Micronutrient supplementation of young stunted Beninese children: effects on appetite and growth performance

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Propositions

1. An appetite test consisting of measuring *ad libitum* intake of a culturally appropriate and well preferred test food offered to children after an overnight fast is a valid tool for appetite measurement provided a standardised procedure is used *(this thesis).*

2. To improve appetite and growth of stunted children, energy-protein supplementation may be required in addition to multi-micronutrient supplementation *(this thesis).*

3. In anemic children who suffer from a multiple micronutrient deficiency, iron supplementation alone will be sufficient to improve iron status *(this thesis).*

4. Nutrition research alone is not sufficient for solving the main nutrition problems of the poor.

5. Hunger and malnutrition are unacceptable in a world that has both the knowledge and the resources to end this human catastrophe *(World Declaration on Nutrition, International Conference on Nutrition, FAO, Rome 1992).*

6. Promotion of breastfeeding should not be continued in those parts of the world where the prevalence of HIV infection is increasing.

7. By investing in children in their early years of life, a country serves not only a child and a family but also the cause of sustainable development. Investing in children is among the most far-sighted decision leaders can make *(The State of The World's Children 2001, UNICEF, New-York 2001).*

8. Poverty is the worst form of violence *(Mahatma Gandhi).*

Propositions belonging to the thesis entitled *"Micronutrient supplementation of young stunted Beninese children: effects on appetite and growth performance".*
Romain Anselme Marc Dossa

Wageningen, 16 May 2001.

A mes parents Angèle et Vincent: ceci est le couronnement de vos efforts

A mes frères et soeurs

A mes grands-parents: ceci en hommage posthume

Abstract

Micronutrient supplementation of young stunted Beninese children: effects on appetite and growth performance

PhD thesis by Romain A.M. Dossa, Division of Human Nutrition and Epidemiology, Wageningen University, The Netherlands. May 16, 2001.

The nutritional status of young children in the Republic of Benin, West-Africa, is poor. More than 40% of the preschool children are stunted and more than 60% of these children suffer from iron deficiency anemia. Traditionally, nutritional problems in young children in developing countries are attributed to lack of food at household level and/or inadequate maternal care and feeding practices. However, nutritional problems may also be due to loss of appetite. A reduced appetite may be caused by micronutrient deficiencies due to imbalanced and monotonous diets. Therefore, we have performed a research to study whether micronutrient supplementation can improve appetite and linear growth of young stunted children in southern Benin. We carried out three micronutrient supplementation studies preceded by a methodological study to set up a procedure for appetite measurement in 2-3 year old children.

The methodological study revealed that an appetite test consisting of measuring the *ad libitum* intake of a culturally appropriate and well-preferred test food offered to the children after an overnight fast might be a valid tool provided a standardised procedure is used (Chapter 3). The first intervention study, a 3-month iron supplementation in combination with deworming, resulted in a significant increase of hemoglobin level by 5 g/L. This increase was still observed 7 months after the intervention. However, there was no improvement of growth performance (Chapter 2). The two other interventions, 6-week multivitamin-multimineral supplementation alone or in combination with an additional iron treatment did not result in an improvement of appetite or growth performance but hemoglobin level was significantly improved by 5 g/L in the children who received iron supplements (Chapters 4 and 5). Our findings suggest that despite the suspected multi-micronutrient deficiency in the children, iron supplementation alone was sufficient for improving their iron status and hemoglobin level. Because the habitual food intake of the children was not appropriate to meet their nutritional requirements (Chapters 2 and 5), energy and good quality protein supplementation may be required in complement to the micronutrient supplementation.

Content

1

General introduction

In most developing countries, inadequate food intake is still one of the major causes of child malnutrition (UNICEF, 1998). Malnutrition may also result from a situation in which an unbalanced food intake and infections co-exist (Golden and Golden, 1991). In most malnourished children, a combination of multiple micronutrient deficiency, loss of appetite and growth retardation is found. However, possible interactions between all these parameters are not yet clearly established. A common model with hypothesised links between all these parameters is given in Figure 1.1. This model suggests that loss of appetite may play a crucial role in the mechanism of linear growth retardation and that an improved nutritional and health status may contribute substantially to an improvement of linear growth through increased appetite. In the present thesis, it will be discussed whether appetite and/or linear growth may be improved by means of micronutrient supplementation.

In this chapter, after pointing out the severity of the problem of linear growth retardation in children in developing countries (paragraph 1.1), the possible relation of linear growth retardation to diet and appetite (paragraph 1.2) and to infections and appetite (paragraph 1.3) will be reviewed. Subsequently, the missing links between linear growth retardation, diet, infections and appetite will be discussed (paragraph 1.4). Next, appetite will be defined and common methods to approach appetite in humans will be presented and briefly commented (paragraph 1.5). Finally, the objectives of the research presented in this thesis and the outline of the thesis will be given (paragraphs 1.6 and 1.7).

1.1. Prevalence of linear growth retardation in children in developing countries

Linear growth retardation or stunting is a chronic and common form of malnutrition in children in poor environments. It occurs mainly in the first 2 to 3 years of life and it may be the outcome of insufficient energy and nutrient intakes or it may result from infections, alone or in combination with insufficient intakes (Golden and Golden, 2000; Waterlow, 1994). It may already be present at birth because of malnutrition of the mother during pregnancy, but it is a phenomenon that often begins in early childhood, usually at the age 4-6 months. Stunting is associated with delays in many functions, for instance mental and motor development, and with an increased risk of morbidity and mortality. In case a child survives childhood, the consequences of being stunted may continue to be apparent in the individual during his or her adult life (Waterlow, 1994).

The number of stunted preschool children in the world has increased during the past two decades. Alarming is the fact that the number of stunted preschool children in the sub-Saharan Africa has increased by 62% from 1980 to 1995 (ACC/SCN, 1998). The global prevalence of stunting in preschool children in developing countries and in the whole African continent was about 33 % in 2000 (ACC/SCN, 2000). The highest levels of stunting are observed in Eastern Africa, where on average 48 % of preschool children are affected. In the Western African sub-region, 35 % of preschool children are stunted. In the Republic of Benin, the reported

