GROWTH AND MICRONUTRIENT STATUS OF CHILDREN 6-15 MONTHS OF AGE FED WITH BIOFORTIFIED TRADITIONAL FOODS

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INTRODUCTION

Background of the Study

Cambodia is one of the poorest countries in the South East Asia region with an estimation of total population of around 14.2 millions, a growth rate of about 1.752% per annum in 2007 and population below poverty line 35% in 2004 (UNDP, 2007). The GDP per capita (PPP): US$ 1,800 (UNPD, 2009) and the GDP shared from three main pillars: Agriculture sector 31%, Industry sector 26% and Service sector 43% in 2007 (World Bank, 2008). Agriculture is the major occupation for around 85-90% of the total population. On the other hand, the human development index (HDI), a human welfare indicator, is rising, from 5.33 in 1995 to 5.71 in 2003. This is attributed to the peace and order in place and considerable foreign assistance (UNDP Cambodia, 2006).

Agricultural productions (or cereal-based diets) are the main sources of diets of rural Cambodian people as many developing countries, which are commonly consumed are rich in phytate and low in animal products, predisposing people to insufficient absorption of iron, and low intakes of several vitamins. (*Cornelius M. S. et al., 2005*). The low micronutrient content of supplementary foods in many disadvantaged population has been associated with growth faltering, increased morbidity, and delayed motor milestone acquisition (*Seth Adu-Afarwuah, et al., 2005).*

Traditional foods such as other aquatic animals (OAAs), aquatic plants, edible wild plants/ semi-cultivated vegetables, edible insects, wild mushroom, and fish species “especially small indigenous fish species (SIS)”, play an important role in the poor households’ food security and nutrition in rural Cambodia, where it is estimated that more than 30% of its 14.2 millions live on less than US$ .50 per day (UNDP, May 2009). These kind of SIS are low value and

available, and hence are accessible to the poor families. In addition, it is well documented that SIS is high bioavailability of micronutrients (*Nanna Roos, 2003, 2006, 2007*)

The aim of the study is to assess the impact of an improved complementary food based on locally available foods on children's growth (weight, length/height, iron status and functional outcomes in children age 6 to 15 months of age. Rice and indigenous fish are core food items in the Cambodian diet along with a diverse selection of other indigenous animals. Based on analysis of the contents of critical nutrients in common fish species and other indigenous foods, a complementary food based on rice, fish and an edible spider has been developed within the WinFood project.

The study will be conducted in Pea Raing Commune, Prey Veng province (90 km from Phnom Penh) which one of the highest prevalence of child malnutrition. Two different formulations of CSB (CSB+ and CSB++) will be used in this study to feed children in control groups. These will serve as the standards which the Winfood food is compared (CSB+ and CSB++ are respectively representing current practice and 'golden' standard). To date, no research has conducted as such a study.

Statement of the Problem

Micronutrient malnutrition remains a problem of public health concern in most developing countries, partly due to monotonous, cereal based diets that lack of diversity. Agricultural productions (or cereal-based diets) are the main sources of diets of rural people as many developing countries, which are commonly consumed are rich in phytate and low in animal products, predisposing people to insufficient absorption of iron, and low intakes of several vitamins. (Cornelius M. S. et al., 2005). The low micronutrient content of supplementary foods in many disadvantaged population has been associated with growth faltering, increased morbidity, and delayed motor milestone acquisition (Seth Adu- Afarwuah, et al., 2005).

Malnutrition and micronutrient deficiencies are still critical problems in Cambodia, which is among the most critical in the Asia-Pacific region. Malnutrition, especially in rural areas, is widespread, particularly among preschool-aged children and lactating women. The country's malnutrition rates are among the highest in the Asia-Pacific region. The infant mortality rate at 60 per 1000 live birth (CDHS, 2008), while under-five aged children mortality rate 83 per 1000 live births (CDHS, 2008). Malnutrition and micronutrient deficiencies, such as protein-energy malnutrition, vitamin A deficiency (VAD), and especially, iron deficiency anemia (IDA) are widespread among under 5 year- aged children. IDA among children under-two years of age it is over 60%.

Iron is selected for study because they are high prevalence among two years aged children. It is well documented that it is rich in small indigenous fish species (SIS).

Objectives of the Study

In general, the objective of the study is to assess the effect of the improved processed locally available complementary foods, which are acceptable for young children to improve on growth, iron status and other functional outcomes.

Specifically, objectives of the study are:

1. To describe the differences in baseline characteristic between the intervention groups, the controlled groups; and pooled intervention groups and pooled controlled groups;
2. To determine the mean energy and nutrient intakes of infants in cross-sectional study;
3. To examine the breast-feeding intake and contribution of project foods to daily energy and nutrient intakes among the intervention groups and the controlled groups;
4. To examine relationships between dietary and other variables measured of individual of intervention groups and controlled groups;
5. To determine the whether there are differences between the intervention groups and the controlled groups in weigh gain, length gain, or changes in hemoglobin concentration and other functional outcomes form 6-15 months of age.

Significance of the Study

With the overall picture shown and all factors pointed out in the intervention study of complementary foods fed by biofortified local available traditional foods the study would be able to contribute in updating research on complementary feeding in the context of nutrition in Cambodia. Furthermore, it could serve as a basis for further research on complementary feeding program in a country-wide scale.

Furthermore, the results will contribute to the effective implementation of the guidelines by providing the documentation for the nutritional impact of a complementary food based on local indigenous Cambodian food stuffs. Moreover, the study will provide vital information on the socio-economic characteristic, average daily energy and nutrient intakes of which intake from breastfeeding, contribution from project foods, and the association of dietary intakes with other variables in young children in the study areas. Moreover, the study will give the information on the optimal improved foods which are locally available to improve growth, iron status and other functional outcomes.

The empirical findings of the study will also be useful in planning, monitoring, and evaluating sustainable development programs and other interventions on nutrition in Cambodia. Lastly, the study could serve as a source of information on nutrition for the government and other related institutions.

Limitations of the Study

The study will assess the efficacy on individual of selected young children from optimal improved food, which developed through traditional foods that are locally available. Due too time and financial constrains, the study will be 9 months. The subjects aged from 6-15 months will be as intervention group (220 children) and control group (220 children). The cross-sectional study will cover 110 children in the age of 6-15 months.

Operational Definition of Terms

Socio-demographic Characteristics- refers to the Community Fisheries members' age, sex, marital status, family income, educational attainment, length of residence in the village, and length of Community Fisheries membership .

Age-refers to the respondent's number of years from birth to last birthday at the time of the survey. It will be dichotomized into "younger" and "older" groups. Respondent whose age is below the mean will be considered as the "younger" ones while those with equal or above the mean are the "older" ones.

Sex- refers to the classification by gender of the respondent as either male or female.

Occupation - refers to the respondents' main occupation

Family Income - refers to the gross family income from all sources (in riels) earned by employed family members.

Education attainment - refers to the total number of years spent by respondent in formal schooling. Educational attainment will be grouped into four levels of formal education:

Informal education	-	0 year
Elementary	-	1-6 years
Secondary	-	7-9 years
High school	-	10-12 years
College	-	13 years and above

Marital status - refers to whether the respondent is single, married, separated or divorced.

Family size - refers to the total number of individuals living under the same housekeeping, kitchen, and eating arrangement. Respondents whose number of household members is below the mean will be categorized as having a "small household." Those with

household members equal to and above the mean will be categorized as having a "largest household."

Economic Characteristics

Occupation - refers to the type of work or job exhibited in different types of work of the respondent.

Annual family income - refers to the respondent's estimated total annual income from job activities as well as from other resources, whether in cash or in kind incurred between 2010-2011. Respondents whose gross annual family income is below the mean can be considered as having "lower gross annual family income." Those with gross annual family income equal or above the mean can be considered as having "higher gross annual family income."

Food:

- Biology - Any matter eaten by man to sustain life and nourish the body.
- Nutrition – Any substance which when taken by the body provides

energy, builds, and repairs body tissues ad regulates body processes (WHO)

Nutrition:

- The science that links food to health and diseases. It includes the processes by which the human organism ingests, digests, absorbs, transports, and excreted food substance.

Nutrients:

- Chemical substances in food that contribute to health, many of which are essential parts of diet. Nutrients nourishes by providing calories to fulfill energy needs, minerals for building body parts, and factors to regulate necessary chemical processes in the body
- Substances obtained from foods that are vital for growth and maintenance of a

healthy body throughout life

Health - A state of complete physical, mental, social, emotional and spiritual well-being and not merely the absence of diseases or infirmities (WHO, 1996)

Complementary feeding - When breastmilk is no longer enough to meet the nutritional needs of the infant, complementary foods should be added to the diet of the child. Complementary feeding typically covers the period from six to 24 months of age, and is a very vulnerable period. It is the time when malnutrition starts in many infants, contributing significantly to the high prevalence of malnutrition in children under five years of age worldwide. (WHO/UNICEF, 1996)

Complementary feeding practices - refers to the practices in relation to—1) duration of exclusive breastfeeding and age of introduction of complementary foods; (2) maintenance of breastfeeding; (3) responsive feeding; (4) safe preparation and storage of complementary foods; (5) amount of complementary food needed; (6) food consistency; (7) meal frequency and energy density (8) use of vitamin-mineral supplements or fortified products for infant (9) feeding during and after illness.

Breastfeeding - The child has received breast milk direct from the breast or expressed. (WHO/UNICEF, 1996)

Exclusive breastfeeding - The infant has received only breast milk from the mother or a wet nurse, or expressed breast milk, and no other liquids or solids with the exception of drops or syrups consisting of vitamins, mineral supplements, or medicines. (WHO/UNICEF)

Predominant breastfeeding - The infant ’s predominant source of nourishment has been breast milk. However, the infant may also have received water and water-based drinks (sweetened and flavored water, teas, infusions, etc.), fruit juice; oral rehydration salts solution (ORS), drop and syrup forms of vitamins, minerals and medicines, and ritual fluids (in

limited quantities). With the exception of fruit juice and sugar water, no food-based fluid is allowed under this definition. (WHO/UNICEF, 1996)

Full breastfeeding - Exclusive breastfeeding and predominant breastfeeding together constitute full breastfeeding. (WHO/UNICEF, 1996)

Bottle-feeding - The child has received liquid or semi-solid food from a bottle with a nipple/teat. (WHO/UNICEF, 1996)

Food Security

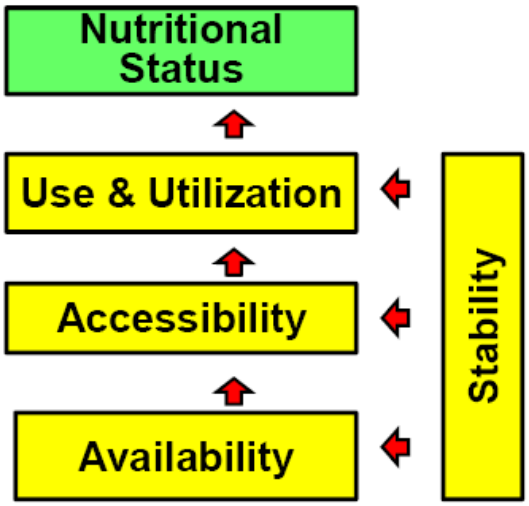
Although the term of food security is very broad, it was defined as:

- "Availability at all times of adequate world food supplies of basic foodstuffs to sustain a steady expansion of food consumption and to offset fluctuations in production and prices" (World Food Summit, UN, 1975)
- "Ensuring that all people at all times have both physical and economic access to the basic food that they need". (FAO, 1983)
- "Access of all people at all times to enough food for an active, healthy life". (WB, 1986)
- "Food security, at the individual, household, national, regional and global levels [is achieved] when all people, at all times, have physical and economic access to sufficient, safe and nutritious food to meet their dietary needs and food preferences for an active and healthy life. (The 1996 World Food Summit adopted a still more complex definition)
- "Food Security" is achieved when it is ensued that "all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food which meets their dietary needs and food preferences for an active and healthy life" (FAO, 2000)

Food and Nutrition Security

- "Food and Nutrition Security is achieved, if adequate food (quantity, quality, and safety, socio-cultural acceptability) is available and accessible for and satisfactorily used and utilized by all individuals at all times to live a healthy and active life" (GTZ, 2004). This definition combines food and nutrition security and emphasizes several aspects, i.e., "**Availability**", "**Accessibility**", and "**Use and Utilization**" of food. The inclusion of the use and utilization aspect underscores the fact that "**Nutrition Security**" is more that "**Food security**"

Figure 1. Food Security and Nutrition



Source: GTZ, 2004

Traditional Food:

Philippines

- Food that has a history of significant human consumption. If defined by a society as food, there is a certainty that no harm will result from the intended use of the food. Example: rice, coconut, root crops, vegetables (*malunggay*, *alugbati*, *saluyot*, *okra*, *sitao*, *ampalaya* and *talong*), fruits (banana, papaya, mango, pineapple), fish and seafood

In the Philippines perspective, food is classified as "**Alternative Food**", it is defined as:

- Food that includes plant and animal sources traditionally utilized as foods in some localities but not commonly consumed in other places
- Food that can substitute or complement the more expensive and conventional protein, calorie, mineral and vitamin sources of food (e.g.: aquatic animals, birds, wild animals, mice, edible insects, wild plants, and other sources)

European Perspective

- Food of a specific feature or features, which distinguish it clearly from other similar products of the same category in terms of the use of "traditional ingredients" (raw material or primary products) or "traditional composition" or "traditional type of product and/or processing method" as defined below

Traditional ingredient (raw material or primary product):

- Raw material (species and/or varieties) or primary product either alone or as an ingredient that has been used in identifiable geographical areas and remains in use today (taking into account cases where use was abandoned for a time and then reinstated) and its characteristics are in accordance with current specifications of national and EU legislation

Traditional composition:

- The uniquely identifiable composition (in terms of ingredients) that was first established prior to the Second World War and passed down through generations by oral or other means (taking into account cases where composition was abandoned for a time and then reinstated) and when necessary is differentiated from the composition defined by the generally recognized characteristics of the wider food group to which the product belongs

Traditional type of production and/or processing:

The production and/or processing of a food that:

- Has been transmitted from generation to generation through oral tradition or other means and:
- Has been applied prior to the Second World War and remains in use (taking into account cases where composition was abandoned for a time and then reinstated) despite its adjustment to binding rules from national or EU food hygiene regulations or the incorporation of technological progress, under the condition that production and/or processing remains in line with methods used originally and that the food's intrinsic features such as its physical, chemical, microbiological or organoleptic features are maintained

WinFood Perspective:

- "Traditional foods" belong to a large and heterogeneous group of raw and processed foods:
    - "Wild" indigenous plants and animals collected from uncultivated land and forest (e.g., leafy plants, roots, berries, small rodents, and insects) and from aquatic environments (e.g., fish, frogs and snail)

- Semi-domesticated, indigenous plants and animals, for example homestead gardening of indigenous plant species such as *Amaranthus* in Africa and the culture of indigenous, small fish species in rice fields in Asia; and
- Locally available staple foods processed using traditional processing technologies. "Traditional foods" have in common that they are: a) culturally acceptable and are part of local food habit, and b) at present, have no or low commercial value, either collected to be used directly for consumption by the household or traded locally.

