Lipoatrophy in HIV-Infected Children on Antiretroviral Therapy

by

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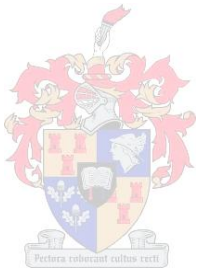

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Declaration

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Summary

Lipoatrophy in HIV-Infected Children on Antiretroviral Therapy

Introduction

Lipoatrophy is a common adverse effect of stavudine and this effect is strongly dose-dependent. Stavudine remains the most commonly used paediatric antiretroviral drug in sub-Saharan Africa, yet when the current study began in 2009, the prevalence and severity of lipoatrophy in children on antiretroviral therapy in sub-Saharan Africa had never been studied. The development of lipoatrophy may have serious and far-reaching consequences for patients and their families. The off-label stavudine dosing method, prescribed to children whose caregivers do not have access to a refrigerator, in which the contents of an adult capsule is mixed into tap water, has potential for over-dosing or under-dosing. In addition, children on stavudine continue to be exposed to a disproportionately high dose out of line with the reduced adult dose.

Aims

1. a) To investigate the prevalence and risk factors for lipoatrophy in HIV-infected children in Southern Africa
   b) To identify a simple anthropometric screening tool to detect early lipoatrophy in children
2. To validate the off-label stavudine dosing method prescribed to children whose caregivers do not have access to a refrigerator, with a view to reducing the recommended dose and thereby the side-effects.

Methods

1. a) We recruited pre-pubertal children on antiretroviral therapy from a family HIV clinic in our facility. Lipoatrophy was identified by two experienced paediatric HIV clinicians using a standardized grading scale. A dietician performed dietary assessment and anthropometric

measurements. Previous antiretroviral exposures were recorded. A subset of recruits received Dual-Energy X-ray Absorbtiometry scanning.

b) Anthropometric measurements in children with and without lipoatrophy were compared using multivariate linear regression adjusting for age and gender. The most discerning anthropometric variables underwent Receiver Operating Characteristic curve analysis to identify the most appropriate diagnostic cut-off.

2. a) Accuracy of the standard off-label stavudine dosing method was investigated using high-performance liquid chromatography to recover active drug from solutions made up using the prescribed method. This was compared to the stated drug content of the capsules.

b) Bioavailability was investigated by performing a randomized crossover pharmacokinetic study wherein healthy HIV-seronegative adult volunteers received one of two generic stavudine capsule formulations, either intact or mixed in water using the prescribed method. Plasma stavudine concentrations were assayed by liquid chromatography tandem mass spectrometry.

Results

1. a) Prevalence of lipoatrophy was 36%, and incidence was 12% per person-year. Adjusted odds ratio for developing lipoatrophy was 1.9 (CI: 1.3–2.9) for each additional year of accumulated exposure to standard-dose stavudine.

b) Baseline biceps skin-fold thickness correlated well with maximum lipoatrophy grading score at any site, giving a partial correlation coefficient of 0.33 (p=0.0006), and a receiver operating characteristic area-under-curve value of 0.75 (CI: 0.64 – 0.84). Biceps skin-fold thickness <5mm at baseline had a sensitivity of 89% (CI: 67–100%) and a negative predictive value of 97% (CI: 91–100%) for predicting which children would go on to develop lipoatrophy by 15 month follow-up. Specificity was 60% (CI: 46–75%) and positive predictive value was 32% (CI: 14–50%).

2. a) Recovery of active drug from solution was 97.1%, 97.4% and 93.8% for the proprietary and two generic formulations respectively.

b) Pharmacokinetic parameters of the off-label dosing method were well within the target range of intact capsule dosing for both generics.

Conclusions

1. a) The prevalence and incidence of lipoatrophy in pre-pubertal children on antiretroviral therapy in South Africa is high. Cumulative exposure to standard-dose stavudine was the greatest risk factor for lipoatrophy.

b) Biceps skin-fold thickness provided reasonable sensitivity and specificity to detect and predict lipoatrophy in pre-pubertal children on antiretroviral therapy.

2. The off-label dosing method for stavudine prescribed to children whose caregivers do not have access to a refrigerator is reasonably accurate and is bioequivalent to intact capsule administration.

Opsomming

Lipoatrofie in MIV-geïnfekteerde kinders op antiretrovirale terapie

Inleiding

Lipoatrofie is 'n algemene nadelige uitwerking van stavudien en hierdie effek is sterk dosis-afhanklike. Stavudien bly die mees algemeen gebruikte paediatriese antiretrovirale medikasie in sub-Sahara Afrika, maar toe ons studie begin het, was lipoatrofie in kinders op antiretrovirale terapie in sub-Sahara Afrika nog nooit voorheen bestudeer nie. Die ontwikkeling van lipoatrofie kan ernstige en verreikende gevolge vir die pasiënt en hul familie hê. Die af-etiket stavudien dosering metode voorgeskryf aan kinders wie se versorgers nie toegang tot 'n yskas het nie het 'n aansienlike potensiäal vir oor-dosering of onder-dosering. Daarbenewens, is kinders op stavudien blootgestel aan 'n disproporsionele hoë dosis uit-pas met die verminderde volwasse dosis.

Doelwitte

1. a) Om ondersoek in te stel na die voorkoms en risiko faktore vir lipoatrofie in MIV-geïnfekteerde kinders in Suid Afrika

b) Om 'n eenvoudige antropometriese instrument te identifiseer om vroeë lipoatrofie op te spoor in kinders op antiretrovirale medikasie

2. Om die af-etiket stavudien dosering metode wat voorgeskryf is aan kinders wie se versorgers nie toegang tot 'n yskas het nie te valideer, met 'n oog op die vermindering van die aanbevole dosis

Metodes

1. a) Ons het 'n groep van onder-puberteitsjarige kinders op antiretrovirale terapie gewerf uit 'n familie MIV kliniek in ons fasiliteit. Lipoatrofie is geïdentifiseer deur twee ervare MIV pediaters deur gebruik van 'n gestandaardiseerde gradering skaal. 'n Diëetkundige het diëet assessering en

antropometriese metings uitgevoer. Vorige antiretrovirale blootstellings is aangeteken. In 'n subset was Dual-energie X-straal Absorbtiometry (DXA) skandering uitgevoer.

b) Antropometriese metings in kinders met en sonder lipoatrofie is vergelyk met behulp van meerveranderlike lineêre regressie aangepas vir ouderdom en geslag. Die mees kieskeurige antropometriese veranderlikes het Receiver Operating Curve analise ondergaan om die mees geskikte diagnostiese afgesnypunt te identifiseer.

2. a) Akkuraatheid is ondersoek deur gebruik te maak van hoë werkverrigting vloeistofchromatografie om aktiewe medikasie vanuit oplossings te herstel, wat gemeng is soos aangedui deur die voorgeskrewe af-etiket dosering metode.

b) Biobeskikbaarheid is ondersoek deur die uitvoering van 'n ewekansige oorgesteekde farmakokinetiese studie waarin gesonde MIV- negatiewe volwasse vrywilligers een van twee generiese stavudien kapsule formulerings ontvang het, óf heel of in water gemeng soos aangedui deur die voorgeskrewe af-etiket dosering metode. Plasma stavudien konsentrasies is gemeet deur vloeistofchromatografie tandem massaspektrometrie.

Uitslae

1. a) Voorkoms van lipoatrofie was 36%, en insidensie was 12% per persoon-jaar. Aangepaste Odds ratio vir die ontwikkeling van lipoatrofie was 1,9 (CI: 1,3-2,9) vir elke addisionele jaar van opgehoopte blootstelling aan standaard dosis stavudien.

b) Biceps vel-vou dikte <5mm het 'n sensitiwiteit van 89% (CI: 83-96%) en 'n negatiewe voorspellende waarde van 90% (CI: 84-96%) vir die opsporing en voorspelling van lipoatrofie.

2. a) Herwinning van aktiewe medikasie uit oplossings was 97,1%, 97,4% en 93,8% vir die oorspronklike en twee generiese formulerings onderskeidelik.

b) Farmakokinetiese parameters van die af-etiket dosering metode was wel binne die teikenband van ongeskonde kapsule dosering vir beide generiese formulerings.

Gevolgtrekkings

1. a) Die voorkoms van lipoatrofie in onder-puberteitsjarige kinders op antiretrovirale terapie in Suid-Afrika is hoog. Die bedrag stavudien waaraan kinders blootgestel is moet hersien word. Die standaard stavudien dosis vir kinders moet herge-evalueer word.

b) Biceps vel-vou dikte het redelike goeie sensitiwiteit en spesifisiteit om lipoatrofie op te spoor en te voorspel.

2. Die af-etiket dosering metode vir stavudien voorgeskryf aan kinders wie se versorgers nie toegang tot 'n yskas het nie is redelik akkuraat en is bio-ekwivalent aan ongeskonde kapsule administrasie.

Contents

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List of abbreviations

AIDS	Acquired Immune Deficiency Syndrome
ART	Antiretroviral therapy
ARV	Antiretroviral drug
AUC	Area under curve
BMS	Bristol-Myer Squibb
CD4	Cluster of differentiation 4
CI	95% confidence interval
C_{max}	Maximum concentration
CT	Computed tomography
CV	Coefficient of variation
DoH	South African National Department of Health
DXA	Dual Energy X-ray Absorptiometry
FDA	Food and Drug Administration
FDC	Fixed drug combination
HIV	Human Immunodeficiency Virus
HPLC	High-performance liquid chromatography
IQR	Inter-quartile range
KID-CRU	Children's Infectious Diseases Clinical Research Unit
NRTI	Nucleoside reverse transcriptase inhibitor
N	number
NLR	Negative likelihood ratio
NPV	Negative predictive value
PK	Pharmacokinetic
PLR	Positive likelihood ratio

PPV	Positive predictive value
ROC	Receiver operating curve
SD	Standard deviation
SEM	Standard error of the mean
SFT	Skin-fold thickness
T_{max}	Time at which blood drug concentration is maximum
$T_{1/2}$	Half-life
WHO	World Health Organization
WHR	Waist-to-hip ratio

Lipoatrophy in HIV-Infected Children on Antiretroviral Therapy

Antiretroviral agents have led to dramatic advancements in life expectancy and quality of life for people living with HIV/AIDS. As a result, the focus of therapeutic management and research has shifted to delayed adverse drug effects and chronic diseases (1). Lipoatrophy is a common adverse effect of stavudine and this effect is strongly dose-dependent (2). Lipoatrophy was previously believed to be uncommon in children, and as a result, very little attention has been given to surveillance, diagnosis and risk factor alteration in children, particularly pre-pubertal children (3). However, European studies have found a progressively increasing prevalence of lipoatrophy in children on antiretroviral therapy (ART), affecting up to 28% in the most recent studies (4). Lipoatrophy has become one of the most common adverse drug effects in children on antiretroviral therapy (ART) (5). When the current study began in 2009, the prevalence and risk factors for lipoatrophy in children living in sub-Saharan Africa had not previously been studied despite the fact that over 90% of the 3.4 million HIV-infected children worldwide live in sub-Saharan Africa (6). Some data was presented by the NEVEREST study group at the 2011 International AIDS Society Conference, which showed consistently greater subcutaneous fat in children on protease inhibitors compared to those on non-nucleoside reverse transcriptase inhibitors, and generally low subcutaneous fat in children on stavudine (7). They did not define a diagnostic cut-off for lipoatrophy and thus no prevalence figures were given.

The development of lipoatrophy may have serious and far-reaching consequences for the patient and their families. Lipoatrophy looks very similar to AIDS wasting syndrome, known as “Slims disease” throughout Africa, and may result in the same stigmatization (8). In contrast to the developed world, stigmatization due to HIV in sub-Saharan Africa may lead to loss of housing, loss of employment or livelihood, denial of schooling, denial of healthcare, secondary stigmatization of family members and

physical violence (9-10). Fear of developing lipoatrophy may cause caregivers to become non-adherent with ART, leading to loss of CD4 cells, subsequent opportunistic infection and possibly death (11). In multivariate logistic regression modelling, fat distribution abnormalities were an independent risk factor for subsequent non-adherence (11). Reports of partial recovery of lipoatrophy are limited to the least severely affected individuals (12). Severe lipoatrophy may not be reversible (13-14). This is understandable since the mechanism of lipoatrophy involves progressive apoptosis of adipocytes, which do not recover (15), as opposed to nutritional wasting where adipocyte fat stores shrink but the cell survives. However, stigmatization may only occur when lipoatrophy is severe enough to be easily recognizable by the broader community. Stigmatization can be prevented if lipoatrophy is diagnosed early and ART is switched to arrest lipoatrophy progression (14). Communities with the highest prevalence of HIV are the most likely to recognize HIV-related or ART-related body changes. Southern Africa has the highest HIV prevalence in the world (6).

While lipoatrophy has been well described in Europe and the United States of America, there is almost no data on this syndrome in pre-pubertal children in sub-Saharan Africa, despite the fact that over 90% of the 3.4 million HIV-infected children worldwide live there (6). In South Africa alone, more than a million children under 15 years of age are HIV-infected, and, despite reductions in vertical transmission, an additional 59,000 new infections in children are added each year (16). South Africa has the largest antiretroviral treatment program in the world, with an estimated 163,000 infants and children on ART by August 2012, and many more added each year (personal communication – South African National Department of Health). The severity and extent of lipoatrophy in pre-pubertal children living in sub-Saharan Africa is unknown (5).

As there is evidence of genetic and ethnic determinants in lipoatrophy (17), it is important to study lipoatrophy specifically in sub-Saharan African populations. Four earlier studies from the developed

world have included immigrant children from sub-Saharan Africa living in Paris (18), London (19), Brussels (20) and a combination of sites in Western Europe (21), and found a lipoatrophy prevalence of 11%, 8%, 20% and 17% respectively. However, many differences exist between immigrant African populations living in the developed world and populations who remain in Africa, and findings may not be generalizable. Specifically, obesity is more common in Europe (22), which may mask early lipoatrophy, whereas under-nutrition is common in sub-Saharan Africa. In addition, stavudine is the most widely used first-line antiretroviral agent for children in the developing world, particularly sub-Saharan Africa, where 49% of children on ART are on a stavudine-based regimen (23). Thus, lipoatrophy in children in sub-Saharan Africa needed to be studied directly.

The prevalence of lipoatrophy in children on ART in Europe appears to be rising over time: Studies from 2005 and 2006 estimated the prevalence of lipoatrophy at 8% to 11% (18-19) whereas the most recent studies have found a prevalence of around 28% (4). Accumulation of cases is not surprising since lipoatrophy changes may persist despite switching of ART regimen (13-14), and survival rates are high in medication-adherent children, who are at highest risk of lipoatrophy.

Mechanism

The mechanism of lipoatrophy is related to (NRTI)-induced mitochondrial damage, particularly in adipocytes (24), which may result in apoptosis. NRTIs such as stavudine can damage adipocyte mitochondria (24) and cause a reduction in functioning mitochondria in adults (25). Other chronic toxic effects such as lactic acidosis and peripheral neuropathy have also been associated with mitochondrial dysfunction (26-27) but are uncommon in children (28-29). It has been suggested that unknown agents released from damaged mitochondria in adipocytes may directly trigger apoptosis, which leads to subcutaneous fat loss. Decline of mitochondrial DNA in peripheral leucocytes may provide an early warning sign of impending lipoatrophy in patients exposed to antiretrovirals (30-31).

In addition, circulating growth hormone levels are significantly reduced in patients with lipoatrophy, and this may contribute to the reduction in sub-cutaneous adipose tissue (32).

Risk Factors

Lipoatrophy does not occur in all children exposed to ART (4, 21, 33). Some show no signs of fat changes despite many years of stavudine or zidovudine exposure, whereas others develop lipoatrophy within 18 months of starting ART (33). This variation may be due to genetic differences that increase or decrease an individual's susceptibility to lipoatrophy (17, 34-35). Particular mitochondrial DNA sub-groups (haplogroups) have been associated with a vulnerability to developing lipoatrophy after exposure to ART (36). A recent study showed that Caucasian American men on ART who have the H mitochondrial haplogroup were at significantly increased risk of lipoatrophy (36). A plausible explanation may be that variations of mitochondrial DNA in adipocytes may reduce the efficiency of energy production or lead to increased oxygen free-radical production, resulting in a reduced mitochondrial functional reserve and an increased vulnerability to apoptosis when exposed to mitochondrial toxins such as antiretrovirals.

Lipoatrophy has been clearly linked to the NRTI drugs stavudine, zidovudine and didanosine (20, 37-41), and the risk appears to be proportional to the dose (42). In comparison, the NRTI drugs abacavir, tenofovir, and lamivudine have minimal or no lipoatrophy-causing effect (43). The non-nucleoside reverse transcriptase inhibitor efavirenz is a less potent cause of lipoatrophy (20). In addition, efavirenz has been associated with lipomastia in some children, although this usually resolves spontaneously without withdrawal of efavirenz (44-45). Protease inhibitors have been linked to dyslipidaemia (46), but have not been convincingly implicated in lipoatrophy. In fact, protease inhibitors have been associated with global increases in fat deposits (47).

The risk of lipoatrophy in patients on stavudine is strongly dependent on the dose. In 2007, an influential review of the previous 15 years' data by Hill *et al* (42) showed that a lower stavudine dose of 20 or 30mg twice daily results in a markedly lower risk of lipoatrophy, while maintaining excellent antiviral efficacy (48-49). This led the World Health Organization to recommend a reduction in the usual dose of stavudine for adults weighing over 60 kg from 40 mg to 30 mg twice daily (50). The current standard paediatric dose of stavudine (1 mg/kg/dose twice daily) was determined by direct extrapolation from the pharmacokinetic parameters of the adult dose of 40 mg twice daily, using data from a few small but well-controlled paediatric pharmacokinetic studies (51-53) which showed that an oral dose of 1 mg/kg/dose twice daily in children under 12 years results in plasma exposure similar to that of adults taking 40 mg twice daily, and that an oral dose of 0.5 mg/kg/dose twice daily in children results in plasma exposure similar to that of adults taking 20 mg twice daily. The WHO recommended dose of stavudine for children, however, has not yet been reduced. Consequently children on stavudine continue to be exposed to a disproportionately high dose, which may result in more rapid accumulation of metabolic adverse effects than adults on the reduced dose.

