Universidade Nova de Lisboa

Beriberi epidemic within rural Muslim fishing communities in Guinea-Bissau

Dominik Metz

DISSERTAÇÃO PARA A OBTENÇÃO DO GRAU DE MESTRE EM MEDICINA TROPICAL

(DECEMBER 2011)

Universidade Nova de Lisboa

Beriberi epidemic within rural Muslim fishing communities in Guinea-Bissau

Dominik Metz

DISSERTAÇÃO PARA A OBTENÇÃO DO GRAU DE MESTRE EM MEDICINA TROPICAL

Orientador: Professor Jorge Atouguia

Dissertação apresentada para cumprimento dos requisitos necessários à obtenção do grau de Mestre em medicina tropical, realizada sob a orientação científica de Professor Jorge Atouguia

(DECEMBER 2011)

AGRADECIMENTOS

Eu queria agradecer todas as pessoas do Instituto de Medicina tropical de Lisboa e as collegas do mestrado pela ajuda e paciencia durante estes dois anos de mestrado. É verdade que foi difícil no início, com o meu português, mas com tanta ajuda e confianca que voces me darem, consegui aprender não só muito de medicina tropical mas também de lingua e cultura portuguesa.

Gostaria igualmente de agradecer o pessoal do Hospital de Bolama em Guinea-Bissau, que diaramente conseguem a fazer um trabalho de qualidade com os poucos recursos que eles têm a disposição. Eles ficaram abertos as minhas ideias e propostas. Encontrei em Guine collegas que tinharam tempo a me oferecer, afim de ajuda-me no dia a dia no trabalho e ensinar me da cultura e lingua guineense. Eu desejaria, particuliarmente, agradecer o meu collega e amigo Afonso Embana, técnico de laboratorio do Hospital de Bolama.

Eu queria agradecer a minha familia toda pelo apoio e a ajuda. Finalmente a pessoa que provavelmente sofreu o mais durante a elaboração da minha tese foi a Eugénie, que sempre ficava ao meu lado mesmo se foi chato muitas vezes!

Thank you all very much and best wishes.

ABSTRACT

BERIBERI EPIDEMIC WITHIN RURAL MUSLIM FISHING COMMUNITIES IN GUINEA-BISSAU

DOMINIK METZ

Beriberi, or thiamine deficiency related cardiopathy and polyneuropathy, was a scourge upon past populations with perhaps the most notable outbreaks occurring with the advent of mass rice milling in parts of Asia at the end of the 19th century. Upon the discovery of the "anti-beriberi" vitamin responsible for the disease, and subsequent food enrichment and public health awareness campaigns, the illness has largely been forgotten by many physicians. However, to this day many outbreaks are still being recorded of the potentially fatal deficiency across the globe amongst vulnerable communities. Certain foodstuffs as well as alcohol are well known to reduce vitamin B1 uptake and the latter is associated with Wernicke's encephalopathy, a condition better known amongst doctors practising in affluent countries. Recent findings of significant neurolinguistic delay amongst infants exposed to thiamine depleted formula milk, as well as the the possible correlation with severe malaria emphasise the importance of the vitamin and even marginal deficiency states on well being.

Plundered by colonial and post colonial conflict, political instability, drug smuggling and disease burden, Guinea-Bissau remains one of the poorest countries in the world with the majority of the population relying on subsistence farming, fishing and the harvest of the valuable cashew nut. Islam is followed by almost half the population with animistic and Christian faiths making up the other main religions. In keeping with the Islamic faith, those Muslims from Guinea-Bissau observe the month of Ramadan with daylight fasting. Comparatively little research has been published on the nutritional and health effects of this ritual and to the authors knowledge this is the first article that relates a micronutrient deficiency to the holy month.

Described are clinical cases of beriberi amongst Muslim rural fishing communities at the end of the rainy season and coinciding with the Ramadan fast. What became evident was that some of the cases described an annual relapse and that this could reflect the tip of the iceberg of vitamin B1 deficiency in Guinea-Bissau. Discussion focuses on the need for further investigation to confirm the extent of the deficiency among the general population, considerations of thiamine deficiency related malaria outcomes and possible long term effects of the problem, followed by practical suggestions of how the disease burden can be reduced.

KEYWORDS:

Thiamin(e), thiamin(e) deficiency, Beriberi, vitamin B1 (deficiency), Ramadan, Ramadan fasting.

RESUMO

SURTO DE BERIBERI NAS COMUNIDADES RURAIS DE PESCADORES MUÇULMANOS DA GUINE-BISSAU

DOMINIK METZ

Beriberi, ou cardiopatia e polineuropatia ligada à deficiência de tiamina, foi, antigamente, um flagelo nas populações. Os mais notáveis surtos de beriberi ocorreram após o advento da moagem de massa de arroz, em várias zonas da Ásia, no final do século XIX. Com a descoberta de vitamina B1, "anti-beriberi", e, subsequentemente, o enriquecimento de alimentos e as campanhas de sensibilização de saúde pública, a doença foi esquecida por muitos profissionais de saúde. Contudo, sabe-se que, hoje em dia, em diferentes partes do mundo, muitos casos de beriberi são os focos primários de epidemias de outras deficiências potencialmentes fatais, em comunidades vulneráveis. Determinados alimentos, assim como o álcool, são conhecidos para reduzir a absorção de vitamina B1. A Encefalopatia de Wernicke, a deficiência de tiamina mais conhecida pelos médicos que trabalham em países desenvolvidos, está associada à ingestão de álcool. Estudos recentes demonstraram, por um lado, atrasos significativos do desenvolvimento neurolinguístico em crianças alimentadas com leite sem tiamina, assim como uma possível correlação entre malária grave e deficiencia em Vitamina B1, o que reforça a importância desta vitamina na saúde das populações.

A Guiné-Bissau, um país fragilizado pelos conflitos coloniais e pós-coloniais, instabilidade política, tráfico de droga e deficiente acesso à Saúde, continua sendo um dos países mais pobres do mundo. A maioria da população vive da agricultura de subsistência, pesca e colheita de cajú. O Islão é practicado por quase metade da população, e as religiões animistas e cristãs compõem as outras religiões principais do país. De acordo com a fé islâmica, os muçulmanos da Guiné-Bissau praticam o jejum, em função da luz do dia, durante o mês de Ramadão. Existe pouca investigação publicada sobre os efeitos nutricionais e na saúde deste ritual. Este trabalho é o primeiro a relacionar uma deficiência de micronutrientes ao mês sagrado do Ramadão.

Esta tese apresenta casos clínicos de beriberi em comunidades rurais muçulmanas, que ocorreram no final da época das chuvas, que coincide com o mês de Ramadão. A análise dos dados mostra que, uma vez que alguns dos doentes apresentavam os mesmos sintomas, repetidamente, todos os anos, estes casos podem refletir a ponta do iceberg da ocorrência de beriberi na Guiné-Bissau. Parte da discussão centra-se na necessidade de mais investigação para confirmar o grau de deficiência na população em geral, efeitos de deficiência de tiamina relacionados com a malária, e consequências possíveis da deficiência marginal, a longo prazo, desta vitamina na saúde. A discussão apresenta ainda sugestões práticas para a redução da incidência da deficiência de vitamina B1 na Guiné-Bissau.

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS AND TABLES

LIST OF ABBREV IATIONS

ATP	Adenosine Triphosphate
BMR	Basal Metabolic Rate
BP	Blood Pressure
BPM	Beats per minute
CSF	Cerebro Spinal Fluid
ETK	Erythrocyte Transketolase
ETKA	Erythrocyte Transketolase Activity
FAO	Food and Agriculture Organization
HIV	Human Immunodeficiency Virus
ICD	International Classification of Diseases
NADPH	Nicotinamide adenine dinucleotide phosphate
RDA	Recommended Daily Allowance
TB	Tuberculosis
TD	Thiamine Deficiency
THTR1/2	Thiamine Transporter protein 1/2
TPN	Total Parental Nutrition
TPP	Thiamine Pyrophosphate
TPPE	Thiamine Pyrophosphate Effect
UN	United Nations
USAID	United States Agency for International Development
WHO	World Health Organization
WK	Wernicke's Encephalopathy

INTRODUCTION

Whilst now occurring less frequently and in more isolated communities, the dietary deficiency of vitamin B1 causing beriberi has burdened large parts of the world for centuries. The introduction of steam powered polishing (removing the outer layers) and subsequent mass distribution of cheap polished rice led the disease to be prevalent amongst many Asian communities just over 100 years ago[1].

The work of Jacob Bontius in 1642 appears to be the first fully documented clinical histories of beriberi[1]. However, documents dating as far back as 800AD give indications of symptoms suggestive of the vitamin deficiency reflecting its prevalence throughout history[2]. The Japanese surgeon, Kanehiro Takaki in 1884 noticed that whilst working on a merchant vessel, providing sailors with a more substantial diet with wholesome foods and meat significantly reduced the prevalence of beriberi on long journeys, a condition that was prevalent among seafarers during that period[2]. Over forty years later, in 1929, Christian Eijkman won the Nobel Prize for his work on beriberi. He found that feeding pigeons polished rice led to neurological deterioration with deranged posturing and eventual death, however by reintroducing unpolished rice, complete recovery was achieved[2,3]. This paved the way to the eventual discovery of the first "vitamine" so called by Funk et al in 1911 and on the presumption that it was a vital amine[3]. The complete structure and synthesis of thiamine was first reported by Williams in 1936[2].

Thiamine is a key cofactor in the metabolic pathway and therefore forms an essential part of energy formation within the mitochondria of many cells[4]. Its deficiency leads to a number of conditions thought to be due to the disruption of these pathways in key organs including the nervous system and heart[4]. Whether the predominant features are that of polyneuropathy (as in dry beriberi), cardiac (wet beriberi) or other (such as Wernicke encephalopathy) is thought to be due to a number of factors including duration of deficiency[2,3,4], concomitant carbohydrate intake and alcohol abuse[3,5]. A genetic predisposition is another suggestion by many researchers explaining why only a

certain percentage of people exposed to diets deficient in thiamine develop overt symptoms[6].

Whilst Vitamin B1 is present in a wide variety of foods, concentrations are normally low and certain cooking habits can further diminish quantities of the heat labile water soluble compound[2,3]. In more affluent societies the deficiency is largely confined to alcohol dependent persons[4] as food variety and widespread fortification of certain staple foods protects the majority[2,3].

Meanwhile, numerous outbreaks reported in recent times highlight vulnerable communities whose poor diets coupled with other risk factors can expose them to disease manifestation[3,4]. Particular "at risk" groups identified include refugee communities[3], prisoners[6] and psychiatric patients[3]. A number of epidemiological studies suggest that marginal deficiency states are endemic in certain areas and that minimal circumstantial changes can lead to outbreaks of overt disease[7, 8].

Guinea-Bissau is a poor West African state that remains heavily dependent on donor country aide[9]. Both infant mortality rates and average life expectancy reflect its poor healthcare and living standards[10]. Guinea-Bissau is ethnically diverse with Islam being practised by nearly half of the 1.6 million population[9]; indigenous beliefs are strong with Christianity (predominantly Catholic) forming a large minority[9].

The holy month of Ramadan is marked by millions of worshippers across the globe with sunlight fasting being upheld by the vast majority[2,11]. Despite this, there remains comparatively little research regarding potential health implications of the ritual. What has been published regarding nutritional intake and body composition during the time remains relatively inconclusive[11,12].

Thiamine can be measured directly in the blood or urine[3], however, given the relatively low free serum levels, erythrocyte transketolase activity and activation coefficients are preferred for confirming diagnosis when available[3]. In reality,

developing countries such as Guinea-Bissau do not have the capacity to do such testing and so diagnosis remains clinical and confirmation can be established with response to treatment. Treatment is simply B1 vitamin replacement, however, precise guidelines of the route of administration and duration of treatment are unavailable and thus dictated by resources and clinical improvement.

The cases reported all presented near the end of the rainy season and within a fortnight of the completion of Ramadan. All sufferers were young Muslim males, usually fit and active. The majority displayed cardiac manifestations, with one also complaining of neurological compromise and one purely neuropathic. All subjects were from the same area of Guinea-Bissau and enjoyed similar monotonous rice-based diets. Once highlighting the epidemic to local and district healthcare workers, nurses and doctors gave reports of additional cases and their subsequent cure with thiamine treatment.

Given the remoteness of the the communities, and lack of financial and laboratory resources, a number of limitations of the reports exist. Unfortunately, even routine tests such as blood film and full blood count could not be done, let alone HIV testing which would have been of interest given related articles that indicate increased TD in infected patients. It would also have been interesting to try and collate with other health centres to see if this epidemic was occurring across the nation as may well be the case given the homogeneity of the population.

The long-term effects of marginal deficiency as well as possible correlations with malarial infection and other disease processes highlight the ongoing importance of this nutritional problem. Further investigation to reveal the true prevalence of thiamine deficiency in Guinea-Bissau and the extent of disease burden this has on the population is of importance and may affect governmental health policy.

THE STATE OF GUINEA-BISSAU

Guinea-Bissau is one of the worlds poorest countries with political instability and a long history of conflict[9,13]. Freedom from Portuguese colonial control was not achieved until 1974 and the the result of a long and bloody battle[9]. Recent exploits by foreign drugs cartels using Guinea-Bissau as a passage to Europe have further strained the political framework[13-15]. This has crippled the small country's development and shackled the majority of the population (~1.6million) in poverty[9]. Subsistence farming (cashew nut being the main exported product) and fishing remains the livelihood of the majority[16], with the gross national income being just over 500 dollars per person per annum[9,13].