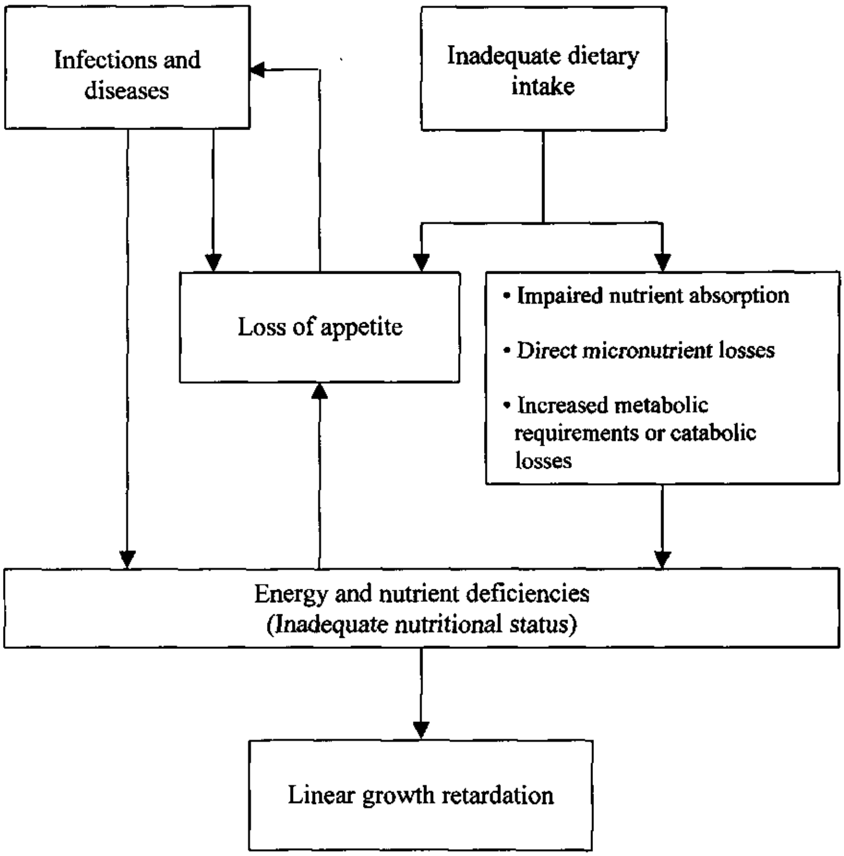

Figure 1.1 Hypothesised interactions between diet, infections, appetite and linear growth

prevalences of stunting among preschool children range between 25 and 40 % (Ategbo, 1993; UNICEF, 1996; ACC/SCN, 2000).

1.2. Linear growth retardation in relation to diet and appetite

It has been established that the causes of linear growth failure are multifactorial. The immediate causal factors are inadequate dietary intake and infections (UNICEF, 1998; Waterlow, 1994). Growth may be affected directly by these factors or through the mediation of a decreased appetite (Figure 1.1).

Both the quantity and the quality of the dietary intakes may be important nutritional factors associated to the phenomenon of linear growth retardation. Most stunted children in developing countries consume monotonous diets. These diets are often poor in animal products and rich in phytates and other dietary factors that may reduce the bioavailability and absorption of nutrients. These diets provide marginal amounts of proteins, vitamins and minerals. An inadequate intake of specific nutrients, such as zinc, might result in an early reduction in appetite and a subsequent decrease in food consumption (Golden and Golden, 2000; Golden, 1995; Wildman and Medeiros, 2000; Cousins, 1986). In fact, reduction of appetite may be a common feature in all forms of malnutrition in a poor environment. The so-called type II nutrients, i.e. zinc, magnesium, nitrogen, sulfur, essential amino acids, potassium, sodium, and phosphorus are defined as the fundamental building blocks of the tissue itself that cannot be sustained without these nutrients. For these nutrients, there is no body store that can be drawn upon (Golden and Golden, 2000; Golden, 1995). Golden and Golden (2000) suggest that reduced appetite or growth faltering may be a response to a deficiency of each so-called type II nutrient and that appetite and growth may be improved if the limiting nutrient is supplied.

Foods are often monotonous in flavour and texture in weanlings in poor areas and this may result in loss of children's appetite (Brown, 1991; Bentley *et al*, 1995; Underwood, 1985; Golden and Golden, 1991). It has been postulated that monotony in the diet in developing countries may explain to some degree the low level of dietary intake that has been observed (Underwood, 1985).

There have been several intervention studies with supplementation of specific nutrients such as energy, protein, zinc, iron, copper, iodine and vitamin A in malnourished children in poor areas. For none of these nutrients, there was a clear evidence that supplementation with the nutrient benefited linear growth (Allen, 1994). Interventions with each specific nutrient had a positive effect on linear growth performance in some studies while no effect was reported in others. For example, iron supplementation over 8 to 14 weeks resulted in a significant increase of height by 0.3 cm in Indonesian and Kenyan school children (Chwang *et al*, 1988; Lawless *et al*, 1994) and by 1.2 cm in Indonesian preschool children (Angeles *et al*, 1993). But linear growth was not improved in Bangladeshi children who received iron supplements over 12 months (Rahman *et al*, 1999). Stunted rural Guatemalan young children treated with zinc during approximately 7 months gained 1.4 cm more than their counterparts who received placebo (Rivera *et al*, 1998). Likewise, the length of stunted Ethiopian infants increased significantly more when supplemented during 6 months with zinc (7.0 cm) than with placebo (2.8 cm) (Umeta *et al*, 2000). But a 12-month zinc supplementation alone or in combination with iron did not improve the linear growth of Mexican preschool children (Rosado *et al*, 1997). There are several other studies reporting linear growth response to iron, zinc or vitamin A supplementation (Hadi *et al*, 2000; Ninh *et al*, 1996) and also studies that did not (Latham *et al*, 1990a; Kikafunda *et al*, 1998). Because multiple deficiencies are likely to occur in many settings, rather than a single nutrient deficiency, failure of growth response to an intervention with a single nutrient may reflect the fact that this nutrient deficiency is not the most growth limiting in the concerned children (Allen and Uauy, 1994; Golden, 1995; Gross *et al*, 2000; Rosado *et al*, 1999; Solomons *et al*, 1999). It is also conceivable that once this nutrient is supplemented, a nutrient imbalance may be created, i.e. an other nutrient may become limiting and may reduce the growth response to the first. Therefore, more emphasise needs to be put on intervention studies that make use of combinations of nutrients likely to be lacking in the children's diet. Indeed, recently, it has been reported that multi-micronutrient supplementation resulted in a significant improvement of linear growth in stunted children (Rosado *et al*, 1999; Thu *et al*, 1999).

1.3. Linear growth retardation in relation to infections and appetite

Acute and chronic infections may impair linear growth by causing micronutrient malnutrition. Micronutrient deficiencies may be generated by infectious diseases in several ways: 1) impairing nutrient absorption; 2) causing direct micronutrient losses; 3) increasing metabolic requirement and catabolic losses; and 4) decreasing food intake (anorexia) (Stephensen, 1999).

Infections usually cause a substantial decrease in food intake and that may result in energy and micronutrient deficiencies. The magnitude of the decrease may be related to the severity of the infection. In Guatemala, children with acute respiratory infections or diarrhea consumed respectively about 8% and 18% less food per day, than children without these infections (Martorell *et al*, 1980). African children consumed 75% less food during the acute phase of measles than after recovery (Duggan *et al*, 1986). Total energy intake from non-breast milk sources in a cohort of 131 Peruvian infants decreased by 20-30% when they had diarrhea or fever (Brown *et al*, 1990). Upper respiratory infections, diarrhea and fevers reduce food intake of preschool Indian children by 15-20% (Pereira and Begum, 1987). The same study revealed that the presence of measles resulted in a prolonged and profound decrease of appetite: the average food intake was reduced by 33% throughout the first two weeks, but also in the following four weeks it remained seriously affected.