Stavudine

Stavudine is now rarely used in the developed world. The 2010 World Health Organization antiretroviral guidelines advise that stavudine should be phased out where possible (54). However, stavudine remains within the nationally recommended paediatric first-line ART guidelines for numerous developing countries. In South Africa, stavudine was the first choice nucleoside reverse transcriptase inhibitor for children, together with lamivudine, from the beginning of the ART access program in 2004 until 2010, when it was replaced with abacavir. Current South African guidelines state that children taking stavudine should continue unless side-effects develop. Thus the majority of children treated for HIV in South Africa remain on stavudine. In most other sub-Saharan African countries the cost of abacavir remains prohibitive and stavudine remains the most commonly used

paediatric antiretroviral there, with around 49% of children on ART on a stavudine-based regimen (23, 55).

Paediatric stavudine solution (Zerit® - Bristol Myer Squibb, Uxbridge, United Kingdom), available as a dry powder, must be reconstituted with a specific volume (202ml) of distilled water. Once reconstituted, the solution must be transported by the caregiver in a cooler box, requires refrigeration at 4^{o}C, and is then only stable for one month (56). Paediatric liquid formulations present significant logistical difficulties in rural or resource-constrained settings. Refrigeration is often not possible in rural or resource-limited areas. Where fixed drug combinations are not available, logistical barriers to the use of the paediatric stavudine formulation in resource-limited areas have forced paediatric clinicians to prescribe an off-label dosing method that makes use of adult stavudine capsules in lieu of the liquid paediatric formulation. The adult capsule formulation is stable at room temperature (<25^{o}C) for two years (57). The South African Department of Health guidelines (58) advise that caregivers should be instructed to disperse the contents of an adult capsule in 5ml water and then withdraw the required fraction of the mixture using a syringe. The contents of Stavudine capsules do not dissolve completely, and rapidly form a sediment at the bottom of the container. The caregiver is advised to re-agitate the mixture carefully in order to re-suspend the sediment that collects at the bottom of the container, before drawing up the aliquot for the child. This administration method has been termed the “opened capsule” dosing method. Manufacturers do not recommend that capsules be opened or suspended in water before consumption (56). As there were no published data on the accuracy or bioavailability of the “opened capsule” dosing method, the potential for over- or under-dosing may be significant. In addition, the South African National Department of Health have prescribed that the volume of mixture that corresponded to the usual dose (1mg/kg twice daily) should be rounded up to the nearest practical volume of mixture, usually 2.5ml, which could result in doses as high as 1.4mg/kg.

Fixed drug combination effervescent tablets incorporating stavudine have been introduced in certain central African countries (Uganda, Zambia, Malawi). These are marketed as Triomune Adult®, Triomune Junior® and Triomune Baby®. These formulations were produced by Cipla Life Sciences (Mumbai, India) as part of a social responsibility response to the dearth of paediatric antiretroviral formulations suitable for sub-Saharan African conditions. Triomune Junior® and Baby® remain unregistered in Southern Africa countries (South Africa, Zimbabwe, Mozambique), although they have been registered in Botswana and Namibia (Personal communication – Cipla Medpro).

The most common alternative to stavudine is zidovudine, with around 47% of children on ART on a zidovudine-based regimen (23, 59), Zidovudine also causes lipoatrophy albeit less severely (60). In addition, the danger of zidovudine-related bone marrow suppression, a common problem with varying degrees of severity, is significant and requires some laboratory monitoring (59, 61). Laboratory facilities are not always reliably available in resource-limited settings. Abacavir is the current preferred first line drug in paediatric care in the developed world (54). However, the cost of abacavir is prohibitive for most resource-limited healthcare budgets in sub-Saharan Africa (62). All antiretroviral drugs carry risks of adverse effects that must be balanced against clinical benefit, particularly in primary healthcare settings in sub-Saharan Africa where surveillance for adverse effects may be limited. Stavudine offers an almost toxicity-free safety profile in the first 6 to 12 months of use (2), with potent antiviral efficacy (42). For unknown reasons, thymidine-related peripheral neuropathy and symptomatic lactic acidosis are rare in pre-pubertal children, with only isolated reports in published literature (28-29, 63-65).

Diagnosis

An objective case definition for lipoatrophy has been established for adults, which incorporates computed tomography (CT) measurements, Dual Energy X-ray Absorptiometry (DXA), and blood lipid and electrolyte values (66). However, this case definition has not been validated in children. Serial DXA is sometimes used in developed countries to monitor fat volume and distribution in children on potentially lipoatrophy-inducing ART. The most robust method of diagnosing lipoatrophy in children is the skilled visual assessment of subcutaneous limb and face fat performed by experienced paediatric HIV clinicians who have been specifically trained to do this (21). In clinical practice, however, the diagnosis is most commonly made by a non-specialist clinician who has been trained to recognise the typical features. Physical signs in children are due to loss of subcutaneous fat in limbs, buttocks and face, with or without accumulation of intra-abdominal visceral fat (20, 39-41). At least 30% of peripheral fat must be lost before lipoatrophy becomes visibly evident (67). Fat loss is slowly progressive and often goes unnoticed until it is severe. Early facial fat loss may be subtle and difficult to detect. In the absence of lipoatrophy, facial muscles are covered in fat and are not normally noticeable. Loss of facial fat results in a lean, muscular appearance of the face with sunken cheeks and deep laugh-lines when smiling. Loss of limb fat results in reduced skin-fold thickness (SFT), prominent limb veins and a well-defined, muscular appearance of limbs in the presence of a normal or enlarged abdomen. A torso-to-arm SFT ratio above 2, calculated as (subscapular SFT + suprailiac SFT) / (biceps SFT + triceps SFT), has been used as a diagnostic criterion in a previous study (68), however, the number of children with lipoatrophy in that study was small (n=4) and were all pubertal, and data was presented as median z-scores without inter-quartile ranges. Loss of buttock fat, with or without enlargement of the abdomen, may result in an increased waist-to-hip ratio (WHR). Breast enlargement and “buffalo hump” (accumulation of fat in the nape of the neck) may occur after puberty (69). Diagnosis of *early* lipoatrophy in children remains difficult. CT and DXA are often not feasible in sub-Saharan Africa, and paediatric-trained HIV specialists are scarce.

Management

Since the disfigurement caused by lipoatrophy may be permanent, the focus of management is on early detection and arresting progression. Once identified, the most likely offending antiretroviral drug is usually withdrawn and is replaced by a less lipoatrophy-inducing antiretroviral. When lipoatrophy is diagnosed, significant benefit in halting progression has been shown by switching from the thymidine NRTI antiretroviral drug to a non-thymidine NRTI such as abacavir (43). Where abacavir is not available, stavudine may be switched to zidovudine with moderate results (12). Tenofovir is generally avoided in children because of its renal and bone toxicity, which are believed to be significantly more common and more pronounced in children than adults (70-72). However, there may be a place for switching to tenofovir in adolescents (73). Switching to an alternative antiretroviral agent typically arrests progression of lipoatrophy, and may result in a small degree of reversal if lipoatrophy is caught early (14). Various authors have demonstrated that the more advanced the lipoatrophy, the less likely it is to reverse when the offending drug is removed (74-75). Intradermal injections of a biodegradable filler such as poly-L-lactic acid (Sculptura®) can ameliorate the aesthetic effect of facial lipoatrophy in adults (76). However the pain and discomfort of this treatment make it inappropriate for children. In addition, the cost is significant and the effect is not permanent and injections may need to be repeated. Uridine (NucleomaxX®) partially reverses the mitochondrial toxicity caused by thymidine NRTIs, and may have a small but beneficial effect on disfiguring lipoatrophy (77). However, uridine is not currently available in South Africa. Growth hormone-releasing hormone analogues may be helpful in the treatment of lipoatrophy (78). The mechanism probably involves reversing the reduced growth hormone levels that are consistently found in patients with lipoatrophy. Although the side-effect profile of growth hormone-releasing hormone therapy is attractive, the cost is prohibitive. Future treatments may involve adipokines such as leptin, but these remain experimental (38).

Gaps in current knowledge

1. The severity and extent of lipoatrophy in pre-pubertal children living in sub-Saharan Africa was unknown (5).
2. Since stavudine is the most common cause of lipoatrophy and the effect is strongly dose-related, it is important to establish the accuracy and bioavailability of the off-label stavudine dosing method prescribed to children whose caregivers do not have access to a refrigerator, with a view to reducing the recommended dose. In addition, as a number of generic alternatives are available and regional purchasing mechanisms may favour switching suppliers, the various generic capsule preparations needed to be studied in order to determine which preparations, if any, are suitable for the off-label "opened capsule" dosing method.

Thus the aims of this PhD were two-fold:

1. To investigate the prevalence and risk factors for lipoatrophy in HIV-infected children on ART in Southern Africa.
2. To investigate the accuracy and bioavailability of the off-label stavudine dosing method prescribed to children whose caregivers do not have access to a refrigerator, with a view to reducing the recommended dose.

These aims have been fulfilled, and are described in chapters 1 to 5. The respective published manuscripts have been added as appendices. Serendipitously during the course of these studies, I realized that a combination of anthropometric measurements might render reasonable sensitivity and specificity to act as a simple screening tool to detect lipoatrophy at an early stage in areas where specialized diagnostic skills and equipment are scarce. This would allow earlier antiretroviral drug switches to be made to arrest the progression of lipoatrophy and prevent potentially stigmatizing face and limb disfigurement. The study that describes that screening tool is presented in chapters 2 and 3 and the published manuscript has been added as an appendix.

Chapter 1

Prevalence and risk factors for lipoatrophy in pre-pubertal South African children on antiretroviral therapy

Abstract

Background: Despite changes in WHO guidelines, stavudine is still used extensively for treatment of paediatric HIV in the developing world. Lipoatrophy in sub-Saharan African children can be stigmatizing and have far-reaching consequences. The severity and extent of lipoatrophy in pre-pubertal children living in sub-Saharan Africa was unknown.

Methods: In this study, children who were 3-12years old, on antiretroviral therapy and pre-pubertal were recruited from a Family HIV Clinic in South Africa. Lipoatrophy was identified and graded by consensus between two experienced HIV paediatricians using a standardized grading scale. A professional dietician performed dietary assessments and anthropometric measurements of trunk and limb fat. Previous antiretroviral exposures were recorded. In a Dual-Energy X-ray Absorbtiometry (DXA) substudy, 42 subjects were scanned. Lipoatrophy assessment was repeated at least one year later.

Results: Among 100 recruits, the prevalence of visually obvious lipoatrophy was 36% (95% CI: 27%–45%). Annual incidence of lipoatrophy was 12% (CI: 5-20%) per person-year of follow-up. Anthropometry and DXA measurements corroborated the clinical diagnosis of lipoatrophy: Both confirmed significant, substantial extremity fat loss in children with visually obvious lipoatrophy, when adjusted for age and sex. Adjusted odds ratio for developing lipoatrophy was 1.9 (CI: 1.3 - 2.9) for each additional year of accumulated exposure to standard-dose stavudine. Cumulative time on standard-dose stavudine was significantly associated with reductions in biceps and triceps skin-fold thickness (p=0.008).

Conclusions: The prevalence of visually obvious lipoatrophy in pre-pubertal South African children on antiretroviral therapy is high. The amount of stavudine that children are exposed to needs review.

Resources are needed to enable low- and middle-income countries to provide suitable paediatric-formulated alternatives to stavudine-based paediatric regimens. The standard stavudine dose for children may need to be re-evaluated. Diagnosis of lipoatrophy at an early stage is important to avoid stigmatization. Diagnosis using visual grading requires training and experience, and DXA and comprehensive anthropometry are not commonly available. A simple objective screening tool is needed that can identify early lipoatrophy in resource-limited settings where specialized skills and equipment are not available.

Aim

We explored the prevalence and risk factors for lipoatrophy in a group of pre-pubertal South African children on ART. We also correlated the visual diagnosis of lipoatrophy with objective anthropometric measurements of body fat in the whole group, and with Dual Energy X-ray Absorptiometry (DXA) findings in a subgroup.

Methods

The Family Clinic for HIV at Tygerberg Children's Hospital is a public-sector clinic providing ART to infants and children from the Northern suburbs of Cape Town. In this study, children who were 3-12 years old, on antiretroviral therapy and pre-pubertal were recruited between February 2010 and January 2011. Pre-pubertal status was defined as Tanner stage 1 for genital and pubic hair development in boys, and Tanner stage 1 for pubic hair development with Tanner stage 1 or 2 for breast development in girls. Using a review of our electronic health record database, we identified 190 subjects that potentially met the inclusion criteria. Of these, 124 attended clinic during the study period and could be approached for screening. A total of 121 provisionally agreed to participate, but 21 did not attend the study visit nor did they respond to attempts at further contact. One hundred subjects were finally recruited. There was no difference in demographic characteristics of the 100

enrolled subjects and the 90 who were not recruited (p>0.20 for age, gender, cumulative time on standard-dose stavudine and CD4).

Lipoatrophy was identified and graded by consensus between two HIV paediatricians who were experienced in identifying lipoatrophy, using the following lipoatrophy grading scale defined by existing literature (33, 66, 68): 0 – No fat changes; 1 – Possible minor changes, noticeable only on close inspection; 2 – Moderate changes, readily noticeable to an experienced clinician or a close relative who knows the child well; 3 – Major changes, readily noticeable to a casual observer. Face, arms, legs and buttocks were assessed for loss of subcutaneous fat resulting in abnormally prominent limb veins, a lean, muscular appearance of limbs and face, and loss of gluteal fat pad with reduction in buttock size and loss of gluteal contour. Where the assessment of the two investigators did not concur, the change was graded as 1. Lipoatrophy was defined as a score of 2 or 3. The parents' subjective assessments of fat changes were recorded.

Durations of previous antiretroviral exposures and demographics were recorded from our electronic health record database. HIV RNA and CD4 values were extracted from our central electronic laboratory results server. Doses of antiretroviral drugs followed nationally prescribed protocols. For stavudine this meant a minimum of 1mg/kg twice daily rounded up to the nearest practical dose. A professional dietician performed dietary assessments and anthropometric measurements of body mass, trunk and limb fat using a non-stretchable tape-measure (model number F10-02DM, Muratec KDS Corporation, Kyoto, Japan), a high-precision Harpenden® skin-fold caliper (Baty International, West Sussex, United Kingdom), a ShorrBoard® stadiometer (Shorr Productions, Maryland, USA), and a precision weighing scale (model number UC-321, A&D Company, Tokyo, Japan) which was calibrated daily. Measurements included mid-upper arm circumference, mid-thigh circumference, chest circumference, waist circumference, hip circumference, biceps skin fold thickness (SFT), triceps

SFT, iliac crest SFT, sub-scapular SFT, mid-thigh SFT, height and weight. The following ratios were derived: waist to hip circumference ratio; body mass index; torso to arm SFT ratio ([subscapular + iliac crest SFT] / [biceps + triceps SFT]). Lipoatrophy assessment was repeated at least one year later.

In a DXA substudy, DXA scans were performed using a Hologic Discovery (Bedford, Massachusetts, USA) to objectively confirm lipoatrophy. Trunk and limb fat mass, lean mass and fat percentage were measured.

Since very little is known about the normal paediatric population in South Africa, additional normative data was collected from healthy age- gender- and socioeconomically-matched HIV-uninfected children from the same community who had been enrolled as part of a different study at our research unit (79). We gathered anthropometry and DXA data from 57 children in order to supplement what is known from published population norms for age-related anthropometric and DXA variables, which are currently only derived from developed country paediatric populations. This local normative data appears in tables 2 and 3 to assist the reader to gauge the magnitude of the changes found in lipoatrophy-affected children.

In univariate analyses, a t-test was used for parametric data, Wilcoxon rank sum test was used for non-parametric data, and Fisher's exact test was used for categorical data. Non-parametric continuous data are quoted as median (interquartile range), and parametric continuous data are quoted as mean (standard deviation) or mean (95% confidence interval) where appropriate. Multiple logistic regression was used to study the risk factors associated with visually obvious lipoatrophy, adjusting for age and CD4. Multiple linear regression models were used to compare fat distributions captured by anthropometry and DXA between children with and without lipoatrophy, adjusting for

age and sex. A least squares approach was used for multivariable modelling. All statistical analyses were performed using R version 2.10.0 (Bell Laboratories, New Jersey).

This study was designed in accordance with the guidelines of the International Conference on Harmonization for Good Clinical Practice and with the Declaration of Helsinki (version 2000), and approved and monitored by the Ethics Committee for Human Research of the Stellenbosch University, approval reference number N08/11/349. Written informed consent was obtained from each caregiver prior to participation, and informed assent was obtained from capable children. All patient-related data were stored in a password-secured database under a patient identifying number and kept strictly confidential. The choice of sample population satisfies the ethical principle of justice as this study will directly benefit the community from which the data is drawn by leading to recommendations about best practice in public-sector HIV clinics in South Africa.

Results

One hundred subjects were recruited. The prevalence of visually obvious lipoatrophy was 36% (95% CI: 27% to 45%). One of the 15 girls with lipoatrophy and 2 of the 33 girls without lipoatrophy were Tanner stage 2 for breast development at recruitment. All others were Tanner stage 1. Overall, children with and without lipoatrophy had similar body mass index, weight-for-age Z-score, height-for-age Z-score, gender distribution, ethnic distribution, WHO clinical stage, viral load and mean CD4 (see table 1). In children with lipoatrophy, face changes were graded as more severe than limb changes in 11/36; the same as limb changes in 24/36; and less severe than limb changes in 1/36. In all cases, lipoatrophy changes were deemed symmetrical. Twelve of the 36 cases had severe changes (grade 3) similar to the child represented in figures 1 and 2. The parents' subjective assessments tended to underestimate the presence and severity of subcutaneous fat changes. In no recruits did the parent assess the changes as more severe than the clinicians' consensus assessment.