The Balanta, Fula, Manjaca and Mandunga tribes form the main ethnic groups along with many others that make up for a diverse mix of cultures, religions and languages within the relatively small country[9]. Islam is practised by nearly half the population[9], with indigenous (animistic) beliefs and Christianity forming the other main religious fractions[9,13]. Situated between Senegal and Guinea (see map below) the country enjoys a tropical climate with the rainy season falling between June and November when most of the annual ~1900mm rainfall occurs[17].

The major hospital of the country is Simão Mendes in Bissau which has capacity to perform basic operations although it is often lacking electricity and a sufficient sanitary system. The state of district hospitals varies depending on foreign donor interest, but can be very basic, lacking diagnostic materials such as X-ray and incubators for culture growth.

Illustration 1: Map of Guinea-Bissau[9]

With only 78 physicians recorded by the WHO to be working in Guinea-Bissau in 2009, each doctor is serving more than 20,000 of the population[10]. Only 4% of government expenditure is on the healthcare system[10], and most services are chargeable (HIV and TB treatment, immunization, certain services during pregnancy being exceptions). Large inequalities in health exist among social and urban/rural divides[10, 14]. This leads to delays in presentation to hospital with people often seeking treatment from traditional healers. The average life expectancy is 47 years (in 2008) with infant mortality rates of 117 per 1000 live births, markers indicating the poor state of health even when compared to neighbouring Guinea (Conakary) and Senegal[10]. Malaria remains the main cause of death among children under five, being responsible for nearly 20% of all deaths in this age group. HIV rates in adults were estimated to be ~1.8% in 2008[10].

Rice is the staple food, accompanied by fish that is often consumed with red palm oil[18]. Imported polished rice from Asian countries, and indeed USAID, is cheaper and thus more widely consumed than that locally harvested. Meat products are limited

to special occasions and wealthier families. Diet is poorest during the rainy period with decreased food variability[18]. It is also during this time that the majority of physically hard labour on the farms is endured[3, 18]. Gonçlaves et al[19] studied nutritional status of 1324 children aged between 7 and 14 in rural Guinea-Bissau, 1992. They found 3% had a weight for height Z score <-2 and stunting (height for age) stood at 8% (Z score <-2)[19]. The nutritional status of the general population is reflected by the WHO estimates that 20% of children under 5 are under weight[19].

RAMADAN FASTING

Fasting is a duty to all Muslims who are medically fit to participate[11]. It is one of the five pillars of Islam and seen in the religion as a way to express self restraint and freedom from desires[11]. Stricter followers can choose to fast twice weekly plus every 13-15th of the month[2]. However the majority of Muslims choose only to follow the ritual of fasting during the month of Ramadan[11]. Its dates follow the 9th lunar month, Hijra, and therefore vary annually in the Gregorian calendar[2,11]. During the 28-29 days of fasting participants may only eat between iftar (sunset) and sahur (sunrise)[2,20]. Exemptions are made for those who are

- under age (12 years in girls and 15 in boys)
- on a voyage/journey
- medically unwell and
- women who are pregnant or who have recently given birth [2, 20]

Lactating women may abstain if they feel fasting may bring ill health upon the infant. However, interpretation of this commandment varies; a study in Turkey finding that most lactating women do commit to fasting[20].

Whilst changes in dietary habits are expected, whether overall intake or indeed nutritional composition of diet reduces during the month remains unclear with researchers expressing divergent conclusions[12]. El-Hourani et al[11] reported a decreased Body mass index post Ramadan amongst 57 fasting female subjects which was in keeping with similar findings amongst professional footballers in Tunis[11,12]. A small study of breast milk nutrient composition pre and post Ramadan in Turkey found reduced zinc and magnesium levels during Ramadan. However, vitamin B1 was not analysed and the study (20 subjects) was very small[21]. There is little research into exact bodily vitamin concentrations during Ramadan, let alone thiamine.

In theory devotees should have little change in vitamin/dietary intake given that large pre-dawn meals are encouraged[2,11]. However, perhaps more so in poorer communities where food variability and stability is already questionable, fasting subjects may not achieve adequate oral intake. In Guinea-Bissau, many people snack on cashew nuts and peanuts among other foodstuffs, in an otherwise marginal thiamine diet, which may well serve to prevent severe deficiency. The prohibition of such comparatively nutrient rich snacks during Ramadan could be a cause for the further depletion of thiamine in the group studied.

THIAMINE (VITAMIN B1)

Thiamine is present in a large variety of foodstuff, however, usually only in small quantities. Thiamine rich foods include legumes and meats especially pork[2]. The concentrations can be significantly depleted prior to ingestion through cooking processes- repeated washing (water soluble) and prolonged boiling (heat labile)[3, 4].

Chemical make up

Vitamin B1 was the first vitamin to be discovered[1]. It is water soluble and is made up of a pyrimidine nucleus and thiazole ring bridged by methylene group[1, 22, 2] (see illustration below). It contains sulphur, lending to the Greek translation of thiamine as vitamin containing sulphur[2]. This is thought to be of importance in certain conditions where cassava based diets consume available thiamine for detoxification (see later)[23].

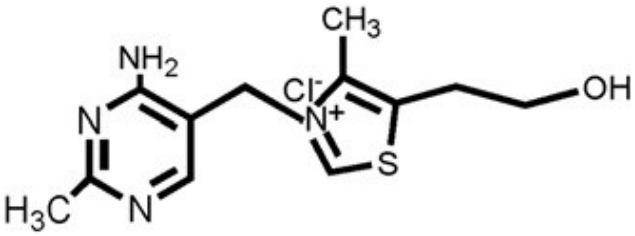


Illustration 2: Chemical structure of thiamine[22]

Absorption and storage

Vitamin B1 absorption occurs in the (mainly proximal) small, and to lesser extent, large intestine where the phosphoralated form is converted into free thiamine by phosphatases[24, 25]. Carrier-mediated uptake occurs when thiamine concentrations are low[24]. This is achieved at the brush boarder barrier via transporters THTR1 and THTR2[22]. At higher concentrations the positively charged compound enters via simple diffusion[22,24]. Inhibition of uptake is documented in enteropathogenic *Escherichia coli* infection as well as chronic alcohol intake (which also inhibits re-absorption at the

kidneys)[24, 25]. The role and extent of bacteria mediated thiamine synthesis at the site of the normal microflora of the colon remains unclear, probable THTR1 mediated plus H^+/thiamine exchange channels being implicated in the vitamin uptake at this location[22,24, 25].

Entry into the circulatory system is coupled with ATP hydrolysis. Thiamine is transported in the blood bound to albumin, and if the capacity of albumin is exceeded excess is rapidly excreted in the urine[2, 24]. Excretion of vitamin B1 is enhanced with the use of loop diuretics and alcohol [2, 26].

Storage of thiamine takes place in various tissues throughout the body with ~40% in skeletal muscle, and the remainder in organs including the heart, brain, liver, and kidneys; totalling 25-30mg in a well nourished adult[2, 22, 27]. Catalyzed by thiamine diphosphokinase (located within cell cytosol), intracellular thiamine is phosphoralated into thiamine diphosphate (TPP)[22]. This is the most abundant form found in body tissue (>80%) while triphosphate and monophosphate, whose biological roles are less clear, can also be found[2]. Once within the cell, a large proportion of TPP quickly enters mitochondria via an antiporter [22]. The half life of thiamine is 18 days and bodily stores can deplete within 2-3 weeks if diet is deficient [27].

Metabolic function

Thiamine (and its derivatives) act as coenzymes in important enzyme driven metabolic pathways[27]. The role of thiamine as cofactor in the dehydrogenase reactions of three mitochondrial enzyme complexes is essential in the oxidative decarboxylation of ketoacids and release of CO_2 [22, 4]. The complexes involved are pyruvate, ketoglutarate and ketoacid dehydrogenases[22]. Thus the enzyme complexes form an integral part of mitochondrial ATP driven energy synthesis[4, 22].

The B1 vitamin is a further cofactor of transketolase in the initial and final steps of the pentose phosphate pathway crucial for cellular function, NADPH synthesis

(redox), and fatty acid and nucleic acid formation[2, 22]. It is this metabolic role that has been the focus of most research of the vitamin and its pathophysiology in deficiency state.

The structural role of thiamine has been described with research suggesting its role in cell membrane structure and stability as well as in neuroconduction (see pathophysiology below)[28].

CONSEQUENCES OF THIAMINE DEFICIENCY

Deficiency of vitamin B1 can lead to a number of conditions including beriberi, Wernicke's encephalopathy, African Seasonal Ataxia, as well as a number of "minor" non-specific symptoms and signs[1].

Beriberi describes the clinical symptoms and signs observed in some classes of thiamine deficiency. Classically the condition is subdivided into "wet", "dry", and infantile beriberi depending on the presentation. There is issue as to the nomenclature of the syndrome with the WHO advocating that the term beriberi be replaced by thiamine deficiency related polyneuropathy (dry beriberi) or TD related cardiopathy (wet beriberi) [3](see below). This definition incorporates all clinical presentations of thiamine deficiency as well as marginal deficiency states that may not cause overt symptoms sufficient to be classed beriberi. Below is a statement of definition of thiamine deficiency from the ICD 1991:

"A clinical syndrome that arises insidiously as a result of a severe, prolonged deficiency of thiamine in the diet, manifested in the initial stages by anorexia, malaise, and weakness of the legs, frequently with paraesthesia; there may be slight oedema and palpitations. The disorder may persist in this chronic state or may at any time progress to an acute condition characterized either by cardiac involvement with oedema or by peripheral neuropathy; forms intermediate between these two extremes may also occur. It is thought that the basic cause is the inhibition of a series of enzyme-catalysed cleavages of carbon-carbon bonds in which thiamine diphosphate is a coenzyme"

**International Nomenclature of Diseases. Vol. IV Metabolic, Nutritional, and Endocrine Disorders. WHO, Geneva 1991 pg 277.*

Initial symptoms of thiamine deficiency can be subtle and non-specific[4] with complaints such as fatigue, anorexia, abdominal discomfort, mouth ulceration,

numbness and tingling sensations reported[2, 4]. However, unless actively sought, these symptoms are rarely diagnosed as TD related[2, 4].

Thiamine deficient cardiopathy (also known as "wet" beriberi)

TD cardiopathy presents either acutely (Shoshin beriberi) or sub-acutely with symptoms including muscular pains, pitting oedema, dyspnoea, chest pains, and difficulty in breathing on lying flat[2]. Signs include those of cardiac insufficiency with tachycardia, tachypnoea, bibasal crepitations, heart murmurs, caridomegaly, hepatomegaly raised jugular venous pressure[3]. Response to thiamine is often dramatic and rapid. These symptoms can be misdiagnosed as other causes of heart failure including ischemic heart disease, rheumatic fever, endocarditis, valvular disease, congenital, idiopathic etc.

Thiamine deficient polyneuropathy ("dry" beriberi)

TD polyneuropathy is an ascending polyneuropathy that typically commences in muscles most used by the cases including the thighs and heels. Both sensation and power are affected with wasting occurring as the disease progresses[3, 4]. Muscle pain often develops and a flaccid paralysis can ensue. Classical signs of ascending polyneuropathy present with decreased reflexes, sensation and power, difficulty in standing from squatting is another typical sign. The presentation may be confused with clinically similar diseases such as Guilian-Barre syndrome. Response to thiamine is slower often requiring weeks to months for complete resolution. A mixed picture of polyneuropathy coupled with cardiopathy, though less common can also occur[3].

Wernicke-Korsakoff Syndrome

Whilst WK sundrome is more often seen in alcohol dependent subjects, it is not exclusive to this group[4]. Symptoms include vomiting, horizontal nystagmus, weakness of extra-ocular muscles, mental impairment, memory loss with confabulation, peripheral

neuropathy, confusion, coma, and death can ensue[4]. Diagnostic confusion can be made with other causes of confusion and coma such as infection, meningitis and dementia.

Infantile beriberi

Infantile beriberi principally (although not exclusively) occurs in babies who are breast-fed by thiamine deficient mothers[1-4]. Presentation usually occurs after the first month or two of life as the neonate has B vitamin reserves from intrauterine stores[1]. Risk of the deficiency should decrease as the infant commences the weaning process with complementary foods of greater thiamine concentrations. Infantile beriberi poses a greater challenge to the clinician as symptoms and signs can be less specific and progression to death faster [1, 29]. Increased screaming, work of breathing, cyanosis and oedema can be due to cardiac related disease, aphonia (secondary to laryngeal nerve palsy) and seizures can be a complication of nervous involvement [29]. These symptoms and signs can be fatally mistaken for many other diagnosis including infections (meningitis), epilepsy and cerebral malaria.