Loss of appetite may not only produce anxiety in the children's mothers or caretakers, but also affect the children's nutritional status and growth rate (Stephensen, 1999). For example, it has been established that the presence of poor appetite was negatively associated with the children's growth increments from 6 to 12 months of age (Brown *et al*, 1991). Infants of 12 months, who were reported to have reduced appetite two to three times more often than other children, gained in weight more slowly, remained underweight and were unable to overcome the growth deficit in later stage of life (Piwoz *et al*, 1994). The association of infections with decreased linear growth have also been established in community studies around the world (Stephensen, 1999). Chronic infections may have substantial cumulative negative effects on linear growth. For example, infection with Ascaris lumbricoides and other gut helminths, that are highly prevalent in developing countries, may impair nutritional status and linear growth (Hlaing, 1993; Stephenson, 1987; Solomons, 1993; Stoltzfus *et al*, 1997a; Tshikuka *et al*, 1997; WHO, 1992).

Several studies have suggested that micronutrient supplementation may result in a reduction of infections in children (Angeles *et al*, 1993; Black, 1998; Lira *et al*, 1998; Rosado *et al*, 1997; Roy *et al*, 1999; Umeta *et al*, 2000). However, in these studies, it has not been clearly established whether an improvement of appetite or linear growth could have resulted from a reduced morbidity. There have also been studies that assessed the impact of a deworming treatment on appetite and/or physical growth of children severely infected by helminths. Their results were not conclusive regarding whether a meaningful improvement of appetite and/or linear growth could be obtained by the deworming treatment. Indeed, some of these studies reported increased appetite and/or linear growth (Adams *et al*, 1994; Hadju *et al*, 1996; Hadju *et al*, 1998; Latham *et al*, 1990b; Stephenson *et al*, 1989; Stephenson *et al*, 1993) while some did not (Donnen *et al*, 1998; Forrester *et al*, 1998; Latham *et al*, 1990a; Stoltzfus *et al*, 1997b).

1.4. Missing links between linear growth retardation, diet, infections and appetite

Most of previous studies were not conclusive regarding the effects of micronutrient supplementation or deworming treatment on appetite and/or linear growth of malnourished or infected children. In addition, whether the improved growth reported by some studies really resulted from an improvement of appetite remained unclear. Therefore, new studies on micronutrient supplementation and deworming treatment should measure simultaneously appetite, food intake and linear growth. A question of great importance is whether an improvement of linear growth by micronutrient supplementation may occur directly or through an improvement of appetite. To be able to address this question, appetite should be clearly defined and an appropriate tool for appetite measurement in young children in field studies should be identified. Intervention studies should preferably be performed in malnourished young children less than 3 year old, because they are more likely to give growth response to nutrient supplementation because of their rapid growth rate and high nutrient needs.

1.5. Appetite measurement in humans

Definition, expression and control of appetite

Appetite is the process which directs eating and guides the moment-to-moment selection of foods (Blundell, 1991). It is a conscious sensation that determines which particular foods will be chosen, the rate of ingestion, and the amount that will be consumed. The human appetite system contains central and peripheral mechanisms that interact with environmental features, especially with the physical and nutrient composition of the food supply (Blundell *et al*, 1996). Foods varying in nutrient composition exert different physiologic effects, some of which function as satiety signals. Although the physiological mechanisms for controlling food intake, i.e. hunger and satiety, exist in man, appetite is a powerful and poorly controlled stimulus to eat even when not hungry (Schutz and Garrow, 2000). Thus, the total energy ingested in a day is determined by the interaction of exogenous and endogenous factors, among which is appetite. Although no standard for appetite measurement exits, appetite of a subject may be reflected by the habitual daily food intake. It may also be assessed by means of rating scales that reflect subjective sensations.

Common methods for appetite assessment in humans

Appetite has been assessed in various studies. These studies may be classified into three groups.

1. Studies investigating possible mechanisms of regulation of appetite in health and disease, for example in obesity. An example of methods used to assess appetite in these studies is energy intake per time unit (Zipf and Berntson, 1987).
2. Studies investigating the effect of food characteristics, exercise or anorexic drugs on appetite in western, adult populations. In these studies, appetite was generally measured by means of energy intakes from *ad libitum* consumption of meals or snacks (Hulshof, 1994; de Graaf *et al*, 1992; Stubbs *et al*, 1996), graphical ratings scales (Hulshof, 1994; Burley *et al*, 1993; King *et al*, 1994; de Graaf and Hulshof, 1996; Rosen, 1981; Stubbs *et al*, 1996; Thompson et al, 1988; Teghtsoonian *et al*, 1981), the length of time between the end of a physical exercise and the voluntary onset of eating (King *et al*, 1994).
3. Studies investigating children's appetite as related to health and growth in developing countries. In this last group of studies, appetite has been assessed by means of energy intakes from *ad libitum* food consumption and/or numerical rating scales (Hadju *et al*, 1996; Hadju *et al*, 1998; Latham *et al*, 1990b; Lawless *et al*, 1994; Stephenson *et al*, 1993; Pereira and Begum, 1987) or by means of observation of eating behaviour (Brown *et al*, 1995; Cohen *et al*, 1995; Garcia *et al*, 1990).

Most of the methods from the first and second group of studies do not seem to be relevant for appetite assessment in young children in field studies in developing countries. The numerical scales, the observation method and the measurement of energy intakes from the *ad libitum* food consumption may be more appropriate for use in field studies. However, little is known about the validity and reproducibility of these methods when used to assess appetite of young children of 2-year old. For example, important issues to consider in the use of the *ad libitum* food consumption approach are the choice of an appropriate test food, the standardization of the test procedure, the validity and reproducibility of the method. These aspects will be addressed in the present thesis.

1.6. Objectives and research questions

The main objective of the research presented in this thesis is to study whether micronutrient supplementation improves appetite and growth in stunted young children in southern Benin.

Results from previous studies in southern Benin (Hercberg *et al*, 1986; unpublished) suggest that iron is possibly the most growth limiting nutrient in most children in the area and that prevalence of parasitic infections mainly by helminths is high in these children. Therefore, we started the research program by studying the impact of iron supplementation and deworming on nutritional status and growth performance of preschool children. In that study, appetite of the children has been approached in a sub-sample by assessing their habitual dietary intakes using the observed weighed records method. The study revealed that the nutritional quality of the children's usual diet was low, suggesting the presence of a multiple micronutrient deficiency. Therefore, iron supplementation alone might not be enough to generate an improvement of the children's nutritional status and linear growth. Then, further intervention studies have been performed with supplementation of a mix of micronutrients. Because young malnourished children are more likely to give substantial growth response to micronutrient supplementation than 3-5 year old children, stunted 2-year old children were recruited for further studies. To be able to assess whether micronutrient supplementation will result in an improved appetite, appetite must be measured in a valid and reproducible way.

Therefore, the following research questions have been addressed:

- Can appetite of young children be assessed validly and in a reproducible way in field studies?
- Does multivitamin-multimineral supplementation improve appetite and linear growth of stunted young Beninese children?
- Is improvement of appetite and linear growth linked to anemia and helminth infection?