On formal dietary assessment, 11 children without lipoatrophy (18%) versus 5 children with lipoatrophy (15%) had an inappropriate diet: 6 (10%) versus 5 (15%) were over-consuming refined carbohydrates; 1 (2%) versus 0 (0%) had inadequate carbohydrate consumption; 3 (5%) versus 1 (3%) were over-consuming fat; 2 (3%) versus 0 (0%) had inadequate fat consumption; 4 (6%) versus 0 (0%) had inadequate total daily calorie consumption. Dietary data was missing in 2 children from each group.

Table 1 reflects that, on univariate analysis, overall time on ART, time on standard-dose stavudine, cumulative lamivudine exposure, cumulative efavirenz exposure, and greater age were associated with visually obvious lipoatrophy. Zidovudine and didanosine were not included in the analysis as too few children had been exposed to these drugs (33 and 6 children respectively, with p=0.56 and 0.58 respectively). WHO clinical stage did not correlate with the presence or absence of lipoatrophy (p=0.78). A multivariate logistic regression model controlling for age and CD4%, incorporating all antiretroviral exposures found significant in the univariate analysis, identified cumulative time on standard-dose stavudine as the predominant risk factor independently associated with lipoatrophy (adjusted odds ratio = 1.9 for each additional year of accumulated exposure to standard-dose stavudine; 95% CI: 1.3 to 2.9; p=0.002), while efavirenz and lamivudine were no longer associated. Abacavir exposure was not included in the multivariate model as it was only used for children who had developed a complication of stavudine therapy. Cumulative time on standard-dose stavudine was significantly associated with reductions in biceps and triceps SFT (p=0.008). Thirty-five of the 36 children with lipoatrophy and 53 of the 64 children without lipoatrophy had ever been exposed to stavudine, and 51/64 (80%) of children without lipoatrophy were on stavudine-based regimens at recruitment. Table 1 shows the comparative antiretroviral exposures and data from the univariate and multivariate analysis. All but one of the children with lipoatrophy had been exposed to more than 18

months of stavudine therapy. At the time of recruitment, 23/36 children with lipoatrophy had been off stavudine for at least six months with only minimal or mild resolution of their fat abnormalities.

Adjusting for age and sex, anthropometrics confirmed significant, substantial extremity fat loss in children with visually obvious lipoatrophy (see table 2). There were no statistically significant differences in anthropometric measurements of fat amount and distribution between children without lipoatrophy and the local HIV-uninfected paediatric population. Biceps SFT, triceps SFT, torso-to-arm SFT ratio and waist-to-hip circumference ratio correlated with maximum lipoatrophy grading score, giving regression coefficients of 0.34 (0.17 to 0.65, p=0.002), 0.31 (0.17 to 0.59, p=0.0006), 3.36 (1.57 to 7.16, p=0.002) and 4.23 (0.37 to 8.10, p=0.03) respectively when adjusted for age and sex.

In the DXA substudy, there was no difference in gender, cumulative time on standard-dose stavudine or CD4 between the 42 subjects who underwent DXA scanning and the 58 who did not (p>0.50 for all). The children who underwent DXA were marginally younger than those who did not (7.1 versus 8.0 years, p=0.03). Measures of fat amount and distribution confirmed significant, substantial extremity fat loss in children with visually obvious lipoatrophy, when adjusted for age and sex (see table 3). There were no statistically significant differences in DXA measures of fat amount and distribution between children without lipoatrophy and the local HIV-uninfected paediatric population. Limb fat versus limb lean ratio, limb fat versus total body weight ratio, limb fat versus body mass index ratio and total limb fat (converted to grams) correlated with maximum lipoatrophy grading score, giving regression coefficients of -2.24 (-0.86 to -3.62, p=0.003), -0.02 (-0.01 to -0.03, p=0.002), -0.015 (-0.006 to -0.024, p=0.003) and -0.8 (-0. 3 to -1.4 grams, p=0.006) respectively when adjusted for age and sex.

Lipoatrophy grading scores at baseline and at follow-up are presented in table 4 and figure 3. Follow-up was completed on 34 of the 36 children with lipoatrophy and 60 of the 64 children without lipoatrophy at baseline. Median follow-up time was 14.9 months (interquartile range: 14.5 to 15.6 months). At follow-up, 9/60 children had developed new lipoatrophy, giving an incidence rate of 12% (CI: 5% to 20%) per person-year of follow-up. Lipoatrophy had resolved in 5/34 children, all of whom had no more than moderate (grade 2) lipoatrophy signs at baseline and had been switched to abacavir >18 months before follow-up.

Discussion

This was some of the first data from sub-Saharan Africa to show the severity and extent of antiretroviral-related lipoatrophy in children and was first presented at the 2011 International AIDS Society Conference. At the same meeting, data was presented by the NEVEREST study group in Johannesburg, which showed consistently greater subcutaneous fat in children on protease inhibitors compared to those on non-nucleoside reverse transcriptase inhibitors, and generally low subcutaneous fat in children on stavudine (7). They did not define a diagnostic cut-off for lipoatrophy and thus no prevalence figures were given.

Visually obvious lipoatrophy was present in a third of pre-pubertal children on ART. Children with visually obvious lipoatrophy had 25-40% less extremity fat (depending on the choice of measurement) than HIV-infected children without lipoatrophy. Children with lipoatrophy were not sicker and did not start ART at a different age, compared to those without lipoatrophy. Since cumulative time on standard-dose stavudine is the greatest risk factor for lipoatrophy, it is not surprising that older children, who had amassed more stavudine exposure, had more lipoatrophy. Other potential confounding effects associated with the difference in age were adjusted for in the multivariate model.

Although DXA and anthropometry may be more precise measures of subcutaneous fat amount and distribution, a visual grading scale was chosen as the primary outcome measure in this study because the greatest danger of lipoatrophy in sub-Saharan Africa stems from stigmatization. Therefore, subtle changes in fat distribution that are not visually obvious are less relevant since they are unlikely to result in stigmatization.

Two previous studies have found that some partial improvement of lipoatrophy may occur after drug switching, with the more severe cases experiencing the least improvement (12-13). In line with this, the 5 individuals with lipoatrophy at baseline who resolved by follow-up had all been graded as having no more than moderate (grade 2) changes at baseline and had been switched to abacavir more than 18 months earlier, during which time some improvement in subcutaneous SFT occurred.

As there is evidence of a genetic determinant in lipoatrophy (17), it is important to study lipoatrophy specifically in sub-Saharan African populations. Our data suggest that the prevalence of visually obvious lipoatrophy in pre-pubertal South African children on ART is higher than the prevalence among most non-African cohorts (typically 10 to 20%) (18, 20-21, 33, 68). In the largest and most comprehensive study to date, Alam et al (4) found a 28% prevalence in a cross-sectional study of 426 European children, including 85 pubertal and 154 post-pubertal children and 107 black children. The authors of that study noted that their prevalence was significantly higher than in previous paediatric studies, in line with our data. Since survival rates are high in medication-adherent HIV-infected children, and established lipoatrophy changes are largely irreversible, prevalence can be expected to increase progressively if incidence remains constant. Alam et al found that Caucasian rather than African ethnicity was a risk factor for lipoatrophy. However, none of our recruits were Caucasian and our pre-pubertal South African group had a higher prevalence than Alam's cohort. This is most likely

due to higher rates of stavudine exposure in our context. The magnitude of this difference suggests that data from immigrant African populations in Europe cannot be extrapolated to populations living in sub-Saharan Africa and emphasizes how crucial it is to study sub-Saharan populations directly, as >90% of HIV-infected children live in these regions.

Ten percent of the cohort reported by Alam et al was currently exposed to stavudine (4), compared to 62% of ours. While Alam et al found an association between lipoatrophy and current stavudine exposure, our results go one step further to show that the risk of lipoatrophy increases progressively as exposure to stavudine accumulates. This finding is in line with that of the Asian cohort reported by Aurpibul et al, which found an increasing prevalence of fat distribution abnormalities as cumulative exposure to ART increased (33). This is significant since Asian cohorts have similar conditions to sub-Saharan Africa in that malnutrition is common, access to ART for children is incomplete, and stavudine has been the most widely used first-line antiretroviral agent (12).

Objective anthropometric and DXA measurements confirmed the clinical assessment of visually obvious lipoatrophy. Thus, diagnosis of lipoatrophy in African children by skilled visual assessment is reliable and, whilst it requires training and experience, no additional investigations are needed in a developing country context. Specific training to recognize lipoatrophy in children should include supervised practice in a clinic setting over a period of time until competence is reached to the satisfaction of the trainer. However, basic competence might be gained through review of an array of photographs of mild to moderate lipoatrophy at varying ages, combined with diligent vigilance during clinical practice. The authors have not studied the adequacy of photograph-based training.

The prevalence of visually obvious lipoatrophy in pre-pubertal South African children on ART is higher than previously anticipated, affecting a third of children on ART. This is most likely due to

more extensive use of stavudine than elsewhere. Whilst ART is life-saving for HIV-infected children, surveillance and early diagnosis of lipoatrophy with appropriate drug-switches is critical. The use of agents associated with potentially stigmatizing face and limb changes is undesirable. The antiretroviral agent most commonly associated with lipoatrophy in adults is stavudine, affecting up to 32% of patients after two years of exposure to 40mg twice daily (2). Our findings substantiate this in the pre-pubertal South African population.

When considering the association of lipoatrophy with zidovudine exposure, it is important to incorporate the confounding effect of prior stavudine exposure and remember that the odds of finding lipoatrophy in a child exposed to stavudine for 18 months was nearly three times higher than finding lipoatrophy in a child not exposed to stavudine. Of the 33 children ever exposed to zidovudine, 18 had also been exposed to more than 18 months of stavudine therapy, of which 15 had lipoatrophy. By comparison, of the 15 children ever exposed to zidovudine who had not been exposed to at least 18 months of stavudine therapy, only 2 had lipoatrophy. Due to limited sample size, the effect of zidovudine exposure on lipoatrophy did not reach statistical significance after correction for cumulative stavudine exposure.

This study has a number of limitations:

1. Of the 190 possibly eligible patients appearing on the clinic database, 66 could not be found and 21 did not arrive for their study visit and either could not be traced or did not respond to attempts at further contact. This is not unexpected since patients in our setting live in a socio-economically-deprived environment and patients are often unable to attend on the planned appointment day. Predicting the day of their visit is difficult and it often requires a number of attempts to meet up with the patient. Since doctor visits occur once every three months, there were only 3 or 4 potential visits for each patient during the 12 month study recruitment

window. Due to their socio-economic instability, patients often don't keep the same cellular telephone number for more than a few months, and the telephone number recorded on the database is often out of date. In addition, the Family Clinic for HIV is based in a tertiary hospital and has a high turn-over rate as children are down-referred to peripheral clinics to make space for new referrals. The clinic's database runs on limited funding for maintenance and there is often a significant lag time between clinical event and data capture. A large number of apparently eligible children appearing on the database may have already been transferred out or lost to follow-up.

2. Although age, gender, cumulative stavudine exposure and absolute CD4 count of our sample were not significantly different to those who were not recruited, it is possible that some selection bias may have occurred. We selected subjects for enrolment who attended a routine clinic appointment during the study-screening period. This could represent a biased subset of all available subjects in our clinic population, since lipoatrophy is more likely in adherent patients. In addition, caregivers who had previously noticed subcutaneous fat changes in their children, or whose children had previously been diagnosed with lipoatrophy, may have been more inclined to participate. This may have falsely raised the found prevalence of lipoatrophy in our study.
3. The two clinicians who performed the visual assessment could not be blinded to the children's ART status, which may have biased their assessment. However, since the children were well known, the clinicians had a longitudinal perspective on which to base their assessment, which may have made their assessment more accurate.
4. Our Family Clinic for HIV is a tertiary-hospital-based clinic in a highly urbanized environment, and our patient population may be dissimilar to patient populations at primary healthcare clinics or in less urbanized or rural settings. Our clinic population may be sicker and have a higher prevalence of ART-related complications, possibly leading to a higher

prevalence of lipoatrophy. However, the use of antiretrovirals *other than* stavudine is more common in our clinic than in less urbanized secondary or primary care settings where supply chains for paediatric formulations of alternative antiretroviral drugs may not be reliable. More common use of stavudine in those settings may lead to an even higher incidence of lipoatrophy than was found in our clinic population.

5. Medical staff at our facility have great experience and thus an excellent ability to detect lipoatrophy at an early stage. Therefore, children at our facility may be switched to alternative ART earlier, resulting ultimately in less severe lipoatrophy and a greater likelihood of resolution after switching. This may have led to a lower prevalence of lipoatrophy in our clinic population compared to patient populations in primary healthcare clinics.
6. Although DXA was requested for all recruits, as DXA is a rare facility in the developing world, DXA was not always available. The individuals who received a DXA scan were those who were present when the DXA scanner was available. The number of children who received a DXA scan was small and the possibility of selection bias in the DXA substudy cannot be excluded.

Conclusions

The prevalence of visually obvious lipoatrophy in pre-pubertal South African children on antiretroviral therapy is high, and is likely to continue rising as stavudine continues to be used extensively at an unnecessarily high dose. The amount of stavudine that children are exposed to needs review. Resources are needed to enable low- and middle-income countries to provide suitable paediatric-formulated alternatives to stavudine-based paediatric regimens. The standard stavudine dose for children may need to be re-evaluated. Diagnosis of lipoatrophy at an early stage is important to avoid stigmatization. Diagnosis using visual grading requires training and experience, and DXA and comprehensive anthropometry are not commonly available. A simple objective screening tool is needed that can identify early lipoatrophy in resource-limited settings where specialized skills and

equipment are not available. The screening tool should detect lipoatrophy in pre-pubertal children before it causes stigmatizing disfigurement, allowing appropriate antiretroviral switches to be made early to arrest progression. The tool should require no special equipment or training and should be easy for primary care nurses and ancillary healthcare workers to use.

Table 1: Comparison of HIV-infected children with and without visually obvious lipoatrophy

	Children with lipoatrophy N=36	Children without lipoatrophy N=64	Univariate p-value (two-tailed)	Multivariate p-value (two-tailed)[a]
Median age (months) at antiretroviral therapy (ART) initiation, with inter-quartile range (IQR)	24 (9 – 43)	19 (9 – 37)	0.74	_
Median age at recruitment (months) (IQR)	89 (71 – 112)	71 (50 – 92)	0.001	0.75
Gender: Male / Female	21 (58%) / 15 (42%)	31 (48%) / 33 (52%)	0.41	_
Median nadir absolute CD4 before ART initiation (IQR)	694 (439 – 798)	802 (447 – 1131)	0.29	_
Median nadir CD4% before ART initiation (IQR)	15% (7 – 21%)	17% (14 – 25%)	0.05	_
Absolute CD4 at recruitment (IQR)	1213 (919 – 1556)	1129 (792 – 1524)	0.57	_
CD4% at recruitment (IQR)	37% (30 – 40%)	31% (26 – 37%)	0.03	0.34
Median $\log_{10}$ viral load at recruitment (IQR)	1.85 (1.60 – 2.54)	1.85 (1.60 – 2.54)	0.10	_
Number with HIV RNA viral load below 400copies/ml	35 (97%)	55 (86%)	0.07	_
Maximum WHO clinical stage ever reached: 1 / 2 / 3 / 4	25% / 11% / 39% / 25%	17% / 9% / 46% / 28%	0.78	_
Median weight for age Z-score (IQR)	-1.0 (-1.8 – -0.5)	-1.0 (-1.6 – -0.3)	0.39	_

Median height for age Z-score (IQR)	-1.1 (-2.0 – -0.5)	-1.3 (-2.3 – -0.8)	0.49	–
Median body mass index Z-score (IQR)	-0.6 (-1.1 – 0.0)	-0.2 (-0.8 – 0.6)	0.008	–
Number on 2nd line therapy, defined as switch of ≥2 antiretroviral drugs (%)	3 (8%)	4 (6%)	0.58	–
Any antiretroviral exposure, median months (IQR)	56 (44 – 75)	43 (25 – 60)	0.002	0.52
Number ever exposed to stavudine (%)	35 (97%)	53 (83%)	0.04	–
Stavudine, median months (IQR)	41 (27 – 48)	30 (7 – 49)	0.02	0.002[b]
Number ever exposed to zidovudine (%)	17 (47%)	16 (25%)	0.02	–
Zidovudine, median months (IQR)	12 (5 – 21)	0 (0 – 0) [c]	0.56	–
Lamivudine, median months (IQR)	52 (41 – 72)	41 (25 – 58)	0.01	0.66
Number ever exposed to lopinavir/r (%)	22 (61%)	50 (78%)	0.07	–
Lopinavir/r, median months (IQR)	26 (0 – 56)	36 (6 – 51)	0.58	0.82
Number ever exposed to efavirenz (%)	17 (47%)	19 (30%)	0.09	–
Efavirenz, median[c] months (IQR)	0 (0 – 44)	0 (0 – 4)	0.003	0.48

[a] Variables with dashes were not included in the multivariate model

[b] Adjusted odds ratio: 1.9 (95% CI: 1.3 - 2.9) for each additional year of accumulated exposure

[c] Since fewer than half of the subjects in these groups had been exposed to these drugs, the median exposure was 0 months. Mean efavirenz exposure was 23 months in children with lipoatrophy versus 7 months in children without lipoatrophy. Mean zidovudine exposure was 8 months in children with lipoatrophy versus 10 months in children without lipoatrophy.