What makes some patients develop wet beriberi, others develop dry beriberi and others still develop central nervous system symptoms and signs (Wernicke's encephalopathy) is still not completely understood but factors that are thought to influence this include;

1. Whether the deficiency is acute or chronic and marginal,
2. The ratio of glucose and other food intake comparative to thiamine
3. Concomitant use of other substances (thiaminases, alcohol)
4. Genetic predispositions
5. Climate (hotter areas favouring development of cardiac manifestations) [30]

African Seasonal Ataxia

African Seasonal Ataxia is a condition thought to be due to TD induced by

ingestion of thiaminase containing silkworm larvae[31, 32]. Presentation can be with nausea and vomiting, tremor and disturbed gait[1, 31, 32]. Signs of cerebellar involvement can be elicited. The disease is usually self limiting with gradual improvement in condition occurring with reintroduction of more thiamine rich products and cessation of silkworm consumption[16, 31, 32].

Long term effects of marginal thiamine deficiency

The long term effects of TD was investigated by a group of neurologists who followed the development of children exposed to TD formula milk during infancy in Israel[33] (see section epidemiology section). They initially found a significant difference in language development when compared to control subjects[33]. Indeed, these infants who had been fed the milk for an average of 3.8 months exhibited significant delay in language abilities at two years post incident[33]. Fattal et al [34] conducted further follow-up analysis finding 57 of the 59 reviewed displayed significant language impairment five years post incident in comparison to only 9% of control subjects[34]. This reflects similar observations in animal studies of thiamine depleted rats[33]. The scenario created by the Israeli disaster provided a fairly unique opportunity to explore the ongoing or more permanent effects of TD given that other factors such as social background, other nutritional intakes, cultural differences etc could be standardized and thus the isolation of vitamin B1 deficiency and its effects more stringently examined. These findings are significant when considering the implications on neuro-psychological development of chronic marginally thiamine deficient communities.

Thiamine and malaria

Malaria remains one of the greatest burdens in developing countries, killing up to a million children annually[35]. It is endemic in many parts of the world including Guinea-Bissau[9]. Areas where malaria is prevalent are also those poorer states where diets can lack variety and therefore susceptibility to thiamine deficiency can also be high[35]. TD can cause a number of physiological processes as well as symptoms that

mimic malaria, such as, haemodynamic instability acidosis and encephalopathy[35]. Further, given that plasmodium infection increases basal metabolic rate and glucose consumption, questions concerning TD and malaria infection have been raised. In particular, is there a relationship with plasmodium prognosis and thiamine status? Or does malaria infection produce clinically significant thiamine deficiency that requires treatment?

Two recent studies in Thailand both found TD, diagnosed biochemically, to be prevalent among patients admitted with malaria[35,36]. Mayxay et al[36] discovered activation coefficient levels >31% in 12% of patients presenting to a Hospital in Laos with uncomplicated malaria[36]. Krishna et al[35] measured activation coefficient in adults presenting with both severe and uncomplicated malaria, confirmed by blood film, at Paholpolpaynhasena Hospital. 52% of patients with severe malaria and 19% of uncomplicated malaria sufferers had severe TD (activation coefficient >31%)[35]. This compared to no patients being identified biochemically to have deficiency in the control group, who were age matched family members (and thus deemed to have similar diets and lifestyles)[35].

These findings present important questions, namely, were those patients with severe malaria more likely to acquire severe TD (acutely) as they were more unwell? Possibly due to increased thiamine utilization with increased BMR, plus perhaps decreased oral intake whilst unwell. Or did TD predispose to developing malaria and complicate the disease to worsen the prognosis? This would imply that thiamine supplementation may reduce malarial attack or at least improve prognosis, and warrants further investigation.

Bates et al[37] speculate that multivitamin supplementation actually increase parasite load in plasmodium infected children in The Gambia[37]. Their study, conducted on 190 children of which half were given nutrient supplementation (containing iron, riboflavin, thiamine and vitamin C), found that those in the intervention group had a significantly higher parasiteamia[37]. However, given the concoction of substances given,

and in particular iron, based on this study it can not be concluded that thiamine supplementation would increase malaria parasiteamia.

Malaria is prevalent in Guinea-Bissau, with peak incidences within the communities studied occurring between October and November[10]. All of the patients were tested with rapid diagnostic tests for malaria (Paracheck) that were all negative. This test only detects *Plasmodium falciparum* and therefore, although none of the cases reported fever on presentation, it can not be confirmed whether they had concurrent or recent infections.

Other diseases where vitamin B1 is implicated

Aside from the above mentioned conditions, thiamine deficiency and thiamine therapy have been implicated in numerous other disease processes that include Alzheimer's disease, cataract development, diabetes melitus, fetal alcohol syndrome and sudden infant death syndrome, colon cancer, atherosclerotic vascular diseases, breast cancer, and maple syrup urine syndrome[22]. These are areas of current interest with relation to vitamin B1. However, more detailed analysis of each association falls out of scope of this review.

PATHOPHYSIOLOGY

The pathological findings related to TD are generally thought to be due to the metabolic role of thiamine and subsequent inhibition of a series of enzyme catalysed cleavages of carbon-carbon bonds where thiamine diphosphate is a coenzyme as discussed previously[1, 2]. More recently, the structural role of thiamine has also been implicated in the pathophysiology of disease process, with emphasis on mitochondrial and axonal membrane structure and stability, together with nerve conductance being influenced by absence of the essential vitamin[28].

Cardiac changes found on autopsy show dilatation with concomitant hypertrophy and microscopic fragmentation of cardiac fibres with fatty infiltration, cloudy swelling and frank necrosis[1,29]. Damage is predominantly found in the atria as opposed to the ventricles[1,29]. Early rat studies found increased cardiac pyruvate and decreased ATP secondary to reduced pyrovate dehydrogenase activity, this intern leads to decreased cardiac contractability[1]. Meanwhile, endomyocardial biopsies conducted on two patients with thiamine responsive Shoshin beriberi revealed interstitial fibrosis and presence of myocytes[29]. It has been suggested that tropical climate increases susceptibility to cardiac involvement given the general increased vasodilation causing a high output state[1], although other researchers point to concomitant carbohydrate rich intake and duration of deficiency as variables in to the aetiology of presentation.

Peripheral nerve biopsy has shown signs of myelin loss, increased collagen fibres and nerve fibre axoplasm disruption with further degeneration in the spinal cord[1]. Changes are described as “biochemical lesions” a term given to the character of reversibility if given treatment with thiamine[1]. Frequent blood findings include a raised lactate, pyruvate and blood volume together with an increased thiamine pyrophosphate effect[1].

RISK FACTORS FOR DEVELOPING THIAMINE DEFICIENCY

The WHO recognises that there remain large populations in the world today who do not have adequate thiamine quantities in their diet (marginal diets) and thus are at risk of developing deficiency related conditions[3]. Typically this can be observed in poorer communities whose diet is made up largely of rice or other high carbohydrate concentrated meal, without much food variety. Other risk factors identified to date include:

Increased demand

This can be secondary to acute or chronic illness that dictates the need for an increased energy requirement, or physiological in pregnancy and lactating mothers[38]. Also an at risk group identified for the same reason are particularly active subjects such as farmers during rainy season[38-40]. The cases in Guinea-Bissau were all young active males who either enjoyed regular sport or worked on farms.

Decreased absorption

Malabsorptive states including chronic diarrhoea, short bowel syndrome, and concurrent parasitic (helminth) infections, can cause reduced intestinal thiamine absorption. These have been frequently found as contributory factors in the development of deficiency [1].

Alcohol use

High alcohol usage is the main cause for thiamine induced disease (Wernicke's encephalopathy) in developed countries and many developing countries alike[39]. There is a combined aetiology for this; with alcohol misuser's having generally poor diets, more likely to suffer from liver disease affecting thiamine storage and reduced absorption secondary to alcohol [2, 25].

Thiaminase ingestion

Chastak paralysis was a condition first described in foxes (1937) who were eating raw fish (carp) products and found to develop neurological signs (paralysis). This led to to the discovery of thiaminases present in some foods that can diminish thiamine levels[1-3].

Two thiaminases are found in food: Thiaminase I catalyzes a base exchange reaction between thiazole and another base. It can be found in certain fish (usually viscera), shellfish, ferns, as well as some bacteria [1-3]. Thiaminase II is mainly found in bacteria and is a hydrolytic enzyme breaking the vitamin at the methylene bridge. Both types are heat labile and therefore found in raw or fermented food. Proper cooking of food thus destroys the thiaminase and can protect the subject from the potential hazard[2].

In North Western Nigeria, the epidemics of African Seasonal Ataxia have been attributed to the seasonal habit of eating roasted larvae of Anaphe venata which was also found to have thiaminase I present[16, 31, 32].

Antithiamine factors

Antithiamine factors have been found in a number of products including betel nut, tea, ferns and various plants and vegetables. They act by binding to thiamine and interfering with the bioavailability of the compound[1-4].

Betel nut chewing is common practice in many parts of south east Asia, and has been found to contain antithiamine factors. Indeed in 1975 Vimokesant et al[41] found in a study in Thialand, conducted on habitual Betel nut chewers (+/- raw fermented fish paste) that the case group of betel nut chewers had a higher thiamine pyrophosphate effect indicating that the nut, as well as raw fermented fish paste both had a depleting effect on human thiamine levels[41].

The effects of tannic acid, present in tea, have also been experimented in both human and rat models[42]. Interestingly, it was found that if tea consumption was delayed by one hour post eating a meal, the thiamine reducing effect of tannic acid was significantly reduced[42]. Whilst the polyphenol compounds in tea do possess these antithiamine factors, it remains unclear the extent to which they can induce thiamine depletion[2]. Ascorbic acid containing substances (in this study orange and papaya) taken with a meal also protected against the thiamine reducing effect of tannic acid[42]. The subjects of this report all drank copious amounts of tea or "warga" averaging four times per day.

Certain foods (eg cassava) contain cyanogenic glycosides and lack certain sulphur containing amino acids (cysteine) which is required for detoxification. Thiamine (containing sulphur) can serve for the detoxification process and thereby consumption of such foodstuffs may also deplete vitamin B1 levels[2, 31-32].

Male predominance

Most epidemics of beriberi occur in male populations[2]. Increased physiological demands secondary to traditional work/lifestyle patterns of male gender (labour) is believed to be the main cause of this[2]. Although this has not been proven and other factors (genetic predispositions, utilization and storage of thiamine etc) have not been extensively researched. All the cases in the series described were male.

Infection

Any process that increases the basal metabolic rate, BMR, will in turn increase body thiamine consumption. Body temperature rise of 1'C increases BMR by 10%, therefore it is not rare to find many cases of beriberi that are triggered or preceded by a non-specific pyrexial episode[2]. Certainly symptomatic severe TD is found more commonly in patients with acute attacks of malaria, and many patients diagnosed with beriberi in the epidemics described also had concomitant infections[35,43,44]. The exact

nature of this relationship is not entirely clear, consideration needs to be emphasised on the possibility that TD is in itself a predisposing risk factor for developing infection.

Diuretics

Loop diuretics (namely furosemide and spironolactone) have been shown to decrease renal thiamine re-uptake, therefore depleting bodily concentrations[26]. This creates a particular difficulty given that such medications are first line treatment of heart failure which therefore will worsen the condition if it is in fact TD induced cardiopathy[26]. Indeed, nutritional guidelines suggest the routine vitamin B1 supplementation to all heart failure patients even in developed countries[26].

Seasonal variations

The seasonality of food availability and physical exertion in many developing countries exposes communities at increased risk during the rainy season. This is the time when fewest products are available from the farms, transportation to nearby towns becomes more difficult and the work force exert most energy on the farms[39,40]. No wonder that this has been associated with numerous epidemics[32,39, 40]. The rainy season in Guinea-Bissau runs from June to October[17] which coincides with the onset of the cases reported below.

HIV infection

Thiamine deficiency has been found to have an increased prevalence in HIV infected patients, both early and late on in disease progression[45]. This is thought to be due to a combination of factors including the catabolic nature of the disease, poor nutrition and other malabsorptive states associated with HIV[45]. Guidelines suggest routine supplementation of HIV infected patients with vitamin B[45].

RECOMMENDED DAILY INTAKE OF THIAMINE

The daily requirement of thiamine is not precise and is influenced by numerous factors including physical activity as well as caloric intake[2]. There is a slightly higher RDA for men than for women. The dietary reference intake for an adult male is 1.0mg/day whilst the recommended daily allowance is 1.2mg/day[2]. Another perhaps more appropriate value is 0.4mg/1000kcal which accounts for caloric intake[2]. Meanwhile the intake requirement of infants is 0.30mg/day[2]. In infants less than 6 months this is expected to be achieved through breast milk alone, whilst those from 12-23 months are expected to source an additional 0.39mg/day from complementary food[2].

Food containing Thiamine

As mentioned previously, the greatest number of cases and fatalities attributable to beriberi were seen in Asia at the turn of the 19th century. This was largely due to the advent of steam powered milled rice that became available to the masses. Milled rice contains ~ 80µg/100g rice where parboiled whole rice contains more than double that amount[2]. Many foods contain thiamine. It is difficult to predict exact quantities of thiamine within many products given the heterogeneous reporting made in food composition tables[2]. Table 1 references some staple foods with thiamine levels[2].

Foodstuff:	Brown rice		White rice		White rice flour	Maize meal	Cornflakes (fortified)	White bread
	uncooked	cooked	prior to cooking	cooked				
Thiamine (mg/100g)	0.59	0.14	0.08	0.01	0.14	0.4	1	0.21

Table 1: Thiamine quantities of some staple foods (adapted from reference 2)

In industrialized countries cereals have become one of the main sources of thiamine given the routine fortification of this product[2].

