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List of PhD Papers

1. **Paper I:** Sanne Sigh, Nanna Roos, Daream Sok, Bindi Borg, Chhoun Chamnan, Arnaud Laillou, Marjleine A. Dijkuizen and Frank. T. Wieringa. Development and acceptability of locally made fish-based ready-to-use products for the prevention and treatment of malnutrition in Cambodia. First published online 9th August, *Food and Nutrition Bulletin* 2018;39(3):420–434.
2. **Paper II:** Sanne Sigh, Nanna Roos, Chhoun Chamnan, Arnaud Laillou, Sophanneary Prak and Frank T. Wieringa. Effectiveness of locally produced, fish-based food product on weight gain among Cambodian children in the treatment of acute malnutrition: A randomized controlled trial. *Nutrients* 2018;10(7):909–925.
3. **Paper III:** Sanne Sigh, Lotte Lauritzen, Frank T. Wieringa, Arnaud Laillou, Chhoun Chamnan, Nheb Angkeabos, Duch Moniboth, Jacques Berger, Ken D. Stark and Nanna Roos. Whole blood polyunsaturated fatty acids and associations with markers of nutrition and health status in acutely malnourished children in Cambodia. Submitted to the *British Journal of Nutrition*. Manuscript id: BJN-RA-19-0090.

Related work

The following publication is not included in this PhD thesis:

1. Bindi Borg, Seema Mirshahi, Arnaud Lailou, **Sanne Sigh**, Daream Sok *et al.* Development and testing of locally-produced ready-to-use therapeutic and supplementary foods (RUTFs and RUSFs) in Cambodia: lessons learned. Under review in *BMC Public Health* (PUBH-D-18-04290).

Oral and poster presentations (academic and non-academic audience) related to the dissemination of the results of this PhD research and the PhD research itself:

1. **Sanne Sigh** (3rd-4th March 2015). *Effectiveness of locally produced RUTF for management of severe acute malnutrition in Cambodian children*. Poster presentation at the national nutrition conference, Phnom Penh, Cambodia.
2. **Sanne Sigh** (18th November 2016). *Acceptability and efficacy of locally-produced ready-to-use foods in Cambodia*. Oral presentation together with Bindi Borg and Daream Sok (PhD Fellows), at the acute malnutrition course, Kampala, Uganda.
3. **Sanne Sigh** (22nd February 2017). *The NumTrey effectiveness study of the treatment of severe acute malnutrition*. Oral presentation at the dissemination workshop on the findings from the locally produced food supplements, Phnom Penh, Cambodia
4. **Sanne Sigh**, Nanna Roos, Chhoun Chamnan, Arnaud Lailou, Sophanearay Prak and Frank Wieringa (5th-8th December 2017). *Can fish replace milk powder in products for the treatment of severe acute malnutrition in Cambodia?* Poster presentation at the global workshop: Nutrition-sensitive fish argi-food systems, Siem Reap, Cambodia.
5. **Sanne Sigh** (10th October 2017). *The effectiveness of NumTrey in the treatment of severe acute malnutrition*. Oral presentation at the French Institute for Development, Montpellier, France.
6. **Sanne Sigh** (1st-2nd March 2018). *Management of severe acute malnutrition in Cambodian children 5-59 months*. Invited speaker at the 14th Lao Pediatric continuing medical education conference, challenges in prevention of childhood morbidity and mortalities, Vientiane, Laos PDR.
7. **Sanne Sigh**, Nanna Roos *et al.* (28th-30th November 2018). *Can fish replace milk powder in products for the treatment of severe acute malnutrition in Cambodia?* Poster presented as a flyer at the accelerating the end of hunger and malnutrition, Bangkok, Thailand.

English summary

Introduction

Undernutrition has been estimated to cause 3.1 million child deaths annually. Severe acute malnutrition (SAM) is a life-threatening condition requiring immediate treatment. Therapeutic foods are used in hospital treatment of SAM - where clinical complications are present -, and Ready-to-Use Therapeutic Foods (RUTFs) are used in outpatient treatment for SAM. Approximately 2% of all children under 5 years suffer from SAM in Cambodia. Commonly used imported RUTFs are not well accepted, which may compromise SAM treatment in Cambodia. A collaborative initiative led by the French National Research Institute for Sustainable Development and UNICEF Cambodia, together with the University of Copenhagen and national partners, initiated the development of a locally produced product in 2013. An initial version of a locally produced product, a fish-based paste, was pilot-tested in pre-school children in 2014.