Table 2: Anthropometric measurements in children with and without visually obvious lipoatrophy, adjusted for age and sex. P-value compares HIV-infected children with and without lipoatrophy

	Children with lipoatrophy, N=36	Children without lipoatrophy, N=64	HIV-uninfected local population norms, N=57	p-value, adjusted for age and sex (two-tailed)
Biceps skin-fold thickness (SFT), mm, mean with 95% confidence interval (95% CI)	4.2 (3.6 – 4.7)	5.3 (4.9 – 5.7)	5.5 (5.0 – 5.9)	0.002
Triceps SFT, mm, mean (95% CI)	7.1 (6.2 – 7.9)	8.9 (8.3 – 9.6)	8.7 (8.1 – 9.4)	<0.001
Torso-to-arm SFT ratio: mean (95% CI)*	1.05 (0.96 – 1.14)	0.88 (0.81 – 0.94)	0.84 (0.78 – 0.91)	0.002
Waist-to-hip circumference ratio: mean (95% CI)	0.97 (0.96 – 0.99)	0.95 (0.93 – 0.96)	0.91 (0.90 – 0.93)	0.009

*Torso to arm SFT ratio = (subscapular + iliac crest SFT) / (biceps + triceps SFT)

Table 3: Dual Energy X-ray Absorbtiometry measurements in children with and without visually obvious lipoatrophy. P-value compares HIV-infected children with and without lipoatrophy

	Children with lipoatrophy, N=15	Children without lipoatrophy, N=27	HIV-uninfected local population norms, N=34	p-value, adjusted for age and sex (two-tailed)
Limb fat versus limb lean ratio: mean with 95% confidence interval (95% CI)	0.36 (0.25 – 0.46)	0.62 (0.54 – 0.70)	0.63 (0.56 – 0.70)	<0.001
Limb fat versus total body weight ratio: mean (95% CI)	9.9 (8.5 – 11.4)	13.7 (12.7 – 14.8)	14.7 (13.7 – 15.7)	<0.001
Limb fat versus body mass index ratio: mean (95% CI)	0.12 (0.10 – 0.13)	0.15 (0.14 – 0.17)	0.17 (0.16 – 0.19)	0.001
Total limb fat, kg, mean (95% CI)	1.7 (1.4 – 2.1)	2.3 (2.1 – 2.6)	2.7 (2.4 – 2.9)	0.01

Table 4: Lipoatrophy grading scores at baseline and at follow-up

Baseline		Follow-up	
Maximum lipoatrophy score	n	Maximum lipoatrophy score	n
0	58	0	39
		1	11
		2	5
		3	0
1	6	0	1
		1	0
		2	3
		3	1
2	24	0	2
		1	3
		2	13
		3	4
3	12	0	0
		1	0
		2	6
		3	6

Figure 1: Stigmatizing lipoatrophy in a child – Front view

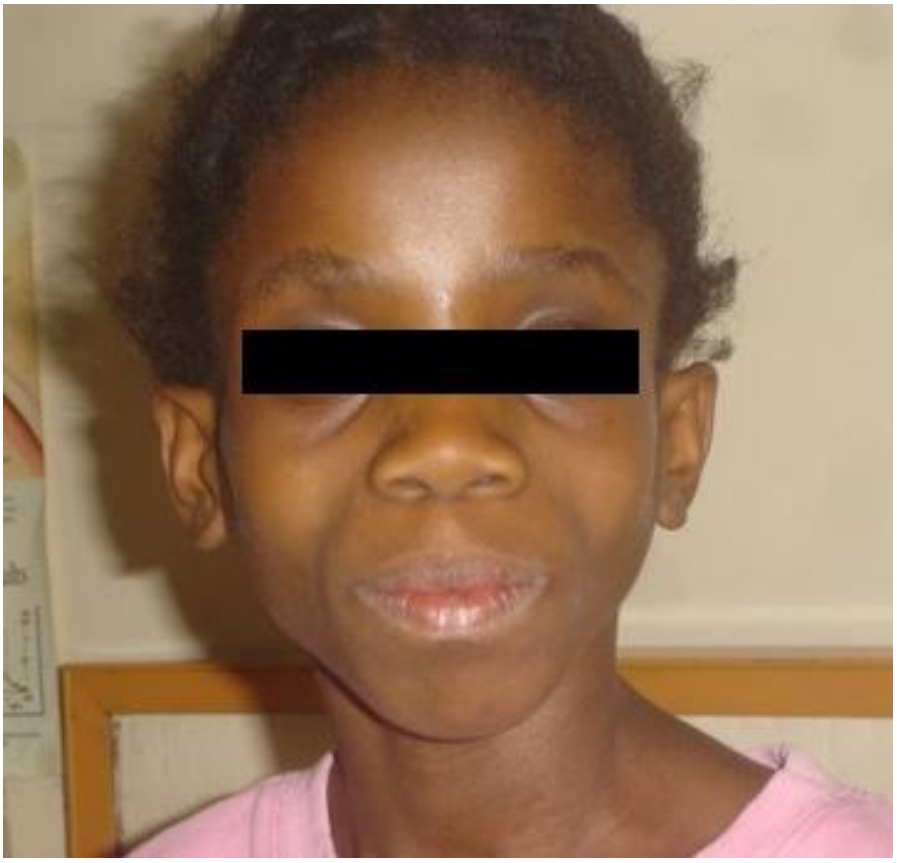
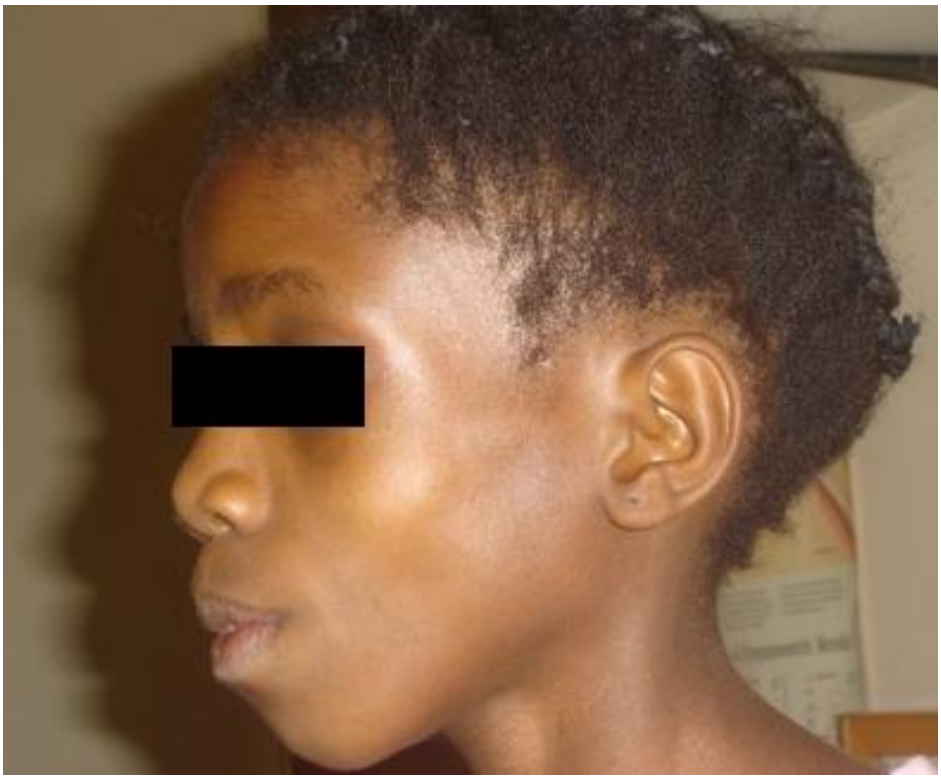
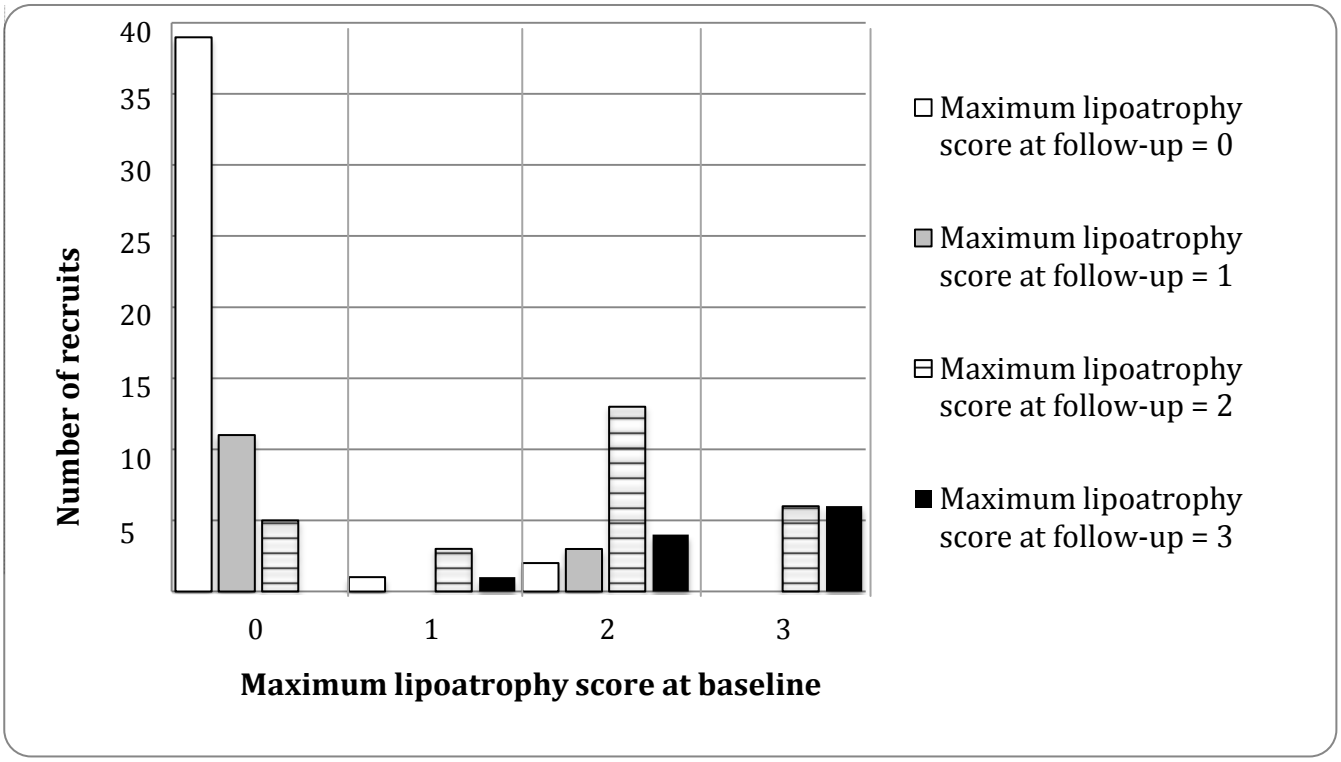


Figure 2: Stigmatizing lipoatrophy in a child – Side view



Both photo's are the author's own, and have been previously printed in Innes S, Levin L, Cotton MF. Lipodystrophy syndrome in HIV-infected children on HAART. South African Journal of HIV Medicine 2009; 10(4): 76-80. (NIHMS218910) (PMCID2919754). They are reprinted here with permission from South African Journal of HIV Medicine. Consent for publication of both photo's was obtained from the relevant legal guardian.

Figure 3: Lipoatrophy grading scores at baseline and at follow-up



Chapter 2

Biceps skin-fold thickness may detect and predict early lipoatrophy in HIV-infected pre-pubertal children on antiretroviral therapy

Abstract

Background: The prevalence of lipoatrophy in children on antiretroviral therapy in Southern Africa is high, affecting around a third of children. Early diagnosis of lipoatrophy is essential for effective intervention to arrest progression.

Methods: This was a sub-analysis performed on data from a previous study wherein pre-pubertal children on antiretroviral therapy were recruited from a hospital-based family HIV clinic in Cape Town and followed up prospectively. Lipoatrophy was identified and graded by consensus between two HIV pediatricians. A dietician performed anthropometric measurements of trunk and limb fat. Anthropometric measurements in children with and without lipoatrophy were compared using multivariable linear regression adjusting for age and gender. The most discerning anthropometric indicators of lipoatrophy underwent Receiver Operating Characteristic curve analysis.

Results: 36/100 recruits had lipoatrophy at baseline and a further 9 developed lipoatrophy by 15 month follow-up. A biceps skin-fold thickness <5mm at baseline had a sensitivity of 89% (CI: 67-100%) and a specificity of 60% (CI: 46-75%) for predicting which children would go on to develop lipoatrophy by 15 month follow-up. Negative and positive predictive values were 97% (CI: 91-100%) and 32% (CI: 14-50%).

Conclusion: Biceps skin-fold thickness <5mm in pre-pubertal children exposed to thymidine analogue-based antiretroviral therapy may be a useful screening tool to identify children who are likely to go on to develop lipoatrophy.

Introduction

Long-term use of antiretroviral therapy (ART), particularly the nucleoside reverse transcriptase inhibitors stavudine and zidovudine, may result in disfiguring loss of subcutaneous fat, termed lipoatrophy. While programmatic changes are starting to phase out stavudine use in adults, stavudine remains the most commonly used antiretroviral for HIV-infected children in sub-Saharan Africa (55, 80). Even in South Africa, while children initiating ART after 2010 have been initiated on abacavir, the majority of children treated for HIV remain on stavudine, and current guidelines state that children taking stavudine should continue unless side effects develop, at which stage the child is switched to abacavir (81). In most other sub-Saharan African countries the cost of abacavir remains prohibitive and the most common alternative is zidovudine, which also causes lipoatrophy albeit less severely (2, 60).

The prevalence of lipoatrophy in children on ART appears to be rising over time: Studies from 2005 and 2006 estimated the prevalence of lipoatrophy at 8% to 11% (18-19) whereas the most recent studies have found a prevalence of around 28% (4). Accumulation of cases is not surprising since lipoatrophy changes may persist despite switching of ART regimen, and survival rates are high in medication-adherent children who are at highest risk of lipoatrophy. Recently we have shown that the prevalence of lipoatrophy in pre-pubertal African children on any ART in South Africa is 36% (82).

Lipoatrophy looks very similar to AIDS wasting syndrome, termed “Slims disease” throughout Africa, and may confer the same stigmatization. In contrast to the developed world, stigmatization due to HIV in the communal cultures of sub-Saharan Africa may lead to loss of housing, loss of employment or livelihood, denial of schooling, denial of healthcare, secondary stigmatization of family members and physical violence (9-10). Patients who develop recognizable ART-related fat distribution abnormalities may become non-adherent to ART in order to avoid stigmatization (83-84), which will

result in declining CD4 cells, development of opportunistic infections and possibly death. This is particularly true of adolescents who are piquantly concerned about body image and social acceptance, and who may become non-adherent even in the absence of ART-related body changes (85). In previous reports, partial recovery occurred in the least severely affected individuals (12, 86). Severe lipoatrophy may not be reversible (13-14, 86). This is understandable since lipoatrophy is due to progressive apoptosis of adipocytes, which do not recover (15), as opposed to nutritional wasting where adipocyte fat stores shrink but the cell survives. However, stigmatization only occurs when lipoatrophy is easily recognizable by the broader community. Communities with the highest prevalence of HIV are the most likely to recognize HIV-related or ART-related body changes. Southern Africa has the highest HIV prevalence in the world (87). Stigmatization can be prevented if lipoatrophy is diagnosed early and appropriate ART switches are made, which will arrest lipoatrophy progression (14).

Diagnosis of early lipoatrophy is difficult. While an objective case definition for lipoatrophy has been established for adults (66), this has not been validated in children and the international gold standard for diagnosing lipoatrophy in children is the skilled visual assessment of subcutaneous limb and face fat performed by experienced pediatric HIV clinicians who have been specifically trained to do this (21). In developed countries, serial magnetic resonance imaging (MRI) and serial dual emission X-ray absorptiometry (DXA) scanning are available to monitor the amount and distribution of subcutaneous fat in HIV-infected children exposed to thymidine analogue ART. Radiographic methods are not feasible in resource-constrained settings and pediatric-trained HIV specialists are scarce. A simple screening tool is needed to detect lipoatrophy in pre-pubertal children before it causes stigmatizing disfigurement, allowing appropriate antiretroviral switches to be made early to arrest progression. The tool should require minimal equipment or training and should be easy for primary care nurses and ancillary healthcare workers to use. Preliminary evidence from two previous

studies has indicated that anthropometric measures of subcutaneous fat might be able to differentiate HIV-infected children with and without noticeable lipoatrophy (19, 68). However, those studies had a number of limitations: Hartman et al included only 4 children with lipoatrophy, all of whom were pubertal, and data was presented as median z-scores without an indication of range (68). Dzwonek et al included only 5 children with lipoatrophy and did not record pubertal stage (19). Both studies relied on a visual assessment to identify lipoatrophy.

The aim of the current study was to develop an anthropometric screening tool to detect lipoatrophy in pre-pubertal children before it causes stigmatizing disfigurement. We performed a second study to test the impact of imprecision in anthropometric measurements performed by an inexperienced healthcare worker on the diagnostic performance of the screening tool.

Methods

This was a sub-analysis performed on data from the study described in chapter 1, wherein children who were 3-12 years old, on antiretroviral therapy and pre-pubertal were recruited between February 2010 and January 2011. Pre-pubertal status was defined as Tanner stage 1 for genital and pubic hair development in boys, and Tanner stage 1 for pubic hair development with Tanner stage 1 or 2 for breast development in girls. Using review of our electronic health record database, we identified 190 patients that potentially met inclusion criteria. Of these, 124 attended clinic during the study period and could be approached for screening. A total of 121 provisionally agreed to participate, however, 21 did not attend the study visit nor did they respond to attempts at further contact. 100 subjects were finally recruited. There was no difference in demographic characteristics of the 100 enrolled subjects and the 90 who were not recruited ($p>0.20$ for age, gender, cumulative stavudine exposure and CD4, data not shown). Sampling bias is therefore unlikely.