- WinFood are improved foods based on traditional foods and traditional food processing technologies, as defined above, and developed, promoted and disseminated to improved diet quality and thus improved child nutrition.

Traditional food processing technologies:

- A wide range of household level, small-scale and semi-scale and semi-industrial processing technologies applied to improve food properties (stability, safety and organoleptic properties). These technologies are:
  - Spontaneous fermentation, comparing a high diversity of indigenous micro-organism and starter culture
  - Physical treatment, e.g. soaking, germination, salting, heating, drying and smoking.
- "Traditional food processing technology" have in common that they are: a) based on traditional knowledge and local food habits and b) used at local production sites, households and SMEs for trading in local markets.

Hypothesis of the Study

The hypothesis will formulate:

- The null hypothesis (H_o) is that there is no difference in the mean energy and nutrient intakes between intervention groups and controlled groups. The alternative hypothesis (H_1) is that the mean intake between the two group is different (two-tail test);
- The null hypothesis (H_o) is that there is no relationships (association) between dietary intakes and other variables measured of individuals of the intervention and controlled groups subjects such as sex; age; geographical area; household composition; socioeconomic status; education; and anthropometric, biochemical, and physiological functional indexes. The alternative hypothesis (H_1) is that there is a relationship between dietary intakes and other variables measured of individuals.
- The null hypothesis (Ho) is that the nutritional improved food has not an effect on growth and iron status for young children. The alternative hypothesis (H1) is that the nutritional improved food has an effect on growth and iron status for young children.

REVIEW OF RELATED LITERATURE

Complementary feeding interventions are usually targeted at the age range of 6–24 months, which is the time of peak incidence of growth faltering, micronutrient deficiencies and infectious illnesses in developing countries. After 2 years of age, it is much more difficult to reverse the effects of malnutrition on stunting, and some of the functional deficits may be permanent. Therefore, interventions that are effective at reducing malnutrition during this vulnerable period should be a high priority. Although several types of interventions can be targeted to this age range (e.g. micronutrient supplementation), a food-based, comprehensive approach may be more effective and sustainable than programmes targeting individual nutrient deficiencies. For this review, a broad definition of 'complementary feeding interventions' is used so as to capture the full range of strategies that can be used. According to World Bank, 2005 provides a framework for the period of supplementary feeding intervention, from 6 to 24 months of age, is one of the most critical times for preventing malnutrition, which is the time of peak incidence of growth faltering, micronutrient deficiencies and infectious illnesses

Guiding principles for complementary feeding

In recognition of the need for greater consistency in child feeding guidelines, the Pan American Health Organization (PAHO) and theWorld Health Organization (WHO) published the Guiding Principles for Complementary Feeding of the Breastfed Child in 2003 (PAHO/WHO 2003). The 10 guiding principles cover: (1) duration of exclusive breastfeeding and age of introduction of complementary foods; (2) maintenance of breastfeeding; (3) responsive feeding; (4) safe preparation and storage of complementary foods; (5) amount of complementary food needed; (6) food consistency; (7) meal

frequency and energy density; (8) nutrient content of complementary foods; (9) use of vitamin-mineral supplements or fortified products for infant and mother; and (10) feeding during and after illness. The interventions described in this review generally included one or more components related to these guiding principles.

Energy and nutrients needed from complementary foods

Breastmilk intake continues to make a substantial contribution to the energy and nutrient intakes of infants and young children in developing countries after the age of 6 months, but nutrient needs from complementary foods increase as breastmilk intake declines with age. Previous documents have reviewed the amounts of energy and other nutrients needed from complementary foods, taking into account the average breastmilk intake and its nutrient composition during each age interval among children in developing countries (Dewey & Brown 2003). These recommendations will be briefly reviewed below.

Energy, protein and lipids

According to WHO, 2003, start at six months of age with small amounts of food and increase the quantity as the child gets older, while maintaining frequent breastfeeding. The total energy requirements of healthy, breastfed infants are approximately 615 kcal/day at 6-8 months, 686 kcal/day at 9-11 months, and 894 kcal/day at 12-23 months of age. Among breastfed children in developing countries, average breast milk energy intake is 413, 379, and 346 kcal/day at 6-8, 9-11 and 12-13 months, respectively (WHO/UNICEF, 2003).

The needs from complementary foods are estimated by subtracting average breast milk energy intake from total energy requirement at each age. The energy needs from complementary foods for infants with “average” breast milk intake in developing

countries are approximately 200 kcal per day at 6-8 months of age, 300 kcal per day at 9-11 months of age, and 550 kcal per day at 12-23 months of age. In industrialized countries these estimates differ somewhat (130, 310 and 580 kcal/d at 6-8, 9-11 and 12-23 months, respectively) because of differences in average breast milk intake.

Complementary feeding of family foods for breastfed children which have a composite energy density ranging from 1.07 to 1.46 kcal/g, the approximate quantity of complementary foods that would meet the energy needs described above is 137-187 g/d at 6-8 months, 206-281 g/d at 9-11 months, and 378-515 g/d at 12-23 months. (WHO, 2003).

Total daily average energy requirements for healthy children are 615 kcal at 6–8 months, 686 kcal at 9–11 months and 894 kcal at 12–23 months of age (Dewey & Brown 2003). In developing countries, the average expected energy intake from complementary foods is approximately 200 kcal at 6–8 months, 300 kcal at 9–11 months and 550 kcal to 12–23 months. These values represent 33%, 45% and 61% of total energy needs respectively. Achieving these energy intakes requires that both feeding frequency and energy density of complementary foods be adequate. An energy density of <0.6 kcal g-1 is generally considered low.When energy density is at least 0.8 kcal g-1, the recommended feeding frequency is two to three meals at 6–8 months and three to four meals at 9–24 months, with the option of including additional nutritious snacks once or twice per day, depending on the child's appetite and responding to the child's signs of hunger and satiety (PAHO/WHO, 2003)

The amount of protein needed from complementary foods increases from about 2 g/day at 6–8 months to 5–6 g/day at 12–23 months, with the percentage from complementary foods increasing from 21% to about 50%. There is uncertainty about the optimal intake of fat during the first 2 years of life. Breastmilk is usually rich in fat (approximately 30–50% of energy), so little additional fat from complementary foods is needed while breastmilk intake is still high. However, the fat content of complementary foods becomes more important as breastmilk intake declines with age. To achieve at least 30% of energy from fat in the total diet, the amount of fat needed from complementary foods (assuming average breastmilk intake) is zero at 6–8 months, approximately 3 g/day at 9–11 months and 9–13 g/day at 12–23 months, or 0%, 5–8% and 15–20% of the energy from complementary foods respectively (Dewey 2005) (a range is given because of variability in breastmilk fat concentration). The quality of the fat may be even more important than the quantity. Infants and young children need good sources of essential fatty acids in their diet, such as fish, egg, liver, nut pastes and vegetable oils.

Micronutrients

Micronutrient needs are high during the first 2 years of life, to support the rapid rate of growth and development during this period. The percentage of the recommended nutrient intake needed from complementary foods varies widely, depending on the concentration of each nutrient in breastmilk. The nutrients that are most problematic – for which at least 75% must come from complementary foods – are iron (97–98%), zinc (80–87%) and vitamin B6 (80–90%) (Dewey 2005). Thus, complementary food diets need to contain foods rich in these nutrients (generally animal-source foods), or be fortified in some way.

Nutritional Status in Cambodia

Iodine Deficiency Disorders (IDD)

Results of the first national goiter survey (MOH, 1997) indicated a projected national average total goiter rate of about 12% in the age group 8-12 years, but with some areas having as much as 45% total goiter rate. The gross goiter rate from the survey was 17%. It is estimated that there are nearly 1.3 million individuals at risk of IDD. This makes Cambodia fall into the category of countries with a mild IDD problem. A national program to achieve Universal Salt Iodization (USI) by the end of 1998 was planned and pursued with UNICEF support. However, due to many operational problems, the objective has not been achieved to date. The CDHS 2000 shows that, nationwide, only 12% of households are adequately using iodized salt; a high proportion of the salt sampled has inadequate levels of iodine.

Vitamin A Deficiency Disorders (VADD)

Micro surveys conducted between 1990-1996 in about three-quarters of the provinces all suggest the presence of a significant problem of VADD. Prevalence rates of up to 12% of night blindness have been recorded in Takeo (WHO cut-off in 1%). Low consumption of vitamin A rich foods by children is seen, particularly during the dry season when lack of water prevents the growing of vegetables in home gardens. Vitamin A rich foods (fruits and vegetables) do not form a regular part of the children's diet. Only 29% of the children <5 years of age nationwide received a vitamin A supplement six months prior to the survey in 2000 (CDHS). The high prevalence of diseases known to predispose children to VADD, particularly Acute Respiratory Infections (ARI), diarrhea and especially measles serve as a proxy measure of the severity of the problem. In some places, prevalence of

these illnesses is over 40%. The importance of the measles vaccination in the control of VADD cannot be overemphasized. Measles vaccination coverage in Cambodia reached a national average of only 55% in 2000 (CDHS). 11 out of 21 provinces had lower coverage rates. While vitamin A supplementation using vitamin A capsules has been given prominence in the Expanded Progam on Immunisation (EPI) in Cambodia, so far there are limitations in the promotion of dietary approaches as a strategy and this needs to be strengthened. Breast-feeding is fortunately a universal phenomenon in Cambodia with median breast-feeding periods of about 24 months (CDHS). Colostrum, a highly precious source of vitamin A for the newborn, is traditionally discarded, but in recent years, nutrition education seems to have slightly increased the proportion of women breastfeeding colostrum. As regards mothers, 11% of them take postpartum vitamin A supplements (CDHS). The CDHS also revealed that only 5% of babies are exclusively breastfed up to 5 months of age.

Iron Deficiency Anemia (IDA)

Regarding IDA, in 2000, the prevalence in children was 63%; for women the rate was 58% (CDHS); for children under two years of age it is 70% (HKI). IDA prevalence in pregnant women is higher, at 65% (CDHS 2000) and anemia is considered to be one factor associated with a high maternal mortality rate, estimated at 473/100,000 live births (United Nations Common Country Assessment). Only 16% of pregnant women took iron supplementation during pregnancy in 2000 (CDHS). Prevention of anemia in pregnant women is included in the MOH Minimum Package of Activities (MPA) to be provided at all health units. Iron and folic acid tablets are available to all health units. Apart from iron tablet supplementation, dietary based approaches have not been emphasized. The coverage of iron/folate tablets is still too low and does not reach the rural women who are at greatest risk of maternal anemia.

Protein-Energy Malnutrition (PEM)

Maternal Nutrition and Low Birth Weight

A 1993/94 UNICEF survey of 12 provinces found that 34.5% of women restricted their diet during pregnancy and 77.8% during lactation. In Cambodia, women continue working as hard during pregnancy as when non-pregnant. The combined effect of dietary restrictions and a heavy workload results in low birth weight babies. Although there is no available data on the level of weight gain during pregnancy, it is likely to be significantly below the recommended weight gain of 10-12 kilograms. A low Body Mass Index (BMI <18.5 kg/m2) is reported for 20% of women in 2000 (CDHS). Low birth weight figures (less than 2500gm) of around 18% are reported, but are mostly estimated given the low percentage of newborns that get weighed. Low birth weight babies have been reported as high as 26% in individual hospitals. Low birth weight reflects maternal malnutrition and is associated with high neonatal and infant mortality. A high prevalence of low BMI in women has also been proposed as a proxy indicator for household food insecurity. Since good maternal health and nutrition provides the first line of defense for the child, improving the nutritional status of women must be an important priority for investing in child nutrition.

The Nutritional Status of Children under the Age of Five.

Data from CDHS (2000) and from MICS (1996) showed the prevalence of malnutrition (for both underweight and stunting) for children of 0-59 months of age in 2000 was around 45%, which is a slight improvement from a prevalence rate of 48% reported in 1996. Wasting, however, increased from 13% in 1996 to 15% in 2000. Severe malnutrition (<-3 SD) is alarming as severe underweight is reported in 13% and severe stunting in 21% of children in 2000. However, it is an improvement from 1996 where

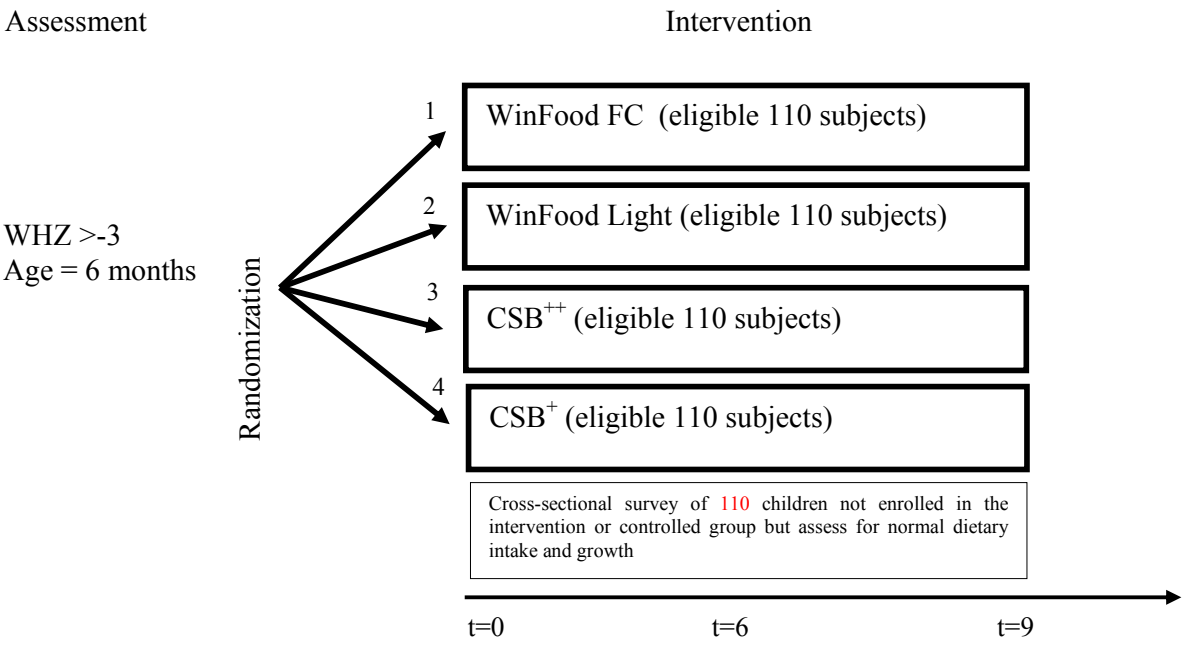
severe underweight and severe stunting were reported at 14% and 28% respectively (MICS). Severe wasting doubled from 2 % in 1996 to 3.8% in 2000. It appears that socio-economic development efforts have not equally benefited all strata's of society.