Polyunsaturated fatty acids (PUFAs) are important for children's growth, as well as cognitive and brain development. Children with SAM might be vulnerable to insufficient intakes of PUFA caused by a low food intake. Children with SAM often suffer from inflammation and other health challenges which impact the composition of blood cells. When PUFAs are measured in whole blood - a mixture of cells and plasma - any changes in blood cells may have implications for the interpretation of whole blood PUFA status.

The aim of this PhD thesis was to optimise the initial fish-based paste to a RUTF and to assess the acceptability of two versions of the product in Cambodian children and their caregivers. Furthermore, effectiveness of the fish-based RUTF was assessed in children during SAM treatment, in comparison with a standard milk-based RUTF. Lastly, whole blood PUFA status and their potential associations with markers of nutritional and health status were assessed in children with SAM.

Methods

This PhD thesis includes three papers. **Paper I** report the optimisation of the pilot-tested paste. In a taste trial, the acceptability of two versions of the fish-based product, the 'NumTrey-Paste' - a pure paste - and 'NumTrey-RUSF' - a wafer filled with paste - was assessed in comparison with a milk-based RUTF (BP-100™). The taste trial was a non-blinded crossover study using sensory score

testing, which was followed by a ranking of the products. The trial was conducted among children age 6mo-18 years ($n = 52$) and their caregivers. The results were used to further modify the NumTrey-Paste into NumTrey-RUTF, a RUTF version of 'NumTrey-RUSF'. Last, longitudinal acceptability assessed every 2nd week during SAM treatment was reported.

The two additional papers were based on the SAM trial; a randomised, single-blinded, controlled trial. **Paper II** report the effectiveness of NumTrey-RUTF (intervention, $n = 60$), compared with BP-100TM (active control, $n = 61$), on weight gain (g/kg/day) in 6-59-month-old children receiving eight weeks home-based treatment for SAM. Secondary anthropometric outcomes were: changes in weight, height, mid-upper-arm circumference (MUAC) along with z-scores for weight-for-height (WHZ) and height-for-age (HAZ). Eligibility for SAM treatment was a WHZ ≤ -2.8 and/or a MUAC ≤ 115 mm. Children were scheduled for follow-up visits every 2nd week. Anthropometric measures were assessed using standard methods.

Paper III was a cross-sectional study on PUFA status, using baseline data from the SAM trial, and also including older children not included in **Paper II**. Whole blood fatty acid composition in children with SAM age 6mo-18 years ($n = 174$), and PUFAs potential association with markers of nutritional- and health status were assessed. Fatty acid composition was assessed in dried blood spot samples by the standard gas chromatography method. Their nutritional and health status were assessed by anthropometry and biochemical markers (haemoglobinopathies, C-reactive protein (CRP), α_1 -acid glycoprotein (AGP), and total blood cell count) using standard methods. Correlations were analysed using simple or multiple linear regression analysis.

Results

Paper I showed it was possible to optimise a fish-based paste to meet the specifications for RUTF products. In the taste trial, 21% of children categorised NumTrey-Paste as overall acceptable, compared with 43% for BP-100TM and 36% for the NumTrey-RUSF. There was no statistically significant difference in the proportion of children ranking the NumTrey-RUSF and BP-100TM as the product they "liked the most" (Pearson χ^2 test, $p > 0.05$). The acceptability evaluated by children receiving NumTrey-RUTF during 8 weeks treatment increased from 72% to 85%, whereas the acceptability for children receiving BP-100TM remained around 90% during the treatment.

Paper II found no statistically significant difference in mean weight gain between the two RUTFs (1.06 g/kg/day (95%CI: 0.72; 1.41) for BP-100TM and 1.08 g/kg/day, (95%CI: 0.75; 1.41) for NumTrey-RUTF). No statistically significant difference between the two RUTFs was found for weight, WHZ, HAZ or MUAC at endline. Statistical difference was found for height gain in favour of BP-100TM, from baseline to endline ($p=0.016$).