The studies were carried out in Agblangandan (in the Ouémé Province) and in Zè (in the Atlantique Province), both located in southern Benin (Figure 1.2).

1.7. Outline of the thesis

Chapter 2 investigates the impact of a combination of iron supplementation and deworming treatment on growth and health status of 3-5 year old Beninese children. Chapter 3 reports on tools developed for appetite assessment in young children in field studies. Chapters 4 and 5 examine the question whether appetite of young stunted and/or anemic children may be improved by means of a short-term (6-week) multivitamin-multimineral supplementation. Chapter 6 discusses the findings from the various studies, including their implications for nutrition policy actions and future research.

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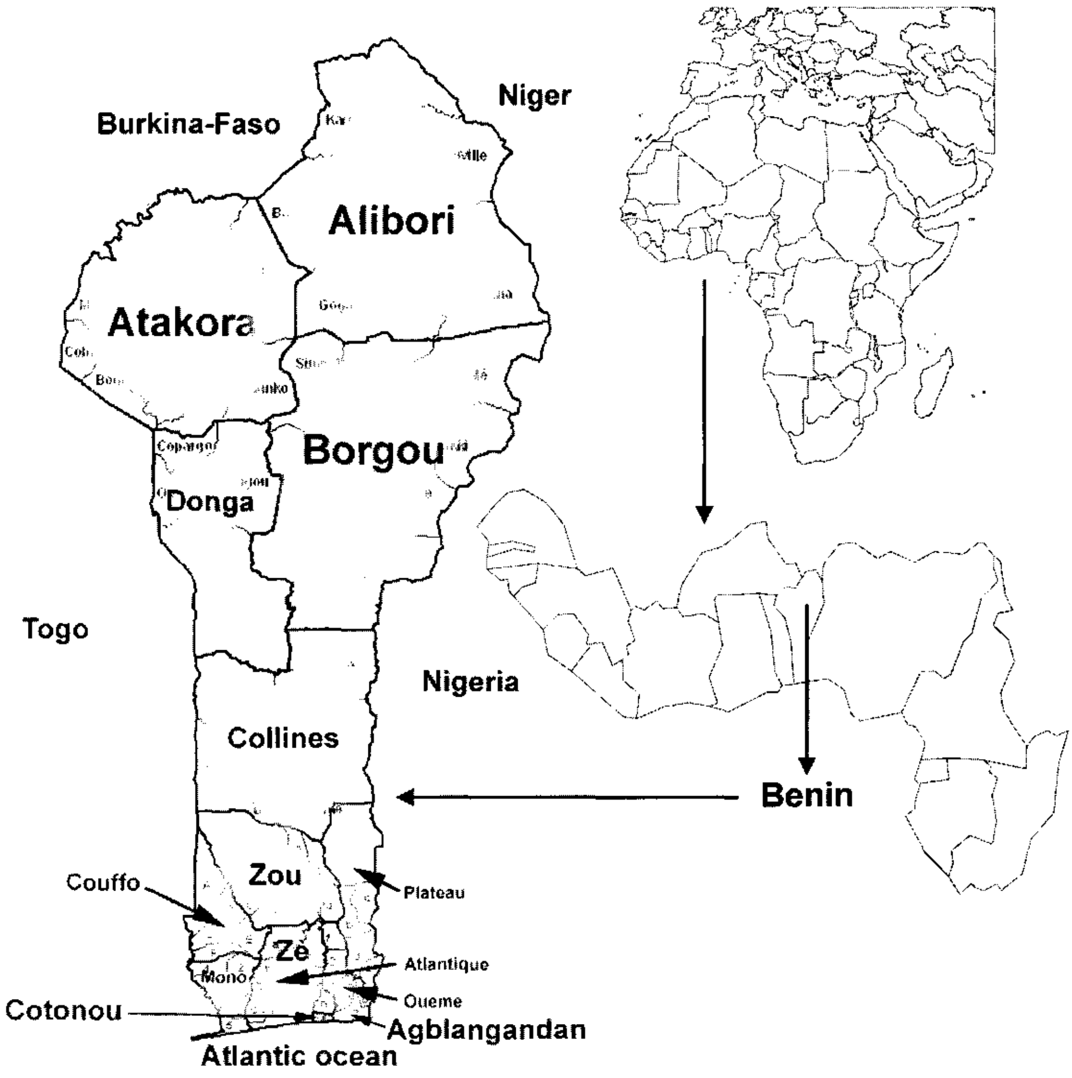

Figure 1.2. Map of the Republic of Benin indicating the study areas Agblangandan and Zè

2

Impact of iron supplementation and deworming on growth performance in preschool Beninese children*

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Abstract

Objective: Assess the effects of iron and deworming on linear growth performance of preschool children.

Subjects and methods: The study was a three-month randomized, double-blind and placebo-controlled trial. A group of 177 children aged 3-5 years was selected from low-income households in a rural area in southern Benin. The children were allocated to one of four treatments: iron (60 mg elemental iron/day) + albendazole (200 mg/day for 3 consecutive days, at start of study and after one month), iron + albendazole-placebo, albendazole + iron-placebo or placebos. The supplementation was supervised. Anthropometric parameters, hemoglobin concentration and eggs per gram feces were measured. A complete data set was analysed for 140 subjects. The children were stunted (58% had height-for-age Z-score < -2) and anemic (76% had Hb < 110g/L), but not wasted (2% had weight-for-height Z-score < -2).

Results: No significant difference in changes in anthropometric parameters was observed between study groups, and also not in a sub-sample of stunted and anemic subjects. Changes in hemoglobin concentration were highest in the iron-treated subjects at the end of the 3-month intervention period (p=0.032). The difference between the iron and the placebo groups remained significant even 7 months later (p=0.022). The difference was 5 g/L in both periods. *Ascaris lumbricoides* and hookworm infections decreased significantly in albendazole-treated subjects (p < 0.05).

Conclusions: In addition to recurrent parasitic infection burden, the children may have multiple micronutrient deficiencies. Therefore, it may be interesting to study appetite and food intake of young toddlers in relation to health and linear growth performance in poor environments.

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Introduction

Anemia is still a large public health problem throughout the world, especially in children in developing countries (ACC/SCN, 1998). A previous study from our group carried out in Benin in 1995 revealed that 57% of the preschool children were anemic (Hb < 110 g/L). These children were also severely infected by a variety of parasites such as *Ascaris lumbricoides* (53%), *Trichuris trichiura* (51%) and hookworms (12%) (unpublished).

The consequences of anemia are well known: alteration of the immune status, adverse effects on morbidity, delayed behavioral and mental development, below average school achievements and growth retardation (Hallberg and Asp, 1996; DeMaeyer *et al*, 1989). Parasitic infections are reported to affect the host's nutritional status and to cause growth retardation in children (Stephensen, 1999; Forrester *et al*, 1998; 1993; Tshikuka *et al*, 1997; Solomons, 1993). However, the consequences of helminth infections in preschool children are less documented. Health policies have been formulated both to reduce anemia prevalence and to tackle the heavy load of intestinal helminths especially in children.

