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EFFECT OF LOCAL FOOD ON LUMEFANTRINE BIOAVAILABILITY AND POPULATION PHARMACOKINETICS IN UGANDAN CHILDREN WITH MALARIA

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Effect of local food on lumefantrine bioavailability and population pharmacokinetics in Ugandan children with malaria

Thesis for Doctoral Degree (Ph.D)

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DEDICATION

I am thankful to GOD (as is the translation of my “mother given name” “MWEBAZA”) for the gift of life and mankind.

This thesis will never have been accomplished without input from many people acknowledged.

I was at the verge of giving up but I never gave up because you held onto my ailing hands and pushed me on as I crawled.

Besides I always had earthly reminders:

“Never give up” GAYAZA HIGH SCHOOL Motto

“If you can't fly then run, if you can't run then walk, if you can't walk then crawl, but whatever you do you have to keep moving forward.”
Martin Luther King Jr.

ABSTRACT

Background. Artemether-lumefantrine (AL) is widely adopted as first-line treatment for uncomplicated malaria. Lumefantrine (LUM), the long acting partner drug is critical for cure by eliminating malaria parasites left after artemether exposure. Absorption of LUM is dependent on dietary fat and the basis for the pediatric dose recommendations is unclear.

Aim. To explore effect of local foods on oral bioavailability of LUM and describe its population pharmacokinetics (PPK) among under five year old children in Uganda treated for malaria with the aim of optimizing use and provide basis for AL rational dosage guidelines.

Methods. In an intensive pharmacokinetics (PK) study, 13 healthy adult volunteers were randomized to participate in an open-label four period crossover design and received a single oral dose of AL (80mg A/ 480mg of LUM) with water, milk, maize porridge or maize porridge with oil on separate occasions. Peak concentrations (C_{max}) and area under concentration-time curve (AUC) truncated at 48 hours after a single dose ($AUC_{0\text{-}48h}$) were compared using average bioequivalence techniques (I). Relevance of the findings was assessed among children < 5 years with uncomplicated *falciparum* malaria who were randomized in a parallel study design to receive standard weight-based AL treatment (*Coartem*®), 6 doses over 3 days, with either milk or maize porridge with oil (n= 33) (III). Parametric two-sample t-test was used to compare relative oral LUM bioavailability, 0 to 8 h after the first dose ($AUC_{0\text{-}8h}$) (III). This bioavailability study (III) was nested in a population pharmacokinetic (PPK) study (IV) in the same pediatric patient group. After treatment, sparse plasma samples were collected during 28 days' follow up in all children (n=55). NONMEM was used to describe the PPK profile of LUM and its metabolite, desbutyl-lumefantrine (DBL) (IV).

A liquid chromatography tandem mass spectrometry (LC-MS/MS) method was developed for determination of low concentrations of LUM and DBL in small amounts of plasma (II).

Results.

The LC-MS/MS method was simple, fast and sensitive requiring only 100 µl of plasma with limits of quantification of 21 and 1.7 ng/ml for LUM and DBL respectively (II).

Lumefantrine exposure was comparable in milk and maize porridge plus oil study groups (I & III). In adult healthy volunteers, the bioequivalence criteria was met [maize porridge plus oil group ranges for means ratios (90% CI) of 0.84 –1.88 and 0.85 – 1.69 for C_{max} and $AUC_{0\text{-}48h}$ respectively, relative to milk (90%CI, 0.80 – 1.25)]. Among pediatric patients, LUM ($AUC_{0\text{-}8h}$) for those dosed with milk (n=16) was comparable to maize porridge plus oil (n=17) arm (GM {95%CI}: 6.01 {3.26-11.1} vs 6.26 {4.5 -8.43} h*µg/ml, p=0.9).

A two-compartment PK model with lag time using first order processes characterized the PPK of LUM (IV). Inter-subject variability in apparent oral clearance (CL/F) was explained by body mass index (BMI) and age, while that in apparent volume of distribution of the central compartment (V_C/F) was explained by weight. Lumefantrine population mean CL/F, inter-compartment clearance (Q/F), V_C/F and apparent volume of distribution of peripheral compartment (V_P/F) were 3.19 L/hr, 0.176 L/hr, 28.1 L, and

58.4 L, respectively. Our results indicate that LUM CL/F decreased with age from two to just less than five years (≈20.6%. p=0.04) and LUM CL/F increased with decreasing BMI.

Conclusions. The LC MS/MS method is suitable for pediatric studies with repeated sampling and long time follow up. Oil fortified maize porridge can be an alternative to milk in augmenting absorption of LUM. Our findings provide a structural basis for consideration of age and BMI in evaluation of rational AL dosing guidelines among under five year old children.

LIST OF SCIENTIFIC PAPERS

I. Mwebaza N, Jerling M, Gustafsson LL, Obua C, Waako P, Mahindi M, Ntale M, Beck O, Hellgren U. Comparable lumefantrine Oral bioavailability when co-administered with oil fortified maize porridge or milk in healthy volunteers. *Basic & Clinical Pharmacology & Toxicology* 2013; 113: 66-72.

II. Silva AV, Mwebaza N, Ntale M, Gustafsson LL, Pohanka A . A fast and sensitive method for quantifying lumefantrine and desbutyl-lumefantrine using LC- MS/MS. *Journal of Chromatography B* 2015; 1004: 60–6.

III. Mwebaza N, Jerling M, Gustafsson LL, V Silva A, Pohanka A, Obua C, Waako P, Beck O, Vafa Homann M, Färnert A, Hellgren U, Oil-fortified maize porridge increases absorption of lumefantrine in children with uncomplicated falciparum malaria.
Basic & Clinical Pharmacology & Toxicology, in press 2016.

IV. Mwebaza N, Hellgren U, Ojara FW, Gustafsson LL, V Silva A, Pohanka A,Obua C, Waako P, Ette EI. Estimation of the population pharmacokinetics of lumefantrine and its metabolite desbutyl-lumefantrine in children under 5 years of age with malaria.
(Submitted to *Journal Of Clinical Pharmacology*)

Papers will be referred to by their roman numbers (I-IV)

CONTENTS

LIST OF ABBREVIATIONS

A	Artemether
ABC	ATP (Adenosine Triphosphate) -binding cassette superfamily of transporters
ABE	Average bioequivalence
ACPR	Adequate Clinical and Parasitological Response
ACT	Arteminisin-based combination therapy
ADVAN4	Routine specified by a program for a two Compartment Linear Model with First Order Absorption
ADVAN6	Routine specified by a program for General Nonlinear Model
AL	Artemether- lumefantrine
ALAG1	Population mean absorption lag time
AQ	Amodiaquine
ART	Antiretroviral therapy
AS	Artesunate
AUC	Area Under the plasma concentration-time Curve for a specified time period (as a subscript e.g. from 0 time to infinity $AUC_{0\text{-}inf}$)
BE	Bioequivalence
BMI	Body mass index
BS	Blood Smear
CL/F	Apparent oral clearance of drug from plasma
CL_M	Rate of elimination of the metabolite
$C_{(LUM)}D7$	Lumefantrine concentration on day 7
C_{max}	Peak or maximum concentration attained after specified dose
CQ	Chloroquine
CRCL	Creatinine Clearance
CYP	Cytochrome P450 enzymes
DBL	Desbutyl-lumefantrine
DMSO	Dimethyl sulfoxide
DP	Dihydroartemisinin plus piperaquine
EFV	Efavirenz
EIR	Entomological Infection Rates
ESI+	Positive electro spray ionization
ETF	Early Treatment Failure
FOCE	First order conditional estimation method
GIT	Gastrointestinal
HPLC	High Performance Liquid Chromatography
IIV	Inter-individual variability
IV	Intravenous
KA	Absorption rate constant
K45	Transfer rate constant from central to peripheral metabolite compartments
K54	Transfer rate constant from peripheral to central metabolite compartments
LOQ	Limit of Quantification
LLOQ	Lower Limit of Quantification
LUM	Lumefantrine
LCMS/MS	Liquid Chromatography Tandem Mass Spectrometry

LCF	Late Clinical Failure
LFT	Liver function tests
LL	log likelihood
LPF	Late Parasitological Failure
LPV	Lopinavir
MoH	Ministry of Health
MSP	Merozoite surface proteins gene
mw	Molecular Weight
NONMEM	Nonlinear Mixed Effect Model
NP	Non-parametric methods
NVP	Niverapine
PCR	Polymerase Chain Reaction for *P. falciparum* genotyping
PD	Pharmacodynamics
pfmdr-1	*Plasmodium falciparum* multidrug resistance gene
PK	Pharmacokinetics
PPK	Population Pharmacokinetics
Q/F	Inter-compartment clearance
REF	Reference
RSE	Relative standard error
SP	Sulphadoxine –Pyrimethamine
SSA	Sub-Saharan Africa
T_{lag}	Time of onset of absorption
T_{max}	Time to peak concentration
TRANS1	Default translation of set of microconstants in NONMEM
TOL	A command in NONMEM that tells "the number of accurate digits that are required in the computation of drug amounts, i.e., the relative tolerance"
TV	Typical value
UV	Ultraviolet Detection
V_C/F	Apparent central volume of distribution
V_P/F	Apparent volume of distribution of the peripheral compartment
WHO	World Health Organization
WT	Weight
WWARN	Worldwide Antimalarial Resistance Network

OPERATIONAL DEFINITIONS

Bioavailability: the amount or fraction (measured by both rate and extent) of the administered dose of unchanged form of therapeutically active agent that reaches the systemic circulation and is available at the site of action [127, 190].

Absolute bioavailability: the amount of a therapeutically active agent that reaches the systemic circulation relative to that given by the intravenous route. Ideally the intravenous dose is assumed to be 100% available [190].

Oral bioavailability: rate and extent of absorption of an orally administered therapeutically active agent that reaches the systemic circulation and is available at the site of action. [190].

Relative bioavailability: estimate of the fraction of a dose administered through another route that is absorbed into the systemic circulation when compared to the fraction available when given as an intravenous dose form, or comparisons between fractions absorbed when a drug is administered in 2 different forms (different route of administration or formulation- solution, suspension or same form but different product /brands) or when given with different supplements [190].

Bioequivalence: the absence of a significant difference in bioavailability of therapeutic equivalents or alternatives at the site of drug action or in systemic circulation when administered at the same molar dose under similar conditions [190].

Pharmacokinetics: the way the body handles the therapeutic agent after it has been administered; once the therapeutic agent has been released it undergoes subsequent processes including absorption, distribution, metabolism and excretion [192].

Population pharmacokinetics: the study of variability in plasma drug concentrations in patient population when standard dose regimens are administered [127].

Apparent oral clearance (CL/F): the volume of plasma cleared of the therapeutic agent (which has been administered orally) per unit of time [133].

Apparent volume of distribution (V_C/F): "the theoretical volume that would be necessary to contain the total amount of an administered drug at the same concentration" as that being observed in the blood plasma [133].

1 INTRODUCTION

Malaria remains one of the leading causes of death among children below the age of 5 years living in sub-Saharan Africa (SSA) [1]. Uncomplicated *Plasmodium falciparum* malaria accounts for above 95% of clinical attacks and if not well treated, rapidly progresses to severe disease among children < 5 years of age [2]. Effective case management is still the cornerstone in malaria control. Artemisinin based combination therapies [ACTs] are recommended to optimize treatment response and reduce the risk of development of drug-resistant parasites [3]. Artemether-lumefantrine [AL] was selected as first-line ACT for uncomplicated *falciparum* malaria in Uganda in 2004 and is a widely adopted policy in SSA countries [3-5].

Lumefantrine (LUM) is the long acting antimalarial partner agent responsible for ensuring cure [3]. In SSA including Uganda, global financial initiatives [6, 7] through World Health Organization (WHO) and governments' commitment have scaled up availability of ACT at public health facilities for treatment of childhood diseases at community level [8-12]. However oral bioavailability of LUM is highly dependent on intake of dietary fat and the basis for the AL dosage regimen among children is still unclear.

This thesis sought to optimize use of AL during treatment of uncomplicated malaria, to benefit the most vulnerable users and provide a pharmacokinetic basis for evaluation of rational AL dosage guidelines for children under five years of age.

1.1 MALARIA BURDEN

Malaria is one of the commonest mosquito-borne diseases in humans [1]. The infection is caused by six *Plasmodium* species namely *P. falciparum, P. malariae, P. vivax, two species of P. ovale* (classic type *P.ovale curtisi and* variant type *Plasmodium ovale wallikeri*) [13, 14] and *P knowlesi* [15]. *P. falciparum* species is the commonest infection, responsible for the highest disease burden and fatality [1, 2].

Globally, 3.3 billion people are estimated to be at risk of malaria, with disproportionately high risk among those with poorly developed immunity and living in tropical climate especially in resource limited settings in SSA [1]. In 2015, WHO estimated "214 million cases of malaria globally, 88% of which occurred in Africa" and "438, 000 deaths, 90% of which occurred in Africa" and children < 5 years accounted for about 70 % of all deaths [1]. In 2015, Uganda was ranked 4th highest malaria burdened country in Africa and 10th global contributor to malaria related mortality [1]. Though mortality among children aged < 5 years reduced by 34% from 137 to 90 /1,000 live births between 2004 and 2011 [16]. Malaria remains one of the leading causes of illness and death for this age group in Uganda [17]. Available data in 2013 by the Uganda ministry of health (MoH) estimated 16 million malaria cases to have contributed to 30%-50% of all outpatient visits, 15%-20% of hospital admissions and 12.8% hospital deaths [17, 18]. Surveys conducted among Ugandan children < 5 years of age, registered reduction in malaria prevalence by microscopy from 42 (range: 5 – 63) % in 2009 [19] to 19 (range: 6-22) % in 2014 [20]. Of note, malaria was reported to be "four times more prevalent in rural than in urban areas" and "seven times as common among children in the lowest compared to those in highest wealth quintile" [20].

Thus the need to optimize AL use in resource limited settings where malaria is most prevalent.

1.2 HISTORY AND TREATMENT OF UNCOMPLICATED MALARIA

For long, treatment of uncomplicated falciparum malaria was effective with monotherapy using 4-aminoquinolines such as chloroquine (CQ), amodiaquine (AQ) or co-formulated antifolate drugs such as sulphadoxine -pyrimethamine (SP). Parasite resistance to CQ was reported in 1950s in South America [21], later in 1960s in East Asia [22] and reported to have increased between 1973 and1981 in Southeast (SE) Asia and South America [23-25]. Worldwide, malaria transmission heightened between 1982 and 1995 [26]. Resistance to CQ was first reported in East Africa (Kenya & Tanzania) in 1978 [27, 28] but documented much later in Uganda in 1988 [29, 30]. Uganda MoH provisionally adopted CQ+SP in 2000 but confirmed the combination as first line treatment of uncomplicated *falciparum* malaria, later in 2002 [30]. In Uganda, CQ monotherapy for *P. falciparum* continued beyond 2002 and remained efficacious in a few low transmission areas [31]. However increased treatment failure rates were reported especially in children < 5 years [32, 33]. Though resistance to SP had been reported as early as 1985 [34, 35], SP was effective when used in combination with 4-aminoquinolines [36-38]. Nevertheless use of CQ + SP was short-lived with increasing treatment failure [37-45]. In 2004, Uganda adopted AL as first line treatment of uncomplicated malaria but was not used widely until 2005 [4].