In **Paper III**, around 14% of the children appeared to have a very low PUFA status. Weight and wasting were not associated with any PUFAs. However, stunting and low height were consistently associated with low PUFAs. The presence of inflammation, anaemia, and haemoglobinopathies were not associated with any PUFAs. Elevated platelet counts were correlated with high levels of linoleic acid and appeared to be influenced by both anemia ($p=0.010$) and inflammation ($p=0.002$). Monocyte counts correlated with n-6- and n-3 long-chained PUFAs and appeared high during inflammation ($p=0.052$).

Conclusion

Small dried fish can be used in the development of a RUTF optimised to tastes and preferences in Cambodia, to be used in treatment of SAM. A taste trial confirmed that a pure paste-like RUTF is unlikely to succeed in Cambodia, whereas filling the paste into a wafer made the product as acceptable as a commercial milk-based RUTF among children and their caregivers. The effectiveness trial showed that neither BP-100TM nor NumTrey-RUTF were superior in weight gain after 8 weeks of home-based treatment of SAM. The results support that a wafer filled with a fish-paste, NumTrey-RUTF, is highly relevant and is a potential alternative to the imported milk-based RUTF currently used in treatment of SAM in Cambodia. Low PUFA levels was found in children with SAM. The associations between low PUFA levels and stunting needs further investigation. Lastly, elevated counts of platelet and monocyte were associated with PUFA levels, which is why future field studies assessing PUFA status using whole blood and dried blood spot tests should consider co-morbidities associated with SAM, there may change the cellular composition of whole blood.

Dansk resumé (Danish summary)

Baggrund

Underernæring er estimeret til at forårsage død af 3.1 millioner børn årligt. Svær akut underernæring (severe acute malnutrition, SAM) er en livstruende tilstand, som kræver øjeblikkelig behandling. Terapeutiske fødevarer anvendes i sygehusets behandling af SAM, hvor kliniske komplikationer er tilstede og klar-til-brug-terapeutisk fødevarer (Ready-to-Use Therapeutic Foods, RUTFs) anvendes i ambulant behandling af SAM. Omkring 2% af alle børn under 5 år lider af SAM i Cambodja. Almindelig anvendte importerede RUTF er ikke vel accepteret, hvilket kan have konsekvenser for behandlingen i Cambodja. Et samarbejde ledet af det Franske Nationale Forskningsinstitut for Bæredygtig Udvikling og UNICEF Cambodja sammen med Københavns Universitet og nationale partnere, igangsatte udviklingen af et lokalt produceret produktet i 2013. Den første udgave af et lokalt produceret produkt, et fiskebaseret smørlignede pasta (fremover benævnt; paste), blev testet blandt før-skole børn i 2014.

Flerumættede fedtsyrer (polyunsaturated fatty acids, PUFAs) er vigtig for børns fysike vækst, så vel som udviklingen af kognitionen og hjernen. Børn med SAM kan være sårbare overfor et utilstrækkelig indtag af PUFA. Børn med SAM lider ofte af inflammation og andre sundhedsmæssige udfordringer, hvilket kan påvirke sammensætningen af blodceller. Når PUFA er målt i fuldblod, der en blanding af celler og plasma, kan enhver ændring i blodcellerne påvirke fortolkningen af PUFA status.

Formålet med denne ph.d.-afhandling, var at optimere den første fiskebaserede paste til en RUTF, samt vurderer at vurdere om to versioner af det fiskebaseret produkt var acceptable blandt Cambodjanske børn og deres forsørgere. Ydermere var effekten af det fiskebaserede RUTF vurderet blandt børn i SAM behandling sammenlignet med et standard mælkbaseret RUTF. Slutteligt blev fuldblods PUFA status og deres potentiel association med markører af ernærings- og sundheds status vurderet i børn med SAM.