Despite the active secretion of thiamine in to milk by the lactating mother, breast milk is a relatively poor source of thiamine (0.49-0.79μmol/l)[2]. Whilst thiamine is actively channelled to breast milk, Ortega et al[38] confirmed that maternal thiamine deficiency (detected biochemically in pregnant Spanish subjects) is associated with lower thiamine content in breast milk and that supplementation leads to increased secretion[38]. However, Nail[46] and other researchers found that breast milk thiamine concentrations plateau, despite higher maternal thiamine consumption[46].

DIAGNOSING THIAMINE DEFICIENCY

In practice, the diagnosis of beriberi is usually made on a clinical basis. This is particularly the case in resource poor settings, where the condition is most prevalent. Clinical diagnosis can be confirmed by the dramatic and rapid clinical response to thiamine therapy, especially seen in subjects with cardiac manifestations[1-4]. Response is somewhat slower in polyneuropathy. A detailed dietry history can be very helpful as well as epidemiological awareness of the condition in diagnosis and indeed when considering deficiency attributable to thiaminase or antithiamine factors[1,3].

The diagnosis of infantile beriberi is somewhat more challenging to the clinician as symptoms and signs are very unspecific and if the lactating mother is not showing any signs herself (which is often the case), the delay or lack of diagnosis can prove fatal[47,48]. Further, sub or marginal thiamine deficiencies with less specific or no symptoms/signs cannot be diagnosed without analysis[3].

Tests that can be used, resources permitting, include blood erythrocyte transketolase activity, thiamine pyrophosphate effect, blood and urine thiamine levels, as well as less specific tests of lactate and dehydrogenase levels[3].

Erythrocyte transketolase activity (ETKA)

Transketolase is a thiamine dependent enzyme essential to energy production (pentose pathway) within erythrocytes[49]. Measure of the ETK can provide a sensitive measure of tissue thiamine level availability[3]. Soukaloun et al[49] reviewed the use of biochemical markers (including ETK activity basal/activated; activation (α) coefficient, as well as, some cardiac makers (brain natriuretic peptide, N-terminal pro-brain natriuretic peptide and troponin T) for detection of infantile beriberi[49]. The group investigated in Lao, where food avoidance behaviours in post-partum mothers, and thus infantile beriberi is relatively common[49]. The study conducted on 47 clinical cases of infantile beriberi and 100 paired controls (both febrile and afebrile) found that reduced

basal ETK activity (<0.59 micromoles/min/gHb) best correlated with clinical infantile beriberi[49], yielding sensitivity values of 75% and specificity 85.2%, and this was stronger than the correlation of activated ETK. Meanwhile, the activation (α) coefficient was a poor test which did not significantly distinguish cases from controls[49]. The investigators found the only reliable (sufficiently sensitive) cardiac marker was Troponin T with 78.6% sensitivity and 56.1% specificity[49]. However, given the numerous cardiac related adult diseases (ischeamic heart disease, rheumatic fever, congestive heart failure etc), specificity of troponin T would likely be further reduced in adult practice rendering its usefulness questionable. The authors do highlight the potential for basal ETK to be influenced by diabetes and liver dysfunction (lowered), whereas vitamin B12 deficiency can give increased levels[49].

Thiamine pyrophosphate effect (TPPE)

Thiamine pyrophosphate effect is defined by the activation coefficient, calculated by the equation[49]:

$$\alpha = \frac{\text{ETK activated} - \text{ETK basal}}{\text{ETK basal}} \times 100$$

Thiamine pyrophosphate is added to the sample after initial ETK activity is measured and then a further measurement is made that reflects the amount of apoenzyme present without the coenzyme[49,3]. It is expressed as percentage with levels greater than 25% (although lots of literature use >31% as cut off)[1] thought to indicate deficiency and 15-24% marginal deficiency[3]. The function test is measured by the disappearance of pentose or appearance of hexose by spectrometry[2]. The advantage of TPPE is its sensitivity at low levels of thiamine[2]. Whilst this is seen as one of the most reliable forms of testing tissue thiamine levels[2,3], in Lao Khounnorath et al[16] argue its appropriateness in infantile cases who may have had low level in-utero exposure to thiamine, and thus falsely low levels of the activated coefficient[16]. There is also debate as to its accuracy in chronically deficient subjects who may also not display a markedly raised activation coefficient[2].

Thiamine levels (blood)

Blood thiamine levels are relatively low (0.8%) given that much of the thiamine in the body is stored in tissues. Therefore blood thiamine levels do not accurately demonstrate whether somebody is truly thiamine depleted, which renders this method practically futile[3]. Detection is based on the conversion of thiamine to flourescent thiochrome derivatives which can then be detected by spectrometry[2]. Once collected, samples need to be placed in ice immediately and the storage time is short, further rendering this process infeasible[2].

Thiamine levels (urine)

Thiamine is excreted in the urine, and levels present in the urine can be used to estimate dietary intake of thiamine. However, given that thiamine is stored in tissue, again absent urinary thiamine does not necessarily signify overt thiamine deficiency[3]. Urinary excretion ranges from 40 to 90μg per day when intake is adequate[3]. 24 hour urine collection would provide a more accurate assessment of thiamine intake adequacy, however, collection would be more difficult to obtain. Fasting (morning) samples are better for analysing thiamine status if one off samples are used. Urine collected needs to be acidified to avoid degeneration and then stored at -20'C[2]. The converted thiochrome is detected fluorometrically and expressed in relation to the creatinine content[2].

Whilst urinary collection may be of limited use in the diagnosis of beriberi, it may be of benefit for larger epidemiological field studies of population thiamine intake estimates given that it is non invasive and simple to obtain. Dose retention tests can also be performed where urine thiamine levels are measured following thiamine administration, if levels remain very low this provides further evidence of thiamine deficiency[3].

Breast milk thiamine

Infantile beriberi is most often seen in breast-fed babies where maternal thiamine intake is deficient. Thiamine levels in breast milk can be evaluated to assess at risk groups for developing thiamine related illness[3]. Below is a table of breast milk concentration of thiamine and corresponding status of the infant.

Breast milk thiamine (µg/l)	Thiamine status of infant
100-200	Normal range
50-99	Marginally deficient
<50	Severly deficient

Table 2: Breast milk concentrations of thiamine and corresponding thiamine status[3]

Delayed weaning remains prevalent in many parts of Guinea-Bissau with Colombatti et al contributing it to poor health education and maternal illiteracy, and found it to be a cause of infantile malnutrition in the older age groups[15]. This would predispose older infants who remain entirely breast fed to thiamine depleted mothers to develop beriberi at a later than characteristic time period.

Lactate and pyruvate

Although thiamine deficiency increases both lactate and pyruvate levels in the blood as their metabolism is thiamine dependent, assays of pyruvate/lactate are not generally used for diagnosing thiamine deficiencies given that many infections can cause these non-specific markers to rise[3].

Whilst methods for assessing thiamine status are available, as mentioned above, high technological equipment and skilled staff are required and thus put these tests out of reach to many locations where they may be most at need. Developing a rapid and simple test that is sensitive enough to detect marginal deficiency state would be of great benefit in assessing the true extent of the burden in developing countries.

TREATMENT

There is no consensus as to the exact dose, preparation or duration of treatment for thiamine deficiency. The British National Formulary advices for the treatment of Wernicke's encephalopathy three times daily 2 injections of Pabrinex intramuscular or intravenous infusion[51.] This is continued for two days and if there is response, further daily injections are given for a further two days. Pabrinex contains 250mg of thiamine hydrochloride along with other B vitamins[51].

Mild deficiency states can be treated with oral supplementation of vitamin B compound ~ 5-10mg per day in divided doses for the period of anticipated deficiency[3]. Preventing thiamine deficiency with medication can be achieved with 1mg daily supplementation during the period of time the person is at increased risk[3].

Toxicity from oral intake is unrecorded as thiamine is not actively absorbed at high concentrations[2]. Reports of side effects at higher parental doses (up to 3g per day) include headache, irritability, insomnia, tachycardia, weakness, contact dermatitis and pruritus[2,51]. There has been a reported fatality through anaphylaxis[51] meaning that ideally resuscitation equipment should be available if parental route is required[51].

In the cases presented in this article, once daily 100mg thiamine intramuscular injections were given for five days following one month supply of x5 daily multivitamin tablets and x5 daily vitamin B compound tablets. The treatment above was dictated by clinical response and resource constraints (only able to stay at location five days, no vitamin B1 oral preparations available).

Vitamin B compound contains 1mg of thiamine per tablet as does the multivitamin tablets used. All of the patients treated reported polyuria (expected) and headaches whilst receiving the thiamine via intramuscular injection, though these were minor side effects and well tolerated.

Certainly in a poor-nutrition case, education is of importance. Other micronutrient deficiencies should be considered as likely and a course prophylactic multivitamins should be included in all treatment follow-ups.

PREVENTION OF THIAMINE DEFICIENCY

As mentioned previously, the reason thiamine deficiency is rare in more affluent countries is a combination of factors that include; availability of varied foodstuffs; awareness of importance of varied diets; fortification and enrichment of many staple foods with thiamine; and supplementation of vulnerable groups with multivitamins. Each of these approaches can be targeted to increase thiamine status in poorer communities.

Public health nutritional education

Ideally micronutrient dietary deficiencies should be overcome with health-promotion campaigns designed at improving nutritional standards. This is a long-term project that requires investment in agriculture and education. There is little value in providing nutritional education to a population if affordable food alternatives are not available. This requires agricultural investment to provide sustainable farming of other such crop[1,2]. Meanwhile cultural habits need to be addressed and overcome in food preparation, and of consumption of different or newly introduced foods[44]. Another aspect to be addressed in this section would be the advise on preparation and cooking of food in order to preserve thiamine content (e.g. minimise milling, rewashing and over boiling of rice)[2]. Educational programmes can have a very positive impact as reported by Adamelokun et al[32] who saw dramatic reductions in cases of African Seasonal Ataxia post public health awareness campaigns[16]. In Western Nigeria, where the epidemics were reported, public health campaigns were instigated using radio broadcast messages and leaflet distribution encouraging people to avoid consumption of the silkworm believed to be responsible as well as highlighting the importance of a varied diet especially during the rainy season[16].

Vitamin supplementation programmes

Another intervention that could be considered is the widespread distribution of

multivitamin or vitamin B complex tablets. Distribution could be timed to coincide with the rainy season, or coupled with other existing interventions such as vaccine campaigns to reduce costs. Community members or Imams could assume responsibility of vitamin distribution to followers during morning or evening prayer for the month of Ramadan in a novel approach to specifically target this group. Compliance may be poor if there is no perceived benefit to the group, and cost would be an issue. Sustainability issues of this approach would suggest it to be a short term measure for at risk communities. National multivitamin, vitamin B complex and folic acid distribution was instigated following the Cuban epidemics of B vitamin related neuropathies that affected approximately 50,000 people[52-54]. This mass supplementation appeared to curtail the outbreak from affecting more civilians and reversed symptoms in those already affected[52,53].

A recent study into the effects of routine multivitamin supplementation by Andersen et al[18] is of interest as given its potentially negative results. The study conducted on pregnant women in Guinea-Bissau and published in 2010 found subjects provided with twice the recommended daily allowance of micronutrients (multivitamin tablets) increased birth weight by 95g[18]. However, contrary to prediction it had no effect on perinatal mortality[18]. Further, follow-up of the 2100 cases studied showed a marginal increase in infant mortality (92-365 days of life) in the two study groups, this was contrary to prediction[55]. This was a surprising finding that questions the possibility of unrecognised negative effects of multivitamin prescription, and would need further consideration were such an intervention be deemed appropriate.

Food fortification

Food enrichment with thiamine in order to prevent beriberi is not a new concept[56]. Robert Williams (1886-1965) a nutritional scientist devoted most of his working life to the disease and campaigned vigorously for the supplementation of rice with thiamine[56]. He helped create a fortified pre-mix rice which was then added to rice for distribution in parts of the Philippines, 1948-50[56]. Despite its apparent clinical success, the project was marred by organizational and political problems which resulted

in the UN to steer away from such a policy globally[56]. Indeed, the overwhelming opinion of policy makers in the FAO and WHO at the time were that beriberi and other such micronutrient deficiencies were products of socio-economic deprivation, and it was this as well as food education that needed to be addressed as opposed to "quick-fix" fortification implementation[56]. This is a somewhat basic summation of food fortification given the now widespread practise in various parts of the world.

Food fortification has been common place in developed countries since iodinization of salt in the 1920's, with other targeted campaigns including cereal enrichment with B vitamins to prevent beriberi and pellagra[2]. Food fortification has greatly improved micronutrient intake in developed countries, thiamine intake from cereal consumption rose from 31% to 59% of total intake[2].

Whilst this appears an attractive option, there are a number of obstacles, especially when considered for commodities in developing countries. For food fortification to be used as a public health intervention a number of aspects must be addressed;

a) An appropriate food vehicle must be selected that is consumed by the majority of the target population in constant amounts (staple food). In Guinea-Bissau rice would be the most likely candidate.
b) Fortification levels must be adequate to correct any dietary deficiency [2, 57]
c) Adverse nutrient interactions must be addressed.
d) There should be evaluation of the intervention and infrastructure should exist to assess this.
e) The actual process requires costly technological equipment that increases the price of production and could therefore bring up the price to become unaffordable to those most at need[57].
f) Compromise as to which nutrients to include in fortification often needs to be made as most staple commodities have a low saturation level [2.]