Lipoatrophy was identified and graded by consensus between two experienced HIV pediatricians using the following lipoatrophy grading scale as defined by existing literature (33, 66, 68): 0 – No fat changes; 1 – Possible minor changes, noticeable only on close inspection; 2 - Moderate changes, readily noticeable to an experienced clinician or a close relative who knows the child well; 3 – Major changes, readily noticeable to a casual observer. Face, arms, legs and buttocks were assessed for loss of subcutaneous fat resulting in abnormally prominent limb veins, a lean, muscular appearance of limbs and face, and loss of gluteal fat pad with reduction in buttock size and loss of gluteal contour. Where the assessment of the two investigators did not concur, the change was graded as the lower score. Lipoatrophy was defined as a score of 2 or 3. Lipoatrophy assessment was repeated at 15 month follow-up. The duration and details of prior ART and demographics were recorded from our electronic health record database. HIV RNA values and CD4 values were extracted from our central electronic laboratory results server. Doses of antiretroviral drugs followed nationally prescribed protocols. For stavudine this meant a minimum of 1mg/kg twice daily rounded up to the nearest practical dose (88). A professional dietician performed formal dietary assessment and anthropometric measurements of trunk and limb fat using a non-stretchable tape-measure (model number F10-02DM, Muratec KDS Corporation, Kyoto, Japan), a high-precision Harpenden® skin-fold caliper (Baty International, West Sussex, United Kingdom), a ShorrBoard® stadiometer (Shorr Productions, Maryland, USA), and a precision weighing scale (model number UC-321, A&D Company, Tokyo, Japan) which was calibrated daily. The stated accuracy of the Harpenden® skin-fold caliper is 99%, with a dial graduation of 0.2mm and repeatability of 0.2mm (89). Measurements included mid-upper arm circumference (MUAC), mid-thigh circumference, chest circumference, waist circumference, hip circumference, biceps skin fold thickness (SFT), triceps SFT, iliac crest SFT, sub-scapular SFT, mid-thigh SFT, height and weight. All anthropometric measurements were performed three times and averaged. The following ratios were derived: waist to hip circumference ratio; body mass index; torso to arm SFT ratio ([subscapular + iliac crest SFT] / [biceps + triceps

SFT]); waist-to-MUAC ratio, and weight-to-MUAC ratio. Diet assessment variables included in the analysis were total daily carbohydrate consumption, total daily fat consumption and total daily calorie consumption. The dietician graded each as inadequate, appropriate or excessive. Data was stored in a secure electronic database using REDCap® software (https://redcap.vanderbilt.edu, Vanderbilt University, Nashville, Tennessee).

Analysis

Baseline data were compared between patients with and without lipoatrophy using T-test for continuous variables and Chi-square test for categorical variables. Univariate Spearman's correlation analysis was performed between maximum lipoatrophy grading score and each of the anthropometric measures. To adjust for age and gender, multiple linear regression models were conducted to assess the associations between each of the anthropometric measures and 4-point lipoatrophy grading scores. SFT data was log-transformed for analysis. The model used the anthropometric measure as the dependent variable and lipoatrophy grading score, age and gender as the independent variables. Partial Spearman's correlations were calculated between the 4-point lipoatrophy score and each of the anthropometric measures adjusted for age and gender, using the variance-covariance matrix.

Receiver Operating Characteristic (ROC) curve analysis was performed on anthropomorphic variables that were significant in the multiple regression analyses. ROC analyses (using pROC package in R) were conducted to compare the performance of these anthropometric measures in predicting lipoatrophy. Two sets of analyses were performed. The first used baseline anthropometric measures to predict baseline prevalent lipoatrophy diagnosis. The second used baseline anthropometric measures to predict the 1-year incident lipoatrophy outcome. The latter analysis only included patients without lipoatrophy at baseline. For each variable, a threshold was determined at which the values of sensitivity and specificity for detecting early lipoatrophy were optimized. Positive

predictive value (PPV) and negative predictive value (NPV) were calculated. For each of these anthropometric measures, empirical ROC curves and partial AUC (pAUC between 80% and 100% sensitivity) with correction by McClish (90) were calculated. 95% confidence intervals were determined by bootstrap for ROC AUC, sensitivity and specificity, and by Gaussian approximation for NPV and PPV. Statistical analyses were performed using R version 2.10.0 (Bell Laboratories, New Jersey).

This study was designed in accordance with the guidelines of the International Conference on Harmonization for Good Clinical Practice and with the Declaration of Helsinki (version 2000), and approved and monitored by the Ethics Committee for Human Research of the Stellenbosch University, approval reference number N08/11/349. Written informed consent was obtained from each caregiver prior to participation, and informed assent was obtained from capable children. All patient-related data was stored in a password-secured database under a patient identifying number and kept strictly confidential.

Results

Baseline anthropomorphic measures that were significantly different ($p< 0.001$) between those with versus without lipoatrophy were biceps SFT (3.8± 1.1 [mean, standard deviation] versus 5.3 ± 2.2, respectively), triceps SFT (6.6 ± 1.9 vs. 9.0 ± 3.0) and torso-to-arm SFT ratio (1.1 ± 0.3 vs. 0.9 ± 0.3) while body mass index Z-score and waist-to-hip circumference ratio were not different between groups. Separate multivariable models, using the three significant SFT measures as the dependent variables and the full 4-point lipoatrophy score, age and sex as independent variables, showed significant correlations between SFT and lipoatrophy score after adjusting for age and gender. When adjusted for age and gender, partial correlation coefficients between the three significant SFT

measures and 4-point lipoatrophy score were 0.33 (biceps, p=0.0006), 0.37 (triceps, p=0.0001) and 0.39 (torso-to-arm ratio, p=0.0001).

In order to determine the optimal differentiating anthropometric variable, partial ROC area-under-the-curve (pAUC), for 80%-100% sensitivity, were done; these were nominally higher for biceps (0.7; 95% CI: 0.59 – 0.80) than for triceps or torso-to-arm ratio SFT (0.68 and 0.66 respectively, figure 1). As the biceps measurement is also clinically easier to perform, this was selected as the optimal measure. The total ROC area-under-curve value was 0.75 (CI: 0.64 – 0.84). An ROC area-under-curve measurement of 0.5 for a diagnostic test indicates zero diagnostic power, whereas a value of 1.0 indicates perfect diagnostic power. The biceps SFT ROC curve revealed that the threshold value for biceps SFT that gave optimal screening sensitivity and specificity for differentiating children with and without lipoatrophy at baseline was 4.85mm. A clinically measurable biceps SFT threshold of 5mm had a sensitivity of 89% (CI: 78 – 97%) and a specificity of 61% (CI: 48 – 72%) to detect lipoatrophy at baseline. NPV was 90% (CI: 80 – 99%) and PPV was 57% (CI: 42 – 68%).

Follow-up was completed on 34 of the 36 children with lipoatrophy and 60 of the 64 children without lipoatrophy at baseline. Median follow-up time was 14.9 months (interquartile range: 14.5 – 15.6 months). At follow-up, 9/60 children had developed new lipoatrophy, giving an incidence rate of 12% (CI: 5 – 20%) per person-year of follow-up. Lipoatrophy had resolved in 5/34 children, all of whom had been switched to abacavir more than 18 months before and had no more than moderate (grade 2) lipoatrophy signs at baseline.

In children without lipoatrophy at baseline, ROC analysis of baseline biceps SFT revealed an area-under-curve value of 0.83 (CI: 0.67 – 1.00) and a partial ROC area-under curve value for sensitivity between 80% and 100% of 0.69 (CI: 0.54 – 0.93) for predicting which children would go on to

develop lipoatrophy by 15 month follow-up (Figure 2). In comparison, the ability of triceps SFT to predict lipoatrophy at follow-up was limited, with a partial ROC area-under-curve value for sensitivity between 80% and 100% of 0.56 (CI: 0.46 – 0.91). Figure 2 shows ROC curves of biceps SFT, triceps SFT and torso-to-arm SFT ratio to predict new lipoatrophy at follow-up. In children without lipoatrophy at baseline, a baseline biceps SFT <5mm yielded a sensitivity of 89% (CI: 67 – 100%) and a specificity of 60% (CI: 46-75%) for predicting which children would go on to develop lipoatrophy. NPV was 97% (CI: 91 – 100%) and PPV was 32% (14 – 50%). Post-test probabilities were as follows: In the presence of a negative test, the post-test probability of developing lipoatrophy by 15-month follow-up was 0.10 (CI: 0.02 – 0.18), whereas the probability of not developing lipoatrophy was 0.91 (CI: 0.84 – 0.99). In the presence of a positive test, the post-test probability of developing lipoatrophy by 15-month follow-up was 0.59 (CI: 0.46 – 0.71), whereas the probability of not developing lipoatrophy was 0.41 (CI: 0.29 – 0.54).

Only three recruits had Tanner stage 2 breast development at baseline. Repeating the analyses without these three children marginally improved the performance of the screening tool both at baseline and at follow-up. Proportions of ethnic subgroups were very similar between those with and without lipoatrophy (p>0.30).

Discussion

Children with and without lipoatrophy had similar immunologic and clinical presentation at ART initiation and at recruitment (p >0.2 for absolute CD4 and WHO clinical staging). They did not start ART earlier and a similar proportion was on second-line ART. The median age of children with lipoatrophy was higher than that of children without lipoatrophy (7.4 versus 5.9 years, p=0.001). This difference in age was expected since the risk of lipoatrophy increases with cumulative ART use (82), which increases with age.

Some partial improvement of lipoatrophy may be experienced after drug switching, with the more severe cases experiencing the least improvement (12). The biceps SFT at baseline was less informative for detecting existing cases than for predicting new cases at follow-up. Individuals known to have lipoatrophy prior to recruitment had been switched to abacavir months or years before recruitment and may have experienced some improvement in subcutaneous SFT before recruitment as a result of the switch. This may explain the limited performance of the screening tool in detecting lipoatrophy at baseline. The ROC analysis for predicting new cases at follow-up may be more helpful since new cases at follow-up had not been influenced by interventional drug switches.

The ROC analyses at baseline did not confirm that any of the three SFT contenders (biceps, triceps and arm-to-trunk ratio) was statistically superior to the others. However, the biceps SFT technique is the most simple to perform, and biceps SFT measurements had the least inter- and intra-observer variability. These strengths led us to choose biceps SFT over triceps SFT and torso-to-arm SFT ratio. Using a threshold of 5mm for biceps SFT yields a high sensitivity and NPV, making it effective for screening. The relatively low specificity is clinically acceptable; as the next step after screening is referral, not change of therapy. The screening tool can be employed by nurses and ancillary healthcare workers who can then refer children with suspected early lipoatrophy to pediatric HIV doctors for confirmation and antiretroviral switching as necessary. Experienced HIV pediatricians who have been specifically trained to identify early lipoatrophy will typically use the formal visual grading scale described above to confirm the diagnosis.

Lipoatrophy does not occur in all children exposed to ART (4, 21, 33). Some show no signs of fat changes despite many years of stavudine or zidovudine exposure, whereas others develop lipoatrophy within 18 months of ART initiation (86). This variation may be due to genetic differences that

increase or decrease an individual's susceptibility to lipoatrophy (17, 34-35), which would explain why viral load did not clearly differentiate between children with and without lipoatrophy in our study. Ethnic differences may also play a role (4). In the face of this unpredictability, our screening tool provides an objective, easy-to-use method to identify children who may be developing lipoatrophy.

The current study was performed using a Harpenden® calliper, which although highly precise, is expensive ($340). A Slimguide® skin-fold calliper (Vancouver: Rosscraft) is marginally less precise (repeatability 0.5mm (91)) but is cheap ($20) and durable, and may be a more suitable device to roll out in resource-limited primary healthcare settings in sub-Saharan Africa. Before implementation of the Slimguide® can be recommended, a further study is needed in which repeated measurements are performed by an experienced anthropometric dietician using both the Harpenden® and Slimguide® callipers to quantify the additional imprecision contributed by using the less precise device.

For broad implementation of this screening tool, a brief written description of how to perform a biceps SFT measurement may be all the training that is necessary. For children on ART, the effort required to perform a single annual biceps SFT measurement is likely to be logistically feasible even in busy community healthcare clinics. Serial annual biceps SFT measurements also give the added benefit of identifying changes from baseline: A biceps SFT that has dropped from a higher baseline to below 5mm, in the absence of an obvious nutritional or other cause, is probably a more convincing indication of impending lipoatrophy than a static measurement below 5mm.

Inclusion of girls with Tanner stage 2 breast development was allowed in this study since early minimal thelarche is not uncommon in very young girls in whom it does not herald the onset of true puberty (92-93). However, since the presence of early breast tissue may be associated with a higher

body fat proportion, the analyses were repeated without the children with stage 2 breast development. Only three of the 100 recruits had Tanner stage 2 breast development at baseline, and repeating the analyses without the three children in fact marginally improved the performance of the screening tool.

Since proportions of ethnic subgroups were very similar between those with and without lipoatrophy, it is unlikely that ethnicity confounded our results.

Our data on the use of biceps SFT with a cut-off of 5mm as a screening tool for lipoatrophy has a number of limitations:

1. These findings apply only to pre-pubertal children between 3 and 12 years of age. While this period of life has relatively little fluctuation in SFT and body fat distribution (94), age and gender do have some impact on global subcutaneous fat and biceps SFT. Our limited sample size did not allow stratification of ROC cut-off thresholds by age or gender. It is possible that the most appropriate diagnostic cut-off value may be different for younger versus older children and for boys versus girls. Converting biceps SFT to an age- and gender-related percentile would adjust for these differences, however, calculation and interpretation of percentiles is complex, particularly for the minimally-trained primary care nurses and ancillary healthcare workers for whom the screening tool is intended. In addition, population reference charts for biceps SFT in African children 3 to 12 years of age have not previously been compiled and building percentile charts de novo would be difficult. Change in biceps SFT over time from each individual's own baseline may be a more useful marker of impending lipoatrophy, and analysis of longitudinal data from this cohort is underway.
2. It is important for clinicians to keep in mind that the onset of puberty will substantially alter subcutaneous fat amount and distribution due to the influence of sex hormones, and an

absolute value is unlikely to be useful in that setting. For pubertal children, a change in biceps SFT percentile should be investigated as a more appropriate indicator of impending lipoatrophy.

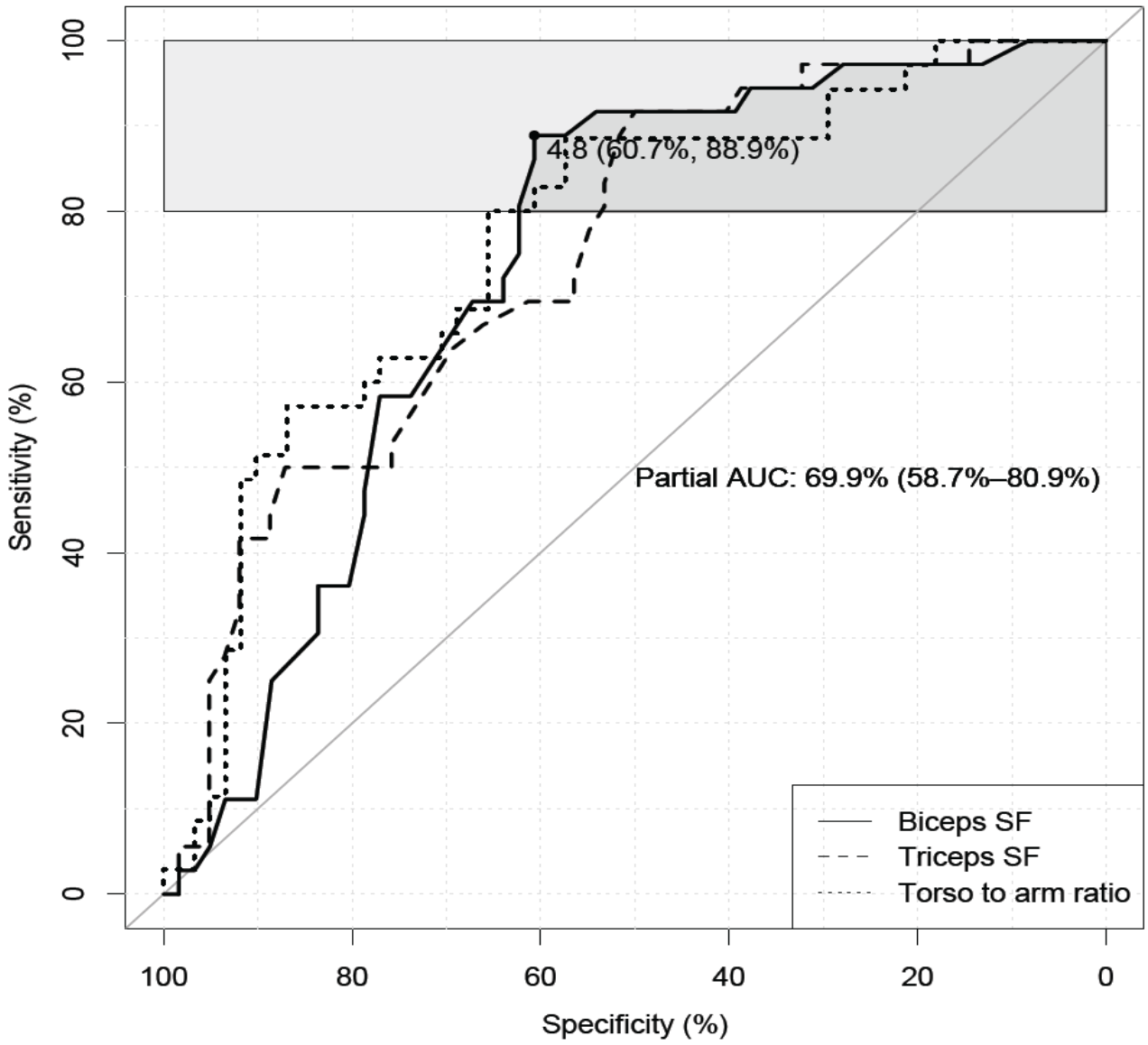
3. While biceps SFT is a reasonable surrogate measure of global subcutaneous fat, it does have a non-Gaussian population distribution, with percentile lines above the median being more widely spaced than percentile lines below the median. This suggests that biceps SFT is more variable in fatter children than thinner children. In addition, the accuracy of SFT callipers is reduced at higher readings. However, lipoatrophy is typically a condition of thinner children in whom these limitations have the least impact.
4. The current study had a limited sample size, resulting in broad confidence intervals and possibly misleading results. A larger study is needed to confirm these findings. Where this study is repeated, it may be wise to include a control group of ART-naïve children of similar ethnicity, age, gender, socio-economic setting and WHO clinical stage whose baseline body mass index is normal for age and whose weight z-score has remained stable for the 12 months preceding recruitment.
5. The low specificity may lead to a significant number of unnecessary referrals, which may overburden secondary referral services.
6. Precision of biceps skin-fold thickness is technique-dependent, which requires some training. An inconsistent method of measurement may lead to increased intra-observer variability. Differences in method between healthcare workers may lead to increased inter-observer variability. Both intra- and inter-observer variability may have the consequence that a patient's change in biceps skin-fold thickness from baseline may go unnoticed.
7. Although durable, if the Slimguide® calliper is used, the spring strength will eventually deteriorate which may not be obvious and may lead to continued use when the calliper should be replaced, resulting in inaccurate and possibly misleading measurements.