Metoder

Denne ph.d.-afhandlingen kombinerer tre artikler. **Artikel I** rapporterer om forbedringen af det prøve-testet (den originale) paste. I et smags studie undersøges smagsoplevelsen. To versioner af det fiskebaserede produkt, ('NumTrey-Paste', som er en ren paste, og 'NumTrey-RUSF' en sprød

vaffel fyldt med paste), sammenlignes med et mælkebaseret RUTF (BP-100TM). Smags studiet var et ikke-blindet krydsover-studie, som anvendte en sensorisk scoringstest, som var efterfulgt af en rangering af produkterne. Studiet var udført på børn mellem 6mdr.-18år ($n = 52$) samt deres forsørgere. Resultaterne var anvendt til yderligere at modificerer 'NumTrey-Paste' til 'NumTrey-RUTF', en RUTF udgave af NumTrey-RUSF. Sluttelig blev ændring i samagsoplevelsen vurderet hver anden uge i løbet af SAM behandling.

De to øvrige artikler var baseret på SAM studiet, et randomiseret enkelt-blindet kontrolleret studie. **Artikel II** rapportere effekten af NumTrey-RUTF (intervention, $n = 60$) sammenlignet med BP-100TM (aktiv kontrol, $n = 61$) i øgning af vægt (g/kg/dag) blandt 6-59mdr. gamle børn i løbet af 8 ugers hjemme-behandling af SAM. Sekundære antropometriske resultater var ændringer i vægt, højde, midt-overarm omkreds (mid-upper-arm circumference, MUAC) sammen med score af vægt-for-højde (weight-for-height z-score, WHZ) og højde-for-alder (height-for-age z-score, HAZ). Berettigelse til SAM behandling var en WHZ ≤ -2.8 og/eller MUAC ≤ 115 mm. Børnene havde planlagte opfølgings besøg hver anden uge. Antropometriske målinger blev evalueret ved anvendelsen af standardiseret metoder.

Artikel III var et tværnsnitsstudie på PUFA status baseret på baseline data fra SAM studiet som også inkluderer ældre børn som ikke er inkluderet i **Artikel II**. Fuldblods fedtsyre sammensætning i børn med SAM imellem 6mdr. -18år ($n = 174$), samt PUFA potentielle association med markører af ernærings- og sundhedsstatus blev vurderet. Fedtsyrer sammensætningen blev vurderet ud fra tørrede blod pletter ved anvendelse af en standard gaskromatografi metode. Deres ernærings- og sundhedsstatus blev målt ved antropometri og biokemiske markører (hæmoglobinpatier, C-reaktiv protein (CRP), α_1 -syre glycoprotein (AGP) samt total antal af blodceller) ved anvendelse af standardiseret metoder. Korrelationer blev analyseret ved anvendelse af simple og flere lineær regressions analyse.

Resultat

Artikel I viste at det var muligt at optimerer et fiskebaseret paste så det opfyldte FN's specifikationer for RUTF. I smags studiet, 21% af børnene kategoriserede NumTrey-Paste som overordnet acceptable sammenlignet med 43% for BP-100TM og 36% for NumTrey-RUSF. Der var ingen statistisk signifikant forskel i andelen af børn, som vurderede NumTrey-RUSF og BP-100TM som det produkt de "kunne lide bedst" (Pearson χ^2 test, $p > 0,05$). Accepten evalueret af børn

som modtog NumTrey-RUTF i løbet af 8 ugers behandling steg fra 72% til 85%, mens accepten blandt børnene som modtog BP-100TM forblev omkring 90% under behandlingen.

Artikel II viste ingen statistisk signifikant forskel i den gennemsnitlige øgning af vægt imellem de to RUTF (1,06 g/kg/dag (95%CI: 0,72; 1,41) for BP-100TM og 1,08 g/kg/dag (0,75; 1,41) for NumTrey-RUTF). Ingen statistisk signifikant forskel imellem de to RUTF blev fundet for vægt, WHZ, HAZ eller MUAC i slutning af studiet. Statistisk forskel var fundet for øgning af højde til fordel for BP-100TM fra starten til slutningen af studiet ($p=0,016$).