More recently, mandatory maize meal and wheat flour fortification with a

number of micronutrients (including thiamine) in South Africa, 2003, led to considerable improvement in micronutrient intakes amongst previously susceptible populations[58].

Fortification has often been more difficult than planed with objections from the manufactures, given increased cost and claims that it changes the taste of the product[57]. In Australia beer fortification with thiamine was met with resistance from both breweries (increased cost) as well as other lobbying groups who campaigned that it would be ethically questionable to fortify a product such as alcohol with thiamine[57].

RECENT EPIDEMICS

Beriberi is thought by many to be a disease of the past, with its highest prevalence being over a 100 years ago in Asia, coinciding with the advent of mass production and utilization of polished rice[3]. Following discovery of and subsequent large scale public health interventions with food fortification policies across developed countries, increased food variability and the decreased importance of rice as part of the diet[1], thiamine deficiency related disease has become largely confined to alcohol abusers (Wernicke's encephalopathy), sporadically seen in food faddists and TPN dependent patients alike or its presentation associated with inherited disorders such as Leigh's Disease[3].

In developing countries and certain at risk communities, nutrition related thiamine deficiency remains burdensome and deadly with numerous epidemics recorded in recent times[3]. Patterns of vulnerable "at risk" communities and time periods are being identified and will be the focus of discussion of this section looking closer at some of the recorded outbreaks, which are highlighted on the map below (illustration 3).

Illustration 3: Map indicating locations of recent outbreaks (map adapted from reference 50)

Refugees and internally displaced persons

Refugee communities have been highlighted as a particular at risk group given the often poor nutritional status on arrival at camps, co-infections and the frequently nutritionally deficient rations provided[3]. Recorded outbreaks of thiamine deficiency related disease in refugee communities include: Cambodian refugees in Thailand (1980), Bhutanese refugees in Nepal 1993-95, Liberian refugees in Guinea (Conakary) 1990, Djibouti 1993-94, Karen refugees in Thailand 1992[3].

Over 12,000 cases of TD related symptoms were reportedly suspected in the Bhutanese refugees, of which 1300 were classed as severe cases (limb weakness, ataxia, oedema breathlessness and cardiac compromise)[3]. The remaining larger group were defined as having mild deficiency type symptoms including tingling/burning sensations and numbness[3]. 80% of all cases reported resolution following vitamin B complex administration[3]. Mild cases were not actively screened for in the affected population described in this case report.

Investigators found that up to 40% of the infant mortality rate in a population of Karen refugees in the late 1980's was due to infantile beriberi[43]. Following vitamin B1 supplementation and treatment in the affected group, infant mortality dropped from 183 to 78 per 1000 live births, the difference occurring principally in post-neonatal deaths[43].

McGready et al[60] evaluated thiamine status of pregnant and postpartum mothers within the same population in 1995 using blood sample ETKA and breast milk thiamine levels[60]. Findings also supported previous evidence that thiamine is preferentially transported to the fetus and breast milk[60].

Detention centres and isolated groups

Ahoura et al[61] described 712 cases of beriberi, 597 definite (improvement with thiamine) and 115 probable, in a detention centre in the city of Abidjan, Ivory Coast,

between October 2002 and April 2003[61]. Prisoners were provided with food rations containing a mere 20% of the recommended thiamine allowance[61]. Usually these prisoners received further food supplementation from family members, however for a period of time this was prohibited due to political reasons[61]. Thus the prisoners soon fell ill to non-specific and specific symptoms of the deficiency[61]. The authors also reported diarrhoea to be a specific risk factor for disease progression[61]. Another prison in Ivory Coast saw a further outbreak of beriberi disease in 2008 highlighting detention centres as particular risk given the poor food rations provided[2].

Chen et al describe an outbreak of beriberi among illegal Chinese immigrants at detention centres in Taiwan in 1999, with 27 inmates requiring hospital treatment, again highlighting institutionalized groups as vulnerable to developing TD[29] .

17 (of which 2 died) cases of beriberi were reported in march 2005 from a crew of a commercial fishing vessel docked in Thailand[59]. The crew had difficulties with food supply and resorted to eating just ocean caught fish and rice for the previous 2 months[62]. This situation reflects those of historical outbreaks of nutritional deficiency related illness amongst sailors.

Beriberi in African communities

The Gambia, a small west African country geographically close to Guinea-Bissau has had numerous reports of beriberi epidemics within the general population. Tang et al[40] describe an outbreak of beriberi during the rainy season of 1988 in a rural community of The Gambia reportedly killing 22 and affecting a further 118[40]. Affected individuals were predominantly young men and all were Muslim. Treatment induced full recovery in hospitalized patients and led to community based oral thiamine distribution[40]. The authors contribute the outbreak to increasing tendency of the population to eat imported rice as opposed to locally produced (more enriched) cereals, as well as high rainfall, labour, cooking methods and the possibility of thiaminase antagonists[40]. Interestingly, the month of Ramadan in 1988 was from 18th April- 17th

May, a few weeks before the first reported cases of beriberi, could this have been another unaccounted factor in that outbreak?

In 1990-91 25 cases were reported by Rolfe et al[62] in the capital, Banjul with a peak incidence occurring at the end of the rainy season[62]. Ramadan would appear to have had no apparent relationship on these cases as it fell march of 1990. These cases highlighted pregnancy as a risk factor[62]. Rolfe discusses the nutritionally poor diet of urban dwellers in the Gambia suggesting there to be a large number of sub-clinical thiamine deficient inhabitants[7]. The author suggests addition of thiamine to wheat flour in place of rice given the cooking practices observed in the community (repeated washing and prolonged boiling) that will likely deplete any supplemented thiamine [7].

Dry (polneuropathic) beriberi appeared to be the prominent presentation amongst 70 patients with diagnosed thiamine deficiency in Reunion from a eight year case study conducted by Darcel et al[63]. Again an association to chronically poor diets was found and hypothesis upon the genetic predisposition to developing beriberi debated[63].

Recent outbreaks of beriberi in Brazil

Brazil was the focus of recent beriberi epidemics from 2006-2008[8,64]. The Northern state of Maranhão reported more than 1200 cases over the three year period with peak incidences occurring soon after the rainy season from may-august[39]. Interestingly, the majority (87%) had polyneuropathy (dry beriberi) form of the disease[39]. Most were young males of poor socio-economic backgrounds with history of smoking and alcohol intake[39]. In all cases, a comparatively monotonous rice-based diet was observed[39]. Whether citreoviridin production by fungi (penicillium citreonigrum) contaminated rice was associated with the outbreak is unclear with two separate groups supporting and discrediting the motion[39,65].

Infantile beriberi in Asia

Fifty-five cases of infantile beriberi were recently reported in Hyderabad, India[47]. Presenting features included tachypnoea, tachycardia, recessions, hepatomegaly, cough and fever[47]. The authors highlight the difficulties in clinical diagnosis of beriberi disease in infantile cases given that specific signs of heart failure such as pedal oedema are rarely present[47].

The People's Democratic Republic of Lao was the focus of another investigation into infantile beriberi. Upon realization that cardiac failure was attributable to vitamin B1 deficiency in many sick infants in 1991, subsequent diagnosis and treatment of infantile beriberi increased in the area[48]. Indeed, over 600 cases were recorded at Mahosot Hospital between 1990-2000[48]. Khounnorath et al[44] measured ETK (basal) and activation coefficient levels of 778 sick infants without clinical evidence of beriberi over 1 year of admissions. They discovered 13.4% of all infants had basal ETK <0.59 (cut off for severe thiamine deficiency) and 5.1% with an activation coefficient >31% (greater than 31% classed as severe thiamine deficiency)[44]. There was a significant relationship between mortality and biochemical markers of thiamine depression[44]. They proposed the widespread deficiency of thiamine to be due to food avoidance behaviours amongst many women, especially of lower social class[48], pre and postnatal. The authors also noted the contribution of increased demand during physiological stress incurred on a sick child[44,48].

Thiamine related African Seasonal Ataxia epidemics

Adamolekun et al[32] describe the epidemics of African Seasonal Ataxia in the North and Western regions of Nigeria in 1993[16,31,32]. This population saw an epidemic of symptoms including nausea and vomiting followed by tremor, ataxia, confusion, stupor and coma during and near the end of the rainy season[16,32]. It was observed that symptoms would classically commence following a large carbohydrate meal and though debilitating, were usually self limiting[16]. A trial of thiamine treatment versus control

found that almost all patients given thiamine showed complete symptomatic resolution within 72 hours, unlike those in the control group[16,31,32].

Adamolekun et al related the disease with the custom of eating roasted silkworm larvae (Anaphe venata) as a source of protein during the rainy season[32]. These insects have been shown to contain a thiaminase (type I base exchanging type)[1,32]. Large scale health promotion and education within the affected populations by the research group led to a great reduction in ASA[16,1].

Adamolekun[23] goes on to implicate TD in two other conditions previously thought to have a different aetiology: Tropical ataxic neuropathy and Konzo (spastic paraperisis)[23]. These two neurological conditions are associated with the monotonous consumption of Cassava and numerous epidemics have been reported in many part of Africa[23]. Cassava is a carbohydrate rich substance that contains a cyanogenic glycoside (linamarin), it lacks many proteins and in particular the sulphur amino acid methionine[23]. Detoxification of cyanide is sulphur dependent. Thiamine contains sulphur and therefore can be utilised for this process if the diet is otherwise deficient of other sulphur containing amino acids[23]. The author concludes that this usage of thiamine could be implicated in the cause of the diseases in Cassava based diets[23].

However, Nzwalo argues this concept unlikely (thiamine deficiency as the cause of Konzo) given the heterogeneous presentations in Konzo (spastic paraparesis) when compared to typical thiamine deficiency states, the author also highlights the lack of cardiac cases which should be expected were it thiamine related[66]. What is agreed by both parties is the need for further investigation into the aetiology and treatment of these conditions[23,66].

B vitamin related neuropathy epidemic in Cuba

The largest outbreak of vitamin related neurological disorders in recent times occurred in Cuba from January 1992 to September 1993[52-54,66-68]. This period saw

financial difficulties in the communist country, affected by the breakdown of the Soviet Union and the American Economic Embargo[66,68]. This led to food shortages with diets deficient in B Vitamins and sulphur containing amino acids[53]. Some 50,000 people were affected by symptoms ranging from sensory deafness, sensory peripheral neuropathy, optic neuropathy, dorsolateral myeloneuropathy, spastic paraparesis and dysphonia[54,67].

Borrajero et al[67] biopsied sural nerves of 34 patients and found changes ranging from axonal dystrophy to severe axonal damage and loss of myelinated fibres in 92% of the biopsies[67]. This was concluded to be in keeping with a nutritional, toxic or metabolic aetiology[67] and not of an infective or degenerative nature[67]. Marcias-Matos et al[68] measured urine thiamine along with blood transketolase activity in 107 clinical cases and 106 controls[68], whilst there was no significant difference between case-control studies, they did find widespread thiamine deficiency[68]. The exact contribution this had to the epidemic remains unclear, with a mixed nutritional deficiency being more likely[66-68]. Risk factors included alcohol usage, fasting, and sugar consumption, those that are similar to that of developing TD related conditions[54]. Symptomatic improvement occurred in the vast majority with vitamin B and folic acid treatment[54,67]. This was followed by countrywide multivitamin allocations.

Israeli thiamine deficiency outbreak amongst formula fed infants

A very tragic and controversial outbreak of thiamine deficiency, that lead to a police investigation, occurred in Israel affecting formula fed infants[69]. On the 6th of November 2003 a diagnosis of Wernicke's encephalopathy was made in a 5 month old infant at Sourasky medical centre[6,70]. Symptoms improved dramatically with thiamine administration[70]. This led to the same diagnosis being made in similar cases in the same hospital and thus a national alert was triggered[70]. The only common factor was the brand of soya based (Remedia Super Soya 1, manufactured by Humana milchunion, Germany) formula milk they were being fed with[6]. Laboratory analysis, conducted three days later, revealed the formula to be thiamine deficient, contrary to the manufacturer's label[69,71].

Infants presented with non-specific symptoms such as vomiting, failure to thrive and lethargy, with the more severe cases developing symptoms and signs suggestive of Wernicke's encephalopathy[6,70]. Final estimates by the ministry of health reveal that approximately 3,500 infants were fed the depleted formula, with 20 requiring hospitalization for severe symptoms, and 2 deaths (cardiac compromise)[70]. All severely affected cases were found to have a raised TPPE and of those who had testing, raised serum and CSF lactate were present[70]. Hospitalized severe cases demonstrated remarkable resolution in all but one of the cases who was admitted prior to index case recognition and suffered long term disability with severe developmental delay and persistent seizures[70]. The authors of the case reports suggest a genetic predisposition to be the cause of why only 20 of the 3500 exposed infants developed symptoms[70]. Further, the increased glucose content of the formula is thought to be why most cases presented with Wernicke type symptoms and not cardiac beriberi like in other cases of infantile thiamine deficiencies[72]. What is interesting to note is that all 9 patients admitted with severe deficiency at Sourasky medical centre also had concomitant infections[6]. Whether they were more susceptible to infection being thiamine deficient or whether their infections propagated the thiamine deficiencies remains unclear.

