1.15 | 0.90 | 448 | 2.7 | 2.7 | 4.0 | 3.4 | 205 |
| <i>Prevalence of high risk (%)</i> | | | | | | | | | | | |
| | Age Group | | | | Total | N | Age Group | | | | N |
| | 40–49 | 50–59 | 60–69 | 70+ | Total | N | 40–49 | 50–59 | 60–69 | 70+ | N |
| Body Mass Index: BMI \geq 30kg/m ² | 3.1 | 1.7 | 2.6 | 0.0 | 2.1 | 381 | 3.1 | 8.3 | 2.3 | 1.9 | 477 |
| Total cholesterol: TC \geq 240mg/dL | 0.0 | 0.7 | 0.0 | 0.0 | 0.2 | 472 | 0.0 | 0.0 | 0.0 | 0.0 | 203 |
| High-density cholesterol: HDL $<$ 40mg/dL | 81.9 | 82.4 | 73.6 | 84.3 | 80.8 | 465 | 59.7 | 68.1 | 57.7 | 68.2 | 172 |
| Low-density cholesterol: LDL \geq 160mg/dL | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 471 | 0.0 | 0.0 | 0.0 | 0.0 | 170 |
| Triglycerides: TG \geq 200mg/dL | 0.0 | 0.0 | 0.0 | 1.4 | 0.2 | 471 | 15.5 | 12.0 | 13.3 | 3.8 | 203 |
| Albumin: ALB $<$ 3.5g/dL | 40.4 | 44.5 | 43.5 | 49.2 | 43.6 | 460 | – | – | – | – | – |
| C-reactive protein: CRP \geq 3mg/L | 22.6 | 23.8 | 26.7 | 32.4 | 25.3 | 448 | 49.0 | 45.1 | 60.0 | 53.7 | 205 |

Notes: Source for Tsimane data: [105]; totals for biomarkers across all age groups are not available for Tsimane data.

Future research using the MLSFH biomarker samples will help to answer these questions, as well as help to better understand health and disease, and their relation to social, demographic and environmental risk factors, in Malawi and sub-Saharan Africa.

Limitations of the MLSFH biomarker data collection include that the present study was conducted in only one area of rural Malawi, Balaka in the south of the country. This region is predominantly Muslim and has the highest HIV/AIDS prevalence in the country. Without evidence from other regions in Malawi, it is difficult to assess to which extent these data reflect an overall health pattern for the rural population in the country. For instance, Muslim populations are characterized by different dietary and life style habits, and evidence suggests that they are characterized by different health and mortality patterns compared to Christian populations [113]. Thus, it is reasonable to expect differences in biomarker characteristics between the different Malawian regions and ethnic groups. Despite existing cultural, dietary and life style differences, people in rural Malawi live at large in high poverty and have been exposed to a high risk disease environment since birth. These latter factors may be more powerful determinants of health patterns in a poor context compared to cultural, dietary and life style norms. Based on this pilot biomarker data collection, we are not able to distinguish between the determinants of health as reflected by biomarkers in Malawi.

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