I **Artikel III** havde omkring 14% af børnene tilsyneladende et meget lavt PUFA status. Vægt og tyndhed (wasting) var ikke associeret med nogen PUFA'er, men lav HAZ (stunting) og lav højde var konsekvent associeret med lav PUFA. Tilstedeværelsen af inflammation, anæmi og hæmoglobinopati var ikke associeret med nogle PUFA'er. Forhøjede blodplader korrelerede med høje niveauer af linolsyre og var tilsyneladende påvirket af både anæmi ($p=0,010$) og inflammation ($p=0,002$). Antallet af monocytter korrelerede med n-6- og n-3 langkædede PUFA og de forekom tilsyneladende høje under tilstedeværelsen af inflammation ($p=0,052$).

Konklusion

Små tørrede fisk kan anvendes i udviklingen af et RUTF der er tilpasset smag og præferencer i Cambodja til behandlingen af SAM. Et smags studie bekræftede, at et rent paste-lignende RUTF sandsynligvis ikke kan lykkes i Cambodja, men hvis pasten var fyldt i en vaffel var produktet lige så acceptabelt som et kommerciel mælkebaseret RUTF iblandt børn og deres forsørgere. Effektivitetsstudiet viste, at hverken BP-100TM eller NumTrey-RUTF var mere effektiv i forhold til vægtøgning efter 8 ugers hjemmebaseret SAM behandling. Resultaterne støtter, at en vaffel fyldt med fiske-paste, NumTrey-RUTF, er særdeles relevant og det er et potentielt alternativ til det importerede mælkebaseret RUTF, der anvendes i behandlingen af SAM i Cambodja. Lav PUFA niveauer blev fundet hos børn med SAM. Associationerne imellem lav PUFA og stunting behøver yderligere undersøgelse. Slutteligt, et forhøjet antal af blodplader og monocytter var associeret med PUFA niveauer, derfor bør fremtidige studier der vurderer PUFA status ved anvendelsen af fuldblod og tørrede blod pletter overveje følgesygdomme associeret med SAM, som kan ændre den cellulære sammensætning af fuldblod.

1. Introduction

In this chapter, acute malnutrition, therapeutic foods, along with essential fatty acid status and deficiency are introduced. Lastly, the thesis objectives are presented.

Undernutrition is a major health concern throughout the developing world, resulting in increased morbidity and mortality. Undernutrition was estimated to be an underlying cause of 3.1 million child deaths in 2011, or 45% of all child deaths [1]. Besides a higher risk of death, children affected by undernutrition are also at increased risk of impaired cognitive development and chronic diseases later in life [1,2].

Several classifications of undernutrition exist; the most commonly used is the division of undernutrition into moderate or severe acute malnutrition (MAM or SAM, respectively). SAM is a critical condition requiring immediate treatment with specialised therapeutic foods, and - if complications are present- hospitalisation [3]. However, hospitalisation is at the expenses of the caregiver's work, the family and it predisposes the child to hospital-acquired infections [4,5]. These barriers and risks have been resolved with the introduction of community-based management of acute malnutrition and the use of ready-to-use therapeutic foods (RUTFs), which help to improve the survival of children with non-complicated SAM [5–7]. RUTFs are energy-dense products, providing all the nutrients required for rehabilitation of SAM [8,9], and are practical in settings with limited hygiene. Furthermore, the lower treatment cost in community-based management of SAM per child has meant that more children could be treated, and therefore more child lives saved.

Despite improvements in food security in Cambodia over the last decade, the prevalence of acute malnutrition remains high at around 9-12%, and has not changed since 2005 [10]. In 2008, there was an attempt in Cambodia to introduce the peanut-based-paste RUTF Plumpy`Nut[®], which is successfully used to treat SAM globally. However, the introduction of Plumpy`Nut[®] was unsuccessful, due to its low acceptability and uptake by the health system in Cambodia [11]. The Cambodian government is currently using the imported BP-100TM bar in SAM treatment [12], even though BP-100TM not appear to be well accepted (F.T. Wieringa, personal communication). The lack of access to a well-acceptable RUTF suggests that the current SAM treatment in Cambodia may not be optimal. Therefore, a locally produced RUTF - adapted to taste and preferences in Cambodia - has been urgently needed. In 2013, an initiative led by United Nations Children's Fund