STUDY METHODOLOGY

Study Design

The study will be assigned a community-based randomized controlled interventional trial involving two intervention groups and two nonintervention groups. After selection of eligible children for intervention trial which use of WinFood CF and WinFood CF "Lite" for intervention group and CSB+ and CSB++ as controlled groups.

Figure 2. Study design



Study sites and Participants

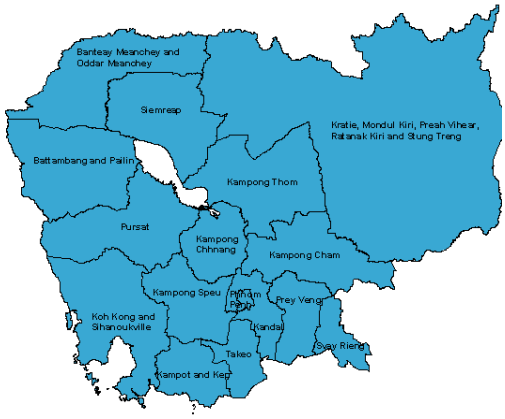


Figure3. Mape of Cambodia

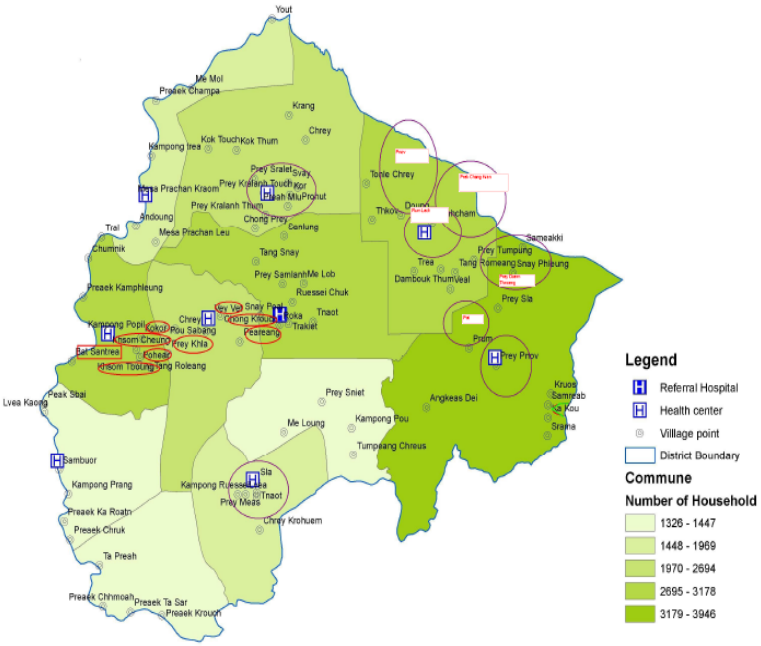


Figure 4. Mape of Pea Raing commune

The study will be conducted in selected villages in Pea Raing commune, Prey Veng province (90 km from Phnom Penh), where RACHA/WFP MCH programme is being implemented. The selection criteria of households for the intervention study are identical to the selection of beneficiaries for the RACHA/WFP MCH programme. The households will be selected from villages which are identified by RACHA/WFP as being food insecure, but in which the MCH program has not yet been implemented. This will ensures that the advice regarding food choices and child health disseminated through the on-going implementation of the MCH programme is not interfered with the WinFood intervention, nor have that households to change from an existing MCH food basket to the WinFood-specific food basket.

Within the selected villages, "poor households" will be identified according to the existing criteria applied by RACHA to identify MCH beneficiaries. Qualified households will be invited to participate in the WinFood intervention study.

A cross-sectional survey of basic anthropometric measures (height, weight and age) of non-enrolled children will be conducted in villages adjacent to the villages selected for the intervention study and which not are enrolled in the RACHA MCH program. The cross-sectional survey will cover 110 children in the age of 6 to 15 months. Among the 110 children, a dietary assessment based on questionnaires will be carried out in a sub-group. The purpose of this survey is to compare the children enrolled in the intervention study with the general population in the same area.

Recruitment and enrolment for the intervention study

Mothers with children aged 6 months in households which are qualified for enrolment will be informed about the study, and invited to a screening session. Enrolment of the child will be based on the following criteria:

Inclusion: Children are 6 months old and have a weight-for-height z-score > -3 and the mothers are planning to stay in the study area for the next 9 months, and are consenting to participate in the study.

Exclusion: Children with weight-for-height z-score <-3 and/or bilateral pitting oedema, or with anaemia (Hb< 80 g/L) or clinical signs of vitamin A deficiency (xerosis or Bitot spots). These children will be referred for treatment to hospitals.

The complementary foods in intervention and control groups

Food in intervention group 1: Winfood Complementary Food (WinFood CF)

The complementary food - referred to as WinFood CF - is developed based on the traditional complementary food in Cambodia, rice-fish based porridge "Bor-Bor Trey" with addition of locally available foods identified to have a high density of nutrients. The WinFood CF consist of rice added two species of common Cambodian fish species, Trey Changwa Plieng (Esomus longimanus) and Trey Slak Russey (Paralaubuca typus), and the common edible sandy spider, Aping (Haplopelma albostriatum). WinFood CF is processed as a partially pre-cooked instant food using dry extrusion of pre-dried and powdered foods. WinFood CF will be processed to be similar to CSB products in terms of cooking and texture.

Food safety: WinFood CF will be processed and packed by the food manufacturing company "So Nutritious", Phnom Penh. The production will be planned to ensure a maximum shelf-life of 2 months. The final product will be controlled for microbiological contamination meeting the same limits as specified for CSB+ and CSB++. During processing, sampling and processing of fish and spider is conducted under the quality control of Department of Fisheries Post-Harvest Technologies and Quality

Control (DFPTQ). Cooling chain from sampling to processing is ensured and food components are stored at -20 °C. Fish are sundried under controlled hygienic conditions before the final processing at the manufacturing site of So Nutritious.

The WinFood CF has been tested for acceptability in the target group of 23 mothers, using semistructured interviews, focus-group-discussions and observations. The field work of the acceptability study was conducted in May 2010. The preliminary results indicated high acceptability of the product among mothers and children in the target group

Table 1. Contents of key nutrients in a portion of 100 g processed WinFood-CF

			Content, g		E%		Mineral content, mg/100g (3)			Density (mg/1000 kcal)		
Processed food	Dried %	Energy	Protein	Fat	Protein	Fat	Fe	Zn	Ca	Fe	Zn	Ca
Rice	84,6	308	7,1	1,2	9	3	1,0	1,4	42,3	3,3	4,7	137
Fish												
Trey changwa phlieng	6,3	35	5,6	1,4	64	36	2,8	1,3	209	80	38	6000
Trey sloeuk russey	6,3	58	5,0	4,2	35	65	0,6	1,0	209	9,7	17	3623
Other animal foods												
Spider (aping)	2,1	9	1,5	0,3	71	29	0,2	1,0		27	118	494
Oil (1)	0,6											
WinFood product	100,0	409	19,2	7,0	18,8	15,5	4,6	4,8	461	11	12	1127
To be added after processing												
Oil (1)	5,0	45	0	5,0	0	100	0	0	0	0	0	0
Sugar (2)	5,0	20	0	0,0	0	0	0	0	0	0	0	0
Total	110	474	19	12	16,2	22,9	4,6	4,8	461	10	10	972
Recommended					15	20-30				9	13	600

Notes:

(1) 0,6 % oil added during processing, 5 g at preparation in HH

(2) Sugar to be added after processing

(3) Values are calculated from contents contributed from each food. The measured contents in a test batch of WinFood product: Fe 5.2 mg/100g, Zn 3.8 mg/100g.

Food in intervention group 2: Winfood CF "Lite"

This complementary food which is a similar rice-fish based porridge "Bor-Bor Trey", but the fish component which in WinFood CF are species specifically selected for high nutritional value are substituted by unspecified mixed small indigenous fish. The simplified product does not contain Aping (spider). The compensate for less micronutrients being contributed from the simplified composition, this Winfood product will be added a premix of vitamins and minerals which is matching the nutrition content in CSB++. This complementary food product will be called "Winfood-Lite". The Winfood Lite is based on the Winfood approach, but with a simplified set of criteria. The Winfood Lite is aimed as an example of a possible product suitable for immediate scaling up as it can more easily be adapted by the producers. This will greatly facilitate promotion and speedy implementation of the Winfood approach. WinFood Lite is processed as a partially pre-cooked instant food using dry extrusion of pre-dried and powdered foods. WinFood Lite will be processed to be similar to CSB products in terms of cooking and texture.

Food safety: WinFood Lite will be processed and packed by "So Nutritious", Phnom Penh. The production will follow the exact same procedure as the Winfood CF production.

Table 2. Contents of key nutrients in a portion of 100 g processed WinFood Lite

Food	Dried protion g	Energy kcal	Protein g	Fat g	Prot E%	Fat E%	Fe mg	Zn mg	Ca mg	Fe mg/1000kcal	Zn mg/1000 kcal	Ca mg/1000 k
Rice	84,8	309	7,1	1,2			1,0	1,4	42			
Mixed fish species	10,0	55	8,8	2,2			0,3	0,3	308			
Oil	5,0	45	0	5,0								
Premix	0,2						7,0	2,8				
Total WF Lite	100,0	409	15,9	8,4	15,6	18,5	8,3	4,5	350	20	11	857
Added sugar	5,0	20										
Total WF-Lite + sugar	105	429	15,9	8,4	14,9	17,6	8,3	4,5	350	19	11	817

Foods in the control groups

The recruited participants will be randomized to receive the intervention Winfood CF, Winfood CF "Lite" or one of two control foods. The control intervention foods are either 1) Corn-Soy-Blend Plus (CSB+) with added sugar and oil according to standard practice for the WFP (RACHA MCH programme), or 2) Corn-soy-Blend Plus-Plus (CSB++), a newly developed improved CSB with 8% milk powder and additional vitamins and minerals.

All foods (Winfood CF, Winfood CF "Lite", CSB+ or CSB++) will be packed in daily rations which will be adjusted to the age of the child. Instructions and labels will inform that the food is for babies, to restrict intra-household sharing. The daily rations packets will be delivered in monthly rations. The distribution will be coordinated with clinical examination and nutritional status assessment of the child. The food and nutrition composition for the Winfood Lite, CSB+ and CSB++ are presented in table 3, 4 and 6.

Table 3. CSB++ formula (copied from WFP technical specification for CSB++, version 1.1)

N°	Ingredients	Percentage (by weight)
1	Corn (maize white or yellow)	57.05 – 62.05
2	De-hulled soya beans	15-20
3	Dried skim milk powder	8
4	Sugar	9
5	Refined soya bean oil	3
3	Vitamin/Mineral FBF-V-10	0.20
4	CaCO3 (calcium carbonate)	1.19
5	$Ca(H_2PO_4)_2$. H_2O (mono calcium phosphate)	0.80
6	KCl (potassium chloride)	0.76

Table 4. CSB+ formula formula (copied from WFP technical specification for CSB+, vers.1.6

N°	Ingredients	Percentage (by weight)
1	Corn (maize white or yellow)	72.05- 77.05
2	Whole soya beans	20-25
3	Vitamin/Mineral FBF-V-10	0.2
4	CaCO3 (calcium carbonate)	1.19
5	$Ca(H_2PO_4)_2$. H2O (mono calcium phosphate)	0.80
6	KCl (potassium chloride)	0.76

Table 5. Micronutrient rate and chemical form for CSB++ and CSB+ (copied from CSB++/CSB+ technical specifications version 1.1/1.6, respectively)

	Target	Chemical forms
Vitamin/Mineral *FBF-V-10*		
Vitamin A	1.664 IU	Dry vitamin A palmitate 250 n.s
Thiamine	0.128 mg	Thiamine mononitrate
Riboflavin	0.448 mg	Riboflavin
Niacin	4.8 mg	Nicotinamide
Pantothenic acid	6.7 mg	Calcium d-pantothenate
Vitamin B6	1.7 mg	Pyridoxine hydrochloride
Folate	60 mcg	Folic acid
Vitamin B12	2 mcg	Vitamin B12 – 0.1% spray dried
Vitamin C	100 mg	Ascorbic acid
Vitamin D	4 mcg	Dry vitamin D3 100 CWS
Vitamin E	8.3 mg	Vitamin E 50% CWS
Vitamin K	100 mcg	vitamin K1 5% CWS
Iron (a)	4 mg	Ferrous fumarate
Iron (b)	2.5 mg	Iron-sodium EDTA
Zinc	5 mg	Zinc oxide
Iodine	40 mcg	Potassium iodate (KIO3)
Carrier	Qs	Malto dextrin
Other minerals		
Calcium (a)	470 mg	Calcium carbonate (CaCO3)
Potassium	400 mg	Potassium chloride (KCl)
Phosphorus	200 mg	Mono calcium phosphate $Ca(H_2PO_4)_2$. H2O
+ Calcium (b)	130 mg	

Table 6. Comparing the WinFoods, CSB++ and CSB+ nutrient composition

	WinFood CF	WinFood Lite	CSB++	CSB+ (oil+sugar)
Portion				
Total dried food, g/portion	100	100	100	100
Animal ASF (% of tot. dried)	14	10	8	0
Added oil	5	5	3	10
Added sugar	5	5	9	10
Total portion, g	110	105	112	120
Energy and maconutrients				
Energy, food+oil+sugar (kcal/portion)	474	428	420	510
Protein E%	16	15	15	11
Fat E%	23	18	19	27
Total protein, g/portion	19	16	16	14
Animal protein, g/portion	12	9	3	0
Fat, g/portion	12	8	9	16
Minerals				
Fe, total mg/portion	5.2	9	6.5	6,5
Fe, mg/portion "high" bioavail. (from ASF or NaEDTA)	3.4		2.5	2.5
Fe, estimated mg absorbable/portion[1)]	0.8		0.7	0.7
Fe, mg/1000 kcal	11	20	15	13
Zn, mg/portion	3.6	4.5	5.0	5.0
Zn, mg/1000 kcal	8	11	12	10

1) Based on assumptions of bioavailability: 20% from ASF, 12% from NaEDTA and 10% from Ferrous fumarate

Randomization, allocation concealment and blinding

Individual randomization will be used. The children will be randomized to receive Winfood CF, Winfood CF "Lite", CSB+ or CSB++. The study includes the children defined as normal nourished or moderate malnourished children (Figure 1). To ensure complete blinding, there will be four different codes for each group. Hence, in total there will be 16 codes. Random allocation sequences will be generated using a block size of 24. The complementary food packets will be labeled by So Nutritious using a concealment key according to whether it contains the Winfood CF, Winfood CF " Lite" or CSB+ or CSB ++. The key of codes will be kept in a sealed envelope until preliminary analyses is completed. The study will be single-blind, in that staff assessing the outcome will be kept unaware of treatment allocation but because of the taste and smell will differ between the products it cannot be regarded as truly double-blinded.

Baseline and follow-up examinations in intervention study

The baseline and follow-up examinations are described in Table 7.