The present study reports the effects of iron and deworming treatments on physical growth performance, hemoglobin level and intestinal helminth egg loads in preschool children in a poor rural African environment. It is a placebo-controlled intervention study, which investigates whether iron supplementation and deworming, separately or in combination, can improve the linear growth performance of malnourished preschool children.

Subjects and methods

Study area

The present study was carried out in Agblangandan, a semi-rural area in the Département de l'Ouémé in southern Benin. It is situated at about 10 km from Cotonou, the economic capital of the republic of Benin. The main income-generating activities are fishing, trading and rearing small livestock such as pigs, sheep and chicken. The living conditions are characterised by poor sanitation and limited access to tap water. A health centre, a welfare centre, a primary school and a kindergarten are available in the area.

Study design

The study was double-blind and placebo-controlled. One-hundred and seventy-seven children aged 3-5 years were selected and randomly assigned to one of four treatments: 1. iron + albendazole; 2. iron + placebo; 3. albendazole + placebo; and 4. placebos. Two types of placebos were used: iron-placebo and albendazole-placebo. Iron supplements (60 mg elemental iron as ferrous sulfate) were provided daily throughout 3 months. The supplements were taken under supervision between breakfast and lunch in the presence of an observer who also checked that they were really swallowed by the children. Albendazole treatment was given at baseline (200 mg per day for 3 consecutive days) and one month later (200 mg per day for 3 consecutive days).

Hemoglobin concentration and anthropometric parameters were measured before and after 3 months of supplementation. Stool collections and stool examinations (for qualitative and quantitative determination of intestinal helminths) were done before supplementation, at 1 month (just before the second albendazole treatment) and after 3 months of supplementation. At the end of the supplementation period, food intake was measured during 3 consecutive days, in a subsample of 30 children (from each study group, seven or eight children were randomly selected). Blood hemoglobin and anthropometric parameters were measured again 10 months after baseline measurements (136 children).

The study was approved by the ethical committee of the National University of Benin through the Centre National Hospitalier et Universitaire in Cotonou.

Subjects and sampling criteria

The study sample consisted of 177 children aged 3-5 years. Ages were confirmed by birth certificates. None of the children suffered from acute diseases. Informed consent was obtained from parents for participation of their children in the study.

Although 175 children finished the study, the data from 140 children were included in the final analyses. One child was treated for severe worm infection and 34 children received other pills during the study period (iron, vitamins/minerals or deworming pills that were not provided by our research team). Baseline characteristics of the 140 subjects are presented in Table 2.1. Of the overall sample, 76% have anemia (Hb less than 110g/L), 58% was stunted (Z- score height-for-age less than –2) and only three children (2%) have Z-score weight-for-height less than –2). The group of children excluded was not different from the rest of the sample with respect to baseline characteristics.

Measurements

Anthropometric parameters. Body weight was measured early in the morning at the welfare centre. The measurement was done using a beam weighing scale (babies and toddlers scale model 625 T, CMS Weighing Equipment Ltd, London). The scale was placed on a table and was calibrated before each measurement session using a standard weight of 10 kg. Children were measured wearing a minimum of clothing. Readings were done to the nearest 0.1 kg.

Height was measured with the child standing without shoes on a horizontal surface against a wall with heels together, chin tucked in and body stretched upwards to full extent and head in the Frankfurt plane. Heels, buttocks and shoulders were in contact with the wall to which a microtoise was attached. Height was read to the nearest 0.1 cm.

Mid-upper arm circumference (MUAC) was measured on the left side of the body half-way between the tip of the shoulder and the elbow with the subject's arm hanging freely along the body using a flexible non-extensible tape. Readings were done to the nearest 0.1 cm.

Triceps skinfolds were measured in triplicate on the left arm using a Holtain calliper

Table 2.1 Baseline characteristics of the subjects

	Treatment groups			
	Iron and albendazole	Iron (n=36)	Albendazole (n=38)	Control (n=32)
Age (months)	46±6	46±5	44±6	47±5
Height (cm)	92.0±5.6	92.8±4.5	90.5±4.3	91.4±4.1
Weight (kg)	12.7±1.6	13.2±1.6	12.6±1.3	12.7±1.5
HAZ [a]	-2.16±0.84	-2.03±0.85	-2.26±0.84	-2.48±0.88
HAZ<-2 (%)	53	50	63	66
WHZ [b]	-0.72±0.08	-0.53±0.81	-0.55±0.82	-0.66±0.84
WHZ<-2 (%)	2	0	0	1
MUAC [c] (cm)	14.9±1.1	15.1±1.2	15.0±1.2	15.0±1.2
Triceps (mm)	7.6±1.6	8.2±2.1	8.9±2.3	8.2±1.8
Hemoglobin (g/L)	101±12	100±10	98±12	102±9
Hemoglobin < 110g/L (%)	74	79	76	78

Mean±SD; [a] HAZ: height-for-age Z-score; [b] WHZ: weight-for-height Z-score; [c] MUAC: mid-upper arm circumference

(Holtain Ltd, Briberian, UK; pressure 10 g/mm^2 precise at 0.2 mm). This measurement was performed half-way between the tip of the shoulder and the elbow with the child's arm hanging relaxed at the side.

Z-scores Weight-for-height (WHZ) and height-for-age (HAZ) were calculated using the National Centre for Health Statistics (NCHS) reference data (WHO, 1983) and Epi info software. Changes in anthropometric parameters over 3 and 10 months were calculated.

Hemoglobin concentration. Hemoglobin level (g/L) was measured using a finger prick blood sampling technique and a photometry analysis method: the HemoCue device (Laifer *et al*, 1990). Changes in hemoglobin level over 3 and 10 months were calculated.

Stool collection and stool examination. Each mother was given a plastic bottle in which she collected a sample of her child's stool. On the stool collection day, a single stool sample was taken from each child. The sampling was done on the first stool provided early in the morning. Samples were brought the same day to the Laboratory of Parasitology in the Centre National Hospitalier et Universitaire in Cotonou. Intestinal parasites in each sample were identified by direct stool examination using a microscope. As the main parasites observed were Ascaris lumbricoides, Trichuris trichiura and hookworms, their eggs were counted using the Kato's concentration method (Martin and Beaver, 1968). The outcome was expressed as number of eggs per gram of stool (epg) for each type of helminth. Arithmetic and geometric means were calculated for each type of helminth and per study group.

Dietary intakes. Food intake was measured during 3 consecutive days in a sub-sample of 30 children randomly selected from each treatment group (seven or eight from each treatment group). Intakes were measured using the observed weighed record method (Cameron and van Staveren, 1988). Every food was weighed before and after cooking as well as the subject's portion and leftovers, using a digital weighing scale (Soehnle type 8000.00.090) for weights up to 1 kg. For weights between 1 and 10 kg a spring weighing scale (Soehnle type 1203) was used. Measurements were carried out by well-trained local assistants every day from 7 a.m. until subjects had eaten their last meal (often around 8 p.m.). Foods consumed when the assistant was not present (after diner and before breakfast) were assessed by recall using household measures. Individual energy, protein and iron intakes were obtained by averaging intakes of the 3 consecutive days. Energy and nutrients intakes were calculated based on a food composition database compiled for foods consumed in Benin and using a software for dietary studies (Scholte, 1995).