The fatal mistake by the manufacturer's was due to a misunderstanding that thiamine, present in the soya and thought to be sufficient, was in fact destroyed when the mixture was heated (thiamine being heat labile)[69,71]. This led to ministerial change within Israel, requiring all formula preparations to be double checked by the government[69].

CASE REPORTS

Routine monthly medical consultations were being provided by myself as part of an international non-governmental organization within the region of Bolama-Bijagos. The total population of the region is approximately 10,000. Transport to the capital Bissau is via public canoes three times per week or an eight hour road trip. Sanitation facilities are basic with each village relying on a covered pump well for water. As all the areas lie close to the sea, fishing and therefore fish based meals were the prominent food source accompanied by rice (either locally harvested or imported).

Consultations were conducted in the communities;

Ilha de Bolama (Caboupa Cabral, Madina & Watu)
Ilhas das Galinhas (Ametite & Ambacana) and
São João (Gã Minjor, Gambacar & Berculum) (see illustration 4 below)

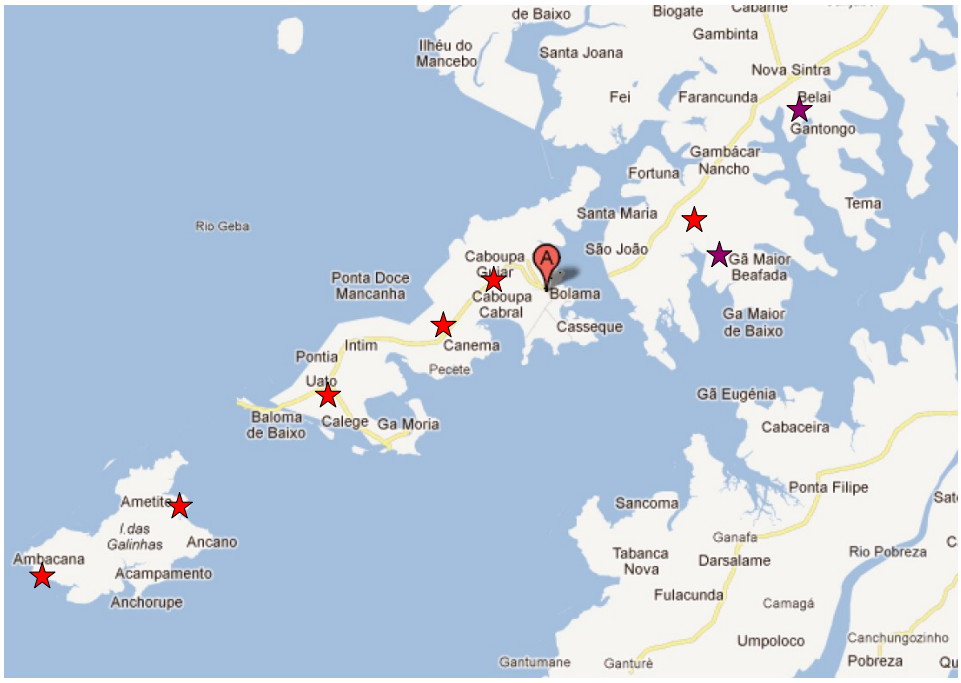


Illustration 4: Map of Bolama with red stars indicating locations of consultations and purple stars highlighting origins of cases (map adapted from reference 50)

Consultations were aimed at providing outreach medical assistance to more isolated communities with poor access to healthcare.

During consultations in September 2010 six patients with symptoms and signs which confer with the classical deficiency state of beriberi were detected. All patients were from the region of São João: four from Gã Mindgor and two further had travelled from Gantongo to Berculum to seek medical help. All were Muslims, males and of Fula ethnicity.

The diet of the subjects was similar as they all lived in the similar communities with similar resources, and available foods. Diets varied wit the season and included:

Months of rain (June-November):

Staple rice (both polished and unpolished) with fresh fish and oysters. Cucumber, sweet corn (once weekly) pumpkin (once monthly) meat (halal chicken) rarely (not more than once monthly at festive occasions). Casava was also occasionally eaten. Rice would be cooked with palm oil, lime, salt and chillies. Snacking with unroasted peanuts and sweets from local shops was common. Large amounts of sweet green tea, "warga" (~5 cups per day) are consumed daily.

Dry season (November-May):

As above plus mango, tomato, cashew nut, banana, avocado, lettuce, papaya, coconut, kidney beans.

Month of Ramadan (August):

As for months of rain but with reduced dietary intake- one large meal at sunset and small breakfast without snacking of any sort during the day.

Gã Mindgor

Case 1

A previously healthy 23 year-old male, presented with a one month history of bilateral leg oedema, nocturnal dyspnoea and palpitations. Alcohol, medication and drug consumption were denied. He was a non-smoking, Muslim farmer usually very active working in the fields and playing football twice weekly. The symptoms commenced within a week of finishing fasting for Ramadan which was during the month of August, near the end of the rainy season in Guinea-Bissau.

On examination the patient was tachycardic (100 bpm, regular) amd normo-tensive with a blood pressure of 130/80. A systolic murmur was present along with peripheral pitting oedema which extended to the waist with ascitis. There was no evidence of hepatosplenomegaly. Test of the urine (urinalysis) did not reveal any heamaturia or proteinuria and rapid detection test for Plasmodium falciparum malaria (paracheck) was also negative.

Once daily intramuscular thiamine 100mg injections were commenced with symptomatic improvement being noticed on the second day of treatment (reduction in oedema). Intramuscular thiamine injections were continued for five days with a further one month supply of 10 per day multivitamin/vitamin B complex supplementation. Complete resolution of symptoms and signs were achieved by day five. The patient remained symptom free at monthly follow-up for six months. Telephone consultation in October 2011 revealed that he remained symptom free one year post. The subject stated that in 2011 he divided the month of Ramadan and only fasted for 15 days, with the option to make up the remaining days of fasting during the year.

Case 2

A 37 year old normally fit and well farmer presented with leg weakness, which had been progressive over the previous month. Both legs were affected with decreased sensation also noticed. The patient recalled he had a similar problem the previous year at a similar time which resolved spontaneously over a period of four to five months without treatment. Alcohol, medication and drug consumption were denied. He was a non-smoker, Muslim farmer who was usually very active. The symptoms commenced within days of finishing fasting for Ramadan.

On examination bilateral muscle wasting of legs was noted with decreased power in both legs (particularly plantar dorsal flexion), and reduced sensation (fine, blunt and sharp, proprioception and vibration) in both feet extending to the mid thigh. Reflexes in both knees and ankles were reduced and plantar reflexes were normal. Cardiovascular examination did not reveal any abnormalities, pulse was 70bpm regular, BP 126/82, he did not exhibit any peripheral oedema and heart sounds were normal. Rapid malaria detection was negative, urinalysis did not reveal any abnormalities.

Treatment was commenced with intramuscular thiamine 100mg for five days, and a 1 month supply of (x10 daily multivitamin/vitamin B complex supplementation). Symptomatic improvement in power and sensation were reported by the patient by day five, however, complete recovery of function and sensation of legs was not achieved until approximately one month. He remained symptom free on monthly follow up for six months and at one year follow-up by telephone consultation.

Case 3

A 20 year old male farmer presented with bilateral leg weakness and increasing difficulty in walking, requiring aide. He also complained of bilateral pitting oedema. All of his symptoms started in early September 2010 and had progressed over the previous 2-3 weeks. This patient also noticed a change in vocal tone, palpitations and wasting.

He was previously fit and well, enjoying regular sport, with no alcohol, drug or cigarette consumption recorded. He was a Muslim and had completed Ramadan at the same time as symptoms commenced.

Cardiovascular examination revealed a tachycardia of 90bpm, a blood pressure of 130/80, an ejection systolic murmur and bilateral peripheral oedema that extended to his ankles. Bilateral muscle wasting of the quadriceps was observed. On neurological examination a reduced power in both legs (~3/5) and hand grasp was evident with difficulty in standing from squatting position. Sensory loss in the lower limbs was in a glove and stocking like distribution. Reflexes were reduced in both knees and ankles. Urine dipstick analysis did not reveal any abnormalities and rapid detection test for *Plasmodium falciparum* was negative.

On commencing treatment with intramuscular thiamine (100mg) oedema resolved within two days and power gradually improved over the course of a month. The patient felt that all symptoms were completely resolved on follow-up review in two months. The same treatment was given as for the other patients (intramuscular thiamine 100mg for five days followed by one month of 10x daily multivitamin and vitamin B complex supplementation).

Telephone consultation in October 2011 revealed a relapse of symptoms in September 2011, again post Ramadan fasting. These were promptly treated with intramuscular thiamine and recovery was observed within two weeks.

Case 4

A 24 year old Muslim male farmer presented with complaints of bilateral leg and facial swelling. The symptoms had been progressive over the previous month with an associated feeling of fullness, difficulty in breathing on lying flat and palpitations. The patient also said he had felt a mild temperature at the time of onset which had passed of its own accord. The symptoms started in mid-september. There was no past medical

history or family history of medical problems. He was a non-smoker who denied alcohol or drug intake. He conducted moderate amounts of physical exercise laboring in the farm over the previous three months.

The patient was found to be in high cardiac output failure with a tachycardia (96 bpm), a BP of 120/70, and had a systolic murmur. Bibasal inspiratory crepitations were heard on pulmonary auscultatio. Oedema to the thighs, with accompanying ascitis and facial swelling present. There was no blood or protein on urinalysis.

Treatment was commenced as for the other cases with intramuscular thiamine (100mg) injections. Five days of once daily injections followed by x5 per day multivitamins with x5 daily vitamin B complex for one month accompanied by dietary advise. Dramatic improvement of oedema was noticed at the second day of treatment with complete resolution of symptoms and signs at one month review. The patient remained asymptomatic at six month and one year follow-up (one year follow-up conducted by telephone interview).

Gantongo

Case 5

A 29 year old farmer, father of two, presented with leg and facial oedema that had been progressive since the last week of August. He found it difficult to sleep at night with paroxysmal nocturnal orthopneoa and was unable to exert himself without shortness of breath. He reported of a similar episode one year previously that lasted until the january and then gradually imporved. During that time period he had sought medical help in Bissau where an echocardiogram was performed and diagnosis of cardiomyopathy made. During this time, treatment with furosemide was commenced but there was little improvement after a month and so the patient stopped buying medication.

Examination findings included tachycardia 110 bpm, a slightly low blood pressure of 110/60 and a load systolic murmur with a raised jugular venous pressure. Bilateral pitting oedema to the thighs were felt and bibasal crepitations could be heard on pulmonary auscultation.

Thiamine 100mg injections were commenced with dramatic improvement in leg oedema noticed within 72 hours, injections were therefore continued for a further three days and a month long vitamin supplementation was provided along with dietary advise. The patient remained asymptomatic at monthly review for 6 months and did not report any symptoms at one year review by telephone consultation (October 2011).

Case 6

Another male resident of the same village of 21 years of age presented with similar symptoms of bilateral leg oedema which had started before the end of Ramadan, in August 2010. He was an occasional smoker, denied alcohol intake and the usage of other medications. This was the first time he had noticed such symptoms, and stated the onset of illness was preceded by a febrile illness for which he took coartem and paracetamol three weeks prior.

Examination findings were similar to the other cases described with cardiac features including tachycardia (98bpm), a heart murmur, and pitting oedema extending above the knees. No investigations were performed.

Intramuscular thiamine 100mg was administered with good clinical response the following day, and thus five days of injections and a months course of oral supplementation was provided as in previous cases. The patient remained asymptomatic for six months, however was not available at one year follow up (migration to Bissau, with change of telephone number).

With the above six cases recorded and the dramatic response to thiamine

treatment observed, a diagnosis of thiamine deficiency related cardiopathy and polyneuropathy (or "wet" and "dry" beriberi) was made. Given the prevalence and timing of the outbreak, an alert was raised with the local director of health with recommendation made to alert the Ministry of Health and the regional department of the World Health Organization. An awareness campaign was triggered locally with all nurses involved in providing consultations in the community as well as the healthcare workers at Bolama Hospital being informed of the outbreak, its cause, symptoms and signs to be aware of and treatment of suspected individuals. Following this a number of further cases were reported by the responsible healthcare workers with reported dramatic responses to treatment (described below). However, these can not be confirmed as I did not personally see any of the cases.

Other cases

The nurse responsible for the Gantongo community reported seven cases with similar features in the community. All were described as having symptoms and signs consistent with cardiopathy type thiamine deficiency (or wet beriberi). All were Muslim and had fasted during the month of Ramadan. Unfortunately one of these cases (male 25 years old) died prior to awareness of the cause of the heart failure had been identified (September 2010). He had been unsuccessfully treated with intravenous frusemide. The other cases were five males in their late twenties and one elderly female. All had symptoms and signs of heart failure with peripheral oedema and shortness of breath being the predominant features. The five males described a brief period of symptoms with onset in late August - early September. The elderly female gave a longer history of symptoms, starting in November 2009. All were promptly treated with thiamine injections and a rapid response was observed in the five males, with little benefit seen in the other case (elderly female) suggesting another cause for her cardiopathy.