Table 7. Detail of baseline and follow-up examinations in intervention study

Time (months)	0	1	2	3	4	5	6	7	8	9
Screening/recruitment [1]	●									
Randomisation	●									
Supplementation		●	●	●	●	●	●	●	●	●
Routine visits/supervision	●	●	●	●	●	●	●	●	●	●
Examinations	●	●	●	●	●	●	●	●	●	●
Questionnaire[2]	●	●	●	●	●	●	●	●	●	●
Clinical examinations[3]	●	●	●	●	●	●	●	●	●	●
Anthropometry[4]	●	●	●	●	●	●	●	●	●	●
Blood sampling	●									●
Haemoglobin	●									●
Serum AGP, CRP, IGF-1	●									●
Serum ferritin, TfRc	●									●
Body Composition	●									●
Child development	●	●	●	●	●	●	●	●	●	●
Physical activity										●

[1] Screening for eligibility, e.g. age 6 month and weight-for-height z-score> -3
[2] Demographic and socio-economic data at baseline. Dietary assessment, compliance and morbidity data at baseline at each follow-up
[3] Measuring temperature, blood pressure and looking for symptoms of malnutrition or other diseases
[4] Weight, Height, Left arm circumference, Head circumference, knee-heel length, skinfold thickness

Questionnaire data

General information will be obtained at baseline. Data on the date of birth, sex of the child, demographic and socio-economic data for the family will be collected. The socio-economic questions are based on the questions from the Demographic and Health Survey Cambodia.

Dietary assessment

Will be conducted by using a 24-hours food recall record to estimate the amount of food has been eaten. The mother will be asked what the enrolled child ate in the last 24 hours. Furthermore, will the frequency of breastfeeding for the last 24 hours also be reported. All food and beverage consumed will be recorded as raw cleaned food in gram per child per day using standard household measurement which was then converted into weight using digital

scales Soehnle digital scale (Art.Nr. 65811) with 1 g accuracy between 0-1 kg, and 2 g accuracy between 1-2 kg.

Compliance: The mother will be asked how much of the Winfood CF, Winfood CF "Lite", CSB+ or CSB++ were consumed by the child. The degree of sharing of the assigned diets with other household members will be assessed by interviewing the mother. In addition, the mothers will be asked to keep all the distributed packets after these are empty so they can be collected and counted on a monthly basic.

Morbidity data will be collected by asking mothers about specific symptoms and clinic visits in the past seven days. Mothers will continuously be encouraged to bring their children to the clinic in the event of severe illness prior to the next visit. Based on serum samples collected at baseline and nine months, the concentrations of the acute phase reactants AGP and CRP will later be measured as objective markers of the presence of illness (See further section 3.6.3 for description of blood sample). These markers have been shown in community studies to be associated with maternal reports of morbidity among Ghanaian (Filteau et al. 1993) and Zambian (Filteau, unpublished observations) children, and to correct for the effects sub-clinical infection and inflammation on markers of micronutrient status (ferritin,).

Clinical and anthropometric examinations

Clinical examinations will be done at baseline and at each month, when the mother comes to collect the complementary food sachets at the distribution site during the intervention period. The clinical examination will include measurement of temperature and blood pressure, as well as signs of disease and malnutrition.

Procedure:

- The child will get temperature measured, with a standard ear thermometer.
- The child will get the blood pressure measured with a standard paediatric sphygmomanometer and auscultation
- A medical doctor will examine the child for different symptoms which can be related to diseases and malnutrition
- The child will be sitting with the mother, throughout the examination

Anthropometry: Weight will be measured to the nearest 0.01 kg, using Seca-UNICEF scales (UniScale). Length will be measured to the nearest 0.1 cm using calibrated length board. Knee-heel length will be measured using an electronic knemometer. The head circumference will be measured by using a fibreglass tape. Mid-upper-arm-circumference will be measured to the nearest 0.1 cm, using a special insertion tape, and triceps (the upper arm) and subscapular (between the shoulders) skinfold thickness (SF, 0.2 mm) will be measured using a Harpenden calliper. Based on these measurements, arm muscle and fat areas will be computed. For all anthropometric measurements and derived variables, the increment from baseline will be computed.

Procedure.

- Weight: The mother will step on the Uniscale, without child and her weight will be calibrated as zero. The mother will step down from the Uniscale and step back up with the child in her arms. The scale will then read the weight
- Length: The child will lie down on a length board and two trained people will measured the child.
- Kneemometer: The child will lie on their back on an examination couch, where right leg is free of clothing. The length of heel to knee will be measured.

- Head circumference and MUAC: The child is sitting with the mother, when the head circumference with a tape and the circumference of the left arm will be measured.
- Skin Fold: The child is sitting with the mother, when the skin on the left arm triceps and between the shoulders will be measured.

Justification: The clinical examination is carried out to make sure that no adverse reaction will happen with the child, and if there are any sign of severe undernutrition or disease, child will be immediately referred to a hospital. Anthropometry measurement is the traditionally outcome indicator in nutritional studies, which makes it possible to compare the results to other nutritional studies carried out in Cambodia or other countries.

Blood sampling and analyses

A venous (3 mL) blood sample will be collected at baseline and at the 9 months follow-up visit. The 13 blood sample will be collected using a field-friendly closed vacutainer system, using trace-element free tubes. Blood samples will be stored a 4 °C until separation. Plasma will be divided into 5 Eppendorf tubes of 200 µL each, and stored at -20 °C before analyses. Haemoglobin will be determined using HemoCue, at baseline and 9 months follow-up visit. A control cuvet will be measured daily to ensure right calibration of the HemoCue. Whole blood fatty acid composition will be determined by gas chromatography. As a measure of the acute phase response, serum acute phase proteins (APP) will be determined: the slow-reacting α1-acid-glycoprotein (AGP) and the fast-reacting C-reactive protein (CRP). As measures of iron stores and deficiency, respectively, serum ferritin and soluble transferring receptors will be determined.

Procedure

- The child will be sitting on the mothers lap, while a trained nurse will take 3 ml venous blood, from the inner elbow of the child, using standard hygienic procedures and a paediatric wingneedle. A trained Winfood staff will assist the mother and the nurse.
- The blood for the Hemocue test, will be taken from the blood which is left in the wing-needle tube, after the blood tube has been filled. No additional procedure is necessary for the infant.

Justification: The blood sample analyses are carefully selected to give insight in the key metabolic mechanisms that underlie the main outcomes of the study. These data will enable a better understanding of the effects observed when children are consuming nutrient-dense food, e.g. linear growth, weight gain, micronutrient status, morbidity/immune activation. The analyses is restricted to the key indicators of the key functions only, in order to restrict the blood sampling to an absolute minimum. The blood sample analyses are necessary for the interpretation of the results of the Winfood study and will enable the formulation of well-founded conclusions and recommendations that can give clear and unequivocal guidance to policy-makers and program designers.

Child development and milestones

Child development involves several domains but we will concentrate on motor development for two main reasons: 1) motor development and tools to assess it are less dependent on culture than cognitive or behavioural development and their assessment tools; 2) motor milestones have been shown to be delayed in malnourished children (Kariger et al. 2005; Grantham-McGregor et al. 2007) and to respond to nutritional supplementation within 6 months. In addition, it is notable that good motor development is required for good cognitive

development (Kariger et al. 2005). Attained motor develop milestones will be measured at the baseline and at each month. Data collected will be based on the six WHO motor development milestone, which include: Sitting without support, Hands and knees crawling, Standing with assistance, Walking with assistance, Standing alone and Walking alone (Wijnhoven et al. 2004). The scale will be piloted in the study populations and adapted as required. The mother will receive a data collection form at baseline, and asked to note the date of when the child performs one of the milestones for the first time in the household. Furthermore, each month when the mother bring the child for data measuring, the child will be asked to demonstrate several milestones which are of relevance for their age, as well a few which they should have achieved already, and a few which they need not to have achieved The observe performed milestone will be recorded by the field staff.

Procedure

- The child will be asked to do the different milestones. It will be noted if the child was able to perform the milestone.
- During the study period the mother will be asked to note which date the child performs the milestone for the first time

Physical activity assessment

Physical activity is a key functional outcome, reflecting health and predicting survival (Olney et al. 2007; Trost 2007). For all children, data on physical activity will only be collected at the 9 month follow-up visits, using both maternal reports based on a standard questionnaire and direct measurements. As for the measurements, it will be done using high-frequency sampled (80 Hz) tri-axial seismic accelerometry (GENEA, Gravity Estimator of Normal Everyday Activity, Unilever, UK). The enrolled children, will be getting the above mentioned accelerometry (in the form of a small plastic box with sensors, appr. 3 x 3 cm) placed on their body for three hours. This will be feasible while the mother-child pairs are around for the

food distribution. The data collection site will be provided with a safe sheltered play/waiting area for mothers and children waiting for their turn to be seen. Toys common to the community, e.g. balls and other will be provided. As mentioned above the physically activity will only be measured at the 9 months follow-up visit and therefore, details will be decided later with colleagues at MRC Epidemiology Unit, Cambridge, UK, with whom The Department of Human Nutrition at University of Copenhagen have a well-established collaboration, and who have experience using this equipment among small children. Therefore we have not yet developed an SOP for this indicator.

Ethical considerations

It is essential to ensure that the intervention and control group is given food that is known to be beneficial and generally acceptable for young children. The intervention group will receive Winfood CF, Winfood CF "Lite" which is nutritiously justified by a composition of rice and animal-source-foods, and that has been tested to be acceptable to mothers in taste and appearance and composition. The control group will be given the standard treatment for the on-going MCH project implemented by WFP/RACHA. Written and oral information will be given to the parents or guardians of all eligible children in the local languages before obtaining written consent. The Winfood CF, Winfood CF "Lite" are not expecting to cause any adverse effects. However, the children will be monitored by clinical examination at each monthly follow-up, allowing rapid identification of any potential adverse effect. As mention in section 3.6.4 the enrolled child will at baseline and the 9 follow-up visits have their body composition measured by using the stable isotope deuterium-labelled water (^2H_2O). The child will be given 3 g or 6 g 99.9% atom deuterium which are a fixed standardized dose recommended by the IAEA for children with a body weight less than 10 kg or with a weight between 10 and 20 kg, respectively. We would like to highlight that the deuterium is non-

radioactive and there are no ethical or health concerns about its use in young age groups (IAEA 2009), and that it has been used safely in infants, children, pregnant women, and lactating women. Studies by J C K Wells has used the technique in over 2000 infants and children, both in the UK and in overseas populations.

The mothers will receive one sarong and a cooking pot as compensation for the time they will need to spend for the examinations and the time used for blood and saliva sampling. The haemoglobin analysis will be done in Cambodia by Winfood staff using the HemoCue method. The further lab analyses of the blood and saliva samples will be done at the Laboratory at Department of Micronutrients, National Institute of Nutrition, Ministry of Health in Hanoi, Vietnam. Before sending to Hanoi, the samples will be stored at the laboratory at the Department of Post Harvest technologies and Quality Control, Fishery Administration, Phnom Penh, Cambodia. The samples will only be used for the terms of the Winfood study, and after the analyses have been carried out, the samples will be destroyed. All samples and data obtained in the study will be kept anonymous. The mother can at any time say no to continue in the study and will still continue to receive the monthly food ration but will not take part in the Winfood study. If any adverse reaction will occur doing the study period, this will be reported to NECHR immediately. The study will also obtain ethical approval from the ethical committee in Denmark.

Statistical Analysis

Sample size consideration

There are no data on the standard deviation of the increase in fat-free body mass among children between 6-15 months, based on the body composition method deuterium dilution, on which to base sample size calculations. The sample size per group, n, required to detect a

change, d, in a continuous variable with standard deviation, s, with 80% power, is $16*s^2/d^2$. Hence, to detect a 0.4 SD higher increase in fat-free body mass, we will need 100 children in each group. To allow for 10% loss to follow up, we will need to recruit 440 children in order to have at least 110 children in each of the groups 1-2 (Fig 1). We are expecting to have an on-going recruitment, which will take three months, which means that about 140 children will be enrolled in the Winfood study each month.

Statistical analysis

A monitor will be appointed to assess the conduct of the intervention study. Case record forms will be checked daily and entered within 2 weeks. EPI-INFO version 3.5.1 or SAS (Statistic Analysis System) version 9.2 (or SPSS version 16.0) will be used for data entry and analysis.

Objective 1

T-test and analysis of variance will be used to examine the differences in baseline characteristics between the intervention groups, controlled groups. Descriptive statistics include measures of central tendency of available such as mean and measures of dispersion, such as standard deviation will be used to express the variability in the data, with the confident interval 95%.

Objective 2

Mean and standard deviation will be used to express the variability in the data (i.e. how much the measurements of the 1-day intakes differ, on the average, from the estimated mean of the population), with the confident interval 95%.

Objective 3

Mean and standard deviation will be used to express on the average of the breast feeding intake and contribution of project foods to daily energy and nutrient intakes from the estimated mean of the population of the cross-sectional stud , with the confident interval 95%.

Objective 4

Pearson's correlation coefficient (r) will use to examine additional variables that may have relationship (associated) with variation in dietary intake such as sex; age; geographical area; household composition; socioeconomic status; education; and anthropometric, biochemical, and physiological functional indexes. The strength of the linear association between two continuous variables that are normally distributed, and ranges between -1.0 and +1.0. Negative values indicate that as one variable increases, the other decrease. The closer the absolute value of r is to 1.0, the stronger the association; the closer to 0, the weaker the association.

Objective 5

T-test and Analysis of Variance will be used to evaluate whether there are significant differences in weight gain, length gain, changes in hemoglobin concentration and other functional outcomes over the study period of intervention groups and controlled groups. Growth status (weight-for-age) and (height-for-age) of intervention groups and controlled groups will be compared each age by using Student's t-test. Statistical significance will be tested at 5% with CI 95%.