Statistical analyses

Changes in anthropometric parameters and hemoglobin concentration (Hb) over 3 and 10 months between treatment groups were studied by means of analysis of variances (ANOVA). In case of significant treatment effects, differences between groups were studied using independent-samples t-test.

Helminths egg counts, which have a skewed distribution, were transformed by conversion to natural log (epg + 1). Egg reduction rates were calculated from both arithmetic and geometric mean egg counts with the formula: percentage egg reduction = [(initial epg - final epg)/initial epg]. Arithmetic means were calculated for positive egg counts. Prevalences of helminth infection were compared within groups by the McNemar test and between groups by the Chi-Square test. Changes in intensity of helminth infection were compared within groups using paired t-test and between groups using group t-test. Paired and group t-tests were performed on log (epg + 1). All statistical tests were two tailed and p values less than 0.05 were considered statistically significant. Analyses were performed with the SPSS Statistical Package.

Results

Anthropometry

Anthropometric parameters are presented in Table 2.2. Analysis of variances revealed that changes in weight, height, mid-upper-arm-circumference (MUAC), triceps skinfold, Z-scores weight-for-height and height-for-age over 3 and 10 months did not vary between treatment groups. Analyses restricted to a sub-group of 63 stunted (Z-score height-for-age < -2) and anemic children (Hb < 110 g/L) also revealed no significant difference between treatments groups.

Hemoglobin concentration

Hemoglobin levels and changes over 3 and 10 months are shown in Table 2.3. Analysis of variances for changes over 3 and 10 months revealed a significant difference between treatment groups ($p < 0.05$). Further t-tests revealed no difference between the two groups of children who were treated with iron and also between the two groups of children who did not receive iron supplements. When the two iron groups and the two non-iron groups were combined, we observed that iron supplementation resulted in a 5 g/L higher blood Hb level at month 3 ($p < 0.05$) and at month 10 ($p < 0.05$). This 5 g/L increase in blood hemoglobin level resulted in a decrease in prevalence of anemia (Hb < 110 g/L) from 76 % to 51% after 3 months and to 45% after 10 months.

Stool examination

For the overall sample, before treatment, the percentages of children infected by *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm were 38%, 47% and 13%, respectively, and 27% were infected by both *Ascaris lumbricoides* and *Trichuris trichiura.* After 3 months, there was a significant decrease in *Ascaris* and hookworm egg loads in the albendazole group in

Table 2.2. Changes in anthropometric parameters during the study

	Treatment groups			
	Iron & albendazole	Iron	Albendazole	Control
3-month changes	(n=34)	(n=36)	(n=38)	(n=32)
Weight (kg)	0.4±0.4	0.3±0.4	0.4±0.4	0.5±0.6
Height (cm)	1.9±0.6	1.9±0.6	2.3±0.8	2.1±0.9
MUAC [c] (cm)	-0.1±0.6	-0.2±0.6	-0.2±0.6	0.0±0.8
Triceps (mm)	0.4±1.1	0.0±1.1	0.3±1.6	0.5±1.2
WHZ [a]	-0.02±0.37	-0.05±0.37	-0.10±0.39	0.06±0.51
HAZ [b]	-0.03±0.15	-0.02±0.14	0.07±0.21	0.02±0.22
10-month changes	(n=31)	(n=33)	(n=37)	(n=28)
Weight (kg)	1.2±0.6	1.2±0.5	1.2±1.0	1.2±1.1
Height (cm)	6.2±1.0	6.2±1.5	6.5±2.6	6.0±2.5
MUAC (cm)	0.1±0.7	0.0±0.8	0.1±0.8	0.1±0.9
Triceps (mm)	0.0±1.5	-0.2±1.6	-0.6±1.3	0.2±1.7
WHZ	-0.15±0.39	-0.15±0.47	-0.24±0.48	-0.09±0.57
HAZ	0.12±0.23	0.17±0.36	0.13±0.44	0.16±0.42

Mean±SD; [a]WHZ: weight-for-height Z-score; [b]HAZ: height-for-age Z-score; [c]MUAC: mid-upper arm circumference.

Table 2.3. Hemoglobin level (g/L)

	Treatment groups					
	Iron and Albendazole (n=34)	Iron (n=34)	Albendazole (n=38)	Control (n=32)	Iron group (n=68)	Non-iron group (n=70)
Baseline	101±12	100±10	98±12	102±9	101±11	100±11
3 months	109±11	108±12	101±12	105±9	108±12	103±11
10 months	113±13	111±9	106±10	106±13	112±12	106±11
3-month changes	8±3 [a]	7±14 [a]	2±16	4±10	8±13 [a]	3±13
10-month changes	13±15 [a]	11±12 [a]	8±13	5±12	12±13 [a]	7±13

Mean ± s.d.; [a] Different from non-iron subjects ($P < 0.05$).

comparison to placebo group ($p < 0.05$; Table 2.4). The difference between the two groups appeared to be significant already 1 month after baseline deworming (changes in log (epg+1) were −0.94±1.89 in albendazole group versus +0.29±1.82 in placebo group for *Ascaris lumbricoides* and −0.15±0.88 versus +0.42±1.15 for hookworm). A similar trend was also observed for *Trichuris trichiura* infection; however, the difference in wormload between albendazole and placebo groups was not statistically significant. The egg reduction rate (geometric mean epg) for *Ascaris lumbricoides,* hookworm and *Trichuris trichiura* egg loads were 74%, 69% and 49%, respectively, in the albendazole group. On the other hand, intensity of *Ascaris lumbricoides* and hookworm infections increased significantly in the placebo group by 438% and 24%, respectively. The intensity of *Trichuris trichiura* infection also increased by 35%. The decreased *Ascaris lumbricoides* and hookworm wormload was also reflected by the lower prevalence of infections by these worms in albendazole group as compared to placebo group ($p < 0.05$). The prevalence of hookworm infection in the placebo group increased by 17% from baseline to month 3 ($p=0.035$).

Dietary intakes

Meals often consist of thick dough made of corn flour that is served with a side dish. The side dish is mostly a sauce prepared from various ingredients such as green leafy vegetables, okra, tomatoes, onions, pepper, vegetable oil and local mustard. Rice, beans, cassava, yam and potatoes are also frequently consumed. Dietary energy, protein and iron are essentially of plant origin. Meat and fish are consumed less frequently and often in very small amounts. Iron intakes ranged from 4.5 to 16.2 mg/day with a mean value of 8.5±2.5 mg/day, about 61% of the RDA to cover basal requirements (WHO, 1988). Energy intakes ranged from 2982 to 8635 kJ/day (713 - 2066 kcal/day); the mean value was 5416±1129 kJ/day (1296±270 kcal/day, about 83% of the RDA) (WHO, 1985). Protein intakes ranged from 17 to 69 g/day; the mean protein intake was 36±13g/day.