(UNICEF), Cambodia, and the French National Research Institute for Sustainable Development (IRD), started the development of such a product, in collaboration with several Cambodian governmental institutions (the Ministry of Health; the Department of Fisheries Post-harvest Technology and Quality, the Fisheries Administration), Vissot, the National Pediatric Hospital and the University of Copenhagen, Denmark. The project “Development of locally produced, fish and lipid-based nutrient supplements for the treatment and prevention of malnutrition in Cambodia” aimed to develop two products: 1) a food supplement for preventing acute malnutrition (ready-to-use supplementary food (RUSF), and 2) a therapeutic RUTF for SAM treatment. In 2014, the first version of a locally produced, fish-based paste was piloted in pre-school children, assessing the acceptability of aforementioned paste compared to BP-100™. The pilot study found that both products had low acceptability and the local paste did not fulfil RUTF specifications for nutrition composition when it was tested [13]. This is why the locally produced, fish-based products needed to be optimised before assessing effectiveness.

Besides the importance of acceptability and the well-known effectiveness of commercial RUTFs, most focus in current research has been given to other factors such as diagnostic criteria and effectiveness of RUTFs for example by partly replacing milk powder with plant protein rather than polyunsaturated fatty acid (PUFA) status. Children with SAM might be vulnerable to insufficient PUFA intake and essential fatty acid (EFA) deficiency, which could be caused by a combination of three factors: a decreased food intake, an impaired nutrient absorption, and the presence of infection leading to higher nutrient requirements [14–16]. PUFAs play an important role in human brain, cognitive, and visual development, along with immune function and are thus vital for healthy growth and development of children [17–19]. Low levels of PUFAs might especially be a concern in children with acute malnutrition [20–28], who also are vulnerable to anaemia, infections, and other severe health challenges [16,29–33]. While PUFAs can be measured in various blood cell pools, whole blood samples have logistical advantages for field research collection due to less sampling processing post-collection, as well as the ability to collect dried blood spots. Therefore, recent studies have used whole blood to assess PUFA levels. However, when using whole blood to assess PUFA levels, the plasma itself and all the different blood cells can differentially impact the overall fatty acid composition [34], and any negative changes in health may also affect the cell counts. The onset of infection, which would result in monocyte cell counts increasing [35,36], or any alteration of blood cells may, for example, change the fatty acid composition of whole blood. Health challenges and/or changes in blood cell counts could therefore

result in a not nutriture PUFA estimate in severely acutely malnourished and health compromised children. This could lead to insufficient nutritional support for the children to reach sufficient PUFA levels which is required to maintain essential body functions, growth and development of the child.

Conclusively: a well-accepted, locally produced, fish-based RUTF - adapted to the taste and preferences in Cambodia - might improve SAM treatment, increasing the likelihood of more children recovering from SAM. Likewise, children with SAM may have low PUFA status, potentially interfering with their rehabilitation from SAM. Using whole blood to measure PUFAs in health compromised children could result in reflecting a not nutriture PUFA estimate, which would lead to a misinterpretation of PUFA status.

Objectives of PhD research

The overall aim of this PhD study is to investigate the effectiveness of a fish-based RUTF in home-based treatment of SAM in children, who are also at risk of poor PUFA status. This has been done as a part of the project, “Development of locally produced, fish and lipid-based nutrient supplements for the treatment and prevention of malnutrition in Cambodia”. The specific objectives of the work described in this PhD thesis are to:

1. Optimise an already piloted, locally produced, and fish-based paste adjusted to Cambodian taste and preferences, along with following UN’s international RUTF specifications for nutrition composition. Furthermore, to include packaging and labelling of the fish-based RUTF (**Paper I**).
2. Conduct a taste trial and assess the acceptability of two locally produced, ready-to-use, fish-based products against a commercial milk-based RUTF among children and their caregivers (Taste trial) (**Paper I**).
3. Conduct an effectiveness trial and thereby assess the effectiveness of the locally produced, fish-based RUTF (named ‘Num Trey-RUTF’) compared to the imported milk-based RUTF (named ‘BP-100™’), for home-based treatment of SAM in Cambodian children (SAM trial) (**Paper II**).
4. Investigate fatty acid composition, particularly PUFA levels of whole blood and their potential associations with markers of nutritional- and health status in Cambodian children with SAM (**Paper III**).