8. Reliance on an objective measurement may lead to reduced clinical vigilance on the part of primary healthcare staff, particularly in busy clinics where patient burdens are large.
9. The high sensitivity may falsely reassure healthcare workers, leading to missed cases. This screening tool should not replace diligent attentiveness during routine follow-up.
10. Specificity and PPV are limited and confirmation of suspected lipoatrophy using visual grading assessment by a skilled operator remains necessary.
11. Adherence was not measured or correlated with lipoatrophy, and this was a weakness of the current study.
12. This screening tool is not appropriate for a child who is clearly non-adherent to ART and developing AIDS wasting syndrome, or is severely malnourished. This does not limit the usefulness of the screening tool since cumulative ART exposure is the most potent risk factor for lipoatrophy (86), and it follows that children who are persistently non-adherent are least likely to develop slowly-progressive cumulative side effects like lipoatrophy. At-risk children are typically those who turn up reliably for their follow-up visits and whose caregivers follow instructions diligently. As a result, implementation of the screening tool at primary healthcare level is likely to reach the target population.

Conclusion

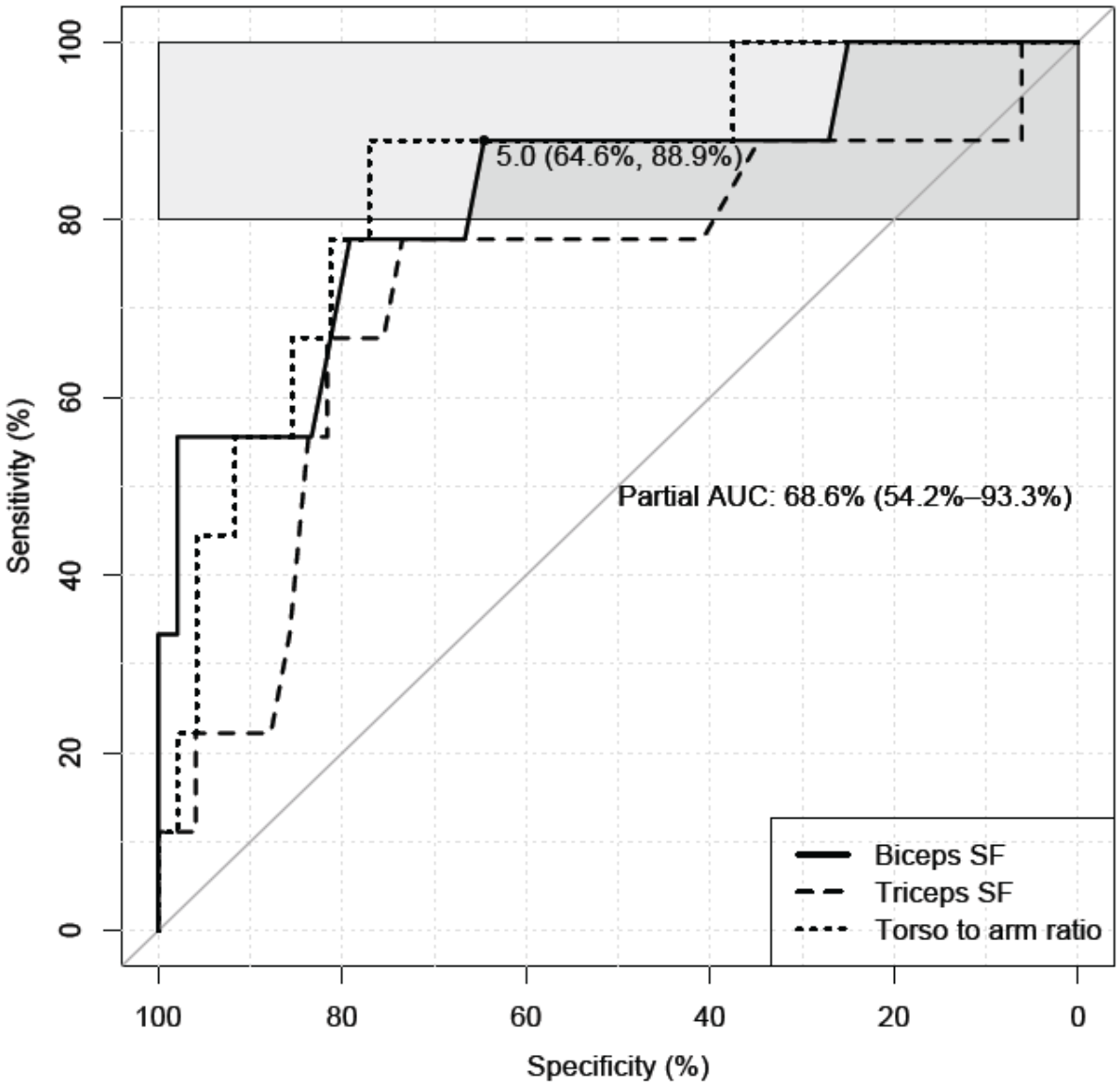
A biceps SFT of <5mm in HIV-infected pre-pubertal children exposed to thymidine analogue-based ART may be an objective, relatively easy-to-use screening tool to identify children who currently have or may progress to develop lipoatrophy, allowing appropriate antiretroviral drug switches to be made at an early stage, which will arrest progression and avoid stigmatizing disfigurement. However, a larger study is needed to confirm these findings before the screening tool is implemented.

Figure 1: ROC curves of biceps SFT (solid line), triceps SFT (dashed line), and torso-to-arm SFT ratio (dotted line) to detect prevalent lipoatrophy at baseline, n = 97. The horizontal light grey shape corresponds to the pAUC region. The pAUC of biceps SFT with 95% confidence interval is printed in the middle of the plot. The best threshold of 4.8mm with corresponding specificity (60.7%) and sensitivity (88.9%) for biceps SFT is also located in the plot.



ROC = Receiver Operating Characteristic curve; SFT = skin-fold thickness; pAUC = partial area-under-the-curve for sensitivity between 80% and 100%.

Figure 2: ROC curves of biceps SFT (solid line), triceps SFT (dashed line), and torso-to-arm SFT ratio (dotted line) to predict which children will go on to develop new lipoatrophy by 15 month follow-up, n = 58. The horizontal light grey shape corresponds to the pAUC region. The pAUC of biceps SFT with 95% confidence interval is printed in the middle of the plot. The best threshold of 5mm with corresponding specificity (64.6%) and sensitivity (88.9%) for biceps SFT is also located in the plot.



ROC = Receiver Operating Characteristic curve; SFT = skin-fold thickness; pAUC = partial area-under-the-curve for sensitivity between 80% and 100%.

Chapter 3

Substudy to determine the precision of anthropometric measurements performed by an inexperienced observer

This substudy was performed together with Eva Schulte-Kemna.

Abstract

Background: The precision of anthropometric measurements performed by an inexperienced healthcare worker may influence the performance of an anthropometric screening tool for lipoatrophy in pre-pubertal children on antiretroviral therapy in Southern Africa.

Methods: Pre-pubertal children were recruited from a hospital-based family HIV clinic in Cape Town. Anthropometric measurements were performed separately by an inexperienced observer and an experienced research dietician on the same children on the same day using the same equipment, without observation or communication between them. Mean absolute pair-wise differences were calculated for each anthropometric variable, and the difference was then incorporated into the threshold anthropometric values used by the screening tool, in order to assess how the difference in precision might impact the effectiveness of the screening tool.

Results: Measurements were successfully completed on 69 children. The mean absolute pair-wise difference in biceps skinfold measurement was less than 1mm. Incorporating this imprecision into the screening tool, the sensitivity, specificity, predictive values and likelihood ratios of the screening tool were only marginally impacted.

Conclusion: The variation in precision of anthropometric measurements performed by an inexperienced healthcare worker only marginally impacted performance of the screening tool.

Aim

To assess the precision of anthropometric measurements performed by an inexperienced observer and examine how the imprecision might impact the performance of the screening tool.

Method

Anthropometric measurements performed by an inexperienced observer (Eva Shulte-Kemna) were compared to measurements performed by a highly skilled and experienced research dietician as a gold standard. Before the study began, the inexperienced observer received basic instruction on the anthropometric method for each measurement. Measurements were then performed separately by the inexperienced observer and the research dietician on the same children on the same day using the same equipment used in the parent study, without observation or communication between the research dietician and the inexperienced observer. Coefficients of variation were calculated. For each anthropometric variable, the difference between the inexperienced observer's measurement and the research dietician's measurement were calculated. This difference was then incorporated into the threshold anthropometric values used by the screening tool, in order to assess how the difference in precision might impact the effectiveness of the screening tool.

Results

77 children from a local paediatric clinic were recruited between April and June 2009 (72 HIV-infected and 5 HIV-uninfected). These subjects had a median age of 3.0 years (interquartile range 2.0 – 3.8 years) and 37/77 were female. Two subsequently refused to co-operate with any measurements and six refused SFT measurements. SFT measurements were successfully completed on 69 children. The measured differences between the inexperienced observer and the research dietician are presented in table 1. The mean absolute difference in biceps SFT measurement between the inexperienced observer and the research dietician was 0.8mm. Taking this variability into

account, the sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio, and negative likelihood ratio of biceps SFT thresholds of <4mm and <6mm were calculated in order to determine how the variability in precision might affect the performance of the screening tool for predicting which children would have lipoatrophy at 15 month follow-up (table 2).

Anthropometric measurements had been performed three times and averaged. Table 3 uses the mean absolute difference between the three values to calculate the intra-observer variability for the inexperienced observer compared to the experienced research dietician. The intra-observer variability of the inexperienced observer compared favourably to that of the experienced research dietician.

Conclusion

The variation in precision of measurements performed by an inexperienced healthcare worker only marginally impacted performance of the screening tool.

Table 1: Precision of anthropometric measurements performed by an inexperienced observer compared to an experienced research dietician (inter-observer variability)

N = 69	Measurements performed by inexperienced observer			Measurements performed by experienced research dietician			Measured pairwise absolute difference between inexperienced observer and research dietician	
	Mean	SD	CV (%)	Mean	SD	CV (%)	Mean	SD
Waist circumference (cm)	50.4	3.9	8%	50.1	3.3	7%	0.9	0.9
Hip circumference (cm)	49.7	3.8	8%	49.1	7.6	15%	1.7	1.5
Mid-upper arm circumference (cm)	15.7	1.8	11%	15.9	1.3	8%	0.5	0.4
Triceps SFT (mm)	9.1	3.4	38%	9.2	2.2	24%	0.9	0.9
Biceps SFT (mm)	5.9	3.8	64%	6.0	1.5	26%	**0.8**	0.6
Subscapular SFT (mm)	6.5	4.1	64%	6.3	1.7	27%	0.5	0.4
Iliac crest SFT (mm)	8.2	4.5	54%	6.7	2.9	44%	1.7	1.5

SFT=skin-fold thickness; CV=coefficient of variation; SD=standard deviation

Table 2: Effectiveness of biceps skin-fold thickness (SFT) threshold of <5mm with variations in precision of +/-1mm, for predicting ***any*** lipoatrophy at 15 month follow-up (incorporating resolved cases, persistent cases and new cases), n = 94. (95% confidence intervals are given in parentheses)

Biceps SFT threshold	Sensitivity	Specificity	Positive predictive value	Negative predictive value
<4mm	68% (59 - 78%)	75% (66 - 84%)	65% (55 - 75%)	78% (69 - 86%)
<5mm	89% (83 - 96%)	63% (53 - 72%)	62% (52 -72%)	90% (84 - 96%)
<6mm	95% (90 - 99%)	43% (33 - 53%)	53% (43 - 63%)	92% (87 - 98%)

Table 3: Intra-observer variability of the inexperienced observer compared to the experienced research dietician.

N = 69	Inexperienced observer measurements			Experienced research dietician measurements		
	Mean	SD	CV (%)	Mean	SD	CV (%)
Waist circumference (cm)	0.49	0.48	97%	0.44	0.38	87%
Hip circumference (cm)	0.33	0.27	82%	0.34	0.32	95%
Mid-upper arm circumference (cm)	0.13	0.11	84%	0.13	0.10	78%
Triceps SFT (mm)	0.42	0.33	79%	0.38	0.38	102%
Biceps SFT (mm)	0.29	0.26	90%	0.24	0.26	106%
Subscapular SFT (mm)	0.25	0.22	86%	0.17	0.16	92%
Iliac crest SFT (mm)	0.41	0.30	74%	0.30	0.33	107%

SFT=skin-fold thickness; CV=coefficient of variation; SD=standard deviation

Chapter 4

Accuracy of the "opened capsule" dosing method for stavudine

Abstract

Introduction: Where a caregiver does not have access to a refrigerator, the South African National Department of Health has advised that stavudine adult capsule formulations be employed using the off-label "opened capsule" dosing method. The accuracy of this dosing method has not previously been validated.

Aim: To assess the accuracy of the off-label "opened capsule" method for stavudine dosing in infants and children. In addition, we assessed the relative ease of dispersion of generic and original capsule preparations in water in order to determine which preparations, if any, are suitable for the off-label "opened capsule" dosing method.

Method: We evaluated ten Zerit®, five Aspen Stavudine®, and five Cipla Stavir® capsules. Each capsule was dispersed in 30ml water, creating 20 separate solutions. Timed dispersion of each generic was compared to original (Zerit®). Each solution was then centrifuged to remove sediment, and the concentration of active drug (in mg/ml) was analysed using high-performance liquid chromatography.

Results: The ease of dispersion of the contents of Aspen Stavudine® capsules was equivalent to that of Zerit®, and resulted in a mean recovery of active drug from solution of over 97%, confirming the accuracy of this dosing method. The contents of Cipla Stavir® capsules, however, were extremely difficult to disperse in water despite prolonged agitation, and the recovery of active drug from solution was reduced as a result.

Conclusion: The accuracy of the off-label "opened capsule" dosing method for stavudine is acceptable. There is no need to instruct caregivers to include sediment in the aliquot given to the infant. However, it is important to avoid supplying generic capsules whose contents do not disperse

easily in water, as this may lead to significant imprecision in the amount of active drug that a child receives.

Aim

The aim of this study was to assess the accuracy of the off-label "opened capsule" dosing method for stavudine dosing in infants and children. In addition, we assessed the relative ease of dispersion of generic and original capsule preparations in water in order to determine which preparations, if any, are suitable for the off-label "opened capsule" dosing method.

Method

In July 2008, we compared two of the commercially available generic adult stavudine capsules, Aspen Stavudine® and Cipla Stavir®, to the original preparation, Zerit® (Bristol-Myers-Squibb). We evaluated ten Zerit®, five Aspen Stavudine® capsules, and five Cipla Stavir® capsules. The generic capsules were obtained through the Western Cape Department of Health ARV depot. The Stavir® capsules were from lot X70669 expiry date 06/2009. The Zerit® capsules were purchased commercially by the Children's Infectious Diseases Clinical Research Unit (KID-CRU) pharmacy, Tygerberg Children's Hospital.

Each capsule was stated to contain 30mg of active drug, and was dispersed in 30ml water, thus creating 20 separate solutions. Rather than use the technical dispersion method for capsules described in the British Pharmacopoeia (95), we intentionally used the manual dispersion technique taught to caregivers in public sector clinics. However we used a larger volume of water than described in the National Department of Health guidelines (58), in order to avoid amplifying the effect of any inaccuracy from the manual technique, ensuring that the recovery of active drug from each solution could be fairly compared. All solutions were made up by the same investigator

using the same standardized method. Timed dispersion of each generic was compared to the original (Zerit®). Each solution was then centrifuged, and the concentration of active drug (in mg/ml) was analysed using high-performance liquid chromatography (HPLC).

Statistical analysis was performed using JMP statistics software, version 7.0 (SAS Corporation – California).

Laboratory method

Undissolved particulate matter was separated from the solution by centrifugation and discarded. The stavudine containing samples were analyzed using an Hewlett Packard 1100 series high-performance binary liquid chromatograph (Agilent Technologies, Waldbronn, Germany) with an Eclipse (XDB-C18) Zorbax analytical column (15 mm x 4.6 mm ID, 5µ particle size). The latter is preceded by a 30mm x 2.1 mm ID C 18 guard column (40 µ particle size). The temperature was maintained at 40°C and the flow rate at 1 ml / min. The mobile phase consisted of a mixture of two solvents, A (50 mM KH_2PO_4, pH 5.42) and B (acetonitrile: isopropanol, 4:1 (v/v)). The following gradient was used: 0 – 1 minute = isocratic at 5 % B; 1 – 7.5 minutes = linear increase to 15% B, 7.5 – 10 minutes isocratic at 15% B. All reagents used for the mobile phase were HPLC grade (E. Merck, Darmstadt, Germany) and were filtered through a 0.45µ filter to remove particulate matter. Deionized water was used for the preparation of all aqueous solution and standards. 200µl of the sample was diluted with 800µl of deionized water to avoid detector overload and to ensure that detector response remained within linear range of the calibration curve. Aliquots of 5 µl from each sample were injected onto the column. The variable wave detector was set at 266 nm. The retention time of stavudine was 4.92 minutes with a total run time of 7.5 minutes. A 3 minute post time was allowed for the column to stabilize at 5% B before each subsequent injection. Recording and integration of the peaks were performed by means of an Agilent Chem Station. Spiked

standards were prepared from pure stavudine compound (Aspen Laboratories, Batch # 13961 V), and were randomly included into each batch over the expected concentration range. The calibration curve of triplicate samples was found to be linear over the entire concentration range, R^2 = 0.999915 (figure 1). The limits of quantification were 0.5 to 2mg/ml.