The WHO recommends ACT regimens. Short acting artemisinins are combined with long acting partner drugs to optimize cure rates, reduce the risk of development of resistant *P.falciparum* and reduce malaria transmission [46-48]. Current treatment options for uncomplicated falciparum malaria (except pregnant women in first trimester) include AL, AQ and artesunate (AS), AS + mefloquine, AS + SP, and dihydroartemisinin plus piperaquine [3, 49]. For pregnant women in first trimester, quinine plus clindamycin is recommended [3].

1.3. CLINICAL TRIALS OF ARTEMETHER-LUMEFANTRINE IN AFRICAN CHILDREN

The current standard AL regimen with 6 doses at 0, 8, 24, 36, 48, 60 hours has been efficacious with day 28 cure rates among African children in clinical trials ranging between 95 and 100% [50-59]. However, a recent meta-analysis indicated low cure rates among Asian children between 10 and 15 kg; and malnourished African children between 1- 3 years [60]. Efficacy may be lower in real life situations. Indeed effectiveness studies in African children have reported varying trends, ranging from those observed in clinical trials to higher failure rates (4%-39%) [61-64]. In Africa, trials comparing AL with another commonly used ACT, dihydroartemisinin plus piperaquine (DP) showed lower Day 28 PCR adjusted parasitological failure rates with DP {range 2-5.8 % vs 0- 2.0% for AL vs DP respectively} [65-70]. This may be explained by better absorption and longer half-life of the long acting partner drug piperaquine compared to LUM.

1.4 RELATIONSHIP BETWEEN LUMEFANTRINE EXPOSURE AND MALARIA TREATMENT OUTCOME

The risk of therapeutic failure during AL treatment is attributed to low PK exposure of the long acting partner LUM [71-73]. Predictors of therapeutic response have been identified as the overall area under LUM plasma concentration- time curve (AUC) and increased time during which LUM concentrations were above minimum inhibitory concentration of 280 ng/ml [72]. Venous plasma LUM concentration on day 7 ($C_{(LUM)}D7$) has been found to be a surrogate marker of the overall AUC [72, 74, 75]. Various $C_{(LUM)}D7$ ranging from 50, 175, 400, 500, 600 ng/ml, have been correlated with therapeutic response in different clinical studies [72, 76-79]. The two most commonly referenced $C_{(LUM)}D7$ as prediction threshold for recrudescence have been 280ng/ml, first described among Thailand patients in an area of highly drug resistant P. *falciparum* [72] and 175 ng/ml among Thailand patients in low malaria transmission area [76]. In another study in Uganda, $C_{(LUM)}D7 < 280$ ng/ml did not predict treatment failure, but re-infections were registered in patients with $C_{(LUM)}D7 < 400$ ng/ml [79, 80]. In this Ugandan population, predictors of low $C_{(LUM)}D7$ and increased risk of malaria re-infection were reported to be age below 5 years and a low total LUM dose between 50 and 79 mg/kg [79]. In a real life study in Tanzanian children, significant correlation between low LUM exposures and increased risk of treatment failure was demonstrated [63]. The WHO recommends "target total dose ranges" of "5- 24 mg/kg of artemether" and "29-144 mg/kg of LUM" to provide adequate drug exposure [3].

1.5 HISTORY AND PHARMACOLOGY OF ARTEMETHER-LUMEFANTRINE

Artemether (A) is co-formulated with LUM in fixed ratios of 1:6 {20mg of A and 120 mg of LUM} administered as weight-based fixed-dose: 5 to < 15 kg receives 1 tablet {20 mg A /120 mg LUM}, 15 to < 25 kg receives 2 tablet {40mg A/240 mgLUM}, 25 to < 35kg {60 mg A / 360 mg LUM}, and ≥ 35 kg {80 mgA/480mg LUM}[81].

Development of AL combination was started in 1981 by Zhou Yiqing and colleagues at Academy of Military Medical Sciences, Beijing, China (www.epo.org/learning-events/european-inventor /finalists /2009/ zhou.html). Artemether lumefantrine was the first co-formulated ACT and was approved for use in China in 1992 [82]; in 1999 in Africa and the European Union and as late as 2009 by the US Food and Drug Administration [83]. The dispersible formulation for use in children was availed in 2009 [83]. Artemether-lumefantrine is effective against all malaria species although it is particularly indicated for treatment of uncomplicated *P. falciparum* and *P. vivax* [3].

1.5.1 History, pharmacokinetics and pharmacodynamics of artemether

Artemether (A), {$C_{16}H_{26}O_5$, molecular weight [mw] 298.38 Da} is a lipid-soluble semi-synthetic artemisinin derivative from *Artemesia annua,* a traditional Chinese medicinal plant. The active component of artemisinins {qinghaosu} was first extracted and chemically isolated in 1971 by Youyou Tu [84-86] and evaluated for clinical use in 1981[87,88]. Youyou Tu received the 2015 Nobel Prize in Physiology / Medicine.

Artemether is currently given orally or intramuscularly. Oral absorption of A is enhanced by fatty food and is rapid with lag time between 0.13 and 2 h and peaks within 1-2 h [74, 89-91]. Artemether {$t_{1/2}$ =1-3 h} is metabolized predominantly by CYP 3A4 and to minor extent by CYP2B6, 2C9, and 2C19 [81, 92]. Artemether is more rapidly eliminated than its active metabolite dihydroartemisinin {$t_{1/2}$ =1 – 5 h} [74, 93, 94] and undergoes auto induction (enzymatic metabolism) with subsequent doses [95]. Artemisinins are sesquiterpene lactone endoperoxides that exert rapid parasite clearance. Direct reduction of their endoperoxide ring by iron in heme generates reactive oxygen radicals which rapidly kill most stages of blood ring forms, trophozoites and schizonts of all *Plasmodium* species [96, 97]. Artemisinins also affect activities of parasite mitochondria, inhibit nucleic acid and protein synthesis [98, 99]. Other pharmacodynamic (PD) effects of *A* include inhibition of adhesions process (cytoadhesion and resetting) associated with severe form of *P. falciparum* malaria [100]. Resistance to arteminisins (K13 gene mutation) has been reported in SE Asia [101-102]. Similar mutations were found in East and West Africa but these isolates were not validated to confirm association with clinical resistance [103-105].

1.5.2 History and pharmacodynamics of lumefantrine

Lumefantrine is the long acting schizonticidal agent in the AL combination, responsible for eliminating the residual parasites surviving short acting A exposure [73]. Lumefantrine (benflumetol {$C_{30}H_{32}Cl_3NO$, mw 528.95 g/mol}, 2-dibutylamino-dichlorobenzylidene-H-fluorenyl-ethanol) is a synthetic aryl amino alcohol. It was synthesized by 1976 under Academy of Military Medical Sciences in Beijing, China and certified for manufacture in 1989 by Kunming Pharmaceutical Company [82, 106]. Lumefantrine has schizontocidal effect on erythrocytic stages of all *Plasmodia* species and also gametocidal activity [3, 107]. The mechanism of action of LUM is not clearly defined but it is thought to be similar to that of quinolines and related aryl alcohols [108]. The drug accumulates in the parasite food vacuole and interferes with heme polymerization by forming complexes. Thus inhibit formation of non-toxic hemozoin. Accumulated toxic heme leads to parasite death and also inhibits parasite nucleic acid and protein synthesis [108]. Although molecular markers associated with LUM resistance (P. falciparum multidrug resistance genes, *pfmdr-1* 86N, 184F, and 1246D alleles) have been found in African clinical samples where AL has been widely used, this has not yet been associated with clinical failure [109- 111].

1.6 PHARMACOKINETICS OF LUMEFANTRINE

Lumefantrine is currently only available as an oral co-formulation with A for human use. However PK comparison of parenteral and oral LUM in rats indicated poor erratic oral absorption with absolute oral bioavailability ranging between ≈ 5 and 12%; and a low extraction with clearance of 0.03 liters/h/kg (< 2% of the hepatic plasma flow) [112].

Lumefantrine is highly lipid soluble with slow and erratic fat dependent absorption [91] with lag time of 2-4 h and peaks between 4 and 10 hr after an oral dose [72, 91, 113]. Lumefantrine binds variably to plasma lipoproteins (77%, 7.3%, 6.6% for high, low and very low density lipoproteins respectively) and minimally (10%) to erythrocytes [114]. Estimates of apparent oral clearance (CL/F) (range 0.077-0.104 L/h/kg) and apparent volume of distribution (V/F) (range: 0.4-8.9 L/kg) of LUM have been summarized by WHO [3]. The half life ($t_{1/2}$) of LUM is variable, ranging from 3 to 10 days among healthy

adult volunteers [113, 115, 116] and malaria patients including children and pregnant women [72, 117, 118]. Some studies reported significantly shorter LUM $t_{1/2}$ (range 1- 4 days) in pediatric patients [119, 120] and among pregnant women compared to non-pregnant adults [121,122]. Lumefantrine undergoes predominantly hepatic excretion, and both parent and metabolites have been found in bile and faeces during animal studies [115]. There is no published data on the extent of renal excretion of LUM.

Lumefantrine is metabolized mainly to desbutyl-lumefantrine {DBL} predominantly by the liver microsome enzyme CYP3A4 [81, 113]. The metabolite has anti-malarial activity [123-124] but systemic exposure of DBL is low ranging between 0.8% and 14 % of that of the parent drug [76, 81, 117, 125, 126]. The half life ($t_{1/2}$) of DBL was reported to be slightly longer than that of the parent drug in one study among children {median 5.3 vs 5.9 days} [117].

1.6.1 Factors influencing lumefantrine exposure

Drug exposure is a result of various PK processes including absorption, distribution, metabolism and excretion [127]. Differences in LUM PK exposure are mostly attributed to absorption (section 1.6.1.1) and metabolism (section 1.6.1.2). Variation in LUM exposure can also be caused by physiological changes including pregnancy [118, 121,122] and pharmacogenetic variations in CYP3A4 enzymes [128].Variation in CYP3A4 activity as a result of physiological maturation changes has been reported to affect exposure of some substrates (section 1.6.2).

1.6.1.1 Effects of food on lumefantrine absorption

Oral bioavailability of LUM is highly variable but dependent on food intake [91, 129, 130]. This was evident in malaria patients as they resumed normal diet [72, 74]. Among healthy volunteers, high fat meals augmented oral bioavailability of LUM as much as 16-fold compared to only two fold increase for A [91, 129]. At least 1.2g of fat is needed to sufficiently improve LUM absorption [130].

The clinical importance of dietary fat was demonstrated in an earlier study, day 28 PCR adjusted cure rates varied significantly between patients who received AL with food and those dosed without food (86.5% vs. 71.1%, p=0.02) [78]. No significant difference in clinical outcomes was observed in studies examining unsupervised and supervised AL therapy in three different countries [64, 77, 79]. However, in two of these studies, significantly lower $C_{(LUM)}D7$ were observed with unsupervised AL treatment compared to the supervised groups [64, 77].

Concern still remains whether optimal LUM levels are attainable in real life situations when unsupervised outpatient AL treatment is inevitable and in areas where milk may not be easily available. A study conducted in 5 African countries reports that only 57.5% of the AL doses were administered with milk [131]. In Tanzania, only 43% of hospitalized pediatric patients receiving AL for treatment of uncomplicated *falciparum* malaria were able to complete their milk portions [120]. Thus, smaller meals but with sufficient fat content may be necessary.

1.6.1.1.1 Factors affecting bioavailability of orally administered drugs

Oral bioavailability of a drug depends on both pharmaceutical (properties, bioequivalence, drug interactions) [132] and patient factors (physiological processes and co-morbidity) [133]. Drug-related factors involved are physicochemical properties of the drug (solubility, size, lipid solubility, stability) and the formulation (design and form) [132, 134]. Patient related factors include gastrointestinal {GIT} physiological processes and food effect on absorption [132, 135, 137, 138]. These include alterations in P^H of GIT contents and hepatic blood flow [132]; drug efflux by ATP-binding cassette (ABC) transporters p-glycoprotein and metabolism at the intestinal mucosa [139, 140]. Gastric emptying varies with dietary content, mechanical effects such as volume and state of food, either solid or liquid and food viscosity [133, 135, 136].

Mechanisms responsible for poor LUM oral bioavailability are attributed to its physicochemical properties and GIT barriers to absorption [139]. The solubility of LUM and availability for absorption is enhanced by dietary fat [91, 129, 130]. Fat delays gastric emptying which prolongs bile secretion necessary for emulsification of fat thus augments absorption of lipid soluble drugs like LUM [133, 135]. Absorption of LUM is further moderated by enterocyte CYP3A4 metabolism and efflux activity by ABC transporters at intestinal epithelial cell level [139].

1.6.1.1.2 Assessment of bioavailability

Oral bioavailability is a measure of drug exposure, estimated by the rate and extent to which an orally absorbed drug reaches the systemic circulation or site of action [127]. Assessment of bioavailability employs direct PK outcomes (estimates of drug levels in circulatory or excretory compartments) and rarely indirect PD outcomes (immediate pharmacological or therapeutic responses) [141]. Pharmacokinetic measures include the rate of exposure {time of onset of absorption (T_{lag}) and to peak (T_{max})} and extent of exposure {peak concentration (C_{max}), in respect to concentrations attained over time (AUC)} of exposure [141].

Bioequivalence (BE) assumes the absence of significant difference in the bioavailability and therefore similar therapeutic and safety effects of innovator drug and therapeutic equivalents (generic drug products) or alternatives (different formulations) [141]. Average bioequivalence {ABE} technique compares PK outcome measures of reference and test drug/pharmaceutical products. The two are bioequivalent if the confidence interval {90%CI} for the ratio of means of the drug exposure is within bioequivalence limits (0.80-1.25) [141]. However it may be argued that bioequivalence may not deem that the products can be used interchangeably, some drugs are highly variable [142] and drug products may display significant differences in variance in PK estimates due to inter-individual and intra-individual variability [143-146]. So, other BE criteria may be considered [143-146].

1.6.1.2 Drug-drug interactions influencing lumefantrine exposure

Drug- drug interactions influencing LUM metabolism has been reported. Cytochrome P4503A4 inhibitors such as ketoconazole [120] and protease inhibitors {lopinavir, lopinavir/ ritonavir, darunavir/ritonavir} [127, 147-151] have been reported to increase LUM exposure when given concurrently. Lumefantrine exposure was reduced when AL was co-administered with CYP3A4 inducer such as efavirenz [147, 149, 152-154], etravirine [151] and rifampicin [155]. Lumefantrine has been reported to inhibit CYP2D6 [81].

1.6.2 Role of ontogeny on pharmacokinetics of drugs

Ontogeny, the course of growth (size change) and development of an individual organism impacts on PK process [156]. Development entails physiological maturation which involves phenotypic (structural changes in size, shape and appearance) and functional changes in body systems including drug metabolizing enzyme systems [157 - 161]. Differences in PK between children ≥2 years of age and infants <2 years of age have been reported [156]. The maturation trends in CYP3A4 have been reported to reach peak at or after 6 months and appeared to decline after infancy or 2 years [162-164]. This trend has been observed for some CYP3A4 substrates including midazolam [165], tacrolimus [166] and lopinavir [167]. Lumefantrine is also a substrate for CYP3A4 [84, 120]. There is evidence pointing to lower LUM exposure concentrations ($C_{(LUM)}D7$) among children compared to adults treated according to the current dosage regimen [79, 80, 117, 119, 131, 168, 169]. Therefore allometric scaling adjusted to physiological functional changes in relation to ontogeny of CYP3A4 with age can be incorporated into elimination PK models to help optimize parameter estimations [170].