RESULTS AND DISCUSSION

Table 8. Baseline characteristics of households of infants in the 2 intervention groups, the 2 controlled groups

Characteristics	WinFood CF (n=110)	WinFood Lite (n=110)	CSB^{+} (n=110)	CSB^{++} (n=110)
Birth Weight (kg)				
Birth Length (cm)				
Weight at age 6 months (kg)				
Length at 6 months (cm)				
Mother age (y)				
Mother age (y)				
Mother's education (y)				
Father's education(y)				
Household income per moths (Riel/$)				
Household income per month				
Household size				
No of Children under 5 years old				

Table 9. Baseline characteristics of households of infants in the pooled intervention groups, the pooled controlled groups

Characteristics	Pooled intervention groups (n=220)	Pooled controlled groups (n=220)
Birth Weight (kg)		
Birth Length (cm)		
Weight at age 6 months (kg)		
Length at 6 months (cm)		
Mother age (y)		
Mother age (y)		
Mother's education (y)		
Father's education(y)		
Household income per moths (Riel/$)		
Household income per month		
Household size		
No of Children under 5 years old		

Table10. Source of drinking water of households of infants in the 2 intervention groups and the 2 controlled groups

Household drinking water	WinFood CF (n=110)	WinFood Lite (n=110)	CSB^{+} (n=110)	CSB^{++} (n=110)
Dry season (%)				
Improve source				
Piped water into dwelling/yard /plot				
Tube well or borehole				
Protected dug well				
Protected spring				
Rain water				
Non-Improve source				
Unprotected dug well				
Unprotected spring				
Tanker truck/cart with small tank				
Surface water				
Bottled water				
Improved source for cooking, washing				
Unimproved course for cooking				
Other source				
Total				
Rainy season (%)				
Improve source				
Piped water into dwelling/yard /plot				
Tube well or borehole				
Protected dug well				
Protected spring				

Rain water
Non-Improve source
Unprotected dug well
Unprotected spring
Tanker truck/cart with small tank
Surface water
Bottled water
Improved source for cooking, washing
Unimproved course for cooking
Other source
Total
Water treatment prior to drinking
Boiled
Bleach/chlorine
While alum
Strained through cloth
Ceramic, sand or other filter
Solar disinfection
Stand and settle
Other
No treatment

Table 11. Household sanitation facilities of infants in the 2 intervention groups, the 2 controlled groups

Type of toilet/latrine facilities	WinFood CF (n=110)	WinFood Lite (n=110)	CSB^{+} (n=110)	CSB^{++} (n=110)
Improved , not shared				
Flush/pour flush to piped sewer system				
Flush/pour flush to septic tank				
Flush/pour flush to a pit latrine				
Ventilated improved pit (VIP) latrine				
Pit latrine with a slab				
Composting toilet				
Not improved				
Any facility shared with other household				
Flush/pour flush not to sewer/ septic tank/pit latrine				
Pit latrine without slab/open pit				
Bucket				
Hanging toilet/hanging latrine				
No facility/bush/field				
Other				
Total				

Table 12. Household distribution by household characteristic of infants in the 2 intervention groups, the 2 controlled groups

Housing characteristic	WinFood CF (n=110)	WinFood Lite (n=110)	CSB^{+} (n=110)	CSB^{++} (n=110)
Electronic				
Yes				
No				
Total				
Flooring material				
Earth, sand				
Wood planks				
Palm, bamboo				
Parquet, polished wooded				
Vinyl, asphalt strips				
Ceramic tiles				
Cement tiles				
Cement				
Floating house				
Other				
Total				
Room used for sleeping				
One				
Two				
Three or more				
Total				
Cooking fuel				
Electricity				
Natural gas				

Biogas	
Kerosene	
Coal, lignite	
Charcoal	
Firewood, straw	
Dung	
Other	
Total	
Type of fire/stove among household using solid duel[1]	
Open fire	
Open fire with chimney	
Other	
Total	

[1]Coal/lignite, charcoal, wood/straw/shrubs, and animal dung

Table 13. Household possession of infants in the 2 intervention groups, the 2 controlled groups

Possession	WinFood CF (n=110)	WinFood Lite (n=110)	CSB^{+} (n=110)	CSB^{++} (n=110)
Household effects				
Radio				
Television				
Mobile telephone				
Refrigerator				
Wardrobe				
Sewing machine/loom				

Mean of transport	
Bicycle	
Animal-drawn cart	
Motorcycle/scooter	
Car/truck	
Ownership of agricultural land	
Ownership of farm animal[1]	

[1] *Cattle, cows, bulls, horses, donkeys, goats, sheep and chicken.*

Table 14. Mean energy intake and micronutrient intake of infants in cross-sectional study (n=110) by 24-hr recall

	Total (n=110)		Male		Female	
Nutrients	Mean	95 CI%	Mean	95 CI%	Mean	95 CI%
Energy (kcal)						
Animal Protein						
Vegetable protein						
Animal Fat						
Vegetable fat						
Carbohydrate						
Free sugar						
Iron						
Zinc						

Table 15. Breast-feeding frequency and contribution of project complementary food to daily energy intake of infants in the 2 intervention groups and the 2 controlled groups from non-breast-milk foods estimating be 24 hr-recall

Intake and age	WinFood CF (n=110)	WinFood Lite (n=110)	CSB^{+} (n=110)	CSB^{++} (n=110)
Brest-milk				
Breast-feeding frequency				
Complementary foods				
6 mo total intake (kcal)				
7 mo total intake (kcal) (% from project food)				
8mo total intake (kcal) (% from project food)				
9mo total intake (kcal) (% from project food)				
10mo total intake (kcal) (% from project food)				
11mo total intake (kcal) (% from project food)				
12mo total intake (kcal) (% from project food)				
13mo total intake (kcal) (% from project food)				
14mo total intake (kcal) (% from project food)				
15mo total intake (kcal) (% from project food)				

Table 16. Contribution of project complementary foods to daily iron and other nutrient intake (zinc) of infants in the 2 intervention groups and the 2 controlled groups from non-breast-milk foods estimating by 24-hr recall

Age and nutrient	WinFood CF (n=110)	WinFood Lite (n=110)	CSB^{+} (n=110)	CSB^{++} (n=110)
Iron				
6 mo total intake (kcal)				
7 mo total intake (kcal) (% from project food)				
8mo total intake (kcal) (% from project food)				
9mo total intake (kcal) (% from project food)				
10mo total intake (kcal) (% from project food)				
11mo total intake (kcal) (% from project food)				
12mo total intake (kcal) (% from project food)				
13mo total intake (kcal) (% from project food)				
14mo total intake (kcal) (% from project food)				
15mo total intake (kcal) (% from project food)				
Zinc				
6 mo total intake (kcal)				
7 mo total intake (kcal) (% from project food)				
8mo total intake (kcal) (% from project food)				
9mo total intake (kcal) (% from project food)				
10mo total intake (kcal) (% from project food)				
11mo total intake (kcal) (% from project food)				
12mo total intake (kcal) (% from project food)				

13mo total intake (kcal) (% from project food)	
14mo total intake (kcal) (% from project food)	
15mo total intake (kcal) (% from project food)	

Table 17. Mean weight for age z score, length for age z score, midupper arm circumference, skinfold-thickness measurements and midupper arm fat and muscle areas of infants in the cross-sectional study

Indicators	Cross-sectional study (n=110)	
	Male	Female
Mean weight for age z score		
Mean length for age z score		
Midupper arm circumference (cm)		
Head circumference (cm		
Triceps Skinfold thickness (mm		
Subscapular skinfold thickness (mm		
Midupper arm fat area (mm^2		
Midupper arm muscle area (mm^2		

Table 18. Mean weight for age z score of infants in the 2 intervention groups, the 2 controlled groups

Infants and age	WinFood CF (n=110)		WinFood Lite (n=110)		CSB^{+} (n=110)		CSB^{++} (n=110)	
	Male	Female	Male	Female	Male	Female	Male	Female
6 months								
7 months								
8 months								
9 months								
10 months								
11 months								
12 months								
13 months								
14 months								
15 months								

Table 19. Mean height for age z score of infants in the 2 intervention group, the 2 controlled groups

Infants and age	WinFood CF (n=110)		WinFood Lite (n=110)		CSB^{+} (n=110)		CSB^{++} (n=110)	
	Male	Female	Male	Female	Male	Female	Male	Female
6 months								
7 months								
8 months								
9 months								
10 months								
11 months								
12 months								
13 months								
14 months								
15 months								

Table 20. Mean weight for age z score infants of combining in the pooled intervention groups, the pooled controlled groups

Infants and age	Pooled intervention groups (n=220)		Pooled controlled groups (n=220)	
	Male	Female	Male	Female
6 months				
7 months				
8 months				
9 months				
10 months				
11 months				
12 months				
13 months				
14 months				
15 months				

Table 21. Mean height for age z score infants of combining in the pooled intervention group, the pooled controlled groups

Infants and age	Pooled intervention groups (n=220)		Pooled controlled groups (n=220)	
	Male	Female	Male	Female
6 months				
7 months				
8 months				
9 months				
10 months				
11 months				
12 months				
13 months				
14 months				
15 months				

Table 22. Weight and length gains from 6-15 months of age, and midupper arm circumference, skinfold-thickness measurements and midupper arm fat and muscle areas of infants in the 2 intervention groups, the 2 controlled groups

Indicators	WinFood CF (n=110)		WinFood Lite (n=110)		CSB^{+} (n=110)		CSB^{++} (n=110)	
	Male	Female	Male	Female	Male	Female	Male	Female
Weight gain (kg)								
Length gain (cm)								
Midupper arm circumference (cm)								
6mo								
12 mo								
Difference								
Head circumference (cm)								
6mo								
12 mo								
Difference								
Triceps Skinfold thickness (mm)								
6mo								
12 mo								
Difference								
Subscapular skinfold thickness (mm)								
6mo								
12 mo								
Difference								
Midupper arm fat area (mm^2)								
6mo								
12 mo								
Difference								
Midupper arm muscle area (mm^2)								

6mo	
12 mo	
Difference	

Table 23. Clinical chemistry measures of infants at 6 and 15 months of age in the 2 intervention groups and the 2 controlled groups

Indicators	WinFood CF (n=110)	WinFood Lite (n=110)	CSB^{+} (n=110)	CSB^{++} (n=110)
Hemoglobin (g/L)				
6mo				
12 mo				
Difference				
Hematocrit				
6mo				
12 mo				
Difference				
Plasma transferrrin saturation(%)				
6mo				
12 mo				
Difference				
Plasma ferritin (ug/L)				
6mo				
12 mo				
Difference				
Plasma Zinc				
6mo				
12 mo				

Difference

Table 24. Percentage of infants in the 2 intervention groups and the 2 controlled groups with low clinical chemistry test values at 6 and 12 mo of age

Indicators	WinFood CF (n=110)	WinFood Lite (n=110)	CSB^{+} (n=110)	CSB^{++} (n=110)
Hemoglobin <100 g/L				
6 mo				
12mo				
Hematocrit <0.33				
6 mo				
12mo				
Transferrin saturation <16%				
6 mo				
12mo				
Ferritin <12 umol/L				
6 mo				
12mo				
Zicn <10.7 umol/L				
6 mo				
12mo				

Table 25. Morbidity incidence and prevalence in infants between 6 and 15 mo of age in the 2 intervention groups and the 2 controlled groups

Indicators	WinFood CF	WinFood Lite	CSB^{+}	CSB^{++}

	(n=110)	(n=110)	(n=110)	(n=110)
Diarrhea				
Incidence				
Prevalence (%)				
Fever				
Incidence				
Prevalence (%)				
Respiratory illness				
Incidence of Cough or Runny nose (%)				
Prevalence (%)				
Cough and Runny nose				
Cough or Runny nose				

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Interviewer ID: ☐ **ANNEX A** Child´s ID: ☐☐☐☐

BASELINE INFORMATION

Village: _____

Date of data collection _____ / _____ / _____ No of data collection : BASELINE

Verbal consent obtained from primary caretaker ..... Yes ☐ No ☐

FIRST, I WOULD LIKE TO ASK SOME GENERAL QUESTIONS ABOUT THE CHILD

1. **CHILD´S NAME:** _____ **FATHER'S NAME:** _____ **MOTHER'S NAME:** _____ **GRANDFATHER'S NAME:** _____	
2. **CARETAKER´S NAME:** _____ **HEAD OF HOUSEHOLD NAME:** _____	
3. **HAVE THE CARETAKER PICKED UP THE FOOD RATION?** *Circle ONLY ONE answer*	Yes .....1 No .....2 Answer refused .....8 Don't know.....9
4. **WHAT IS YOUR RELATIONSHIP TO *(name)*?** *Circle ONLY ONE answer*	Biological mother ..... 1 Grandmother ..... 2 Sister ..... 3 Stepmother..... 4 Aunt ..... 5 Other female relative ..... 6 Brother ..... 10 Father ..... 11 Other (specify) ..... 7 _____ Answer refused ..... 8 Don't know 9
5. **HAVE YOU HAD PRIMARY RESPONSIBILITY FOR TAKING CARE OF (name) FOR AT LEAST THE LAST TWO WEEKS?** *Circle ONLY ONE answer*	Yes .....1 No .....2 Answer refused .....8 Don't know.....9
6. **IS *(name)* A BOY OR GIRL?** *Circle ONLY ONE answer*	Boy ..... 1 Girl 2
7. **WHAT IS *(name)*´S DATE OF BIRTH?** *Even if the mother knows the exact date of birth, ask if she has an immunization card or other document and check that the date is correct.* *(mark 01.01.2099 if not known)*	☐☐ Day ☐☐ Month ☐☐☐☐ Year

83. **SOURCE OF DATE OF BIRTH INFORMATION** *Circle ONLY ONE answer* *If a **date of birth is available**, jump to question 10.*	Immunization or vaccination card ..... 1 Birth certificate ..... 2 Caretaker's recall ..... 3 Other (specify)..... 7 _____
9. **HOW OLD IS *(name)*?**	Age in months ..... ☐☐

Demographic and socio-economic variables

I WILL NOW ASK YOU ABOUT THE PEOPLE WHO LIVE HERE AND OTHER THINGS ABOUT YOUR HOUSEHOLD.