Results

All three capsule preparations produced a sediment that rapidly collected at the bottom of the container when the contents of a capsule were mixed into water (Figure 2).

The contents of BMS Zerit® capsules dispersed easily with minimal agitation, resulting in a mean recovery of active drug from solution of over 97% (Table 1). The ease of dispersion of the contents of Aspen Stavudine® capsules was equivalent to that of Zerit®, resulting in a mean recovery of active drug from solution of over 97% (Table 1). The minor difference in recovery of active drug from solution when comparing Zerit® to Aspen Stavudine® was not statistically significant (p=0.8272), suggesting that these two drugs are likely to be equivalent when given to children using the off-label "opened capsule" administration method.

The contents of Cipla Stavir® capsules were extremely difficult to disperse in water despite prolonged agitation. The contents of Cipla Stavir® capsules appeared to be partially hydrophobic, floating persistently on the meniscus of the sample. Recovery of active drug was reduced as a result (table 1). However, due to the small sample size, the difference in recovery of active drug from solution when comparing Zerit® to Cipla Stavir® did not reach statistical significance (p=0.1730).

Discussion

The lack of appropriate paediatric antiretroviral formulations is a global problem (96). The use of liquid formulations may not be feasible in resource-limited settings, since they are heavy and difficult to transport, have a short shelf-life, and may require refrigeration. Paediatric clinicians are often forced to use medicines in an off-label manner, as pharmaceutical companies tend to avoid the expense of developing paediatric formulations of established adult medicines unless the market demand is high. Although both the United States Federal Drug Administration and the European Medicines Agency now mandate that a paediatric plan be put in place for essential medicines, paediatric formulations are still often not appropriate. For this reason, where suitable solid paediatric antiretroviral formulations are not available, the World Health Organization (WHO) has recommended that off-label use of adult formulations should be investigated (97).

When a drug is employed in an off-label manner in children, the clinical implications should be carefully considered. The dosing accuracy, bioavailability, efficacy and safety of using an adult formulation in children all require investigation. In addition, off-label use of adult formulations requires manipulation that may affect the dosage, such as either cutting adult tablets or emptying the contents of capsules into water and then giving a specific volume. These activities take time and may impact negatively on adherence. However, once tested, the off-label use of adult formulations in children can be extremely effective, as has become well established in fields such as paediatric cardiology (98).

In our study, we found that when the contents of a stavudine capsule are dispersed in water, practically all the active drug is found in the supernatant, and, by inference, only a negligible amount remains in the visible sediment. It is likely that the sediment is composed only of the pharmacologically inactive excipients of the capsule formulation, such as magnesium stearate. This

information is important for caregivers, who do not need to re-suspend sediment when giving the solution to their infant. It is therefore unnecessary to instruct caregivers to agitate the mixture before withdrawing the aliquot to be given to the child. This important finding will help to simplify a technique that is at best difficult for elderly caregivers, who struggle with the dexterity and precision needed for this method.

We found a significant difference in the ease of dispersion of the capsule contents of the two generic capsule preparations. This may lead to a significant reduction in the amount of active drug that a child receives when Cipla Stavir® capsules are supplied to caregivers. Further research into the effect of unskilled caregivers' technique on dose preparation is needed.

In the future, paediatric fixed drug combination (FDC) ARV formulations will greatly accelerate the roll-out of ARV's to children in rural resource-limited settings. However, as yet no paediatric antiretroviral FDC's have been registered in South Africa. Until FDC's become widely available in the public sector, clinicians should use the best available data in prescribing adult ARV formulations in an off-label manner.

The validity of these findings is limited by the following assumptions:

1. The prescribed dose is correct**:** Where the dose is calculated per kilogram body weight, calculation errors may occur. Where weight-based dosing charts are used, a child with a weight at the lower end of the weight band may receive a far higher dose per kilogram body weight than a child with a weight at the upper end.
2. The correct capsules are used**:** Generics available on-code change often and each have a different appearance. Poorly literate patients often depend on the colour of the capsule to

identify the medication. To add to the confusion, caregivers are often on similar-looking capsules themselves.

3. Caregivers open the capsule successfully**:** Although the two halves of the proprietary capsule formulation separate easily, the halves of certain generic capsules are glued and thus very difficult to open without spilling. Precision with this technique requires dexterity, which elderly caregivers may not have. In addition, it is sometimes difficult to extract all the powder contents as some powder may remain in the tips of the capsule halves.
4. Caregivers use the correct volume of water**:** Incorrect volumes may significantly alter the dose given to the child.
5. The capsule's powder contents disperse completely when mixed in water**:** Certain generic capsules contain excipients that are hydrophobic, causing the powder to float on the meniscus of the water and not disperse.

Conclusion

Although the method of dispersing the contents of a capsule in water is off-label, and not condoned by the manufacturers of any of the three preparations, our findings suggest that the accuracy of the off-label "opened capsule" dosing method for stavudine is acceptable. We have also shown that there is no need to instruct caregivers to include sediment in the aliquot given to the infant. However, it is important to use generic capsules whose contents disperse easily in water, as this may otherwise lead to significant imprecision in the amount of active drug that a child receives.

Figure 1: Calibration curve for pure stavudine compound. The dotted line represents the best fit linear curve (y = 2678.121488 x – 6.446728, R^2 = 0.999915)

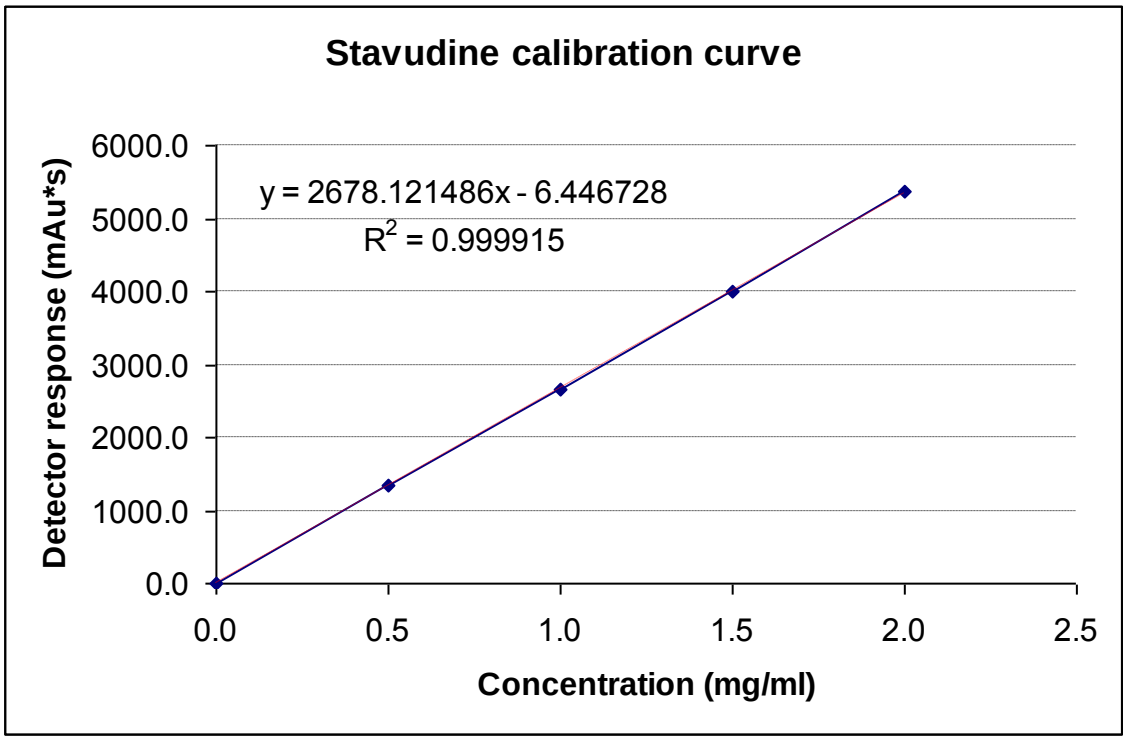


Figure 2: The contents of stavudine capsules do not disperse completely, leaving a visible sediment at the bottom of the container. Z = Zerit®, A = Aspen Stavudine®, B = Cipla Stavir®.

Table 1: Comparison of the dispersibility and active drug content of mixtures made with original and generic brands of stavudine capsules using the "opened capsule" dosing method.

	BMS Zerit® **(N = 10)**	**Aspen Stavudine®** **(N = 5)**	**Cipla Stavir®** **(N = 5)**
Found concentration (mg/ml)			
Mean	0.97136	0.97391	0.93767
Standard deviation	0.02181	0.01862	0.06961
Ideal concentration (mg/ml)	1.00000	1.00000	1.00000
Recovery (% of ideal)			
Mean	97.136%	97.391%	93.767%
Standard deviation	2.181%	1.862%	6.961%
Variation from ideal	- 2.864%	- 2.609%	**- 6.233%**
Time to total dissolution of the contents of a single capsule in water	18 seconds	18 seconds	**>65 seconds**

Chapter 5

Bioavailability and pharmacokinetic profile of stavudine when given according to the "opened capsule" dosing method

Abstract

Introduction: Stavudine, a nucleoside reverse transcriptase inhibitor, is used commonly to treat HIV-infected children in the developing world. The paediatric liquid formulation presents major logistical difficulties in rural and resource-limited areas, and prescribers are frequently forced to employ off-label "opened capsule" dosing methods using the adult capsule. South African Department of Health (DoH) has advised that caregivers should be instructed to disperse the contents of an adult capsule in 5ml water and then withdraw the required dose using a syringe. The bioavailability of stavudine using the "opened capsule" dosing method has not previously been validated.

Aim: to compare the bioavailability of stavudine administered as a solution using an "opened capsule" dosing method with the same dose given as an intact capsule in healthy adult volunteers. In addition, this study aimed to compare the bioequivalence of the two most commonly used generic stavudine capsule preparations available in South Africa.

Method: This was a randomized crossover pharmacokinetic study with each subject serving as his/her own control. 28 healthy HIV-negative adult volunteers were randomized on a 1:1 basis to receive one of the two generic preparations. They were then further randomized to receive either intact or opened 30mg capsules. One week later, those who initially received intact capsules, were given opened capsules and vice versa. The capsule dispersion technique used was identical to that prescribed by DoH. Serial blood samples were collected and plasma stavudine concentrations were assayed by liquid chromatography tandem mass spectrometry. Stavudine pharmacokinetics were analyzed using non-compartmental analysis and formulations were compared using ANOVA.

Result: Plasma drug exposure after stavudine administration as a solution using an "opened capsule" dosing method was found to be bioequivalent to intact capsule administration. This was true for both generics tested.

Conclusion: The "opened capsule" dosing technique is bioequivalent to intact capsule dosing for stavudine in HIV-seronegative adults.

Aim

This study aimed to compare the bioavailability of stavudine administered as a solution using an "opened capsule" dosing method with the same dose given as an intact capsule in healthy adult volunteers. In addition, this study aimed to compare the bioequivalence of the two most commonly used generic stavudine capsule preparations available in South Africa: Stavir® (Cipla Life Sciences, Mumbai, India) and Aspen Stavudine® (Aspen Pharma, Port Elizabeth, South Africa).

Method

Study Design

This was a randomized crossover study with each subject serving as his/her own control. After screening, 28 healthy HIV-negative adult volunteers were randomized on a 1:1 basis to receive one of the two generic preparations. They were then further randomized to receive either intact or opened 30mg capsules. One week later, those who initially received intact capsules, were given opened capsules and vice versa (see table 1). Literature-based variability in exposure (AUC 2568 +/- 454 ng*h/mL) for stavudine was accounted for in calculation of sample size for the study. A sample size of 28 (14 per arm) would have a power of 0.99 to detect a 30% difference in AUC of formulations, or a power of 0.85 to detect a 20%, at an alpha value of 0.05.

This study received approval from the ethics committee of Stellenbosch University and was conducted in accordance with the Declaration of Helsinki.

Study procedures

Participants were fasted from six hours prior until two hours after the dose was given. The dispersion technique used was identical to that prescribed by the DoH (58)**:** Each capsule was opened by the pharmacist and mixed into 5ml water, and agitated until completely dispersed. The mixture was immediately swallowed by the participant, under direct supervision. Stavudine pharmacokinetic blood samples were collected at the following times: Predose, 15 minutes, 30 minutes, 45 minutes, 60 minutes, 90 minutes, 2 hours, 4 hours, 6 hours and 8 hours after dosing. The reason for only sampling to 8 hours relates to the very short half-life of Stavudine (~1hr). With absorption nearly complete by 1 hour, less than 1% of peak plasma drug level is present in the bloodstream after 8 hours. Samples were processed on-site and frozen within 8 hours of collection. Samples remained frozen at -70^{o}C until analysis.

Assay Method

Plasma stavudine concentrations were assayed by liquid chromatography tandem mass spectrometry using a validated method on an API 4000 LC mass spectrometer. The mobile phase consisted of gradient of acetonitrile and 0.5% glacial acetic acid. Chromatography was performed on a Phenomonex Synergi fusion C18 column maintained at 25°C. 2-deoxythymidine was used as an internal standard. For each participant, all pharmacokinetic samples were processed in a single batch. 50µL of each sample was precipitated with acetonitrile containing the internal standard, centrifuged and 5µL of the supernatant injected onto the column. Standard curves in the range 0.02 – 6µg/mL and appropriate quality control samples were run with each batch. The lower limit of

quantification was 20ng/mL. Inter- and intra-day coefficients of variation were below 9% for all quality control samples.

Data Analysis

The Food and Drug Administration (FDA) considers two products bioequivalent if the 90% confidence interval of the log transformed C_{max} and AUC of the test formulation in the fasting state is between 80% and 125% of the reference formulation (99). A formal pharmacokinetic (PK) study is the ideal for determining this. However, the large number of blood draws required for a formal PK study, are impractical in small children. Healthy adult volunteers are an acceptable surrogate for bioequivalence studies of generic medicines, including antiretrovirals (100-102). This principle spares children much unnecessary pain, and is supported by the FDA (99, 103).

The PK analysis was performed using noncompartmental methods and ANOVA bioequivalence analysis using WinNonlin version 5.3. This included a 90% confidence interval of the ratio of log transformed parameters (C_{max} and $AUC_{0\text{-infinity}}$), as per FDA guidance on bioequivalence studies (99). $AUC_{0\text{-infinity}}$ was calculated using the linear/log trapezoidal rule. The maximum observed concentration was taken as C_{max}. If that concentration was not unique, then the first observed value was used in the AUC determination.

Results

This study was planned and performed between April 2008 and March 2010. Baseline characteristics of participants were well matched with an overall median age of 37.5 (range 22-61) years (see table 2). Plasma drug exposure after stavudine administration as a solution using an "opened capsule" dosing method was bioequivalent to intact capsule administration. This was true for both generics tested, i.e. Aspen Stavudine® and Cipla Stavir® (table 3). The median ratio of $AUC_{0\text{-}8}$ to $AUC_{0\text{-}infinity}$ was 0.947, confirming that sampling time was sufficiently long, and that $AUC_{0\text{-}8}$ is a reasonable estimation of $AUC_{0\text{-}infinity}$.

For Aspen Stavudine®, there was no statistically significant difference in the PK parameters (T_{max}, C_{max} and AUC) or the mean concentration-time profiles of the "opened capsule" versus intact capsule dosing methods (see figure 1 and table 4). For Cipla Stavir®, the T_{max} after intact capsule dosing was significantly later than after "opened capsule" dosing (0.87hr vs 0.53hr; p=0.0078) (see table 5). This difference is clearly visible in the superimposed mean concentration-time profiles of the two dosing methods (see figure 2).

Discussion

Our data shows that administration of stavudine as a solution using an "opened capsule" dosing method is bioequivalent to intact capsule dosing. This was true for both generics tested (Aspen Stavudine® and Cipla Stavir®). Although still within the prescribed PK parameters, Cipla Stavir® capsules, when dispersed in water, follow a different PK curve to that of intact Cipla Stavir® capsules. Therefore Cipla Stavir® may not be an ideal product when an "opened capsule" dosing method is prescribed. This finding is in line with our previous study of the physical characteristics of generic stavudine capsules, which found a significant delay in the dispersability of Cipla Stavir® capsule contents compared Bristol Myer Squibb Zerit® capsules and to Aspen Stavudine® capsules. Although

the “opened capsule” dosing method has been prescribed by the South African Department of Health since 2004, none of the three stavudine capsule patient information leaflets condone the use of an “opened capsule” dosing technique (56-57, 104), and it remains an off-label dosing method.

The cumulative amount of stavudine that children are exposed to needs review. The standard stavudine dose for children (1mg/kg twice daily) may need to be re-evaluated in the light of the 2007 reduction in the adult dose (50) following accumulation of evidence that a dose as low as half the previous standard dose results in a significantly lower frequency of delayed toxicity (lipoatrophy, lacticacidosis, peripheral neuropathy) while maintaining excellent antiviral efficacy (42). Stavudine is rapidly cleared from circulation after dosing ($t_{1/2}$ = ~1 hour) (51), and the magnitude of its antiviral and toxic effect depends on the intracellular concentration of its phosphorylated metabolite, stavudine-triphosphate ($t_{1/2}$ = ~7 hours), rather than on the concentration of free drug in plasma (105). Studies are needed to compare the intracellular concentration of stavudine-triphosphate in children on 0.5mg/kg/dose twice daily to that of adults on 20mg twice daily in order to recommend a reduction in the paediatric dose of stavudine.