1.6.3 Challenges for pharmacokinetic studies among children

Traditional PK methods employ intensive sampling designs. As such, studies often involve adult healthy volunteers and patients. These may not usually be valid for pediatric population with their developmental changes which are likely to alter physiological and PK processes [171]. Ethical considerations for not including children intensive PK studies involve avoiding inflicting pain, lack of certainty of consent and safety considerations of volume of blood to be drawn. This constrains research among young children and explains the lack of studies necessary for rational recommendation of pediatric dosage regimens.

1.7 POPULATION PHARMACOKINETICS

1.7.1 Population pharmacokinetics methods

Methodological limitations are associated with use of traditional PK analysis despite availability of dense individual data from few people. Population PK approach uses sparse data from larger populations to characterize population PK mean values, explain sources of variability in PK outcomes and relationships between PK and PD outcomes [172]. Population based PK methods describe both fixed effects (population mean estimates) and the contribution of the random effects [173]. Furthermore PPK models display the extent of variability. An individual estimate differs from the population mean by a value contributed by random effects from inter-subject differences and unknown influences. Unknown influences may include intra-subject differences, inter-occasion differences, measurement errors, and unexplained residual differences [173].

Population PK approaches commonly employ parametric methods (non-linear mixed effect modeling, NONMEM), first described by Sheiner and Beal in 1972 [174]. Non-linear mixed effect modeling assumes a population with approximate normal distribution of parameter estimates. However, non-parametric (NP) methods for population modeling are available {e.g. NP maximum likelihood, NP adaptive grid, NP Bayesian algorithm}, which make no assumptions about the parameter distribution. This makes NP methods suitable approach for characterizing population parameters for populations with uncertain or bimodal or multimodal distributions [175].

1.7.2 Population pharmacokinetics of lumefantrine

There is increasing data on PPK of LUM and its metabolite, DBL profiles. Studies have been done among children [117, 120, 177], children and adults [178], pregnant and non-pregnant women [118, 179], and HIV populations on antiretroviral co-treatment (ART) [154, 180]. Population estimates for central compartment parameters (apparent oral clearance {CL/F} and volume of distribution {V_C/F}) of LUM and DBL varied across similar and different populations (Table 1). Different structural PK models were used to characterize LUM and DBL population parameters (Table 1). Furthermore, different approaches or assumptions were used to model DBL disposition. In some studies, the V_C/F of DBL was fixed to that of parent drug, LUM [178, 179]; whereas others assumed 100% conversion of parent drug to metabolite [117, 180].

The variability in CL/F was partially explained by dose, age and weight during an earlier dose finding trial among adult patients > 15 years [72]. In addition, the influence of the initial level of parasitemia on LUM bioavailability was also reported [72]. Another population PK study that enrolled children and adults with age ranging from 1 to 78 years (median: 9 years) identified body weight, age and height as significant covariates influencing CL/F [178]. They retained weight only in their model because weight correlated well with age and height. Their findings justified the current weight based LUM dosing approach [178]. Studies that involved pediatric malaria patients do not provide information particularly on the vulnerable, < 5 year old population [117, 120, 178]. These

studies in children did not identify any influential patient covariates. Lately, the PPK of LUM has been described in Ugandan children aged ≤ 2 years where age was found to have a positive influence on bioavailability [177].

Table 1. Reported mean population pharmacokinetic estimates of lumefantrine and desbutyl-lumefantrine parameters after six AL doses in children and adults.

Study Ref		Study Population Location (Number)	Age[mr] (years)	Model (cpt)	T_{lag}	Ka (h^{-1})	CL/F (L/h)	V/F (L)	Duration of sampling
Children									
177	LUM	Uganda (207)[#]	1.2 (0.5 - 2)	2	-	-	**2.19**	83.2	21 days
117	LUM	Papua New Guinea (13)	7.7(5-10)	3	-	0.461	**7.29** [(a)]	227 [(b)]	28 days
	DBL			2	-	-	**701** [(a)]	51,100	
120	LUM	Tanzania (n=50)	4(1-10)	1	1.92	0.82	**0.077**[(d)]	8.9 [(b)]	3 days
Children , adults and pregnant women									
178	LUM	Tanzania (143)	9 (1-78)	1	-	0.54	**7.7**	265	7 days
	DBL	Pregnant (3/143)		1	-	-	**4.8**	-	
Pregnant and Non-pregnant women									
179	LUM	Tanzania. Pregnant (33) and	21 (18–41)	1	-	-	**2.89**	134	7 days
	DBL	Not (22)		1		-	**2.6**	-	
118	LUM	Uganda Pregnant (115) and Not (17)	21 (15–38)	2	-	(4.09)[(c)]	**5.09**	123	21 days
HIV Patients on Antiretroviral therapy with malaria co-treatment									
180	**LUM**	Uganda (n=89)	36 (20-70)	2	-	(6.27)[(c)]	**4.77**	68.9	3-5 days
	DBL	{LPV/r or EFV or NVP}		1			**489**	22 800	
154	LUM	Tanzania (194) {EFV or NVP}	43 (21-67)	2	1.45	0.032	**4.54**	25.6	14 days

: 107 children with 207 malaria episodes cpt: Number compartment in disposition model.
mr: median age (range). (a):unit, L/h/70kg . (b) Unit, L/70kg. (d):L/h/kg
LPV, EFV & NVP: Lopinavir, efavirenz & niverapine based antiretroviral therapy (ART)
All first order absorption except (c), mean transit time in hours for the transit absorption model.

1.8 CHALLENGES IN QUANTIFICATION OF LUMEFANTRINE IN BLOOD

Lumefantrine determination is constrained by its physicochemical properties. Lumefantrine is heat labile therefore is easily degraded at room temperatures [181], and may not be stable for more than 6 months in -20°C [182]. This poses a challenge while sampling, during storage and handling of samples. Lumefantrine plasma levels may be potentially lowered during collection in field studies or during sample preparation in case samples are not immediately frozen and kept so until extraction. Furthermore LUM is highly lipid soluble and bound to plasma lipoproteins [114]. Careful choice of solvents for sample extraction and mobile phase is required. In preparation for a study among pediatric patients, capillary samples would be preferred to venous samples. Good correlation without significant variation between capillary and venous LUM concentrations was reported, but capillary levels tend to be slightly higher thus the need for a correction factor [183]. Venous plasma is therefore preferred for precise quantification of LUM concentrations. Furthermore, minimal but adequate volume of drawn blood is a crucial consideration when undertaking studies among young children.

Recent establishment of anti-malarial activity of the metabolite, DBL underlines the importance for concurrent determination with the parent compound [123-125]. However, DBL is present in very low concentrations with a factor of up to 100x lower than the parent compound [76, 81, 117, 125, 126]. Therefore, HPLC-UV detection may not provide the required sensitivity for quantification of low concentrations of DBL [126].

Due to technical limitations including laboriously lengthy extraction process and low sensitivity, earlier high-performance liquid chromatography methods are not adequate for follow up of low LUM levels in small volume samples [126, 182, 184, 185]. Recently, more sensitive liquid chromatography tandem mass spectrometry techniques have been published [186-188]. The availability and use of isotope labeled internal standard is preferred in order to overcome matrix effect and ion saturation (interferences leading to signal suppression or enhancement) [188]. Thus, these isotopes are preferred as internal standards rather than structural analogues to LUM like halofantrine [187] in order to improve accuracy and precision of the quantification method [188].

1.9 RATIONALE FOR THIS STUDY

Effective case management remains the cornerstone in malaria control. With exception of poorly developed host immunity to malaria and parasite resistance, low drug exposure is the other contributor to the increase of risk of malaria treatment failure [75].

Artemether –lumefantrine is a widely adopted first-line ACT for uncomplicated malaria, in SSA including Uganda [3-5]. Furthermore, global financial support through WHO and government commitment has sustained prompt access to effective malaria treatment in SSA [6-10, 12]. However it is important to ensure optimal drug exposure during treatment.

The long acting partner drug, LUM is responsible for adequate treatment outcomes by eliminating residual parasites, left after massive but short-lived clearance of parasites by the most potent short acting artemether [72, 113]. The critical role of food in augmenting artemether - lumefantrine uptake is well established [91, 129, 130]. Dietary fat is essential for augmentation of AL absorption particularly LUM which is highly lipid soluble [129]. Milk or high fat food is recommended food supplement for AL [81]. Milk is scarce and food consumption in most sub-Saharan countries is principally carbohydrate rich with little or poor fat content. It is not known whether adequately uptake of AL is achieved when given with available local foods with minimal fat content. However it has been confirmed that as little as 1.2g of fat sufficient improves LUM absorption [130]. This information can be used to identify appropriate alternative food supplements suitable for user populations.

Children under five are especially vulnerable to malaria and are at higher risk of treatment failure than adults by virtue of their undeveloped immunity [1]. The basis for pediatric AL doses is still unclear [60, 169]. The dose is currently fixed-weight adjusted and probably based on adult data [3], yet maturation may impact on drug disposition [156-161]. Indeed meta-analysis findings indicated lower exposure among young and malnourished children compared to older children and adults treated under the current AL dosage regimen [60]. Scientific data on factors influencing variability in drug concentrations among vulnerable populations is necessary to lend credence to rational dosage guidelines [172]. There is sparse data on PPK of LUM among pediatric malaria patients but studies do not provide information particularly on the vulnerable, < 5 year old population [117, 120, 177, 178].

For the intended bioavailability and PPK studies among pediatric patients with uncomplicated malaria, a sensitive analytical method was required. The method needed to be sensitive to allow determination of LUM and DBL concentrations in small plasma volumes obtained from young children during follow up to the study end point, which was 28 days.

2 STUDY OBJECTIVES

2.1 General objective

To explore the effect of local foods on the pharmacokinetics of lumefantrine (LUM) and describe the population pharmacokinetics of LUM among Ugandan children; in order to optimize AL use and provide basis for suggesting rational dosage for artemether-lumefantrine (AL) adjusted to the user population and conditions in the country.

2.2 Specific objectives

1. To determine the effects of local food (milk, maize porridge, maize porridge plus vegetable oil) on the bioavailability of LUM among healthy Ugandan adults after a single oral dose of co-formulated AL.

2. To develop and evaluate a sensitive LC MS/MS method for determination LUM and its desbutyl-metabolite in small plasma volume.

3. To compare the effects of maize porridge plus vegetable oil versus milk on the oral bioavailability of LUM among under five year old Ugandan children receiving AL for uncomplicated *falciparum* malaria.

4. To describe the population pharmacokinetics of LUM among under five year old children in Uganda receiving AL for uncomplicated *falciparum* malaria.

3 METHODS

3.1 STUDY SETTING

The study was conducted at the Department of Pharmacology & Therapeutics, Makerere University College of Health Sciences, Kampala, Uganda. This is within the Mulago National Referral Hospital Complex Campus. Drug assay was done at Department of Pharmacology & Therapeutics (I) and Division of Clinical Pharmacology, Karolinska University Hospital, Huddinge, Stockholm Sweden (II, III, IV).

3.2 STUDY PARTICIPANTS

3.2.1 Healthy Adult Volunteers (I)

Healthy volunteers were respondent adult paramedical and nursing students attending Mulago Paramedical and Nursing Schools, Kampala, Uganda. The student population is largely of black origin, predominantly originating from Ugandan ethnic groups.

3.2.2 Pediatric patients (II, IV)

Pediatric patients were under five year old children suffering from uncomplicated *P. falciparum* malaria. Parents / guardians were approached at hospital outpatient departments of Mulago Hospital and surrounding public health facilities within 20 Km radius, including Kampala, Mukono, Wakiso and Mpigi districts (Central Uganda). Central Uganda is mesoendemic for malaria with low Entomological Infection Rates (EIR) of about 8 [189].

3.3 GENERAL METHODOLOGY

This scientific work comprised of 4 experimental sub-studies. A bio-analytical method (II) was developed to facilitate drug assays for sub-studies III and IV. Food-drug interaction studies included an initial explorative bioavailability study in healthy adult volunteers (I) followed by a comparative bioavailability study (III) nested in the observational PK study among < 5 year old children treated for uncomplicated *P. falciparum* malaria (IV) (Figure 1). Reference will be made to adult healthy volunteers as "adult volunteers" and < 5 year old sick children as "pediatric patients".

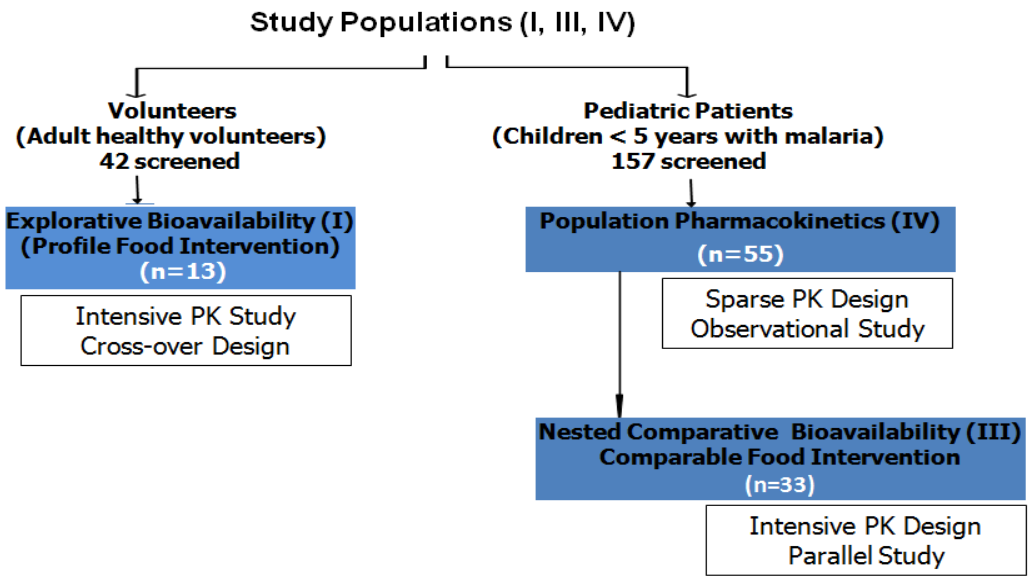

Figure 1. *A schematic diagram showing the study populations and designs for the pharmacokinetic studies.*

3.4 PROCEDURES FOR ADULT VOLUNTEERS BIOAVAILABILITY STUDY (I)

3.4.1 Selection of adult volunteers

The study was conducted between April and August 2009. Eligibility of consented healthy volunteers (n=13) was established based on history, clinical examination, hematological and chemistry laboratory parameters screening on days 21 to 2 prior to the study.

3.4.2 Study design for the adult volunteers bioavailability study

In open-label randomized study, adult volunteers were assigned to participate in a non-replicate four period cross-over study design to explore effects of selected local foods on oral bioavailability of LUM after a single oral dose of AL.