Discussion

The present study was carried out in Beninese preschool children of whom 76% were anemic (Hb < 110g/L) and 58% stunted (Z-score height-for-age < -2). The prevalence of helminth infections in these children was also high (about 40 % for *Ascaris lumbricoides*, 50% for *Trichuris trichiura* and 13% for hookworm). Such a combination of health burden is often observed in children in developing countries (Stephensen, 1999; ACC/SCN, 1998; UNICEF, 1998; Stoltzfus *et al*, 1997). As might be expected, iron supplementation resulted in an improvement of iron status. It is remarkable that the difference of 5g/L in hemoglobin level was still observed between the iron group and the placebo group even 7 months after the supplementation has ended. Deworming treatment also resulted in a significant reduction of egg loads (especially for *Ascaris lumbricoides* and hookworm). However, both treatments did not improve the linear growth performance of these children.

The lack of improvement in linear growth may not be ascribed to the duration or to the age of the children in our study since significant improvements in linear growth were obtained with iron supplementation and also with deworming treatment for a comparable duration as in our study and most of these studies included school age children (Hadju *et al*, 1998; Lawless *et al*, 1994; Angeles *et al*, 1993; Stephenson *et al*, 1993; Chwang *et al*, 1988). Also, the lack of improvement in linear growth of our children cannot be explained by applied doses since appropriate iron and albendazole doses were used based on the World Health Organization's recommendations (DeMayer, 1989; WHO, 1992). So, our study really indicates that a 3-month iron supplementation and deworming did not improve linear growth performance in Beninese preschool children. This outcome is in line with results of some studies (Rahman *et al*, 1999; Rosado *et al*, 1997; Hadju *et al*, 1996) but conflicts with outcomes of other studies (Hadju *et al*, 1998; Lawless *et al*, 1994; Angeles et *al*, 1993; Stephenson *et al*, 1993; Chwang et *al*, 1988).

As indicated before, duration, age and doses cannot explain the lack of improved linear growth in our children. Plausible causal factors for differences in results between our study and other studies may be the low general nutritional status of our children in combination with possible long-term detrimental effects of continuous parasitic infections in these children. With respect to infection burden, it is remarkable that, in spite of the substantial reduction in infections' intensity, worm infections were recurrent. The significant increase in intensity of worm infection in the placebo group and the residual infections that were still present in the albendazole group suggest that reinfection was a continual process as sanitation and hygiene conditions did not improve throughout the study. Moreover, this supports the evidence that, in the absence of frequent deworming treatment, the parasitic infection burden is cumulative and a high wormload may be achieved in a short period. In spite of the residual helminth infections, the deworming treatment might have, in the long term, beneficial impacts on the health status of the children as reported in a recent study (Beasley *et al*, 1999), but did not generate detectable changes on their short-term physical growth performance.

Maybe an improvement in linear growth could have been achieved if the overall nutritional status of the children have been adequate. Indeed, our food consumption survey revealed that they had marginal energy and iron intakes. Their dietary intakes might be of low quality, as mostly of plant origin and containing high amounts of fiber and phytates. It is probable that, although iron supplementation significantly improved the iron status of these children, improvement in linear growth performance could be achieved only if the entire diet was balanced and then adequate amounts and quality of energy, protein and other essential nutrients provided. It is conceivable that, because of their marginal and monotonous diet in addition to infection burdens, these children might have also a decreased appetite and consequently an inadequate food intake and multiple micronutrient deficiencies.

This intervention really differs from most operational health programs in that it was placebo-controlled and supervised. Such input and compliance is mostly not feasible in regular public health programs. Consequently, it provides the information about which health improvements can be achieved if interventions as described in this paper are introduced. As might be expected the intervention was effective in improving hemoglobin level and significantly decreasing the intensity of helminth infections in the children. The long-lasting effect on hemoglobin was surprising, given the low energy and iron intakes of these children. However, these beneficial effects did not result in improvement of linear growth performance. The lack of improved linear growth may be linked to the low quality and quantity of the habitual food intake of the children. The inadequate food intake may be related to marginal food availability at household level or to suboptimal feeding practices. However, low appetite due to several reasons might also be a cause. Therefore, it may be interesting to put more emphasis on studies of appetite and food intake of young children in relation to health and linear growth performance in poor environments. Such studies should not concentrate on supplementation with single nutrients but provide the children with a package of all limiting nutrients needed to correct for the multiple nutrient deficiencies common in stunted children (Rosado, 1999; Solomons, 1999; Golden, 1995; Allen, 1994). In addition, for such studies, young toddlers might be the most appropriate targets as stunting usually starts in early childhood.

Table 2.4 Helminth egg counts

	% positive			Arithmetic mean [a] (epg)			Geometric mean (epg)			Log(epg+1) (Month 3 - baseline) mean±sd
	Baseline	Month 1	Month 3	Baseline	Month 1	Month 3	Baseline	Month 1	Month 3	
Ascaris										
Albendazole	31	8[d,e]	26[e]	26130	480	5438	4292	195	1098	-0.33 ± 2.08 [c]
Placebo	47	49	65	19874	35891	42984	2940	8433	15816	$+1.08 \pm 2.37$ [b]
Hookworm										
Albendazole	11	7[e]	7[e]	733	233	142	369	180	116	-0.16 ± 0.67 [c]
Placebo	14	31[d]	31[d]	781	302	331	186	212	231	$+0.43 \pm 1.21$ [b]
Trichuris										
Albendazole	38[e]	38[e]	44[e]	2426	3664	2075	1159	586	587	$+0.07 \pm 1.27$
Placebo	59	65	65	1918	1671	2956	468	580	634	$+0.24 \pm 1.16$

Epg: egg per gram; Albendazole group (n=61); Control group (n=51). [a] Arithmetic mean was calculated for positive egg counts. [b] Significant difference between baseline and month 3 (paired t-test). [c] Significantly different from placebo group (group t-test). [d] Significantly different from baseline (McNemar test). [e] Significantly different from placebo group (Chi-Square test).

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3

An appropriate tool for appetite testing and evaluation in young children in Benin*

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Abstract

Objectives: Develop a tool for appetite evaluation in young children in nutritional intervention programmes.

Subjects and methods: Appetite measurements were performed in 109 Beninese children aged 18-30 months. Two test foods were identified as appropriate for these children: a maize porridge (aklui) and rice (riz-au-gras). A*d libitum* intakes of the foods served after an overnight fast according to a standardized offering procedure were measured on 3 days. The children's habitual intakes were measured during 3 consecutive days not overlapping with the appetite test days.

Results: Energy intake from the test foods was comparable to breakfast energy intake which was 0.8-1.0 MJ, representing 21% of total daily energy intake. Energy intake from aklui was significantly correlated with daily intake ($n=38$; $r=0.41$, $p < 0.05$) and with energy intake from breakfast ($r=0.52$, $p < 0.01$). Correlations concerning riz-au-gras were less pronounced and non-significant. Reproducibility (as coefficient of variation) of the appetite test as calculated from the triplicate measurements was 40% for aklui and 25% for riz-au-gras. This reproducibility is better than that of the habitual breakfast intake (43-45%).