104. **WHAT IS THE HIGHEST LEVEL OF SCHOOLING YOU** *(the caretaker)* **HAVE COMPLETED?** *Write in NUMBER OF YEARS of school.*	Number of years of school ..... ☐☐ Informal schooling ..... 66 Answer refused ..... 88 Don't know 99
11. **WHAT IS THE HIGHEST LEVEL OF SCHOOLING** *(say the name of the head of household, see question 2)* **HAS COMPLETED?** .....*Write in NUMBER OF YEARS of school*	Number of years of school ..... ☐☐ Informal schooling ..... 66 Answer refused ..... 88 Don't know 99
12. **HOW MANY PEOPLE USUALLY LIVE IN THIS HOUSEHOLD, THAT IS, HOW MANY PEOPLE USUALLY SLEEPS IN THE HOUSE DURING NIGHT TIME?**	Number of people: ..... ☐☐
13. **HOW MANY CHILDREN UNDER 5 YEARS OF AGE USUALLY LIVE IN THIS HOUSEHOLD?** *Including the study child*	Number children under 5 years ..... ☐☐

14. **WHAT IS THE MAIN SOURCE OF DRINKING WATER DURING DRY SEASON FOR MEMBERS OF YOUR HOUSEHOLD?** *Circle ONLY ONE answer*	Piped water Piped into dwelling ..... 1 Piped to yard/plot ..... 2 Public tap/standpipe ..... 3 Tube well or borehold ..... 4 Dug well Protected well ..... 5 Unprotected well ..... 6 Water from spring Water from protected spring ..... 10 Water from unprotected spring ..... 11 Rainwater Surface source (river/dam/stream/lake pond/canal/irrigation channel) ..... 12 Tanker truck or water vendor ..... 13 Bottled water ..... 14 Other (specify) ..... 7 _____ Answer refused ..... 8 Don't know 9
15. **DURING THE WET SEASON, IS THE MAIN SOURCE OF DRINKING WATER FOR MEMBERS OF THE HOUSEHOLD THE SAME AS DURING DRY SEASON?**	Yes ..... 1 No ..... 2 Answer refused ..... 8 Don't know 9
16. **WHERE IS THE WATER SOURCE LOCATED**	In own dwelling ..... 1 In own yard/plot ..... 2 Other (specify) ..... 7 _____ Answer refused ..... 8 Don't know 9
17. **DO YOU TREAT YOUR WATER IN ANY WAY TO MAKE IT SAFER TO DRINK?** *Circle ONLY ONE answer* *If **yes**, continue.* *If **other answer**, jump to question 30.*	Yes ..... 1 No ..... 2 Answer refused ..... 8 Don't know 9

18. **WHAT DO YOU DO TO THE WATER TO MAKE IT SAFER TO DRINK?** *Circle ALL applicable answers*	Boil ..... 1 Add bleach, chlorine or Agar ..... 2 White alum ..... 3 Strain it through a cloth ..... 4 Use a water filter ..... 5 Solar disinfection ..... 6 Let it stand and settle ..... 10 Other (specify) ..... 7 _____ Answer refused ..... 8 Don't know 9
19. **WHERE DO MEMBERS OF YOUR HOUSEHOLD USUALLY GO TO RELIEVE THEMSELVES?** *Circle ONLY ONE answer*	Flush or pour flush toilet Flush to piped sewer system ..... 1 Flush to septic tank ..... 2 Flush to pit latrine ..... 3 Flush don't know where ..... 4 Pit latrine Ventilated improved ..... 5 Pit latrine (VIP) ..... 6 Pit latrine with slab ..... 10 Pit latrine without slab/open pit ..... 11 Composting toilet ..... 12 Bucket toilet ..... 13 Toilet over water ..... 14 No Toilet/field/forest ..... 15 Other (specify) ..... 7 _____ Answer refused ..... 8 Don't know 9
20. **DO YOU SHARE THIS TOILET FACILTY WITH OTHER HOUSEHOLDS?**	Yes ..... 1 No ..... 2 Answer refused ..... 8 Don't know 9
21. **HOW MANY HOUSEHOLDS USE THIS TOILET FACILITY?**	No of household members ..... ☐☐ Answer refused ..... 88 Don't know 99

22. **WHAT IS THE PRIMARY SOURCE OF INCOME FOR THIS HOUSEHOLD?** *Circle ONLY ONE answer*	Fishery ..... 1 Farming, including cash crops ..... 2 Livestock ..... 3 Employment/salary ..... 4 Petty trading (including sale of fire-wood, charcoal, grass, local brewery) ..... 5 Daily labor ..... 6 Handicrafts/artisan ..... 10 Remittances ..... 11 Other (specify) ..... 7 _____ Answer refused ..... 8 Don't know 9
23. DOES THIS HOUSEHOLD OWN ANY LIVESTOCK, HERDS OR FARM ANYMALS?	Yes ..... 1 No ..... 2 Answer refused ..... 8 Don't know 9
24. **I WILL NOW MENTION SOME ANIMALS, AND I WOULD LIKE YOU TO TELL ME HOW MANY ANIMALS OF EACH TYPE YOU HAVE.** *Fill in NUMBER of each type of animal*	**WATER BUFFALO**: ..... ☐☐☐ **COWS/BULLS**: ..... ☐☐☐ **HORSES:** ..... ☐☐☐ **GOATS:** ..... ☐☐☐ **PIGS**: ..... ☐☐☐ **CHICKEN/DUCKS**: ..... ☐☐☐
25. **DOES ANY MEMBER OF THIS HOUSEHOLD OWN ANY LAND THAT CAN BE USED FOR AGRICULTURE?**	Yes ..... 1 No ..... 2 Answer refused ..... 8 Don't know 9
265.**HOW MUCH LAND DOES YOUR HOUSEHOLD OWN?** *Write in number of local units and the name of the local unit*	**SQ METERS**..... ☐☐☐ **A:** ..... ☐☐☐ **HECTARE:** ..... ☐☐☐ **RAY:** ..... ☐☐☐ **KONG**: ..... ☐☐☐ **DON'T KNOW**: 999

27. **DOES YOUR HOUSEHOLD HAVE:** *Circle 1 or 2 for each item*	Yes No **ELECTRICITY/GENERATOR:** ..... 1 2 **A SEWING MACHINE:** ..... 1 2 **A TRIDLE PUMP:** ..... 1 2 **A RADIO:** ..... 1 2 **A TELEVISION:** ..... 1 2 **A MOBILE TELEPHONE:** ..... 1 2 **A NON-MOBILE TELEPHONE:** ..... 1 2 **A REFRIGERATOR:** ..... 1 2 **A TABLE:** ..... 1 2 **A CHAIR:** ..... 1 2 **A BED:** 1 2
28. **DOES YOUR HOUSEHOLD HAVE ANY MOSQITO NETS THAT CAN BE USED WHILE SLEEPING?**	Yes ..... 1 No ..... 2 Answer refused ..... 8 Don't know 9

THANK YOU VERY MUCH FOR TAKING YOUR TIME FOR THIS INTERVIEW.

Signature of Interviewer: _____

Interviewer ID: ☐ **ANNEX B** Child´s ID: ☐☐☐☐

General Data Collection Form-Follow Up

Village: _____

Date of data collection _____ / _____ / _____ No of data coll: 1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9

Verbal consent obtained from primary caretaker ..... Yes ☐ No ☐

6. **CHILD´S NAME:** _____ **FATHER'S NAME:** _____ **MOTHER'S NAME:** _____ **GRANDFATHER'S NAME:** _____	
7. **CARETAKER´S NAME:** _____ **HEAD OF HOUSEHOLD NAME:** _____	
3. **WHAT IS YOUR RELATIONSHIP TO *(name)*?** *Circle ONLY ONE answer*	Biological mother ..... 1 Grandmother ..... 2 Sister ..... 3 Stepmother..... 4 Aunt ..... 5 Other female relative ..... 6 Brother ..... 10 Father ..... 11 Other (specify) ..... 7 _____ Answer refused ..... 8 Don't know 9
8. **HAVE YOU HAD PRIMARY RESPONSIBILITY FOR TAKING CARE OF (name) FOR AT LEAST THE LAST TWO WEEKS?** *Circle ONLY ONE answer*	Yes .....1 No .....2 Answer refused .....8 Don't know.....9

Child morbidity

First there will come some specific questions on fever cough and Diarrhea

5. **IN THE PAST 14 DAYS, HAS (NAME) BEEN:** *Circle ONLY ONE answer*	Well …….....1 Mild illness but recovered without treatment...2 Moderate illness which needed drugs or Clinical visit ..... 3 Serious illness requiring doctor ..... 4 Answer refused …….8 Don´t know 9

69. **IN THE PAST 14 DAYS, HAS (*NAME*) HAD FOLLOWING SYMPTOMS:** 1=yes 2= no

A	**BLOOD IN STOOLE**	1	2
B	**LOOSE WATERY DIARRHEA**	1	2
C	**DIARRHEA WITH BLOOD OR MUCUS**	1	2
D	**COUGHING**	1	2
E	**RUNNING NOSE**	1	2
F	**FEVER**	1	2
G	**DIFFICULTY IN BREATHING**	1	2
H	**VOMITING**	1	2
I	**EYE PROBLEM (REDNESS, DISCHARGE)**	1	2
J	**EAR PROBLEM (DISCHARGE) /PAIN**	1	2
K	**FEEDING POORLY**	1	2
L	**LETHARGIC CHILD**	1	2
M	**SKIN RASHES**	1	2
N	**CONSTIPATION**	1	2
O	**OTHER (SPECIFY) _____**	1	2
N	**MEDICINE TAKEN**		

Medicine codes: 0=nothing; 1=vitamins, tonics; 2=anti-cough, anti-vomiting, anti-diarrhea; 3=painkillers, anti-inflammatories; 4=antibiotics; 5=other medicine supplied by health professionals; 6=other medicine supplied by non-health professionals

THANK YOU VERY MUCH FOR TAKING YOUR TIME FOR THIS INTERVIEW.

Signature of interviewer:_____

Interviewer ID: ☐ **ANNEX C** Child´s ID: ☐☐☐☐

THE WINFOOD INTERVENTION STUDY
24 FOOD RECALL FORM

Village _____No of data collection: Baseline / 1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9

Date of data collection _____ / _____ / _____ , Day of week: _____ Time of Recall: _____ / _____

24 FOOD RECALL FORM

Time	Place	Food & Description	Serving size	Raw ingredients		Weight of total cooked ingredients (g)	Amount Eaten (g)
				Food items	Weight (g)		

Additional questions

Breast feeding (*from WHO standard question on breastfeeding*)

I WILL NOW ASK YOU SOME QUESTIONS ABOUT BREAST FEEDING YOUR CHILD

10. **HAS *(name)* EVER BEEN BREASTFED?** *Circle ONLY ONE answer*	Yes ..... 1 No ..... 2 Answer refused ..... 8 Don´t know 9
2. **SINCE THIS TIME YESTERDAY, HAS *(name)* BEEN BREASTFED?** *Circle ONLY ONE answer*	Yes ..... 1 No ..... 2 Answer refused ..... 8 Don´t know 9
3. **AT WHAT AGE DID *(name)* START EATING COMPLEMENTARY FOOD?** **COMPLEMENTARY FOOD IS ANY FOOD WHICH IS NOT BREASTMILK EXCEPT MEDICATIONS.**	Number of months ..... ☐☐

Compliance

I WILL NOW ASK YOU SOME QUESTIONS ABOUT THE UTILIZATION OF THE SUPPLEMENTARY FOOD IN THE HOUSEHOLD YOU HAVE RECEIVED FROM RACHA.

4. **CAN I PLEASE SEE THE EMPTY RATIONS BAGS FROM THE MONTHLY FOOD RATION?**	No. of empty packets …….. ☐☐ Answer refused ……..……88 Don't know ……99
5. **YESTERDAY, DID YOU PREPARE AND SERVE ANY OF THE MCH FOOD YOU RECEIVED?**	Yes ..... 1 No ..... 2 Answer refused ..... 8 Don´t know 9
6. HOW MANY PACKETS DID YOU USE TO PREPARE THE FOOD YESTERDAY?	No. of used packets…….. ☐☐ Answer refused ……88 Don't know ……99

11. **YESTERDAY, WHO IN THE HOUSEHOLD ATE THE PREPARED MCH FOOD YOU PREPARED?** *Circle ALL applicable answers*	(name) ..... 1 Another child in the HH under 5 years of age ..... 2 Another child in the HH 5 years or older .. 3 Father ..... 4 Mother ..... 5 Pregnant/lactating woman in the household ..... 6 Elderly member ..... 10 Other member of household ..... 11 Other (specify) ..... 7 _____ Answer refused ..... 8 Don't know 9
12. **YESTERDAY, WHAT PROPORTION OF THE PREPARED MCH FOOD YOU PREPARED WAS EATEN BY *(name)*?** *Circle ONLY ONE answer*	None ..... 0 Less than 1/2 ..... 1 About half ..... 2 Most ..... 3 All ..... 4 Answer refused ..... 8 Don't know 9

Responsible Data Collector ID: ☐ **Annex D** Child´s ID: ☐☐☐☐

The Morbidity – Routine Clinical Examination
(Morbidity – Routine Clinical Examination)

Village : _____

Date of data collection **_____ / _____ / _____**

No of data coll: Baseline /**1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9**

_____

1	GENERAL CONDITION	Well/alert ……..... 1 Restless/irritable ..... 2 Abnormally sleepy) .....3 Sleeping, could not be assessed.....4
2	RESPIRATORY RATE/MINUTE *count for 60 sec and repeat*	1:……..…..…… ☐☐. 2:…….……..…… ☐☐
3	COUGHING	Yes…….…….1 No……...2
4	RUNNING NOSE	Yes…….…….1 No……...2
5	NASAL FLARING	Yes…….…….1 No……...2
6	AUDIBLE WHEEZING OR	Yes…….…….1

	GRUNTING	No……..……...2
7	SEVERE CHEST INDRAWING	Yes…….……1 No……...2
8	BULGING FONTANELLE	Yes…….……1 No……...2
9	EYE PROBLEM (redness, discharge)	Yes…….……1 No……...2
10	EAR PROBLEM (e.g. discharge)	Yes…….……1 No…….……...2
11	SKIN RASH OR PUSTULES	Yes…….1 No……...2
12	JAUNDICE	Yes…….……1 No……...2
13	TREATMENT REQUIRE	None…….……1 Home Care without medicine……..2 Hospitalization…….3

Interviewer ID: ☐ **ANNEX E** Child´s ID: ☐☐☐☐

Anthropometric and clinical examination

Village: _____

Date of data collection _____ / _____ / _____

No of data coll: Baseline / 1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9

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1. Anthropometry

1.1 Weight (record three measurements)		
1.1.1 I__I__I . I__I__I kg	1.1.2 I__I__I . I__I__I kg	1.1.3 I__I__I . I__I__I kg
1.2 Lenght (record three measurements)		
1.1.1 I__I__I__I . I__I cm	1.2.1 I__I__I__I . I__I cm	1.2.3 I__I__I__I . I__I cm
1.3 Knee-heel (record three measurements)		
1.3.1 I__I__I . I__I__I cm	1.3.2 I__I__I . I__I__I cm	1.3.3 I__I__I . I__I__I cm
1.4 MUAC (record three measurements)		
1.4.1 I__I__I . I__I cm	1.4.2 I__I__I . I__I cm	1.4.3 I__I__I . I__I cm
1.5 Head circumference (record three measurements)		
1.5.1 I__I__I . I__I cm	1.5.2 I__I__I . I__I cm	1.5.3 I__I__I . I__I cm
1.6 Triceps Skinfold Thickness (TSF) (record three measurements)		
1.6.1 I__I__I . I__I cm	1.6.2 I__I__I . I__I cm	1.6.3 I__I__I . I__I cm

2. Clinical Examination

2.1 Temperature		
I__I__I . I__I C^o		
2.2 Blood pressure		
Diastolic: I__I__I mmHg	Systolic: I__I__I mmHg	
2.3 Signs of undernutrition related diseases		
Oedema: yes no	Vitamin A deficiencies: yes no	
2.4 Hemoglobin		
I__I__I . I__I g/l		

Interviewer ID: ☐ **ANNEX F** Child´s ID: ☐☐☐☐

Data Collection Form To Measure The Child Development Milestone- Staff Report

Village: _____

Date of data collection _____ / _____ / _____

No of data coll: BASELINE, 1, 2, 3, 4, 5, 6, 7, 8, 9

The tool for measuring milestone (ref. WHO – the Multicentre Growth Reference Study)
(The Staff data collection form)

Test items	
1.Sitting without support	No (*inability*) ..... 1 No (*refusal*) ..... 2 Yes ..... 3 Unable to test 9
2.Hands-and-knees crawling	No (*inability*) ..... 1 No (*refusal*) ..... 2 Yes ..... 3 Unable to test 9
3.Standing with assistance	No (*inability*) ..... 1 No (*refusal*) ..... 2 Yes ..... 3 Unable to test 9
4.Walking with assistance	No (*inability*) ..... 1 No (*refusal*) ..... 2 Yes ..... 3 Unable to test 9
5.Standing alone	No (*inability*) ..... 1 No (*refusal*) ..... 2 Yes ..... 3 Unable to test 9