3.4.3 Data collection for the adult volunteer study

Selection of food groups for the bioavailability study. Practical food alternatives were based on findings from a pilot survey (Mwebaza 2009, unpublished). Exit interviews were conducted with caregivers of malaria suffering children < 5 years, as they exited the health facility. Maize flour was the commonest {100%} local food at Ugandan households {n=94} of caretakers of < 5 year old malaria sufferers, given a 3 days' recall of food provided. In Uganda, maize {25g flour constitute 500ml of porridge cost~ $0.01} is cheaper and more available than milk {500 ml cost ~ $0.25}. However maize {0-4% fat: 0-

0.2g of fat / 100 ml of porridge} has much lower fat content than cow's milk {3.4g of fat/100ml} (I). Maize can be cheaply fortified with little vegetable fat. Vegetable cooking oil is more affordable and available than milk, 20 ml of vegetable oil cost ~ $0.06.

Profiled food groups were as follows: A: Fasted, received water {200ml}, B: Standard milk supplement {6.8g fat /200ml}, C: Maize porridge {0.5g fat / 200ml of porridge} D: Maize porridge fortified with vegetable oil {0.5g fat / 200 ml plus 5g/5ml of vegetable oil}.

Data collection. Adult volunteers received a single oral adult treatment dose of AL treatment (Tablet *Coartem*®, Novartis Pharma AG, Basel, Switzerland, 4 tablets of 20 mg A/120 mg LUM) with assigned feed on separate occasions with a washout period of about 4 weeks. They were instructed to fast ≥ 8hours prior to dosing and refrained from food for 3 hours after dosing, but allowed water at liberty. Intensive samples were collected over the first 72 hours, and thereafter weekly till day 28. Plasma was frozen at -38^{0}C until analysis by modified HPLC-UV technique [126].

3.4.4 Data management and analysis for the volunteer bioavailability study

Though samples were available till day 28, PK evaluations were restricted to 48 hours. This was due to unfortunate freezer breakdown following 72 hours' interruption in power supply. Half of the profiles had samples collected beyond 48 hours that thawed before analysis. Subsequently LUM stability was doubtful based on unsatisfactory reproducibility and repeatability of results. Consequently results for thawed samples were invalidated and assessment limited to last point, 48 h at which valid results were available for all individual profiles (I).

Statistical and pharmacokinetic evaluations: Lumefantrine PK outcomes were determined using non-compartmental methods following single extra-vascular dose using WinNonlin Pro software [Phoenix version 6.2, Pharsight Corporation Inc., Mountain View, CA, USA]. These included time of onset of absorption (T_{lag}), peak concentrations in plasma (C_{max}) and time to reach C_{max} (T_{max}) directly from observed data and partial area under concentration-time curve from 0 to 48 hours (AUC_{0-48}) calculated using linear trapezoidal interpolation. Appropriate parametric and non-parametric comparisons depending on the distribution of data {normal or not} were done for multiple followed by two group comparisons for both matched and unmatched data. The level of significance was set for multiple ($p<0.05$) and binary group comparisons (unadjusted $p < 0.0083$ across 4 groups).

Relative oral bioavailability was assessed using the confidence interval approach for evaluation of average bioequivalence (ABE) for non-replicate cross-over studies adopted from standard guidelines [189]. Evaluations were based on log transformed estimates of normally distributed dependent variables namely C_{max} and AUC_{0-48} (I).

3.5 PROCEDURES FOR PHARMACOKINETIC STUDIES IN PEDIATRIC PATIENTS (III, IV)

3.5.1 Selection of pediatric patients

Children aged between 6 months to 5 year old were eligible if they had microscopically confirmed uncomplicated *P. falciparum* malaria mono-infection as defined by WHO [3], (Paper III and IV) and parental/ guardians consent. Exclusion criteria included weight <5 kg; severe / complicated malaria including hemoglobin < 5 mg/dl; mixed plasmodia infection; medication known to influence CYP3A4/5 enzymes; receipt of AL in the past 28 days [3].

3.5.2 POPULATION PHARMACOKINETIC (PPK) STUDY IN CHILDREN UNDER FIVE YEARS (IV)

3.5.2.1 Population pharmacokinetics study design

This was a prospective, non-comparative observational study. Children (6 months to < 5 years) with uncomplicated falciparum malaria were consecutively approached and screened for eligibility. Sample size was based on consideration for a non-comparative observational study, and with a target of 70 patients [191]. However our primary outcome was population PK, where a sample size ≥ 50 is considered reasonable [192]. To increase the power of the study, about 20 per dose group with intense samples or 30 per group with sparse sampling was needed [192].

3.5.2.2 Data collection for PPK study in children under five years

The study was conducted between September 2013 and June 2014.

Clinical and laboratory {malaria microscopy, filter spot for genotyping, hematological, biochemical and pharmacokinetic} baseline data were collected. Thereafter children were treated with standard AL doses {Dispersible *Coartem* ® 20mg A/120mg L: 5 to ≤ 15 kg received 1 *tablet*, and 15 to ≤ 25 kg received 2 *tablets*}. The six doses were scheduled at about 0, 8, 24, 36, 48 and 60 hours. Doses were administered with milk {50ml / tablet} or maize porridge plus oil {50ml plus 1.5 ml oil / tablet} in case of co-enrolled participants in the nested comparative bioavailability study (III). All initial doses and at least one of the doses on subsequent dosing days were supervised. A population PK sampling design was used to obtain sparse samples (0.5 - 1 ml, 1 to 8 venous blood samples/ participant) during a 28 days follow up period at various times. All participants were scheduled for PK sampling, clinical and malaria microscopy follow up on days 0, 3, 7, 14, 21 and 28. The primary outcome was population PK of LUM (IV). Efficacy outcomes and tolerability were secondary outcomes.

3.5.2.3 Population pharmacokinetic analysis

Fifty five children contributed data to the PPK dataset. Several structural PK models (1, 2, and 3 compartmental models with first-order absorption and elimination, with and without T_{lag} for extravascular input) with varied error models were explored using nonlinear mixed effect approach NONMEM Version 7.3.0 (NONMEM, version 7.3.0, ICON, Hanover, MD). The final descriptive model for LUM (using NONMEM by ADVAN4) was a two-compartment PK model with first order absorption with lag time and elimination; parameterized in terms of apparent oral clearance (CL/F), inter-compartment clearance (Q/F), and apparent volumes of distribution of central (V_C/F) and peripheral (V_P/F) compartments, and first order absorption rate constant (KA). First order conditional estimation method (FOCE) was used. Subsequently the parent drug model was adapted with fixed KA and V_C/F fixed to 1 for the metabolite. The parent and metabolite were modeled sequentially (specified to NONMEM by ADVAN6 TRANS1 TOL=5).

Model improvement was done for only the parent drug, LUM. This was achieved by inclusion of significant covariates. These were body mass index (BMI) and age for LUM CL/F. Age was included with incorporation of CYP3A4 ontogeny adapted from Johnson et al., 2006 [170]. Weight was incorporated in the LUM V_C/F model. Incorporation of BMI into the CL/F model explained variability due to weight and stunting.

$$CL/F = CL/F_{REF} \bullet \left((BMI/16.62)^{CL/F_{BMI}}\right) \bullet \left(\left(1 * \left(AGE^{CL/F_{AGE}}\right)\right) / \left(0.31 + AGE^{CL/F_{AGE}}\right)\right)$$

$$V_C/F = V_C/F_{REF} \bullet \left((WT/13.0)^{V_C/F_{WT}}\right)$$

Where CL/F and V_C/F are the typical values centred on reference median BMI of 16.62 Kg/m^2 and median body weight of 13.0 kg. A log additive residual error model was used.

Model discrimination for nonhierarchical base model was based on significance of changes in the objective function {i.e., -2 x log likelihood (LL) which approximates to objective function value, $\alpha < 0.05$ for covariate insertion for the forward step and <0.005 for backward deletion}, diagnostic plots of observed with predicted data and residual-time plots. The developed final model was tested for reliability using bootstrapping methods and evaluated using diagnostic plots, and prediction corrected visual predictive checks by comparing model predictions with observations [174].

3.5.2.4 Assessment of malaria treatment outcome

Malaria efficacy and adverse events were secondary outcomes in the PPK study (IV). Main assessment was day 28 PCR adjusted parasitological response. Malaria treatment outcomes was further categorized by day 28 clinical and parasitological response (PCR unadjusted) as adequate clinical and parasitological response (ACPR), early treatment failure (ETF), late parasitological failure (LPF) and late clinical failure (LCF) as described by WHO

[193]; To assess predictors (covariates included baseline characteristics and PK outcomes) of treatment outcome, logistic regression was done.

3.5.3 COMPARATIVE BIOAVAILABILITY STUDY IN PEDIATRIC PATIENTS (III)

3.5.3.1 Comparative bioavailability study design

A food interventional, comparative bioavailability study (III), was nested in the observational PK study (IV). In an open label, parallel study design, a subset of children was block randomized in successive blocks of 4 to obtain subsequent balanced allocation. Randomization was done in two stages by dose block (1 and 2 tablets) and food intervention arms (milk or maize porridge plus oil) as described under section 3.5.2.2. We aimed at 12 children in each dose block / study food arm. Sample size was empirically set. In intensive pharmacokinetic study designs, 8-12 participants in each group provide sufficient data to test for differences in 2 independent groups, at an adequate power (1-β) of 80%, at α of 5% [194]. This has been previously demonstrated in a LUM bioavailability study [130].

3.5.3.2 *Data collection for comparative bioavailability study*

Additional parental/guardian consent for co-enrollment was sought if a child enrolled for the PPK study was aged >1 to ≤5 years and if parent was willing to stay and have their child participate in intensive sampling activities on Day 0.

Patients received standard AL treatment regimen and supplemented with food with comparable fat content (3.4.3), either milk {A, standard arm} or maize porridge plus vegetable oil {B, experimental arm} as described under section 3.5.2.2.

On Study Day 0, a baseline sample was collected before the first dose (0), thereafter intensive PK sampling (1ml) was done at 1, 1.5, 2, 3, 4, 6, 8 hours after the first dose. Thereafter patients completed their follow up in the PPK study (IV).

3.5.3.3 Data analysis for comparative bioavailability study

Individual PK data were assembled and profiles were examined for patterns of absorption.

Statistical and Pharmacokinetic evaluations: Lumefantrine PK outcomes (T_{lag}, C_{max}, T_{max} and partial areas under concentration-time curve) were determined as described under 3.4.4. The primary endpoint was LUM exposure up to 8 h after the first dose (AUC_{0-8h}). Relative oral LUM bioavailability was assessed with two group mean comparison with unequal variance t-test (Welch test, $p < 0.05$) using log transformed AUC_{0-8h} estimates (STATA version 12.1 (1985-2011, StataCorp LP)). Secondary outcomes included day 7 concentrations ($C_{(LUM)}D7$), LUM exposure between days 7 and 28 (AUC_{d7-28}) and clinical outcome as described in section 3.5.2.4.

3.6 DEVELOPMENT OF ANALYTICAL METHOD FOR LUMEFANTRINE AND METABOLITE (II)

A sensitive method for quantification of LUM and its metabolite, DBL was necessary to allow use of small volume plasma samples among children treated with AL. In anticipation of low LUM levels among pediatric patients beyond day 7, the previous in house, filter paper method with HPLC-UV detection had LLOQ of 300nM for both LUM (159ng/ml) and DBL (128 ng/ml) would not be sensitive enough for the anticipated low levels [126].

A bio-analytical method (II) was developed at the Therapeutic Drug Monitoring Laboratory, at the Division of Clinical Pharmacology, Karolinska University Hospital, Huddinge, Stockholm Sweden.

Chemical use and sample preparation. The target plasma volume was set to 100 µl. The choice of solvents was based on literature and physicochemical properties of LUM, which is highly lipid soluble and highly bound to plasma lipoproteins [114]. Protein precipitation was the preferred method, to avoid laborious extraction procedures. Varying types and concentrations of solvents were explored for extraction and mobile phases in order to achieve the best chromatographic separation. Acetonitrile (100%) yielded the best results for a one step extraction protein precipitation method. Methanol : DMSO (1:1) were used for LUM and DBL (Figure 2i) stock and intermediate solutions, while working calibrators and controls were spiked with known concentrations in previously tested drug-free plasma.

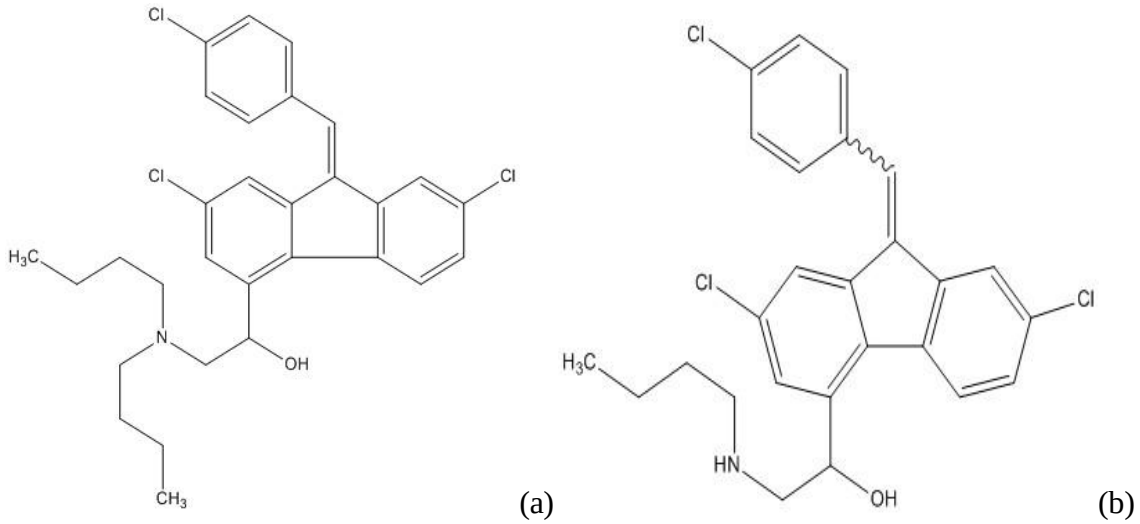

Figure 2i. *Structure of lumefantrine (a) and desbutyl-lumefantrine (b)*

Extraction was done by precipitating 100 µl plasma using 400 µL acetonitrile containing 54.7 ng/mL of deuterated LUM (LUM-d18) and 9.64 ng/mL of deuterated DBL (DBL-d9). These isotopes were labeled internal standard (IS) using deuterium at the butyl chains (II) (Figure 2ii).