The validity of our findings is limited by the following assumptions:

1. The correct capsules are used**:** Generics available on-code change often and each have a different appearance. Poorly literate patients often depend on the colour of the capsule to identify the medication. To add to the confusion, caregivers are often on similar-looking capsules themselves.
2. The capsules have been correctly stored and are not out-of-date.
3. Caregivers open the capsule successfully**:** Although the two halves of the proprietary capsule formulation separate easily, the halves of certain generic capsules are glued and thus very difficult to open without spilling. Precision with this technique requires dexterity, which

elderly caregivers may not have. In addition, it is sometimes difficult to extract all the powder contents as some powder may remain in the tips of the capsule halves.

4. Caregivers use the correct volume of water**:** Incorrect volumes may significantly alter the dose given to the child.
5. The capsule's powder contents disperse completely when mixed in water**:** Certain generic capsules contain excipients that are hydrophobic, causing the powder to float on the meniscus of the water and not disperse.
6. The volume of mixture extracted by the caregiver and fed to the child is correct and that the child swallows it all without spilling.

Conclusion

The off-label "opened capsule" dosing method is bioequivalent to intact capsule dosing for stavudine. A suitable product should be supplied when this method is prescribed.

Figure 1: Mean plasma concentration-time profiles after opened and intact capsule dosing for Aspen Stavudine®. Error bars reflect the standard error of the mean.

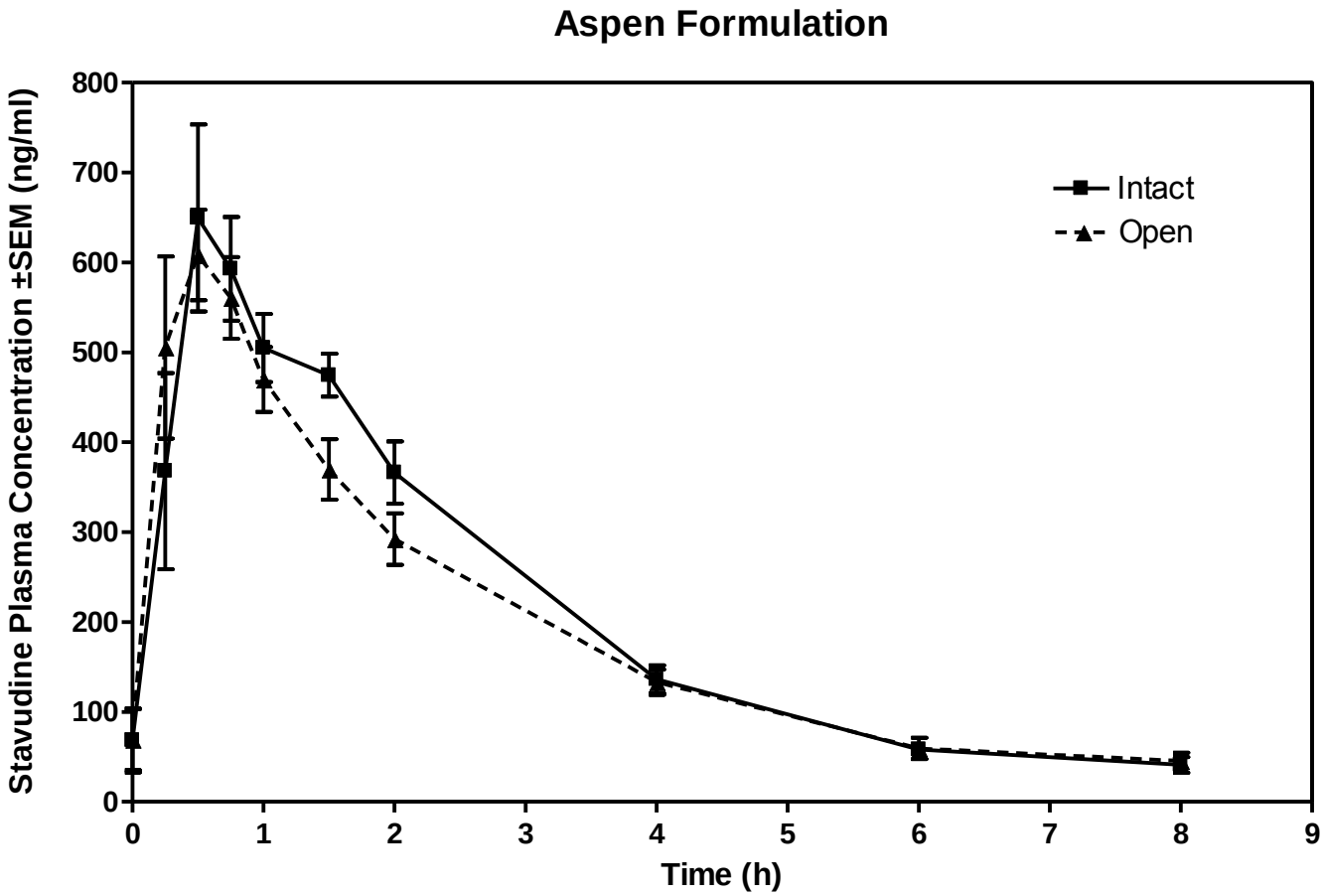


Figure 2: Mean plasma concentration-time profiles after opened and intact capsule dosing for Cipla Stavir®. Error bars reflect the standard error of the mean.

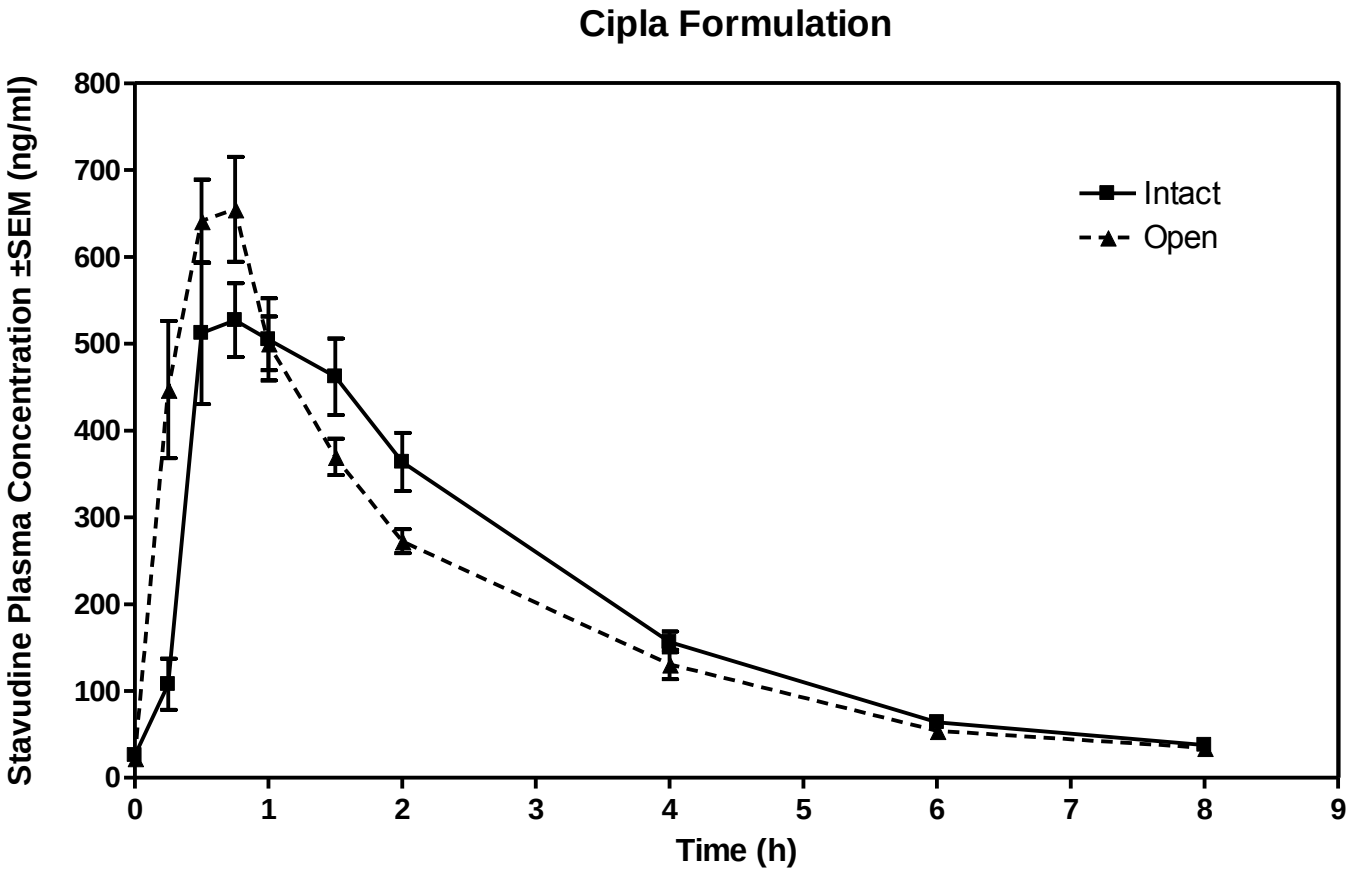


Table 1: Outline of study design

	Study visit 1	Study visit 2
Cohort 1: Aspen Stavudine® (n=14)	Intact capsules (n=7)	Opened capsules mixed with water (n=7)
	Opened capsules mixed with water (n=7)	Intact capsules (n=7)
Cohort 2: Cipla Stavir® (n=14)	Intact capsules (n=7)	Opened capsules mixed with water (n=7)
	Opened capsules mixed with water (n=7)	Intact capsules (n=7)

Table 2: Baseline characteristics of participants

	Participants randomized to receive Aspen Stavudine®	Participants randomized to receive Cipla Stavir®
Gender distribution	3 male / 11 female	6 male / 8 female
Median age (range)	35 years (22 – 54 years)	40 years (21 – 61 years)
Mean weight (range)	74 kg (50 – 117 kg)	82 kg (56 – 138 kg)
Mean body surface area (range)	1.85 m^2 (1.51 – 2.52 m^2)	1.92 m^2 (1.62 – 2.45 m^2)
Mean serum creatinine concentration (range)	83 µmol/L (60 – 114 µmol/L)	84 µmol/L (61 – 106 µmol/L)

Table 3: Bioequivalence of stavudine administered as a solution using an "opened capsule" dosing method, compared to the same dose given as an intact capsule.

	Opened capsule Mean (SD)	**Reference formulation (Intact capsule)** Mean (SD)	**Geometric least squares mean ratio** (90% confidence interval)
C_{max} (ng/ml)			
Aspen Stavudine®	739 (256)	787 (255)	105% (90 – 123%)
Cipla Stavir®	782 (229)	706 (138)	103% (88 – 121%)
$AUC_{0\text{-infinity}}$ (ng.h/ml)			
Aspen Stavudine®	1601 (534)	1787 (467)	112% (102 – 122%)
Cipla Stavir®	1540 (226)	1768 (369)	94% (90 – 99%)

Table 4: Aspen Stavudine® bioavailability data for each participant: Calculated pharmacokinetic parameters Tmax, Cmax, Half life, AUC_{0-8} and $AUC_{0-infinity}$

Participant	*Tmax (h)*		*Cmax (ng/ml)*		*Half Life (h)*		*AUC_{0-8} (ng.h/ml)*		*$AUC_{0-infinity}$. (ng.h/ml)*	
No.	***Intact***	***Open***	***Intact***	***Open***	***Intact***	***Open***	***Intact***	***Open***	***Intact***	***Open***
1	0.5	0.5	1140	752	2.3597	2.2757	1727.31	1522.45	1870.29	1695.14
2	0.75	0.75	695	561	2.2123	2.8631	2330.84	2130.44	2590.95	2485.67
3	0.25	0.25	1030	975	1.4967	1.6909	1496.45	2296.14	1553.46	2367.61
4	0.75	0.75	868	723	1.9823	2.3035	2128.64	1711.16	2305.09	1861.37
5	1.5	0.25	470	880	1.2191	1.2025	1080.46	1213.2	1148.18	1250.15
6	1	0.25	628	771	1.6009	1.901	1799.55	1591.68	1870.22	1791.33
7	0.75	0.5	480	451	2.3961	1.8854	925.9	953.05	1084.57	1027.58
8	1	0.5	603	605	1.5563	1.5426	1446.63	872.98	1501.63	1027.87
9	0.5	1	861	511	1.3662	1.6372	1912.4	1681.53	1953.20	1759.47
10	0.5	0.5	487	454	1.7086	1.36	1205.8	942.88	1316.23	987.61
11	0.5	0.25	987	1370	1.4085	1.2662	1940.05	1843.1	2074.58	1921.83
12	0.5	0.75	1090	651	1.1533	1.2269	1586.65	1209.25	1635.57	1259.34
13	2	1	555	611	-	0.4302	1175.35	778.88	-	854.60
14	0.5	0.5	1120	1030	1.2386	1.195	2268	2063.23	2332.33	2117.70
Mean	**0.7857**	**0.5536**	**786.7**	**738.9**	**1.669**	**1.627**	**1645**	**1486**	**1787**	**1601**
SD	**0.4688**	**0.2627**	**254.9**	**255.7**	**0.4341**	**0.6026**	**446.8**	**499.2**	**467.1**	**534.1**
SEM	**0.1253**	**0.07022**	**68.12**	**68.33**	**0.1204**	**0.1610**	**119.4**	**133.4**	**129.6**	**142.7**
%CV	**59.67%**	**47.46%**	**32.40%**	**34.60%**	**26.00%**	**37.03%**	**27.17%**	**33.58%**	**26.13%**	**33.37%**

* Parametric t test, ** Non-parametric t test both using matched pairs

SEM = standard error of the mean. %CV = coefficient of variation given as a percentage.

Table 5: Cipla Stavir® bioavailability data for each participant: Calculated pharmacokinetic parameters Tmax, Cmax, Half life, AUC_{0-8} and $AUC_{0-infinity}$

Participant	*Tmax (h)*		*Cmax (ng/ml)*		*Half Life (h)*		*AUC_{0-8} (ng.h/ml)*		*$AUC_{0-infinity}$. (ng.h/ml)*	
No.	***Intact***	***Open***	***Intact***	***Open***	***Intact***	***Open***	***Intact***	***Open***	***Intact***	***Open***
15	0.5	0.5	875	991	1.8462	1.6303	2111.28	1635.10	2354.71	1762.34
16	1	0.75	606	1180	1.1995	1.1467	1499.65	1203.93	1548.80	1315.26
17	0.5	0.5	869	956	1.4444	0.8693	1651.00	1347.45	1718.52	1411.04
18	0.5	0.5	812	458	1.4966	1.685	1320.40	1203.38	1409.36	1322.49
19	1	0.25	691	1040	1.5719	1.0925	2106.53	1461.53	2190.66	1500.14
20	1.5	0.5	866	744	1.5652	1.7695	2015.11	1762.68	2112.21	1852.02
21	0.5	0.5	750	928	1.9687	1.4127	1668.65	1569.38	1775.44	1649.88
22	0.75	0.75	874	970	1.6107	1.5522	2300.29	1929.85	2410.20	2033.09
23	1.5	0.5	477	588	1.7236	1.7001	1629.73	1555.98	1707.81	1623.67
24	0.5	0.5	730	438	2.4669	2.0183	1578.85	1367.15	1736.16	1446.35
25	1.5	0.75	569	687	2.3251	1.3949	1529.43	1454.33	1646.50	1535.23
26	1	0.5	553	681	1.2265	1.184	1159.40	1160.40	1205.76	1217.11
27	1	0.75	592	592	2.2249	2.0095	1437.70	1288.40	1549.40	1378.56
28	0.5	0.25	620	701	2.5834	2.6506	1247.03	1366.09	1380.83	1517.90
Mean	**0.8750**	**0.5357**	**706.0**	**782.4**	**1.804**	**1.580**	**1661**	**1450**	**1768**	**1540**
SD	**0.4013**	**0.1657**	**138.1**	**228.7**	**0.4474**	**0.4588**	**348.1**	**222.5**	**368.6**	**226.1**
SEM	**0.1073**	**0.04430**	**36.91**	**61.12**	**0.1196**	**0.1226**	**93.04**	**59.47**	**98.51**	**60.43**
%CV	**45.87%**	**30.94%**	**19.56%**	**29.23%**	**24.80%**	**29.05%**	**20.96%**	**15.34%**	**20.85%**	**14.68%**

* Parametric t test, ** Non-parametric t test both using matched pairs

SEM = standard error of the mean. %CV = coefficient of variation given as a percentage.

Summary of conclusions

Lipoatrophy is a common adverse effect of stavudine and this effect is strongly dose-dependent. Although lipoatrophy was previously believed to be uncommon in children, the current study found that the prevalence of visually obvious lipoatrophy in pre-pubertal children on antiretroviral therapy in South Africa is around 36%, and the incidence is around 12% per person-year. The risk of lipoatrophy increases progressively as time on standard-dose stavudine accumulates.

Biceps skin-fold thickness may provide reasonable sensitivity and specificity as a simple screening tool to detect and predict lipoatrophy at an early stage. This may be a relatively objective, easy-to-use surveillance method to screen for lipoatrophy at primary healthcare level in resource-constrained settings where trained specialists are scarce and complex special investigations are not available. This will assist earlier antiretroviral drug switches to be made to arrest progression of lipoatrophy and prevent potentially stigmatizing face and limb disfigurement. A follow-up study is needed to test the performance of this screening tool on a different group of pre-pubertal HIV-infected South African children on ART.

The off-label dosing method for stavudine prescribed for children whose caregiver does not have access to a refrigerator is reasonably accurate. However, it is important to avoid supplying generic capsules whose contents do not disperse easily in water, as this may lead to significant imprecision in the amount of active drug that a child receives. In addition, the method requires dexterity and careful measuring, which may be challenging for elderly caregivers. The bioavailability of this dosing method is similar to intact capsule dosing for stavudine in HIV-seronegative adults. These findings pave the way for future studies to investigate a possible reduction of the recommended paediatric dose.

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