6.Walking alone	No (*inability*) ..... 1 No (*refusal*) ..... 2 Yes ..... 3 Unable to test 9
7a. Child's emotional state (scale a) *Rate the child's emotional state during the testing of all the milestones*	Drowsy ..... 1 Awake and alert ..... 2 Unable to test 9
7b. Child's emotional state (scale 5) *Rate the child's emotional state during the testing of all the milestones*	Calm ..... 1 Fussy ..... 2 Crying ..... 3 Unable to test 9
Remarks	

Interviewer ID: ☐ **ANNEX G** Child´s ID: ☐☐☐☐

Data Collection Form To Measure The Child Development Milestone- Caretaker Report

Village:_____

The tool for measuring milestone (ref. WHO – the Multicentre Growth Reference Study)
(The Caretaker form)

Test items	
1.Sitting without support Tested and recorded.....1 Recalled……..……..2	☐☐ ☐☐ ☐☐☐☐ Day Month Year
2.Hands-and-knees crawling Tested and recorded.....1 Recalled……..2	☐☐ ☐☐ ☐☐☐☐ Day Month Year
3.Standing with assistance Tested and recorded.....1 Recalled……..2	☐☐ ☐☐ ☐☐☐☐ Day Month Year
4.Walking with assistance Tested and recorded.....1 Recalled……..2	☐☐ ☐☐ ☐☐☐☐ Day Month Year
5.Standing alone Tested and recorded.....1 Recalled……..2	☐☐ ☐☐ ☐☐☐☐ Day Month Year
6.Walking alone Tested and recorded.....1 Recalled……..2	☐☐ ☐☐ ☐☐☐☐ Day Month Year

Interviewer ID: ☐ **ANNEX H** Child´s ID: ☐☐☐☐

Blood sample collection, handling and storage SOP

A. Venous blood sample collection

Preparatory

1. Clean dedicated white coat, powder-free vinyl or nitrile gloves, tourniquet and indelible black pen to be used
2. Sharps box and clinical waste bag are available
3. Supplies of cling film, cotton wool, vacutainers (red top fro serum), butterfly needles (23G Becton Dickinson), cotton wool, ethanol, plasters
4. Racks for holding blood samples stored in the refrigerator
5. Pack for transporting samples to lab is refrigerated. NOTE: Samples must be kept chilled at all times, preferably with 'blue ice' or ice packs from blood collection to arrival at lab, *but not frozen*. For this, a large coolbox, with the icepacks on the bottom is preferred. Direct contact between icepacks and blood tubes should be avoided.

Taking samples

1. Samples to be taken in a private room, each child individually. Hands to be washed between children and a new pair of gloves to be used for each child
2. Apply Ametop gel topical anaesthetic to the area of the infant's arm where blood sample is to be taken. One tube of gel should serve 3 children (~0.5g per child). Wrap cling film around the child's arm at site of venipuncture and leave Ametop gel in place for 30 minutes
3. Wipe away Ametop gel with cotton wool and apply a small amount of ethanol to the site of Venipuncture
4. Apply tourniquet above site of venipuncture
5. A 23G butterfly needle is used to take the blood sample. Insert needle and insert trace vacutainer into collection system barrel when blood is drawn
6. The tourniquet should be loosened during blood collection, *before* the needle is removed from the child's arm
7. The ideal volume of blood to be collected is 3mls. When sample collected removed vacutainer from barrel first and then needle from child's arm. Apply cotton wool immediately and gentle pressure to the site of venipuncture.
8. Dispose of blood collection system into sharps box
9. Remove cotton wool and apply plaster to site of venipuncture
10. Label tubes with subject ID, visit of collection and date of collection
11. Invert tube 8 times and place immediately in the refrigerator in an upright position
12. Note the subject details and collection of blood in log book.

13. Samples are transported to Laboratory at the National Institute for Public Health, Phnom Penh, Cambodia, where it will be kept cold until it is send to the Laboratory at Department of Micronutrients, National Institute of Nutrition, Ministry of Health in Hanoi, Vietnam. The samples will be transported in the refrigerated cool pack in an upright position. If frozen icepacks are used, wrap the sample tubes in cotton wool to prevent them directly touching the frozen ice-pack.
14. The blood samples obtain the current day is copied to the drivers log book.
15. A lab sheet accompanying the sample must display the subject ID, the visit of the blood sample, and the time of blood collection. The sheet must be signed by the nurse in charge. NOTE: The name of the child must **not** be displayed.
16. The driver will take the sample in the cool pack to the Laboratory at the National Institute for Public Health, Phnom Penh, Cambodia. The driver will bring the driver log book.

B. Venous blood samples processing

Preparatory

1. . Dedicated lab coat for sample handling – to be worn at all times in lab, and stored in laboratory only
2. Powder-free vinyl or nitrile gloves (a new pair between laboratory protocols) should be used at all times for the preparation and handling/processing of the blood samples
3. Dedicated cold-pack rack for holding tubes is stored in –20 freezer
4. Ice box with polystyrene tube inserts ready for the vacuutainers when they arrive with the sample
5. Pre-printed labels and fine, indelible black pen available
6. All labelling to be done using the 'permanent' indelible, freezer and water-proof, black marker pens provided
7. Used gloves and used plastic ware in laboratory to be discarded and autoclaved
8. When the driver arrives from the clinic, take samples and record the arrival of the samples in the log book. Make sure the driver takes the clinic ice-pack and log book back to the clinic.

Processing, labeling and storing

1. Place sample immediately to chill in the polystyrene ice-box (in an insert)
2. The sheet accompanying the sample shows the time at which the blood sample was collected from infant. Determine how long the sample may be left to clot and judge the volume of serum that will be available. If the sample is not yet clotted then place immediately in the refrigerator on a pre-cooled rack stored in the refrigerator until clot (estimate 30 minutes)
3. Blood samples to be spun at 2700rpm (calculated equivalent to between 1000-1300g, note must not exceed 1300g or 2700rpm) for 10 minutes in a refrigerated centrifuge at 4°C
4. While samples are in centrifuge remove rack from the –20 freezer, set up appropriate number of tubes (tubes for quantifying volume of serum collected *and* sample tubes) and label sample tubes (cap and pre-printed labels)
5. When sample centrifuged replace blood collection tube in polystyrene ice-box with insert. Check for haemolysis (any pink/orange tints to the sera) or any abnormalities and, if present, note in log book.
6. Remove all the serum to tubes to estimate volume. It is important to avoid the jelly-like phase that may exist between the serum and the clot; therefore pipette gently, and in small volumes, from the top of the serum

7. Determine the total volume of serum that has been removed and note in log book. Divide the serum into storage tubes in aliquots of about 100 l. Tubes should be labeled with ID, visit number, and A, B, C for the sequential aliquots. NOTE: Sample aliquoting and labelling is completely crucial to the research project. Total professionalism must be applied for these important and valued steps. Any mistakes will prevent further analyses and thus are NOT expected from this SOP. If an exceptional problem does happen to occur, it must be recorded.
8. Note in the log book what how many aliquots have been stored.
9. Note in the log book ANY observation which is different from normal, e.g. cloths or fibrin in the serum, large buffy coat etc Calculate and note time of processing in the lab log book.
10. Put samples in -80 freezer and note their location in the freezer log book.

Final check after venous blood processing

1. Log book filled in completely
2. Sample location entered on databases
3. Rack replaced in –20 freezer
4. Tubes and pipette tips replaced in designated cupboard
5. Blood collection tubes, waste tubes and pipette tips disposed of for autoclaving
6. Bench wiped clean with disinfectant

C. Hemoglobin test

1. Cut the tube with a scissor from the butterfly needle.
2. Take an Eppendorf bottle and squeeze the blood from the tube into the Eppendorf bottle.
3. Dip the Hemocue cuvet in the blood.
4. Follow machine instructions to get correct reading. Record on data form.

Interviewer ID: ☐ **ANNEX I** Child´s ID: ☐☐☐☐

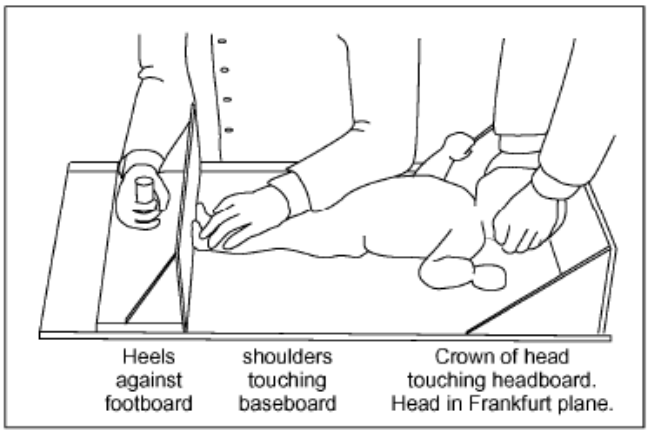
Anthropometry SOP

Measuring Recumbent length

Only children less than 24 months will be enrolled in the Winfood study, therefore always the recumbent length will be measured with a measuring board. Two examiners are required to correctly position the subject and to ensure accurate and reliable measurements of length.

1. Place the subject, face upward, with the head at the fixed end of the board and the body parallel to its long axis. The shoulders should rest against the surface of the board.
2. Apply gentle traction to bring the crown of the subject's head into contact with the fixed headboard and simultaneously position the head so that it is in the Frankfurt plane.
3. Hold the subject's feet, without shoes, toes pointing directly upward, while keeping the subject's knees straight by placing one hand on the knees. Then bring the movable footboard to rest against the heels.
4. Record the length to the nearest millimeter.
5. Repeat 1-4 two times, repositioning the subject between measurements.
6. Record all three measurements on the form.

Note: If the subject is restless, only the left leg should be positioned for the measurement.



Measuring weight with Uniscale

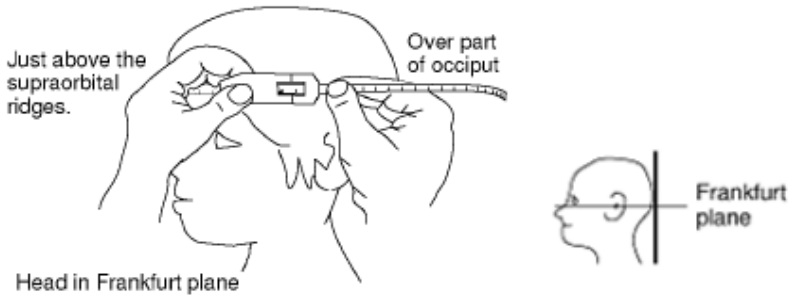
1. Put the scale on the floor. Choose the flattest, most level surface you have.
2. Do not stand on the scale yet.
3. Turn on the scale. Move your foot across switch window **(Figure a)**
4. Ask the mother to step on the scale by herself. She can give her child to you or another person to hold **(Figure b)**
5. Make sure her feet or clothes do not cover the switch window. You will see the mother's weight in the display, for example: **52,4**
6. With the mother on the scale pass your foot slowly across the switch window **(Figure c)**. Then wait a couple seconds and you will see: **0,0**
7. Ask the mother to step off the scale. You should see: **--.-**
8. Ask the mother to step back on the scale with her child **(Figure d and e)**. You should see the child's weight: **5,4**
9. Ask the mother to step off the scale. You should see: **--.-**
10. Repeat 4-9 two times more.
11. Record all three measurements on the form.

Figure a Figure b Figure c Figure d Figure e

Measure head circumference

For the measurement, a fiberglass insertion tape. Any added objects in the hair such a hair pins should be removed for the measurement.

1. The measurer should stand facing the left side of the subject. Gently, place the subject, face upward, on an examination table, allowing the shoulders to rest against the surface of the table.
2. Position the head so that it is in the Frankfurt plane i.e., an imaginary plane which passes through the external auditory meatus (the small flap of skin on the forward edge of the ear) and over the top of the lower bone of the eye socket immediately under the eye, is vertical.
3. Place the tape just above the supraorbital ridges covering the most prominent part of the frontal bulge and over the part of the occiput that gives the maximum circumference. Care must be taken to ensure that the tape is at the same level on each side of the head and pulled tightly to compress the hair.
4. Measure the circumference to the nearest millimeter.
5. Repeat 1-4 two times more.
6. Record all three measurements on the form.



Measure mid-upper arm circumference

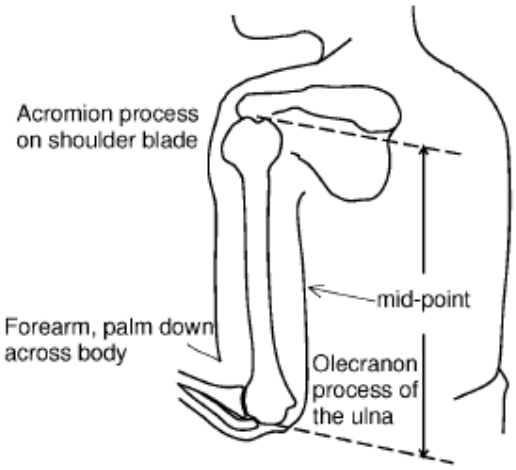
Obtain this measurement with the mother or caregiver seated and holding the infant in her lap. The infant should be wearing loose clothing without sleeves to allow exposure of the shoulder area. Use a fiberglass insertion tape.

1. Gently bend the left arm through 90 degrees at the elbow, and then place the forearm with the palm down across the body.
2. Locate and mark the tip of the shoulder.
3. Locate the tip of the elbow.
4. Measure the distance between these two points using a fiberglass insertion tape, and mark the midpoint with a soft pen or indelible pencil, directly in line with the point of the elbow and shoulder.
5. Relax the arm so that the elbow is extended and hanging just away from the side of the trunk, with the palm facing the thigh. Then wrap the tape gently but firmly around the arm at the midpoint, care being taken to ensure that the arm is not squeezed. Measurements are taken to the nearest mm.
6. Repeat 1-5 two times more.
7. Record all three measurements on the form.

Measure skinfold

Obtain these measurements with the mother seated and holding the infant in her lap. Alternatively, children may be measured lying down. It is helpful to demonstrate the caliper on the hand of the measurer and on the hand of the infant, measuring total palm thickness, before beginning to measure skinfold thickness. Measure the tricep skinfold

1. Gently bend the left arm through 90 degrees at the elbow, and then place the forearm with the palm down across the body.
2. Locate and mark the tip of the acromion process of the shoulder blade at the outermost edge of the shoulder.
3. Locate the tip of the olecranon process of the ulna.
4. Measure the distance between these two points using a fiberglass insertion tape, and mark the midpoint with a soft pen or indelible pencil, directly in line with the point of the elbow and shoulder.
5. Extend the infant's arm so that it is hanging loosely by the side.
6. Grasp a vertical fold of skin plus the underlying fat, 1cm above the marked midpoint, in line with the tip of the olecranon process, using the thumb and forefinger.
7. Gently pull away the skinfold from the underlying muscle tissue, and apply the caliper jaws at right angles, exactly at the marked midpoint.
8. Hold the skinfold between the fingers while measuring.
9. Repeat 5-8 twice more and write the measure in the form



Measure subscapular skinfold

The site is just inferior to the inferior angle of the shoulder, and can be identified more readily by placing the child's arm behind the back (see figure A).