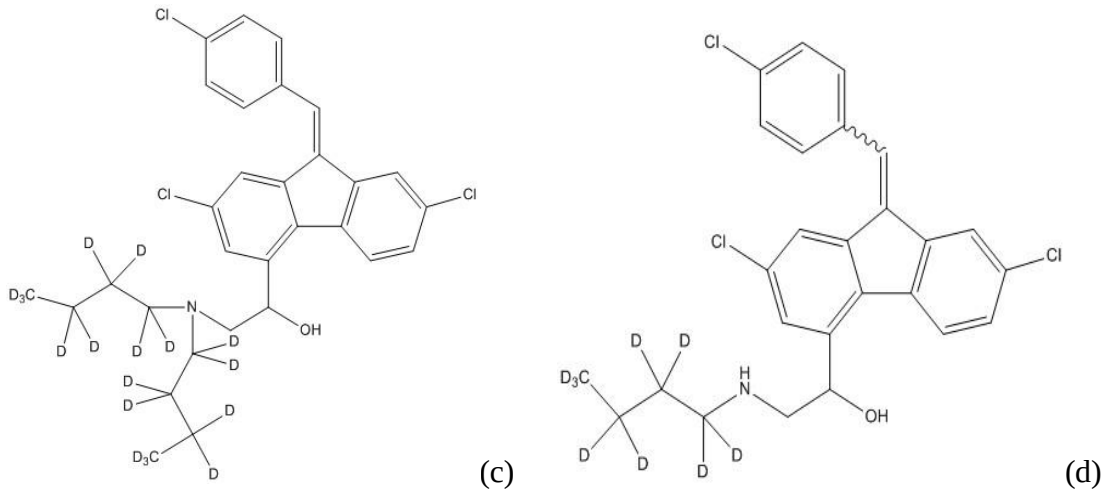

Figure 2ii. *Structure of deuterated lumefantrine (LUM-d18) (c) and deuterated desbutyl-lumefantrine (DBL-d9) (d)*

At the start of method development, halofantrine was tested as an analogue structural IS, but dropped due to subsequent availability of isotope labelled compounds with an advantage of overcoming matrix effects and ion saturation [188]. After 30 seconds of vortexing the samples , these were centrifuged for 5 minutes (4200 G) and 450 µL of the supernatant was transferred to new glass vials. Then 10 µL of the supernatant was injected for liquid chromatographic separation.

Instrumentation and conditions. Chromatographic separation was carried out on a Hypersil Gold C18 column (20 × 2.1 mm, 1.9 µm,Thermo Scientific) with a Hypersil Gold C8 guard column(10 × 2.1 mm, 5µm) at 30◦C. Chromatographic separation was optimized by exploring different solvents' composition for mobile phases, as well as elution mode (isocratic/gradient), column temperature and flow rates. Preferred solvents, acidic water (0.5% formic acid in water) and acidic methanol (0.5% formic acid in methanol) were used as mobile phase A & B using gradient elution at a flow rate of 0.5 ml/min. A stepwise gradient elution was optimized as follows: 32% of solvent A at 0 min, sloped to 12% of A at 2.00 min, then 32% again at 2.01 min until 2.2 min. Total run time was 2.2 minutes. Mass spectrometry conditions were positive electro spray ionization (ESI+) using multiple reaction monitoring, with two product transitions validated for LUM and DBL (II).

Method validation. The quantitative method was validated according to standards guidelines for evaluating a method's performance in regard to precision, sensitivity, accuracy, recovery and stability [195]. Dilution integrity was checked in preparation for calculation of levels exceeding upper limit of quantification.

3.7 OTHER LABORATORY METHODS

3.7.1 High performance liquid chromatography (HPLC) methods

Venous plasma LUM concentrations in the health volunteer study (I) were determined using an HPLC with ultraviolet detection (UV) technique with slight modification for plasma samples [126]. Plasma samples were vortexed them aliquots (100µl) were spiked with halofantrine as internal standard (50 µl, 144 µM in methanol). Acetonitrile (150µl) was then added and mixture vortexed for 1 minute, sonicated for 15 minutes and finally centrifuged at 3500 x g for 10 minutes. The supernatant (20µl) was injected and detection was done under chromatographic conditions as described by Ntale *et al.,* 2008. The plasma method was linear over a range of 52.9 – 2645 ng/ml, with intra-day and inter-day coefficients of variation < 11% and <13% respectively. The lower limit of quantification for LUM (LLOQ) was 100nM (52.9ng/ml) for the modified plasma method.

3.7.2 Malaria microscopy

Blood smears {BS} for malaria microscopy were stained with 2% Giemsa (I, III, IV). In thick BS, parasites were counted against 200 white blood cells (WBC) . Parasite density was calculated as "asexual parasites counted against 200 WBC multiplied by 40" /µl, with an assumption of 8000wbc / µl [193]. Thin BS for species identification was done only on Study day 0 and day of parasite re-appearance (III, IV). Each slide was read independently twice on Study day 0, on day of parasite re-appearance and for some randomly picked slides (10%) on any other day. Discrepancies were resolved by a 3rd read.

3.7.3 Malaria genotyping

To differentiate between recrudescence and re-infection, paired filter paper blood samples [from day of treatment initiation and day of failure] were identified (III, IV). Parasite genotyping was done using nested polymerase chain reaction (PCR) characterization of the highly polymorphic *P. falciparum* merozoite surface proteins 2 gene (MSP-2) [196].

3.8 ETHICAL CONSIDERATIONS

Ethical permits for the study and shipment of specimen were obtained from Makerere University School of Medicine - Research and Ethics Committee (SOMREC 2009-54) and the Uganda National Council for Science and Technology (HS 567). The trial was registered at ClinicalTrials.gov {NCT01944189}. Informed consent was provided from adult volunteers and parent/ guardian of children. Good clinical practice and good laboratory practice standards according to ICH E6 guidelines were observed throughout the entire study period.

4 RESULTS

4.1 EFFECT OF FOOD ON ORAL BIOAVAILABILITY OF LUMEFANTRINE

4.1.1 Effect of food on oral bioavailability of lumefantrine in adult volunteers (I)

4.1.1.1 Adult volunteers

Thirteen Ugandan healthy volunteers (male {84.6%}, age and body weight ranged between 20- 26 years and 43-85 kg) participated in the four period cross-over study. Forty five individual concentration-time profiles (fasted state /water, A: n=12, milk, B: n=11, maize porridge, C: n=11, maize porridge plus vegetable oil, D: n=11) were available for pharmacokinetic analysis (section 3.1.1.2). A total of 10 /13 completed participation in all arms thus provided matched data for relative bioavailability comparisons.

4.1.1.2 Relative oral bioavailability of lumefantrine with food in adult volunteers

The extent of LUM absorption was comparably increased by fat containing food, B and D {median (range): C_{max} , 2 081 vs 3 827 nmol/L , *p=0.059*; and AUC_{0-48}: 47.5 vs 76.8 hr*µmol/L, *p=0.14*, for B and D respectively} (Figure 3, Table 2). Similarly poor absorption of L was observed after intake without fat, with no differences in fasted state, A compared with C (Figure 3, Table 2).

With B (milk) as reference, when AL was given with D (maize porridge with oil), the bioequivalence criteria {ratio of means ranging 0.80 – 1.25} was met and exceeded {ratio of means [90%CI]: 1.20 [0.85 – 1.69] and 1.25 [0.84 – 1.88] using dependent variables $AUC_{(0-48)}$ and C_{max} respectively}. Whereas when compared to groups B, LUM exposures while participating in both A and C did not demonstrate acceptable levels of bioequivalence (Table 2) (I).

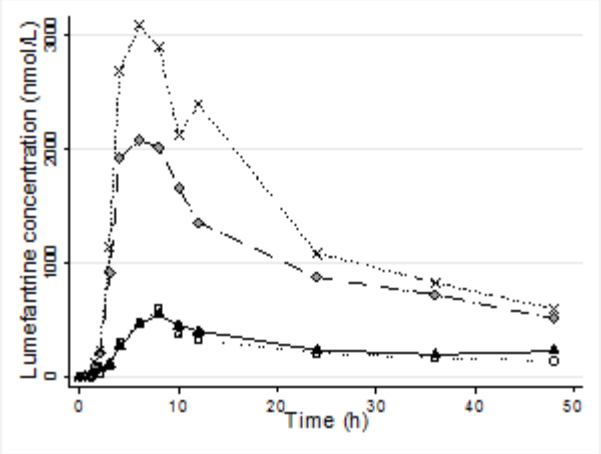

Figure 3. *Median plasma lumefantrine concentrations over time in healthy adults following an oral single dose of artemether (80mg)-lumefantrine (480mg) under different conditions.* {▲: fasted state (A, n=12), ◊ (grey filled) **:** milk (B, n=11), □: maize porridge (C, n=11), X:maize porridge plus oil (D, n=11)}

Table 2. Relative bioavailability of lumefantrine after a single oral dose of AL (80mg, artemether, 480mg lumefantrine) with different food combinations

Dependent Variable	**Formulation Variable : Food**				**Ratio of means (90%CI)**	
	Reference		**Test**			**(0.80-1.25)**
	N=10 [b]		**N=10** [b]			
AUC_{0-48} (hr.µmol/L)	**B**	47.5 (29.7-106.4)	**A**	14.6 (7.3- 78.5)	**0.30**	**0.21 – 0.42**
			C	11.5 (4.8- 29.7)	**0.22**	**0.16 – 0.32**
			D	76.8 (36.4-158.4)	**1.20**	**0.85 – 1.69**
C_{max} (µmol/L)	**B**	2 .08 (1.62 – 4.36)	**A**	0.87 (0.28- 4.00)	**0.34**	**0.22 – 0.51**
			C	0.70 (0.18 – 1.28)	**0.23**	**0.15 – 0.35**
			D	3.83 (2.05 – 5.60)	**1.25**	**0.84 – 1.88**

Median (range) values for estimates

[b]: reduced sample size because of inclusion of only matched data, 10/13 participants completed participation in all 4 food study arms {A: fasted state, B**:** milk, C: maize porridge, D: maize porridge plus oil}.

4.1.2 Comparative bioavailability study in pediatric patients (III)

4.1.2.1 Patients: Pediatric Study Participants (III, IV)

A total of 70 children with uncomplicated P. *falciparum* malaria were enrolled, 41 of who were randomized to participate in the nested comparative bioavailability study (21 to milk and 20 to maize porridge plus oil). Thirteen children were excluded due to significant baseline LUM (Figure 4). Eight of who had been co-enrolled in the comparative bioavailability study. Patients' characteristics are summarized in Table 3 (II –IV).

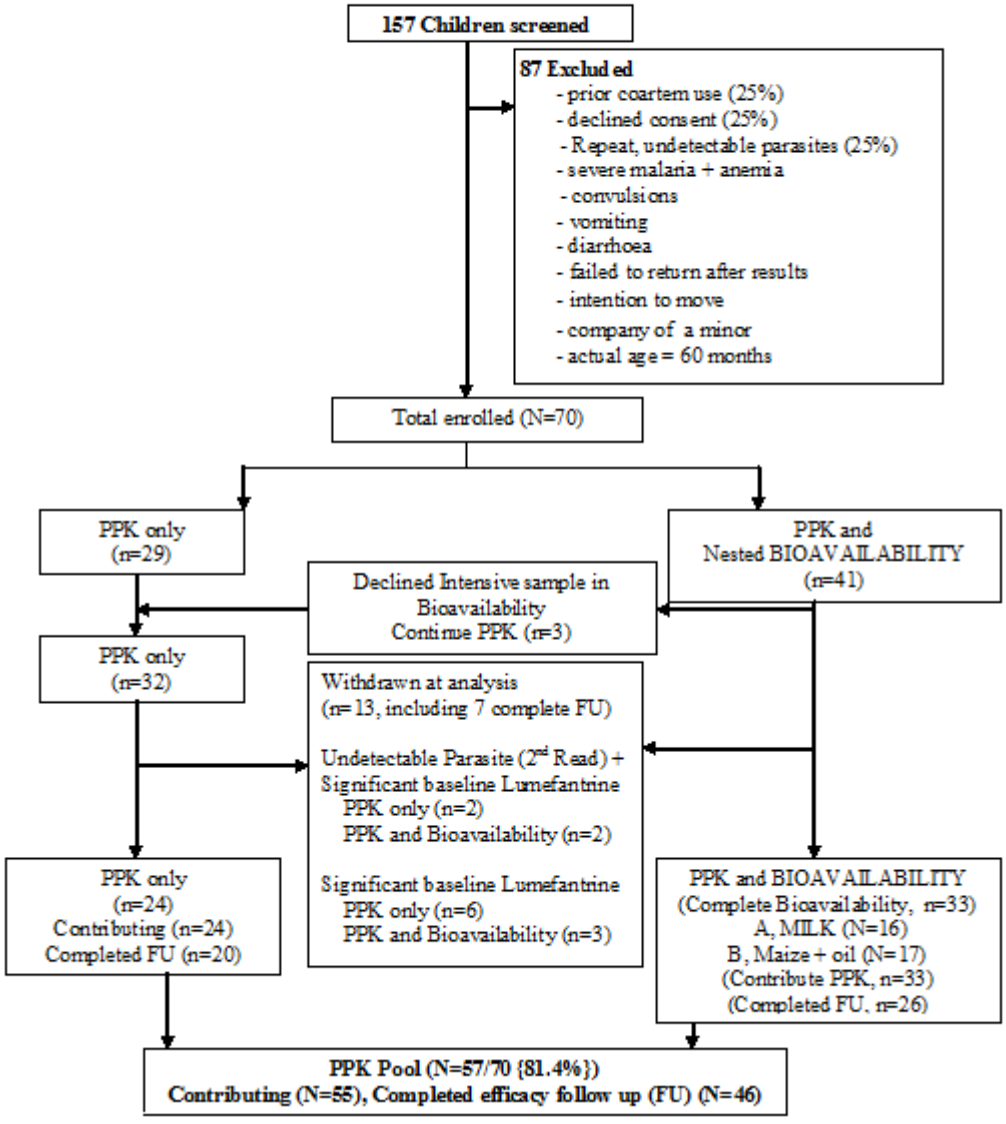

Figure 4. *Trial Profile*

Table 3. Baseline characteristics of pediatric study participants

Description	Pharmacokinetic Study (6 doses)	***Nested Comparative Bioavailability study (Post-first dose)***		
	All, *evaluable*	***Subset evaluable***	***Milk (A)***	***Maize porridge + vegetable oil (B)***
N	55	***33***	***16***	***17***
Dose blocks				
1 tablet, <15kg	38	***19***	***10***	***9***
12 – 24 mo(n)	*n=9*			
>24 – 59 mo(n)	*n=29*			
2 tablets, ≥15kg	17	***14***	***6***	***8***
12 – 24 mo(n)	*n=0*			
>24 – 59 mo(n)	*n=17*			
Male, All	33 (57.9%)		8 /16	11 / 17
Age, months (*mo*)				
All	35.5 (13.5 – 59.7)		35.8 (16.4-57.7)	41.9 (17 -59.7)
1 tablet	28.6 (13.5 - 57.7)*[1a]		29.9 (16.4-57.7)	29.0 (17.0 - 50.6)
2 tablets	47.6 (35.1- 59.7)*[1a]		42.6 (35.1- 48.5)*[2]	53.2 (36.7 - 59.7)*[2]
Weight, kg				
All	13.0 (9.0 – 17.4)		13.6 (9.5-16.0)	14.5 (9.5 -17.4)
1 tablet	12.0 (9.0 -14.8)		12.0 (9.5 -14.0)	11.4 (9.5 -14.5)
2 tablets	15.2 (15.0-17.4)		15.0 (15.0-16.0)	15.6 (15.0-17.4)
Height, cm	88.2 (69.5 – 104.7)			
Total LUM dose, mg/kg			First LUM dose, mg/kg/dose	
All	62.4 (48.6 - 96.0)		11.5 (8.6 - 16.0)	12. 6 (8.3 - 16.0)
1 tablet	60.0 (48.6 -79.8) *[1L]		10.0 (8.6 - 12.6)	10.5 (8.3 - 12.6)
2 tablets	93.0 (82.8 - 96.0)*[1L]		16.0 (15.0 -16.0)	15.4 (13.8 - 16.0)
Baseline parasitemia, /µL	11340 (8 – 688640)		14460 (16 - 503360)	936 (16- 127120)

All values are stated as median (range) *1a, *1b,*1L, *2: p value of <0.0001

Ex: 22 participants participated in only population PK activities

4.1.2.2 Comparative oral bioavailability of lumefantrine in pediatric patients (III)

Primary outcome. Thirty three children were evaluable. (Figure 4, Table 3). After the first AL dose, early LUM exposure ($AUC_{0\text{-}8h}$) was comparable between the two arms A {milk, n=16} and B {maize porridge plus oil , n=17}, (GM {95%CI}: 6.01 h*µg/ml {3.26-11.1} vs 6.26 h*µg/ml {4.5 -8.43}, p=0.9 for A and B respectively) (Table 4). Less inter-individual variability in $AUC_{0\text{-}8h}$ was observed in B (p=0.01) compared to A (Fig. 5, Table 4).