In the town of Bolama, prior to awareness of the outbreak was raised, one hypertensive female (42 years) with a short history of profound oedema and shortness of breath had been admitted for intravenous frusemide therapy on 30th of August 2010,

illness progressed and the lady died four days later. A further woman of 20 years of age died of “heart failure” according to the medical doctor responsible for the hospital one week post-partum. The ethnic and religious backgrounds as well as past medical history of both cases could not be ascertained.

In Summary of cases

Six confirmed cases of beriberi (4 with cardiac features, one with neurological features and one with a mixed presentation) were observed and successfully treated in the communities of Gã Mindjor and Gantongo during consultations conducted in September and October 2010. Table 3 summarizes the principle clinical manifestations of each case.

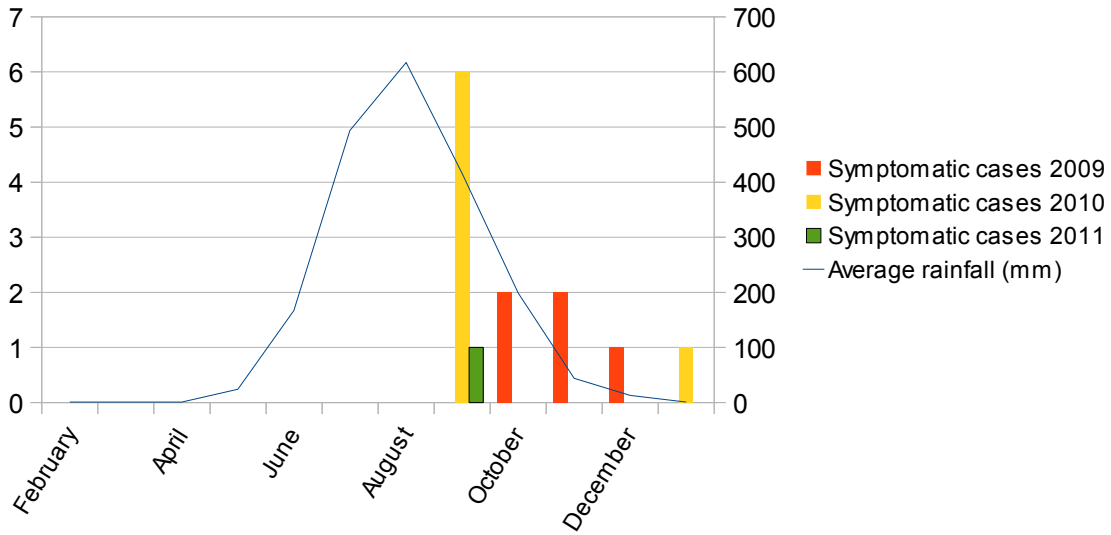
Case	Age	Peripheral Oedema	Palpitations	Heart Murmur	Tachycardia	Dyspnoea	Peripheral neuropathy	Muscle wasting
1	23	+	+	+	+	+		
2	37						+	+
3	20	+	+	+	+		+	+
4	24	+	+	+	+	+		
5	29	+		+	+	+		
6	21	+		+	+			

Table 3: Clinical manifestations of the reported cases

Treatment of five once daily thiamine 100mg injections followed by 10mg of the vitamin B1 oral supplementation (combination of vitamin B compound and multivitamin) for a month was given to the cases reported and dietary advise provided. Side effects to the injections included headache and polyuria (expected) and were reported by all patients. Alerts were raised to local healthcare workers and the regional director of health for further dissemination to the national healthcare authorities. Telephone consultations one year post outbreak, soon after Ramadan (September/October 2011) with previous cases as well as the medical doctor conducting consultations revealed that case 3 reported recurrence that responded rapidly to thiamine

treatment once again. The graph (1) below plots the correlation between annual rainfall and symptom occurrence along with the dates of Ramadan (rainfall figures obtained from reference 17).

Graph 1: Correlation of cases with annual rainfall



Note: Ramadan; 2009 (22 Aug-20 Sept) 2010 (11 Aug- 10 Sept) 2011 (1- 30 Aug)

A further nine unconfirmed cases of which five reported positive responses to treatment and three died prior to treatment were reported within the region. However, these cases were not directly observed by the author.

All cases were Muslims who had observed Ramadan with a period of a month of daylight fasting. The district of Bolama-Bijagos where the consultations were conducted served a mixed community of Muslims, Christians (Catholic and Protestant) and Animists. No cases were found in the non-Muslim populations (which accounted for approximately half of the consultations).

DISCUSSION

Whilst epidemics of thiamine deficiency related conditions have been reported in neighbouring countries[47,62], this article appears to be the first to document such cases in Guinea-Bissau. The poor country, with its fragile infrastructure[9,13] and weak healthcare system[10], may contain many undiagnosed or marginally deficient people with those being identified merely reflecting the "tip of the iceberg". The staple food is rice which is either imported and polished or locally produced. The rainy season, male sex, age, physical activity and the popular consumption of "Warga" (a sweet tea which is consumed throughout the day), are previously documented risk factors[1,2] for the development of TD that were present in the cases.

Ramadan is one of the most celebrated and important events in the Islamic world, observed by hundreds of millions annually[2]. Given that food can be consumed both before and after sunlight, it is not believed to have much effect on the health or nutrition of its participants[20, 21]. However, the little research that has been done on body composition and nutritional intakes of healthy volunteers suggests slight reductions in bodyweight and nutrient intake[21]. These studies were conducted in small group numbers and healthy volunteers of more affluent, well-educated countries which may have an effect on the results. Given the timing of the cases (all within two weeks of completion of Ramadan), religion of all cases (Muslim), and the absence of disease in the non-Muslim population, this report also implicates fasting during Ramadan as a potential risk factor for developing TD. To the authors knowledge this would be the first article to describe this to be the possible cause or aggravating factor in the development of a micronutrient deficiency. It should be noted that the link could be purely coincidental given that the rainy season was a concomitant risk factor of that time. Thiamine status (TPPE/ETKA) of people during Ramadan compared to controls of similar socio-economic background in Guinea-Bissau would support or reject this theory.

Were it found to be a risk factor, specific health interventions aimed at the Muslim community during the holy month could be employed to prevent future

outbreaks. For instance, focused nutritional education forums could be held after or before prayer during the month, or vitamin distribution could be coordinated by local religious leaders to be taken daily before sunrise.

The discovery of thiamine and its casual association with beriberi early in the 20th century led to huge reductions in the prevalence of the disease and resulted in a loss of interest to much of the scientific community[2]. Whilst the structure and metabolic function of the vitamin are described and treatment of its deficiency well known, some old and new questions remain. Namely, the aetiology of why some develop symptoms whilst others remain apparently healthy, and what exactly predisposes to cardiac or polyneuropathic development is not fully understood. Genetic predisposition is likely to be of significance as are the concomitant intakes of other nutrients and carbohydrates and duration of deficiency[70].

TPPE and ETKA are the most widely used tests of thiamine status with best sensitivity and specificity ratios[2,49]. However, concerns over the sensitivity of these tests have been raised in chronically deficient and infantile cases[49]. The relatively sophisticated laboratory and skilled technical staff required render these tests out of reach for poorer countries where dificiency is most likely[2]. A novel, simple and rapid test would be of great benefit for such communities.

Meanwhile, diagnosis relies on clinical suspicion and can be confirmed by response to treatment. This raises the importance of education and awareness raising within the medical and healthcare worker communities in regions susceptible to deficiency. In Guinea-Bissau all patients presenting with symptoms that could be caused by TD should be trialled with vitamin B1 treatment along with other medication as indicated.

Long term prevention of deficiency would likely be achieved by a combination of public health interventions including fortification schemes, nutritional health education, improving living standards and food availability[1,2]. These are all large scale

projects that would require significant financial and political assistance; therefore a true description of the problem in Guinea-Bissau is needed to secure any additional support.

A number of limitations to the study need to be addressed. Unfortunately due to financial and logistical constraints, further investigation of possible cases identified by healthcare workers in near by locations could not be validated. Ideally, screening of further afield healthcare centres would have provided a clearer picture of the extent of the outbreak nationally. If diagnostic material were available, all of the subjects and other community members could have been tested for biochemical confirmation of thiamine deficiency (TPPE or ETKA). Performing other routine tests including blood film (for malaria), Hb and white cell count, renal profiles, stool microscopy/culture as well as HIV testing to see if there was other associated disease that put the people at increased risk, would have also been insightful.

Review of the subject in this article has not looked at some other conditions including Alzheimer disease and colon cancer that have recently been related to thiamine deficiency and treatment[22].

The potential association of malaria and TD is very interesting given the prevalence and mortality of this parasitic disease globally. It appears logical that thiamine levels will deplete during malarial attack as there is an increased basal metabolic rate with pyrexia[36]. However, it needs to be confirmed that this is the case and not that already low thiamine levels predispose to malarial infection. Either way, the evidence suggests that routine supplementation of vitamin B1 to patients presenting with a malarial infection may be advisable, and that in severe (cerebral) infection parental thiamine may be warranted[40].

The suggested negative long term effects on neuropsychological development observed by researchers following the Israeli thiamine deficient formula-fed infants is also of great interest. This study suggests that even patients who did not display symptoms or signs of TD, but were known to have been exposed to the milk, showed

clinically significant delays in lingual development five years post incident[33]. Would that imply that communities where there is chronic suboptimal thiamine intake (such as may be the case in the Bolama region) are at risk of neurodevelopmental delay? Would mass and early long term supplementation of vitamin B1 have an effect on linguistic development? I believe these questions are also of importance and merit further investigation as the potential results may influence national food and health policy.

CONCLUSION

Beriberi is an easily treatable and yet potential fatal vitamin deficiency that was once a great burden in many parts of the world. Whilst clinical diagnosis in the adult can be relatively simple to the trained clinician, that in the infant and that of marginal deficiency requires analyses that are beyond the scope of most resource poor countries.

These case reports identify rural fishing communities in which thiamine deficiency appears prevalent and where small changes in lifestyle or diet are enough to push some into clinical manifestation of the deficiency. In Guinea-Bissau, where Islam is followed by approximately half the population, the cases described all appear to have developed symptoms during or soon after the month of Ramadan. Whether this is merely coincidental or contributory remains unclear and may warrant further investigation.

The problem of TD may be addressed through a number of public health interventions including mass multivitamin supplementation, food fortification, food diversity promotion in agriculture, health education and healthcare worker training. It is likely that a combination of these methods would be most efficient in the short and longer term.

Further investigation into the true prevalence of TD in the country together with the relationship with long term neurodevelopmental delay and malaria prognosis is also needed to better define the true extent of the deficiency and its burden on the population.

REFERENCES

1. McCandless DW. *Thiamine Deficiency and Associated Clinical Disorders*. Humana Press 2010
2. Thurnham DI. Thiamin. In: Caballero B, Allen L, Prentice A eds. *Encyclopedia of Human Nutrition*. 2nd edition. San Diego, USA. Elsevier academic press. 2005. vol 4: p263-278
3. World Health Organization. *Thiamine deficiency and its prevention and control in emergencies*. http://helid.digicollection.org/en/d/Js2900e/8.2.html (accessed 31st August 2010)
4. Berdanier CD, Dwyer J, Feldman EB eds. *Handbook of Nutrition and Food*. 2nd edition. USA. CRC Press. 2008
5. Lonsdale D. A review of the biochemistry, metabolism and clinical benefits of thiamin(e) and its derivatives. *Evidence based Contemporary and Alternative Medicine* 2006;3(1):49-59.
6. Fattal-Valevski A, Kesler A, Sela BA, Nitzan-Kaluski D, Rotstein M, Mesterman R, Toledano-Alhadef H, Stolovitch C, Hoffmann C, Globus O, Eshel G. Outbreak of life-threatening thiamine deficiency in infants in Israel caused by a defective soy-based formula. *Pediatrics* 2005;115(2):e233-8.
7. Rolfe M. Beri-beri: 'Endemic amongst urban Gambians. *Africa Health* 1994;16(3):22-3.
8. Lima HC, Porto EA, Marins JR, Alves RM, Machado RR, Braga KN, de Paiva FB, Carmo GM, Silva e Santelli AC, Sobel J. Outbreak of beriberi in the state of Maranhão, Brazil: revisiting the mycotoxin aetiologic hypothesis. Tropical Doctor 2010;40(2):95-7.
9. US Department of State Diplomacy in Action. *Background note: Guinea-Bissau.* http://www.state.gov/r/pa/ei/bgn/5454.htm (accessed 1st October 2011)
10. World Health Organization. *World Health Statistics 2010*. http://www.who.int/whosis/whostat/EN_WHS10_Part2.pdf (accessed 9th September 2011)
11. El-Hourani HM, Atoum MF. Body composition, nutritient intake and physical

activity patterns in young women during Ramadan. *Singapoure Medical Journal* 2007;48(10):906-10.

