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 2\(^{nd}\) 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-100\(^{TM}\) (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 \(\leq -2.8\) and/or a MUAC \(\leq 115\) mm. Children were scheduled for follow-up visits every 2\(^{nd}\) 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), \(\alpha_{1}\)-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-100\(^{TM}\) and 36% for the NumTrey-RUSF. There was no statistically significant difference in the proportion of children ranking the NumTrey-RUSF and BP-100\(^{TM}\) as the product they “liked the most” (Pearson \(\chi^{2}\) 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-100\(^{TM}\) 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-100™ 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-100™, 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-100™ 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.