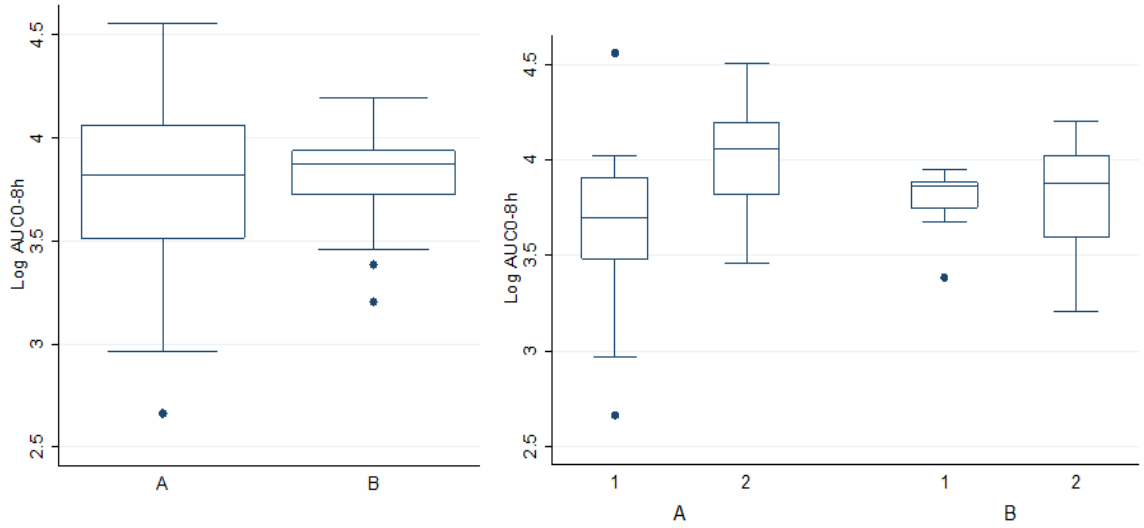

Figure 5. *Distribution of $AUC_{0\text{-}8h}$ [h*ng/mL] estimates (log –transformed), after the first artemether-lumefantrine dose across food arms (A & B) and dose groups (1 or 2 AL Tablets). Reference midline in box is a median value (IQR) with whiskers as non-outlier upper and lower range, and "*"as outliers.*
Food: A: Milk, B: Maize porridge plus oil (all participants dosed with 1 or 2 tablets). Dose groups: 1 =1 tablet containing 120 mg LUM; 2 = 2 tablet total 240 mg LUM

Table 4: Comparison of lumefantrine pharmacokinetic estimates after oral doses of artemether-lumefantrine with different food among under five year old children with malaria

	A Milk	**B Porridge plus Oil**	**p value**
All	16	17	
1 tablet, <15kg	10	9	
2 tablets ≥15kg	6	8	
LUM/dose, mg/kg/dose	11.5 (8.6 - 16.0)	12. 6 (8.3 - 16.0)	0.9 [w]
Parameters after first dose			
AUC_{0-8h}, h.μg/mL			
All, GM(95%CI)	**6.01 (3.26 – 11.1)**	**6.26 (4.65 – 8.43)**	0.9 [t]
Dose Corrected AUC_{0-8h}, h/L/kg			
All, GM(95%CI)	**0.49 (0.27 - 0.86)**	**0.49 (0.36 -0.66)**	0.9972 [t]
Parameters after multiple doses			
Day 7 [o], ng/mL	350 (136 – 9647), n=14 [o1]	376 (158 – 6142), n=13 [o2]	0.73 [w]
AUC_{d7-28}, h μg/mL	50 (20 – 493), n=13	87 (27 – 435), n=13	0.59 [w]

GM (95%CI): geometric mean and its 95% confidence interval
t: t test with unequal variance
w: Wilcoxon rank-sum (Mann-Whitney) test. *=0.02 **=0.006
O: Day 7 including outliers. If excluded o1= 9647, then A (n=13, 343 (136 – 1827 ng/ml)).
if excluded o2=6142, then B (n=12, 342 (158 – 1672) ng/ml).
AUC_{d7-28} : Truncated area under the concentration curve between 7 and 28 days

Secondary outcome. Upon completion of multiple doses, $C_{(LUM)}D7$ and area under the concentratin curve between 7 and 28 days (AUC_{d7-28}) were comparable across the 2 food arms (Table 4). Notably, LUM exposure was significantly higher among bigger children {> 15 kg} receiving 2 tablet dose than smaller children {< 15 kg} dosed with 1 tablet {median $C_{(LUM)}D7$: 505 [192-9647] vs 289 [136-1826] ng/ml, p=0.02 and AUC_{d7-28} : 108 [42-493] vs 41 (20 -167) hr-μg/ml, p=0.006 for 2 and 1 tablet respectively}. However LUM dose-adjusted exposures were not significantly different (III).

4.1.3 Comparison of adult volunteers and pediatric patients findings

Among healthy adults, partial area 8 hours after a dose {$AUC_{(0\text{-}8\ h)}$} correlated well with that truncated at 48 hours {$AUC_{(0\text{-}48\ h)}$} (τ =0.42, *p=0.007*). With reference to early partial exposure of LUM at 8 h, $AUC_{(0\text{-}8\ h)}$ did not significantly differ, when compared across study populations for milk (median: 5.4 μg/ml vs. 6.6 μg/ml, p=0.77 for adults and pediatric patients respectively) and maize porridge plus oil (8.4 μg/ml vs. 7.4 μg/ml, p=0.31, for adults and pediatric patients respectively) (Table 5). Notable differences included weight adjusted LUM dose and delayed T_{lag} in sick children compared to healthy adult volunteers in the milk group (Table 5).

Table 5. Comparison of lumefantrine pharmacokinetics after a single oral dose of AL among healthy adult volunteers and children with uncomplicated *P. falciparum* malaria

Description	**Adult volunteers** ***Single dose***	**Pediatric Patients (< 5 year)** ***After first dose***	***p* value**
n			
Milk	11	16	
Maize porridge plus oil	11	17	
Lumefantrine dose, mg/kg/dose			
Milk	8.0 (5.9 - 10.9)	11.5 (8.6 - 16.0)	***0.0006***
Maize porridge plus oil	8.1 (5.6 - 11.2)	12. 6 (8.3 - 16.0)	***0.0002***
T_{lag}, *h*			
Milk	1.02 (1.00 – 2.00)	1.87 (0.92 - 3.1)	***0.004***
Maize porridge plus oil	1.02 (1.00 – 2.05)	1.55 (0.65 - 3.02)	*0.11*
T_{max}, *h*			
Milk	6.00 (4.00 – 24.0)	6.17 (3.13 - >8.0)	*0.9*
Maize porridge plus oil	6.00 (4.00 – 24.0)	7.85 (4.03 - >8.0)	*0.2*
C_{max}, *ng/ml*			
Milk	1101 (857 - 2308)	1836 (177 – 11545)	*0.69*
Maize porridge plus oil	2024 (1084 - 2963)	1718 (636 - 6684)	*0.62*
$AUC_{(0\text{-}8\ h)}$, *h*μg/ml*			
Milk	5.4 (4.2 - 10.1)	6.6 (0.5 - 36.2)	*0.77*
Maize porridge plus oil	8.4 (3.4 – 14.5)	7.4 (1.6 - 11.9)	*0.31*

Values are Median (range)
AL tablet contains 20 mg artemether and 120 mg lumefantrine
Children: < 15 kg dosed 1 tablet of AL, ≥ 15 - < 25 kg dosed 2 tablets
Adults: ≥35 kg dosed 4 tablet of AL

4.2 PHARMACOKINETICS OF LUMEFANTRINE AND DESBUTYL-LUMEFANTRINE IN PEDIATRIC PATIENTS (IV)

4.2.1 Population pharmacokinetics of LUM and DBL in < 5 year old children (IV)

Fifty five children were included in the population PK evaluations contributing 453 and 233 data points for LUM and DBL respectively (Figure 4, Table 3). According to the current fixed weight-based AL dosage, variation in LUM total dose ranged between 48.6 and 96.0 mg/kg (Table 3). Children {≥ 15kg, n=17} dosed with 2 tablet notably received higher LUM dose {median: 93 vs 60 mg/kg, p<0.00001} and were older {median age: 46.6 vs 28.6 months, p<0.00001) than those dosed under 1 tablet dose group {< 15kg, n=38} (Table 3). Twenty three children (40.3 %) were stunted, and 2 children (3.5 %) were underweight. Overall median BMI was 16.6 (range: 13.6 – 26.2) kg/m^2 and with no difference between children < 24 months {16.6 (range 15.4 – 22.4) kg/m^2} and those > 24 months {16.6 (range 13.6 – 26.2) kg/m^2}.

A two compartment PK model adequately described both the PPK of LUM and DBL (Figure 6). Detailed model development trail and diagnostics are presented in IV.

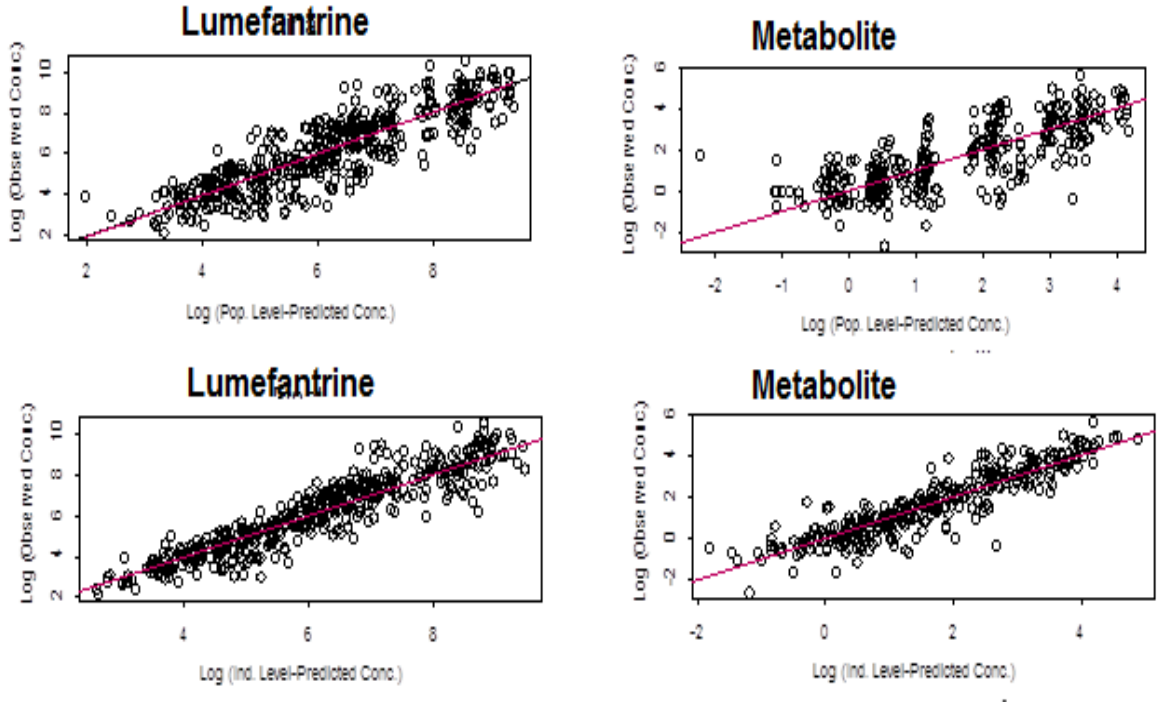

Figure 6. *Goodness of fit plots for final lumefantrine and metabolite in < 5 year old Ugandan children.* Observed against population and individual predicted concentrations on a logarithmic (10) scale.

Inter-subject variability of CL/F was partly explained by BMI and age (incorporating CYP3A4 ontogeny) (Table 6). The CL/F of LUM decreased with increasing BMI (Figure 7). Detailed paired plots showing other covariates and PK outcomes in (IV). The BMI (weight {kg} / (height {m})^2) explained both weight and height thus catered for weight and stunting (height for age) as significant covariates for CL/F. BMI alone in the CL/F model explained 5% of the inter-subject variability (from 29% in base model to 24% with BMI in CL/F model) and when age was also included, they both reduced unexplained inter-subject variability in the final CL/F model by 6.8 % (from 29% in base model to BMI in CL/F model : 24% and BMI and age in CL/F model: 22.2%). Inter-subject variability of V_C/F was explained to a certain extent by weight. The inter-subject variability in V_C/F decreased from 83.7% in the base model to 78.9% in the final V_C/F model with weight included.

Table 6. Summary of significant factors in the covariate analysis

Run	Model	OFV	cf.	df	LLD	Sig.
1	Base model - one residual error model	341.035				
2	Base model - two residual error models	309.077	1	1	-31.958	yes
Forward Stepping *(p=0.05)*						
3	CL/F~WT	308.670	2	1	-0.407	no
4	CL/F~STUNTING	294.445	2	1	-14.632	yes
5	CL/F~BMI	294.401	2	1	-14.676	yes
6	CL/F~BMI & V_C/F~WT	292.746	5	1	-1.655	no
7	CL/F~BMI, CL/F~AGE; V_C/F~WT	287.934	6	1	-4.812	yes
Backward Elimination *(p=0.005)*						
8	"- C/F~AGE"	292.748	7	1	4.814	yes
9	"- C/F~BMI, CL/F~AGE"	307.643	7	2	19.709	yes
9	"-CL/F~BMI,-CL/F~AGE; - V_C/F~WT	309.077	7	3	21.143	yes

CL/F: apparent oral clearance, V_C/F: apparent central volume of distribution,
BMI: Body mass index, WT: Weight (kg) AGE: Age in months,
Sig.: Significant if LLD (change in 2 log likelihood value for compared models) for 1, 2 and 3 df (degrees of freedom) is >7.88, >10.6, >12.84 at p<0.005 or >3.84, >5.99, >7.81 at p<0.05 respectively.