12. Maughan RJ, Bartagi Z, Dvorak J, Zerguini Y. Dietary intake and body composition of footballers during the holy month of Ramadan. *Journal of Sports Sciences* 2008;26(S3):S29-38
13. British Broadcasting Corporation. Guinea-Bissau Profile. http://www.bbc.co.uk/news/world-africa-13444866 (accessed 26th of August 2011)
14. Aaby P. Is susceptibility to severe infection in low-income countries inherited or acquired? *Journal of Internal Medicine* 2007;261(2):112-122.
15. Colombatti R, Coin A, Bestagini P, Vieira CS, Schiavon L, Ambrosini V, Beritinato L, Zancan L, Riccardi F. A Short-term intervention for the treatment of severe malnutrition in a post-conflict country: results of a survey in Guinea-Bissau. *Public Health Nutrition* 2008;11(12):1357-64
16. Adamolekun B, Adamolekun WE, Sonibare AD, Sofowora G. A double-blind, placebo-controlled study of the efficacy of thiamine hydrochloride in a seasonal ataxia in Nigerians. *Neurology* 1994;44(3):549-51.
17. Climate forecaster. *Climate and Temperature of Guinea-Bissau.* http://www.climatetemp.info/guinea-bissau/ (accessed 19th October 2011).
18. Kaestal P, Michaelsen KF, Aaby P, Friis H. Effects of prenatal multimicronutrient supplements on birth weight and perinatal mortality: a randomised, controlled trial in Guinea-Bissau. *European Journal of Clinical Nutrition* 2005;(59):1081-89.
19. Gonçlaves A, Ferrinho P, Aguiar P. Descriptive and comparative analysis on the effect of characterization factors on anthropometric indicators in a population from Guinea-Bissau. *Acta Médica Portuguesa* 2001;14(3):323-9.
20. Rashid H. Ramadan fasting and breast milk. Breastfeeding Medicine 2007;2(1):59-60.
21. Rakicioglu N, Samur G, Topcu A, Topcu AA. The effects of Ramadan on maternal nutrition and composition of the breast milk. *Pediatrics International* 2006;48(3):278-83.

22. Depeint F, Bruce BW, Shangari N, Mehtaq R, O'Brein PJ. Mitochondrial function and toxicity: Role of the B vitamin on mitochondrial energy metabolism. *Chemico-Biological Interactions* 2006;163:94-112.
23. Adamolekun B. Neurological disorders associated with cassava diet: a review of putative etiological mechanisms. *Metabolic Brain Disease* 2011;26(1):79-85.
24. Said HM. Intestinal absorption of water-soluble vitamins in health and disease. *The Biochemical Journal* 2011;437(3):357-72.
25. Halsted CH. Absorption of water-soluble vitamins. *Current Opinion in Gastroenterology* 2003;19(2):113-7.
26. Wooley JA. Characteristics of thiamin and its relevance to the management of heart failure. *Nutrition in clinical practice* 2008;23(5):487-93.
27. Monograph. Thiamine. *Alternative Medicine Review* 2003;(1):59-62.
28. Ba A. Metabolic and structural role of thiamine in nervous tissues. *Cellular and Molecular Neurobiology* 2008;28:923-931.
29. Kawano H, Hayashi T, Koide Y, Toda G, Yano K. Histopathological changes of biopsied myocardium in Shoshin beriberi. *International Heart Journal* 2005; 46(4):751-9.
30. Carpenter KJ. Acute versus marginal deficiencies of nutrients. *Nutrition Reviews* 2002;60(9):277-80.
31. Adamolekun B, Ibikunle FR. Investigation of an epidemic of seasonal ataxia in Ikare, western Nigeria. *Acta Neurologica Scandinavica* 1994;90(5):309-11.
32. Adamolekun B, Ndububa DA. Epidemiology and the clinical presentation of a seasonal ataxia in western Nigeria. *Journal of neurological sciences* 1994;124(1):95-8.
33. Fattal-Valevski A, Azouri-Fattal I, Greenstein YJ, Guindy M, Blau A, Zelnik N. Delayed language development due to thiamine deficiency. *Developmental Medicine and Child Neurology* 2009;51(8):629-34.
34. Fattal I, Friedmann N, Fattal-Valevski A. The crucial role of thiamine in the development of syntax and lexical retrieval: a study of infantile thiamine deficiency. *Brain* 2011;134 (6):1720-1739.
35. Krishna S, Taylor AM, Supanaranond W, Pukrittayakamee S, ter Kuile F, Tawfiq

KM, Holloway PAH, White NJ. Thiamine deficiency and malaria in adults from southeast Asia. *Lancet* 1999;353(9152):546-549.

36. Mayxay M, Taylor AM, Khantavong M, Keola S, Pongvongsa T, Phompida S, Phetsouvanh R, White NJ, Newton PN. Thiamin deficiency and uncomplicated falciparum malaria in Laos. *Tropical Medicine & International Health* 2007; 12(3):363-9.

37. Bates CJ, Powers HJ, Lamb WH, Gelman W, Webb E. Effect of supplemetary vitamins and iron on malaria indices in rural Gambian children. *Transaction od the Royal Soceity of Tropical Medicine and Hygiene* 1987;81(2):286-91.

38. Ortega RM, Martínez RM, Andrés P, Marín-Arias L, López-Sobaler AM. Thiamin status during the third trimester of pregnancy and its influence on thiamin in concentrations in transition and mature breast milk. *British Journal of Nutrition* 2004;92:129-135.

39. Padilha EM, Fujimori E, Borges AL, Sato AP, Gomes MN, Branco Mdos R, Santos HJ, Lermen Junior N. [Epidemiological profile of reported beriberi cases in Maranhão State, Brazil, 2006-2008]. *Cadernos de Sáude Pública* 2011;27(3):449-5.

40. Tang CM, Rolfe M, Wells JC, Cham K. Outbreak of beri-beri in The Gambia. *Lancet* 1989;22(2):206-7.

41. Vimokesant SL, Hilker DM, Nakornchai S, Rungruangsak K, Dhanamitta S. Effects of betel nut and fermented fish on the thiamin status of northeastern Thais. *American Jounral of Clinical Nutrition* 1975;28(12):1458-63.

42. Vimokesant S, Kunjara S, Rungruangsak K, Nakornchai S, Panijpan B. Beriberi caused by antithiamin factors in food and its prevention. *Annals of the New York Academy of Scieinces* 1982;123-36.

43. Luxumberger C, White NJ, ter Kuile F, Singh HM, Allier-Frachon I, Ohn M, Chongsuphajaisiddhi T, Nosten F. Beri-beri: the major cause of infant mortality in Karen refugees. *Transactions of the Royal Soceity of Tropical Medicine and*

Hygiene 2003;97(2):251-5.

44. Khounnorath S, Chamberlain K, Taylor AM, Soukalou D, Mayxay M, Lee SJ, Bounthom P, Luangxay K, Sisouk K, Soumphonphakdy B, Latsavong K, Akkhavong K, White NJ, Newton PN. Clinically unapparent infantile thiamin deficiency in Vientiane, Laos. *PloS Neglected Tropical Diseases* 2011;5(2):969.

45. Muri RM, Von Overbeck J, Furrer J, Ballmer PE. Thiamin deficiency in HIV-positive patients: evaluation by erythrocyte transketolase activity and thiamin pyrophosphate effect. *Clinical Nutrition* 1999;18(6):375-8.

46. Nail PA, Thomas R, Eakin R. The effect of thiamin and riboflavin supplementation on the level of those vitamins in human breast milk and urine. *American Journal of Clinical Nutrition* 1980;33:198-204.

47. Rao SN, Chandak GR. Cardiac beriberi: Often a missed diagnosis. *Journal of Tropical Pediatrics* 2010;56(4):284-5.

48. Soukaloun D, Kounnavong S, Pengdy B, Boupha B, Durondej S, Olness K, Newton PN, White NJ. Dietary and socio-economic factors associated with beriberi in breastfed Lao infants. *Annals of Tropical Paediatrics* 2003;23(3):181-6.

49. Soukaloun D, Lee SJ, Chamberlain K, Taylor AM, Mayxay M, Sisouk K, Soumphonphakdy B, Latsavong K, Akkhavong K, White NJ, Newton PN. Erythrocyte transkytolsae activity, markers of cardiac dysfunction and the diagnosis of infantile beriberi. *PloS Neglected Tropical Diseases* 2011;5(2): e971

50. Google maps. *Bolama, Guinea-Bissau http://g.co/maps/ws835* (accessed 31st of September 2011)

51. *British National Formulary.* 61st edition 2011

52. Papazian O, Duenas D, Tuchman R, Baquero J, Butler k, Alfonso I, et al. Cuban Embargo. *Neurology* 1995;45(5):1033.

53. Epidemic neuropathy in Cuba: A plea to end the United States economic embargo on a humanitarian basis. *Neurology* 1994;44:1784.

54. Roman GC. An epidemic in Cuba of optic neuropathy, sensorineural deafness, peripheral sensory neuropathy and dorsolateral myeloneuropathy. *Journal of Neurological Sciences* 1994;127(1):11-28.

55. Andersen GS, Friis H, Michaelsen KF, Rodrigues A, Benn CS, Aaby P, Kaestel P. Effects of maternal micronutrient supplementation on fetal loss and under-2-years child mortality: long-term follow-up of a randomised controlled trial from Guinea-Bissau. *African Journal Reproductive Health* 2010;14(2):17-26.

56. Hardy A. Beriberi, vitamin B1 and World food policy 1925-1970. *Medical Historv* 1995;39:61-77.

57. Kamien M. The repeating history of objections to the fortification of bread and alcohol: from iron filings to folic acid. *The Medical Journal of Australia* 2006;184(12):638-40.

58. Steyn NP, Wolmarans P, Nel JH, Bourne LT. National fortification of staple foods can make a significant contribution to micronutrient intake of South African adults. *Public Health Nutrition* 2008;11(3):307-13.

59. Doung-ngern P, Kesornsukhon S, Kanlayanaphotporn J, Wanadurongwan S, Songchitsomboon S. Beriberi outbreak among commercial fishermen, Thailand 2005. *Southeast Asian Journal of Tropical Medicine and Public Health* 2007;38(1):130-5.

60. McGready R, Simpson AJ, Cho T, Dubowitz L, Changbumrung S, Bohm V, Munger RG, Sauberlich HE, White NJ, Nosten F. Postpartum thiamine deficiency in a Karen displaced population. *American Journal of Clinical Nutrition* 2001;74(6):808-13.

61. Ahoua L, Eteinne W, Fermon F, Godain G, Brown V, Kadjo K, Bouaffou K, Legros D, Guerin PJ. Outbreak of beriberi in a prison in Côte d'Ivoire. *Food Nutrition Bulletin* 2007;28(3):283-90.

62. Rolfe M, Walker RW, Samba KN, Cham K. Urban beri-beri in The Gambia, west Africa. *Transactions of the Royal Soceity of Tropical Medicine and Hygiene* 1993;87(1):114-5.

63. Darcel F, Roussin C, Vallat JM, Charlin C, Tournebize P, Doussiet E. [Polyneuropathies in vitamin B1 deficiency in Reunion and Mayotte islands in 70 patients of Maori and Comorian descent]. *Bulletin de la Société de Pathologie Exotique* 2009;102(3):167-72.

64. Cerroni MP, Barrado JC, Nobrega AA, Lins AB, da Silva IP, Mangueira RR, da Cruz RH, Mendes SM, Sobel J. Outbreak of beriberi in an Indian population of the Amazon region, Roraima State, Brazil, 2008. *American Journal of Tropical Medicine and Hygiene* 2010;83(5):1093-7.

65. Rosa CA, Keller KM, Oliveira AA, Almeida TX, Keller LA, Marassi AC, Kruger CD, Deveza MV, Monteiro BS, Nunes LM, Astoreca A, Cavaglieri LR. Direito GM, Eifert EC, Lima TA, Modernell KG, Nunes FI, Garcia AM, Luz MS, Oliveira DC. Production of citreoviridin by Penicillium citreonogrum strains associated with rice consumption and beriberi cases in the Maranhão State, Brazil. *Foo Addditives and Containment. Part A Chemistry, Analysis, Control, Exposure and Risk Assessment* 2010;27(2):241-8.

66. Nzwalo H. The role of thiamine deficiency in konzo. *Journal of Neurological Sciences* 2011;302(1-2):129.

67. Borrajero I, Pérez JL, Domínguez C, Chong A, Coro RM, Rodríguez H, Gómez N, Román GC, Navarro-Román L. Epidemic neuropathy in Cuba: morphological characteriszation of peripheral nerve lesions in sural nerve biopsies. *Journal of Neurological Sciences* 1994;127(1):11-28.

68. Marcias-Matos C, Rodriguez-Ojea A, Chi N, Jimenez S, Zulueta D, Bates CJ. Biochemical evidence of the thiamine depletion during the Cuban neuropathy epidemic, 1992-1993.*American Journal of Clinical Nutrition* 1996;64(3):347-53.

69. Vikhanski L. Fatal flaw in baby formula sparks reform in Israeli ministry. *Nature Medicine* 2004;10(1):7.

70. Kesler A, Stolovitch C, Hoffmann C, Avni I, Morad Y. Acute opthalmoplegia and nystagmus in infants fed a thiamine-deficient formula: an epidemic of Wernicke encephalopathy. *Journal of Neuroopthalmology* 2005;25(3):169-72.

71. Siegel-Itzkovich J. Police in Israel launch investigation into deaths of babies given formula milk. *British Medical Journal* 2003;327(7424):1128.

72. Prensky AL. Wernicke encephalopathy in infants. *Journal of Neuroopthalmology* 2005;25(3):167-8.