1. To locate the site, the health professional will run a finger along the shoulder blade until the inferior angle is identified.
2. Relax the shoulder and the arm, and pick up a skinfold on a 45^0 angle from horizontal, in the same direction as the inner border of the shoulder (i.e., medially upward and laterally downward) (See Figure A).
3. Skinfolds should be recorded to 0.2 mm on the Harpenden skinfold calipers three times. Skinfold measurements made with precision calipers should normally agree to within 1mm.

4. Record all 3 measurements on the form

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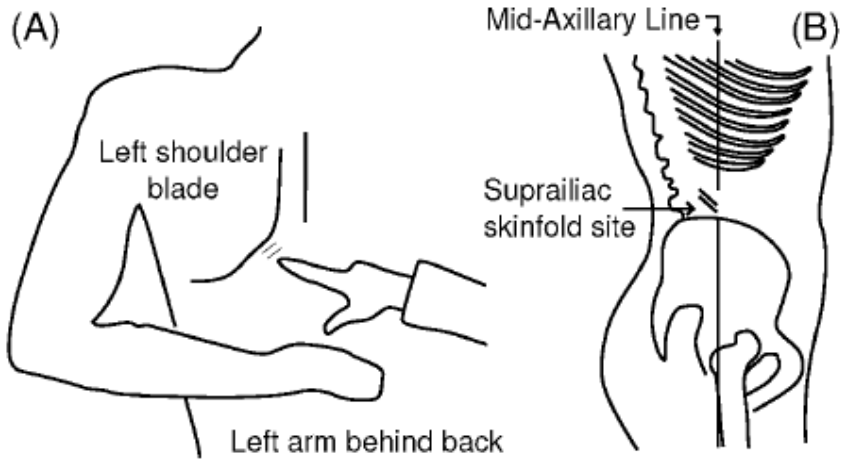

Handheld infant Knemometer

1. Move the two arms of the Knemometer completely together until they touch one another and turn on the display by pressing the ON/OFF button (ZERO/ ON buttons on the old model).
2. Check the zero (every time the display is turned on) by moving the arms of the knemometer a little apart and together again. If the display does not show 0.00, it should be zeroed by pressing the ZERO/ABS button (ZERO/ON button on the old model).
3. Place the child on his/her back on an examination couch. The right leg should be free of clothing including socks. Left leg can be used if there is a strong reason not to use right one (e.g. ulcer). In this case subsequent measurements must be made on the same side.
4. Position yourself on the child's right hand side. Sitting on chair level to the coach and resting the elbows on the coach is preferable.
5. Support the knee with the left hand. Hold the knee with the left thumb and middle finger against the top of " the cap for the knee "with a firm constant pressure. The first and second finger support the knee form both sides, keeping it in the middle of " the cap for the knee'.
6. Support the foot with the right hand. The heel is placed in the ' heel cap' and the foot is held in the position with the thumb and middle finger on the sides of the forefoot. Operate the trigger with the forefinger.
7. Ensure the correct position of the child and apply pressure with forefinger of the right hand, until the trigger is released and the reading is taken.
8. Remove the knemometer after the reading is taken and repeat the measuring procedure until a series of 5 readings have been performed. The mean, range, maximum, minimum and standard deviation of the 5 readings is calculated by pressing the STAT button.
9. A good estimate is a series of 5 readings with a standard deviation (SD) less than 0.8 mm. So repeat the measurement if SD is greater ≥ 0.8mm.
10. Press the CLEAR button before the next child is measured.

DIETARY ASSESSMENT

Dietary assessment will conduct by using a 24-hour food recall to estimate energy and nutrient intakes have been consumed by the child. The mother will be asked what the enrolled child ate in the last 24 hours. Furthermore, the frequency of breastfeeding for the last 24 hours also be reported. All food and beverage consumed will be recorded as raw cleaned food in gram per child per day using standard household measurement which was then converted into weight using digital scales Soehnle digital scale (Art.Nr. 65811) with 1 g accuracy between 0-1 kg, and 2 g accuracy between 1-2 kg.

The cross-sectional study among 440 children aged 6-15 months in the village adjacent to the villages selected for the intervention study; the dietary assessment will be explored. Among the 440 children, a dietary assessment based on the 24-hr recall and baseline data will be carried out in the subgroup as before and after intervention (as single-day 24-hour recall). Moreover, study of the basic anthropometric measures of the non-enrolled children will also be conducted. On the other hand, dietary assessment will also be conducted on the enrolled children in intervention and control group as monthly basic (as 3days 24-hour recall: one working day and 2 weekend days per month).

Preparing for the 24-hour food recall

Errors that affect the quality of the dietary data collected via a 24-hr recall procedure may arise during collection and recording of the food intake data such as:

- Respondent biases occur when the subjects overestimate facts such as income and age. Consumption of meat and refined cereals may also be overreported.
- Interviewer biases may occur if different interviewers probe for information to various degree (i.e. intentionally omit the questions) or record responses incorrectly.
- Respondent memory lapses may result in the unintentional omission of snacks eaten outside the home, such as consumed on the street, neighbors and friend. This may result in food being intentionally added during the recall.
- Incorrect estimation of portion size occurs when the respondents fail to quantify accurately the amount of food consumed. (Interviewer may make assumption about the answer, such as average serving size)
- Use of the nutritional supplements such as multivitamins and minerals may be omitted, particularly of some micronutrients.
- Computation errors may arise when portion size estimates are converted from household measures into grams. Other errors can be occurred when the food consumption values are compiled and nutrient intakes are calculated.

Assembling and calibrating equipment

The selection of local utensils such as glasses, mugs, cup, bowls, plates, and spoons will be purchased for estimating the amount of foods and beverages actually consumed. All of these local utensils will be calibrated with the standard measurement before being used. Glasses will be used to estimate the volume of beverage. Mugs and cup will be used to estimate the volume of any liquid served: sugar cane juice, palm juice, ice juice, etc. Bowl will be used to estimate the volume of soups, dessert, porridges, canned fruits, stew, etc. Spoons will be used to estimate small amount of many kinds of sugar, salt, and cooking oil. The set of calibrating

glass beaker (1000, 500, 250 and 50 ml) will be used to estimate the portion size of liquids and flowing solid. Dietary scale, graduated measuring cylinder, and a set of glass beakers will be bought in advance.

Translating and pretesting

All questionnaires will be translated into Khmer language and the pretest will be conducted in the near study site, using the respondents who are similar to the respondents that will be participated in the actual survey.
The objectives of the pretest are to identify the potential problems:

- The respondents are willing and able to answer the question in the way that they are asked;
- No questions are difficult to answer;
- The question are well understood by respondents;
- The respondents have the same understanding the questions that interviewers have;
- The questionnaires and recall form are designed with adequate space for responses;
- The interview will not interfere with the respondents' ability to performed the necessary daily tasks, and
- The interview does not take too long.

Selecting the recall interviewers

The DFPTQ staff will be selected (3-4 person) as interviewers for conducting questionnaires survey and 24-hour food recall.

Training the interviewers

Adequate training for the interviewers is crucial because the success of the dietary survey depends on the commitment and skill of the recall team. To minimize any interviewer bias on the recalled intakes each interviewer conducts only one recall per respondent, particularly when several interviewers are employed for the same survey.

The training session for the interviewers will be participatory and include discussion, small group exercises, and role playing. Handouts will be prepared and provided for/to the recall team and audiovisual aids will be used during the training. The training will be taken 3 days to train the field staff to carry out the recall interviewer. On day 1 the purpose of the 24-hour food recall will be explained and working arrangement. On day 2 details of the 24-hour recall interviewers and associated questionnaires are discussed, with some practice interviewer and role playing. On day 3 a field exercise will be carried out with emphasis on the procedures used to estimate portion size. Potential problems are also discussed.

Conducting the 24-hour food recall

The success of the conducting of 24 hour recall depends on the respondent's memory, how well the respondent estimates the portion sizes consumed, and the skill and persistence of the interviewer. The interview will be conducted at respondent's home when the person is not too busy, because the familiar environment encourages participation, improve the recall of food consumed, and facilitates calibration of local household utensils by the interviewer. The actual 24-hour recall interview is conducted in four stages by trained interviewers using a standardized protocol.

- In the first stage a completed list of all foods and beverages consumed during the preceding day is obtained.
- In the second stage detailed descriptions of all the foods and beverages consumed, including cooking methods and brand names (if possible) are together time and place of consumption.
- In the third stage estimates of the amounts of all foods and beverages consumed are obtained. Information on the ingredients must also be collected at this time.
- Finally, the fourth stage the recall is reviewed to ensure that all items have been recorded correctly.

Recalling the foods and drinks consumed

For the first stage of the recall interview, a list of all foods and drinks (including water) consumed during the preceding 24-hour period will be obtained. The interviewer will be given the brief introduction about the purpose of the study, during which the name and identification of interviewer will be given to the respondent.

Respondents will be told that questions will cover all the foods and beverages consumed during the preceding day, with emphasis on the pattern of eating. Stress to respondent that all responses will be confidential and emphasize the importance of providing the correct information. Neutral question will be used throughout the interview such as "when did you get up in the morning?" and "Did you eat and drink anything then?" Avoid asking questions about specific meals (e.g. breakfast, lunch, or dinner) or about snack. During the interview the interviewer should keep an open mind and avoid showing signs of surprise, approval, or disapproval of the respondent's eating pattern. The interviewer must always be conducted with as open and pleasant manner with the aim of being friendly, empathetic, and determined, as appropriate.

Describing the foods and drinks consumed

In the second stage of the recall interview, the interviewer go over, in order, each of the responses made by the respondent in the stage 1, probing for more specific description of all foods and drinks consumed, including cooking methods. On the other hand, if the respondents are unable to provide any details listed for the mixed dishes, data for a average recipe for each mixed dish of individual using by household should be used.

Estimating portion size

The third stage of the 24-hour recall interview-estimating portion sizes- is the most challenging part of the recall interview and also one of the most critical for ensuring high-quality results. Tools that will be used in estimating portion size included:

- Salted replicas or actual dietary staples;
- Local household utensils (e.g. glasses, cups, bowls, and spoons) calibrated for use; (Glasses, cups and bowls, estimating items such as beverages, soups, porridges, breakfast cereals, and stews; and spoon can be used to estimate sugar, salt, cooking oil, etc.)
- Modeling clay modeled into the correct size and shape of the food; (modeling clay can be used to estimate the portion size eaten of items such as pieces of meat, fish, vegetables, fruit, cassava, and other roots and tubers)

- Tape measure to estimate linear dimensions (length, width, etc.). The tape measurements are useful for recording linear dimensions for example length and circumference of sugar cane, maize cobs, bananas, and potatoes.
- Monetary value of purchased or street foods. (monetary value can be used to estimate the amount consumed of take-away foods, some commercial foods, and street foods)

Converting portion size to weight equivalents

Procedures will be used to convert the portion sizes of foods consumed into weight equivalents:

- Direct weighing- recording the weight in grams of actual foods or salted replicas directly by using dietary scale,
- Volume equivalents-recording the volume of water that is equivalent to the volume of the food or beverage item consumed and then converting the volume to grams,
- Household measure-recording the portion size of food or beverage in household measure and converting to weight equivalents,
- Clay models-measuring and recoding the volume of a clay model and then in size an shape to the foods item consumed and then converting the volume into weight equivalents of the actual foods,
- Linear dimensions-measuring the linear dimensions (length, width, thickness) of a food item with a nonstretch tape measure and then converting into weight equivalents of the actual food, and
- Monetary value-converting the monetary value of a purchased food item into weight equivalents.

Factors for converting the volumes of the foods items to weight equivalents must be derived. Methods will be used to compile these conversion factors:

- Published specific gravity data can be used to convert volumes into grams,
- Nutrient composition table that provide the weight (in grams), and
- Purchase samples (e.g. five to eight) of different weights of each food item of interest and determine the volume of each by water displacement; calculate the factor for converting volume to grams for each sample and then calculate an average value that can be used for that specific food item.

Assessing the validity and reproducibility (Precision)

The quality of food intake measurements collected by using 24-hour recall depends on the validity and reproducibility. Validity is affected by systematic errors whereas reproducibility is associated with random errors. Systematic errors are especially critical because they can introduce the significant bias into the results. These errors have been minimized in the 24-hour recall by certain quality control procedures and modifications in the recall interview. These include:

- Training interviewers (if possible the respondents) before the recall,
- Provide the respondent with bowls and plates for the recall days and discourage them from eating from a common pot to help them visualize the amount of food consumed,
- Using a standardized interviewing technique and questionnaire,
- Calibrating a set of local household utensils for recording volumes,
- Using probing questions specific for each staple food consumed,

- Preparing salted replicas actual cooked staple foods prepared by local cooking methods to estimate portion sizes,
- Using actual samples of community eaten fruits to estimate the portion sizes,
- Using calibrated dietary scales accurate to 1 g (1kg) and 2g (1-2kg) to weigh the portions of prepared foods actually consumed,

Assessing relative validity

Validity of a dietary method is defined as the degree to which it measure what it is supposed to measure. An absolute validity can not be measured because usual intakes are never known with absolute certainty. Therefore, only relative validity can be measured ("test" dietary method is evaluated against "reference" method.

To assess the relative validity of the 24-hour recall (i.e. the test method), a weighed-food record will be chosen for the reference method and carried out on the same subjects and on the same days as the recall.

Statistical assessment of validity

To assess the extent of agreement on a group basis, mean and standard deviation (normally distribution data) will be used for the intakes derived from the test and reference methods. A paired t-test will be used to test whether the two means are statistically different at some predetermined probability level.

To assess the validity of the dietary intake data collected at the individual level, correlation coefficients will be used to provide data on the association between paired measurements between based on the test and reference method.

Assessing reproducibility

The 24-hour recall method will be considered reproducible (precise and reliable) if it gives very similar results when used repeatedly in the same situation. Reproducibility is a function of random measurement errors and uncertainty that results from true variation in the daily nutrient intakes. Reproducibility is determined by using a test-retest design in which the same dietary method is repeated on the same subjects after a preselected time interval. The effects of season on changes in food habits over time must be avoided. An interval of about 2 weeks between first and second sets of recall should cover both of these problems.

Statistical assessment of reproducibility

To assess the agreement between nutrient intakes on the group basis, paired t-test will be used. No significant difference between means or median intakes of the groups for the two set of data (first vs. second interview) is taken to indicate agreement.

To assess the individual agreement, the percentage of misclassification by comparing the number of pairs with exact agreement or agreement within the defined amount. And the other hand, correlation coefficients will be used to evaluate the extent of agreement in a test-retest design for measuring the reproducibility.