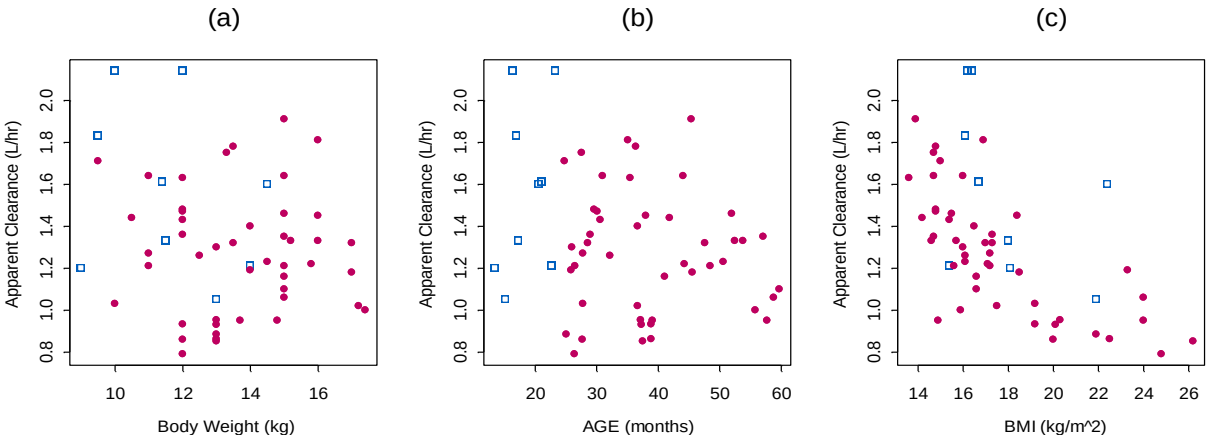

Figure 7. *Plots showing the relationship between apparent oral clearance(CL/F) and significant covariates: (a) body weight, (b) age and (c) body mass index (BMI).* Open blue squares: age = < 24 months; filled red circles: age = > 24 months.

Typical values for LUM and DBL are summarized in the Table 7. The population mean T_{lag} (ALAG1) for LUM was 0.98h and LUM mean absorption rate constant was 0.0383 (RSE of 11.9%).

Table 7. Summary of population pharmacokinetic parameters estimates for lumefantrine and desbutyl-lumefantrine in children under five year old with malaria

Description	Population mean	RSE#	IIV (%CV)	RSE#
Lumefantrine				
CL/F (L h^{-1})	3.19	24.4%	0.0492 (22.2)	31.3%
CL/F_{AGE}	-0.423	27.2%		
CL/F_{BMI}	-1.10	-		
V_C/F (L)	28.1	18%	0.623 (78.9)	30.7%
V_C/F_{WT}	1.06	-		
Q/F (L h^{-1})	0.176	15.6%		
V_P/F (L)	58.4	16.2%		
KA (h^{-1})	0.0383	11.9%		
ALAG1 (h)	0.98	-	0.0029 (5.39)	
Desbutyl-lumefantrine				
CL_M (L h^{-1})	0.0807	-		

CL/F, Q/F, KA, V_C/F, and *V_P/F* are typical values of LUM apparent oral clearance, inter-compartment clearance, absorption rate constant , apparent volume of distribution of central and peripheral compartment at the reference BMI (16.62 kg/m^2)and weight (13.0 kg).
IIV: Inter-individual variability. CL/F_{AGE}, CL/F_{BMI}, V_C/F_{WT} :Effect of age and BMI on CL/F, effect of weight on V_C/F. *CL_M*: rate of elimination of the metabolite.
#: based on 300 bootstrap runs, relative standard errors (RSE = 100 × (standard error/mean)).

In this study, the CL/F of LUM was found to decrease with age, from one to 5 years old (Figure 7). On average, the change in LUM CL/F from ">1 - 2 years old" to "> 2 years old" was approximately -20.6%. The population median CL/F from empiric Bayesian individual parameter estimates which was 1.33 L/hr. Comparing individual LUM CL/F estimates among children (38 / 55) receiving the same dose (< 15 kg, 1 AL tablet), children who were older than 24 months {n = 29} had significantly lower estimates than their younger counterparts {< 24 months, n = 9} (median {range}: 1.27 {0.79 – 1.91} vs 1.6 {1.05 – 2.14} L/h, p=0.046). With reference to the median BMI of 16.62 kg/m^2 for our dataset, the estimated LUM CL/F for a typical 12, 24, 36, 48, and 60 months old were 1.69, 1.46, 1.32, 1.23 and 1.16 L/hr respectively. Thus, LUM CL/F decreased by 31.4% from 12 to 60 months of age.

Desbutyl-lumefantrine. The fraction of DBL formed was estimated to be 0.0542%. The rate of elimination of DBL was estimated to be 0.0807 L/h. Transfer rate constant of DBL from central to peripheral (K45) and from peripheral to central metabolite compartments (K54) were 0.0448 and 0.00804 h^{-1}.

The visual predictive check confirmed good predictive performance of the final pharmacokinetic LUM and DBL model (Figure 8).

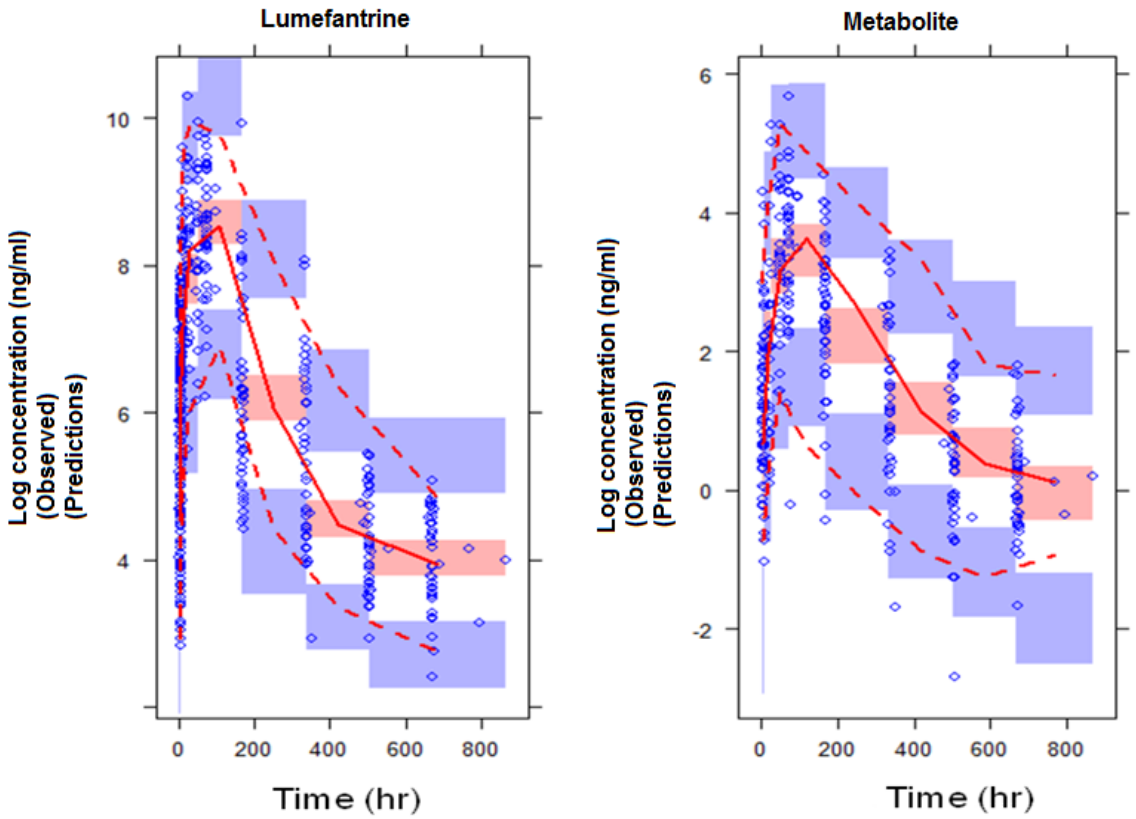

Figure 8. *Prediction Corrected Visual Predictive Check of the final pharmacokinetic lumefantrine (LUM) and desbutyl-lumefantrine (DBL) model.* Open blue diamond shapes: observed concentrations of LUM and DBL. Solid and dotted line: shows 50th, 2.5th and 97.5th prediction intervals with associated 95% confidence intervals.

4.2.2 Secondary pharmacokinetic outcomes

In this < 5 year old population, median $C_{(LUM)}D7$ (n=46) was 441 (136-9647) ng/mL. Notably $C_{(LUM)}D7$ was significantly higher among children dosed 2 tablets compared to those dosed with 1 tablet {median: 586 (192 – 9647) vs 315 (136 – 5418) ng/ml, p=0.04}. For children aged < 24 months and those > 24 months, $C_{(LUM)}D7$ were not significantly different (p=0.09) (Table 3). All the children who received 2 tablets of AL exceeded 280 ng/ml, except for one child (1 / 13) whose $C_{(LUM)}D7$ was 192ng/ml. Of the children who received 1 tablet of AL, 15% (5 / 33) had $C_{(LUM)}D7$ <175 ng/ml and 36% (12 / 33) had concentrations < 280 ng/ml.

Overall computed AUC ($AUC_{0\text{-}inf}$) was significantly higher among children who received 2 tablets compared to those dosed with 1 tablet (median $AUC_{0\text{-}inf}$: 181 vs. 93 hr*µg/ml, p<0.00001) but LUM dose-adjusted $AUC_{0\text{-}inf}$ was not significantly different (p= 0.0544) (Figure 9). The observed non-significant increase in dose adjusted AUC among older children receiving higher doses (240mg vs. 120mg) may imply that absorption does not increase proportionately with increased doses. However notably, comparing children in the same "1 AL tablet" dose group, those aged > 2 years {n=30} had higher $AUC_{(0\text{-}inf)}$ {95 vs. 75 hr*µg/ml p=0.037} and dose adjusted AUC { 0.79 (0.56– 1.26) vs. 0.63 (0.47 – 0.95) hr/mL/kg , p=0.049} than their counterparts aged < 2 years (Figure 9). This is in line with the fact that CL/F is lower among children > 24 months.

The day 7 DBL concentrations ranged from 0 to 74.2 ng/ml (median of 14.7 ng/ml). The ratios of DBL / L UM varied between 1/40 - 1/10.

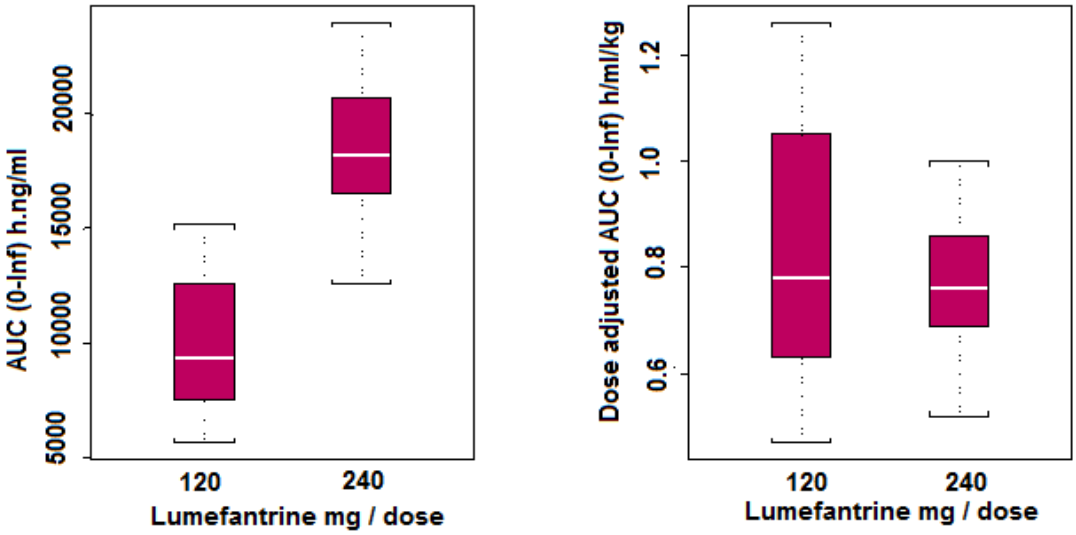

Figure 9. *Distribution of LUM exposure ($AUC_{0\text{-}inf}$ and Dose adjusted $AUC_{0\text{-}inf}$), after standard 6 doses of artemether-lumefantrine among under five year old children treated for uncomplicated malaria.* Reference midline is the median.
Dose groups (1 & 2): 1 tablet = 120 mg LUM, 2 tablets = 240 mg LUM. Age categories: 1= < 24 months, 2= ≥ 24 months.

4.2.3 Treatment outcomes and relationship with pharmacokinetic outcomes

Efficacy assessment as per intention to treat analysis was done. Day 28 PCR unadjusted treatment outcomes were as follows: Out of the 70 children enrolled, 53 (81%) had ACPR, 1 experienced ETF, 3 LPF, 1 LCF and 12 unknown parasitological outcomes, since smears were not done either due to earlier withdrawal or lost to follow up. Per protocol analysis, 9 children had unknown parasitological outcomes; 46 of 55 PPK evaluable children were evaluable for the clinical efficacy outcome (WHO Day 28 PCR unadjusted treatment outcomes) follows: 41 had ACPR, 1 experienced ETF, 3 LPF, and 1 LCF.

Only one child of those with LPF had PCR verified parasite recrudescence on day 28. This was a 47.6 months old child, 17.4kg, BMI = 17.3 kg/m^2, initial parasitemia of 108,680/µl, and dosed 2 AL tablets with milk, total LUM dose of 84.7 mg/kg (14.1 mg/kg/dose). The child who experienced ETF developed danger signs on study day 1, despite reduction of parasite density by 89% and was rescued with intravenous (IV) artesunate. He was 41.9 months old, 10.5 kg, BMI=14.2 kg/m^2 ,initial parasitemia of 63,720 /µl and dosed 1 tablet with maize plus oil, LUM dose of 11.5 mg/kg/dose.

Overall, AL was well tolerated except for 2 children who experienced vomiting, and 2 diarrhea.

Treatment was completed by all the 70 children, including the one with early treatment failure whose AL treatment was resumed after IV artesunate.

Only one of these children had PCR verified treatment failure and neither LUM ($C_{(LUM)}D7$ and $AUC_{0\text{-}inf}$) nor DBL exposure was found to be a significant predictor of parasitologic failure. Among those with parasite re-infection, BMI was a predictor for parasite reappearance.

4.3 ANALYTICAL QUANTITATIVE METHOD FOR LUMEFANTRINE AND DESBUTYL LUMEFANTRINE (II)

A LCMS/MS method was developed for simultaneous determination of LUM and DBL concentrations in human plasma (100 µl). Protein precipitation was the preferred extraction method using acetonitrile (400 µl) containing 54.7 ng/ml of LUM-d18 and 9.64 ng/mL of DBL-d9 as internal standards. The validated measuring range was 21 – 529 ng/mL for LUM and 1.9 – 47 ng/mL for DBL, with a linear regression coefficient > 0.99 for the calibration curves of all runs. The minimum amount of plasma necessary was 100 µl although smaller volumes were successfully tested including diluted samples with concentrations over the upper limit of quantification (II). Inter- and intra-assay precision was < 10% coefficient of variation (CV) for all levels of both LUM and DBL. Accuracy was within -9% to +6% for all levels of both LUM and DBL. The LLOQ for LUM and DBL were 21 ng/ml (40 nM) and 1.7 ng/ml (4 nM), respectively. Retention times of 1.7 minutes for LUM and LUM-d18 and 1.5 minutes for DBL and DBL-d9.

The short total run time of 2.2 minutes, allows a high sample throughput. The method was successfully applied for plasma LUM and DBL determination in children under 5 years of age with uncomplicated malaria, up to 28 days after a standard 3-day treatment with AL.

5 DISCUSSION

This thesis sought to optimize AL use in resource limited settings and also describe population PK of LUM in children < 5 year old with uncomplicated *falciparum* malaria. The overall aim was to provide the structural framework for a rational approach for re-evaluating AL dosage guidelines for children less than 5 years of age.

Effect of food on lumefantrine oral bioavailability

It is well established that dietary fat is essential for augmentation of LUM absorption [72, 129, 130]. In order to reduce the risk for development of resistance, it is essential to optimize drug exposure and avoid sub-therapeutic concentrations.

We demonstrated that LUM absorption was comparably augmented when AL was given with milk (standard) or maize porridge fortified with vegetable cooking oil (experimental) (I & III). This is attributed to similar fat content. This was initially examined among healthy adult volunteers (I). Addition of fat to maize porridge increased LUM $AUC_{(0\text{-}48)}$ by 600% compared with maize porridge alone. Whereas there was no difference between maize porridge alone and intake of AL under fasted state among adult volunteers. Both groups demonstrated lower ranges of LUM exposures relative to milk and maize porridge with oil (I). In agreement with previous studies [74, 75], we observed great inter-subject variability in LUM exposure. Greatest variance in adults was observed after dosing under fasted state (I). Despite the difference in variance of PK parameter estimates across the two food arms, it can be claimed that maize porridge with oil increased LUM absorption to the same magnitude as with milk in children with uncomplicated malaria (III). Notably among young children, less inter-individual variability in $AUC_{0\text{-}8h}$ was observed in the maize plus oil group compared to the milk supplemented group (p=0.01). This could be due to different effect on gastric emptying time for solid feeds compared to liquids [198]. In addition, the observed reduced risk for sub-therapeutic LUM concentrations in the maize plus oil compared to those in the milk group suggests an advantage from an efficacy point of view. The clinical implications need to be further evaluated through larger effectiveness studies.

Though African diet has been considered adequate for LUM absorption [197] and the manufacturer recommends that AL administration "should be followed whenever possible by food/drink (e.g., milk, formula, pudding, broth, and porridge)" [81]; we have demonstrated that commonly available African food with little or no fat content, such as maize porridge cannot be reliably recommended to augment LUM absorption. Addition of fat or cooking oil to porridge should be advised.

Our food strategy aimed at prescribing minimal but sufficient volumes (50-100mL) of food to children, in order to ensure adequacy of fat content (>1.2g of fat) [130]. This is a practical approach as small children are unable to complete big portions of food [120]. Laboratory techniques geared at enhancing solubility of lipophilic oral drugs [199] like LUM have been explored [200, 201]. However new technological innovations are likely

to take a long while to maximally exploit formulations with enhanced solubility. Consequently the importance of dietary fat remains critical to augment LUM absorption.

Methodological considerations for the bioavailability studies

In both adult volunteers and pediatric patients studies, it was not possible to use overall exposure {$AUC_{(0 - infinity)}$} for bioavailability comparisons. Among adult volunteers, $AUC_{(0-infinity)}$ could not be determined in 23/45 individual profiles, because results for thawed samples collected were invalidated. Restriction of evaluations to partial area between 0 to 48 h post-dose {$AUC_{(0-48\ h)}$}, a point with available data in all patients was considered reasonable because it is likely that absorption was completed by 48 h (I). It has been reported that oral absorption of LUM among adults is almost complete by about 18 h [74]. Among adult volunteers' profiles, we observed similar parallel trends in decline of concentration with time regardless of the food group (I). Therefore, it is unlikely that food intake affects elimination process. Among children with malaria, evaluations were restricted to 8 hours {$AUC_{(0-8\ h)}$}, prior to the second dose (III). This was justifiable because interference from cumulative drug absorption sets in with subsequent dosing [74, 75]. In addition, it would not have been ethical and we could not delay the second dose among these pediatric malaria patients. The appropriateness of use of early partial area truncated at 8 hours was evaluated (Section 4.1.3, Table 5). Truncated exposure estimates after a single dose adequately evaluated effects of food on LUM absorption.

The healthy volunteer crossover design provided an advantage of matched comparisons where each participant was their own control. In the crossover study, average bioequivalence techniques were used to assess relative bioavailability (I). For the pediatric patients study which employed parallel design, traditional average bioequivalence techniques could not be employed due to lack of proportional evaluable subjects per dose block in each arm (III). The relative bioavailability was appropriately assessed using the two one-sided tests for comparisons of unmatched two samples [202]. In addition dose adjusted exposures were compared.

In general, wide variation in exposure was observed within and between groups. In addition individual differences while participating in different food arms were observed in the preceding volunteer study (I). However it was not feasible and we could not plan to assess "interchangeability" (using population or individual bioequivalence techniques (Section 1.6.1.1.2)) of use of either food supplement among patients. Selection bias in the pediatric study (III) was minimized by block randomization in two stages, by dose group (weight based) and food arm. Unfortunately we did not recruit targeted numbers for the 2 tablet dose group. This arose because we could not find enough numbers of heavier of children with malaria, weighing > 15 kg. Most children were appreciably small, light and short for their age (40% were stunted), but they didn't qualify to be categorized as underweight or wasted according to WHO standards [203].

It was for obvious reasons, impossible to ensure optimal fasted conditions (8 hours) before participation in the bioavailability study among sick children. Gastric emptying among children may be faster than that in adults [198].

Quantitative bioanalytical lumefantrine and desbutyl-lumefantrine method

A simple, fast and sensitive LCMS/MS method for quantification of LUM and DBL in small plasma volume was developed. The simplicity was achieved by the single extraction step, using acetonitrile for protein precipitation, which allows injecting a 10 µl supernatant aliquote in the LC-MS/MS system. Another advantage is that the developed method requires minimal amount of plasma (100 µl) which is obtained from approximately twice as much whole blood (200 µl) which makes repeated sampling possible in pediatric studies. Further testing proved that it is feasible to use plasma volume as small as 25 µl. The short total run time allows a high throughput. The method was successfully applied for plasma LUM and DBL determination in children under 5 years of age with uncomplicated malaria, up to 28 days after a standard 3-day treatment with AL. Determination of LUM and DBL in dry blood spots would be desirable for field studies.

Population Pharmacokinetics among under five year old malaria patients

The PPK of LUM and its metabolite, DBL was adequately described using a two-compartment PK model with first order processes. This is the first report characterizing the PPK of LUM and DBL among children under five years of age. Few LUM PPK studies among children have been done [120, 178]. However this is the first LUM population-based PK model to incorporate ontogeny of LUM metabolizing enzymes in the CL/F model. The activity of CYP3A4 is very variable especially with age [164]. High inter-individual variability in LUM disposition has been reported [72]. In our study, inter-subject variability in LUM CL/F was partly explained by BMI and age, while that in V_C/F was partially explained by weight. The LUM CL/F was slightly higher among children < 2 years, and decreased with age from 2 to 5 years old. This trend is consistent with the report from the meta-analysis of $C_{(LUM)}D7$ where lower concentration were observed in children below 2 years compared to those above 2 years of age, regardless of nutritional status [60, 169]. The observed trend of decreasing LUM CL/F with age after infancy has also been similarly reported for some other CYP3A4/5 substrates [165-167].

Our findings suggest that stunting is responsible for some of the unexplained variability in CL/F. Weight, on its own, had no effect on LUM CL/F. The effect of malnutrition on LUM PK disposition could not be assessed exhaustively since none of the children could be categorized as wasted, only 2 were underweight and 40% were stunted (height for age z-scores < -2). Incorporation of BMI (a composite of weight and height) into CL/F model explained variability due to weight and stunting among these < 5 year old children. The LUM CL/F increased as BMI decreased. As described above, weight alone did not explain any inter-individual variability in the CL/F model but explained 4.8% of the variability in V_C/F. Weight was not a significant covariate, but the inclusion and retention in the V_C/F model was for allometric purposes (Table 6). This is contrary to what was reported in an earlier study among children and adults, where weight was the most influential covariate and impacted positively on both CL/F and V_C/F [178]. Other significant covariates for CL/F including age, height and in addition sex were also identified in that study [178]. An earlier dose finding trial and one of the very first LUM PPK studies among older children > 15 years and adults showed that CL/F increased with weight above 50 kg and age above 24 years [72]. In a recent study among children less than 2 years, age was shown to have had a

positive influence on LUM bioavailability but it is not reported whether weight affected CL/F. However their CL/F model included allometric scaling in relation to weight. Other PPK studies among children ranging 1 to 10 years did not identify any patient characteristics as being influential on CL/F or V_C/F [117, 120].

Our estimated CL/F for a typical child 1 and 2 year old with reference to median BMI of 16.6 kg/m^2 approximates to the estimates reported by Tchaparian *et al.*, for children between 1 and 2 years of age (Table 1) [177]. Their CL/F population estimates are in agreement with our findings, where children younger than 24 months and with lower BMI had higher CL/F. The children studied [177] weighed much less (median weight was 8.43 kg, range 6.1 -13.0 kg, and no BMI reported) [177] than our children in the age range of 1-2 years (median 11.5 {range: 9-14.5} kg). Other pediatric studies reported much higher CL/F estimates (Table 1) [117, 120]. Some of the difference may have been caused by shorter sampling duration not appropriately encompassing the terminal elimination phase, differences in age, BMI, and the absence of allometric scaling for the PK parameters. For adult populations, other than age, variation may be due to pregnancy [118, 179] and drug- drug interactions [154, 180].

Estimates for V_C/F varied much more than those of CL/F between studies. In our analysis, the inter-individual variability in V_C/F remains greatly unexplained.

Desbutyl-lumefantrine PPK has been described in previous studies [117, 178-180], one of which was among children [117]. Some approaches, assumed 100% conversion of parent drug to metabolite [117, 180]. This may have resulted in biased estimation of DBL population PK parameter estimates. The rationale for assuming 100% conversion of LUM to DBL was not provided by the authors. Thus, we estimated the fraction of metabolite formed as part of the estimation of PK of DBL.

Methodological considerations for the PPK studies

The population analysis lacks information among children less than 1 year as these were not included. Assessment of PK-PD relationships was constrained given the small study population and the fact that we only had a few (n=3) parasite re-infections and only one with parasite recrudescence. The sample size was adequate for PK evaluations, mixed sampling design (intensive and sparse) was performed and several samples (2-8) were provided by most of the patients [204]. It was intended to cater for non-comparative assessment of efficacy in the study group as well. Unfortunately, the sample size was not adjusted for loss to efficacy follow up. Of note is the fact that logistic regression performed with available covariates, only yielded BMI as a predictor for parasite re-appearance. In the logistic regression, both re-infections {n=3} and recrudescence {n=1} were considered parasitologic failure. Note that BMI is also major predictor of CL/F, implying that those with lower BMI had higher CL/F. This suggests that children with lower BMI may need higher dose.

Clinical outcomes and perspectives on clinical implications of our findings

We cannot overlook the efficacy of the current weight-fixed dosing regimens. Given our study population (evaluable n=55), only one recrudescence was registered. This was a child (17 kg, 47.6 months) dosed with 2 AL tablets (total LUM dose of 84.7 mg/kg). The child with the lowest $C_{(LUM)}D7$ was one of those with parasite re-infection, and the only one who experienced late clinical failure and. The 3 children who had PCR confirmed re-infections were older than 24 months but small with BMI much less than the population median (< 15.5 kg/m^2). Given the fact that their weight was < 15kg, they were dosed with 1 AL tablet which resulted in total LUM weight adjusted dose of ≤ 60 mg/kg (≤ 10mg/kg/dose). Our observation is in agreement with an earlier report of total LUM dose between 50 and 79 mg/kg being a predictors of low $C_{(LUM)}D7$ and increased risk of malaria re-infection [80]. This further highlights the fact that children with lower BMI may need higher dose. However LUM has been shown to exhibit dose dependent absorption in animal studies [112] and similarly saturable LUM absorption has been reported in healthy volunteers [130]. We observed slight, non-significant increase in exposure (AUC-dose normalized) with increasing dose which indicates that saturable absorption might exist also in children. Allowing more time for prolonged LUM absorption, before administration of subsequent dose may be beneficial. Extended dosage schemes were earlier suggested for vulnerable populations at risk of lower LUM exposure [121, 149].

Though increasing LUM dose may pose a challenge, our findings highlight that exploration of age specific AL dose schemes, adjusted for BMI may provide rational AL doses for under five year old children regardless of nutritional status.

6 CONCLUSIONS AND RECOMMENDATIONS

Conclusions and implications

The thesis sought to provide and assess suitability of an alternative food supplement for AL. We have shown that if milk is not available, it is possible to recommend fortification of locally available carbohydrate rich staple food with little fat (maize porridge plus vegetable oil) to achieve similar LUM absorption. This is a strategy towards improving appropriate use of AL in resource limited user populations.

A simple, fast and sensitive analytical method for simultaneous quantification of LUM and its metabolite, DBL in human plasma was developed based on liquid chromatography-tandem mass spectrometry. The method was successfully applied for plasma LUM and DBL determination in children < 5 years of age with uncomplicated malaria, up to 28 days after a standard 3-day treatment with AL.

Current AL regimen employs fixed body weight-based dosing. We have demonstrated that LUM CL/F is partly a function of age and BMI while V_C/F of LUM is partly a function of body weight. In this under five year old children population, BMI, a composite variable of weight and height, was more important than weight and stunting alone in explaining the variability in LUM CL/F. The CL/F of LUM was slightly higher in those less than 2 years old when compared with those older than 2 years of age and it decreased with age as subject age increased from two to five years old. Additionally, the CL/F of LUM increased with decreasing BMI.

Our findings provide a structural pharmacokinetic framework for a rational approach to re-evaluating AL dosage guidelines for children less than 5 years of age. The finding further suggests that age specific AL dose schemes with BMI adjustments could be considered for improved AL dosage regimen in under five year old children.

Recommendations for future work

- To explore PK-PD relationship (of LUM and DBL PK, parasitological response and patient characteristics) to address the question of rational dosage regimens for LUM in children under 5 years of age.
- To assess the impact of malnutrition on LUM exposure and malaria treatment outcomes among under five year old children during AL treatment.
    - To further evaluate the influence of BMI on LUM exposure and treatment outcomes.
    - To assess whether BMI can be used as a practical basis for rational AL dose modification among children.
- To further explore the effectiveness of AL in a larger trial, when given with either oil fortified food as a practical alternative instead of milk under real life situations among < 5 year old children with uncomplicated malaria.

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