Conclusion: The appetite test used in our studies can be considered as an appropriate tool for appetite testing and evaluation in young children.

* *Appetite (accepted)*

Introduction

Traditionally, nutritional problems in young children in developing countries are attributed to lack of food at household level, and/or inadequate maternal care and feeding practices. However, nutritional problems may also be due to loss of appetite, which is related to factors such as infections, imbalanced and monotonous diets (Brown *et al*, 1995; Golden, 1995; Golden, 1988; Lawless *et al*, 1994; Pereira and Begum, 1987; Stephensen, 1999; Waterlow, 1994). Appetite stimulation may make a substantial contribution to the improvement of the nutritional and health status of children living in poor environments. Therefore, an appropriate tool for appetite measurement, which can be easily applied in field studies, e.g. in nutrition intervention studies, is needed.

Appetite is defined as the process which directs eating and guides the moment-to-moment selection of foods (Blundell, 1979). It determines the choice for and intake of particular foods. Several methods have been suggested for appetite measurement in adults and children (Bentley *et al*, 1991; Blundell, 1991; Brown *et al*, 1995; Latham *et al*, 1990; Lawless *et al*, 1994; Piwoz *et al*, 1994), but little is known about the validity and reproducibility of these methods, particularly for young children. Although there is no standard for appetite measurement, the habitual daily energy intake seems to be the most appropriate proxy for human appetite. However, a reliable method for estimating the daily energy intake is invasive and time consuming; for these reasons, the measurement procedures to assess the daily energy intake are not appropriate for a quick assessment of children's appetite in field studies. An alternative methodology to estimate appetite in field situation may be through the assessment of energy intake from a test food to which children have free access under standardized conditions. To set up that methodology, several aspects should be carefully addressed such as the choice of the test food, the choice of an appropriate moment of the day for performing the appetite test, the standardization of the offering procedure and whether or not water should be supplied during the test. After making these choices, the reproducibility and validity of appetite assessment should be tested.

We have performed four studies in young Beninese children in West Africa for testing a methodology for appetite measurement. We assessed the reproducibility of energy intake from a morning test food to which the children had free access under standardized conditions, and determined whether or not this value gave a good indication of the children's usual daily energy intakes. Our main objective was to investigate whether energy intake from a test food can be used to assess appetite in a reproducible and valid way and can, therefore, be used as an appropriate tool for appetite measurement in young children in field studies. The first appetite study was carried out in Cotonou in a small sample of 8 children to test the methodology using both solid and liquid test foods. The test procedure was further elaborated in the first and second Zè studies with larger samples. Aspects related to water intake during the test were examined in the third Zè study.

Subjects and methods

Study area and subjects

Our first study was carried out in Cotonou, the economic capital of the Republic of Benin. The other three studies were performed in Zè, a rural area situated at about 50 km from Cotonou. Since our studies involved young children, parental agreement for participating to the studies was obtained. In Cotonou, 8 children aged 22-26 months were selected from middle-income households. None of them were still breast fed. The first and second Zè studies each included 38 children aged 22-26 months. The third Zè study was performed with 25 children aged 18-30 months. The Zè children were from households where farming was the main income generating activity. These households have poor living conditions and a low purchasing power. Approximately one third of the Zè children were still breast fed.

Weight and height were measured only in the Cotonou study and in the third Zè study. The average Z-scores weight-for-height were -0.62±1.04 and –1.14±0.77 for Cotonou and Zè, respectively. The average Z-scores height-for-age were –0.86± 0.66 and –1.73±0.98 for Cotonou and Zè, respectively.

Design of Cotonou and Zè studies

Figure 3.1 presents the design of the studies. The appetite test was performed for three days in each study. Daily food intake was only assessed in the Cotonou study and in the second Zè study over 3 consecutive days not overlapping with appetite test days. Morbidity status was assessed only in the Zè studies. Deworming treatment was done only in the first and second Zè studies. Each child received a weekly dose of 200 mg of albendazole over 3 weeks for the first Zè study and over 2 weeks for the second Zè study.

Measurements

Dietary intakes. Daily food intake of the children was assessed during three consecutive days by the observed weighed record method (Cameron and van Staveren, 1988). An observer stayed in the household every day from 7 a.m. up to the moment the child had the last meal, usually between 7 and 9 p.m. Mothers or caretakers were given detailed explanations and demonstrations on how foods would be weighed by the observer. Parents and caretakers were instructed to maintain the usual feeding habits during these three days. To be able to measure the child's food intake, his or her meals were served on separate plates. All foods were weighed before and after cooking, and also served portions and leftovers were weighed. Between-meals snacks were also weighed.

Eating time, description of each food item and amounts served and eaten were recorded. Less than 5 % of the children, consumed some foods or drinks at night or early in the morning, in the absence of the observer. In that case, food intakes were assessed by recall using household measures (Cameron and van Staveren, 1988).

	Week 1	*Week 2*	*Week 3*	*Week 4*	*Week 5*
Cotonou (n=8)					
Appetite test					
• Aklui	□	□	□		
• Riz-au-gras	□	□	□		
Dietary study	[□□□] [a]		[□□□] [a]		
First Zè study (n=38)					
Appetite test					
• Aklui		□	□	□	
Morbidity		□□	□□	□□	
Deworming treatment	□	□	□		
Second Zè study (n=38)					
Appetite test					
• Aklui		□	□	□	
Morbidity		□□	□□	□□	
Deworming treatment	□	□			
Dietary study		[□□□] [b]	[□□□] [b]	[□□□] [b]	
Third Zè study (n=25)					
Appetite test					
• Riz-au-gras	[□□□] [c]				[□□□] [c]
Morbidity	□□□□				□□□□

[a] During 3 consecutive days not overlapping with appetite test days, twice per child during the study period

[b] During 3 consecutive days not overlapping with appetite test days, once per child during the study period;

[c] Every other day, 3 times in the week

Figure 3.1 Designs of Cotonou and Zè studies

From the weighed observed records, daily energy intakes and energy intakes from breakfast were calculated for each child using a nutrition software, Komeet 2.1 (Scholte, 1995a), based on a composite data base created with Vbs edit 1.0 (Scholte, 1995b). The database was made using data on local foods commonly eaten in the study area and existing food composition tables for foods commonly eaten in Africa (Platt, 1979; West *et al,* 1988; Woot, 1970). Breakfast was defined as the first meal eaten each day between 7 and 10 a.m.

Appetite test. The test foods chosen were culturally appropriate and well accepted by children. They could also be prepared and supplied in a standardized way. They were identified based on oral interviews with the mothers.

In Cotonou both liquid and solid test foods were used. The liquid food was a porridge called aklui, made from a fermented maize product. Throughout the study, it was cooked by an experienced local vendor. Before selecting this food as test food, samples of the porridge were collected over 5 days and were checked on density. The mean density was 1.06 g/mL with a SD of 0.03 g/mL; this small SD suggested that this porridge was quite stable between days. Aklui was consumed with sugar added (10 g of sugar for 100 g of porridge). The solid test food, riz-au-gras, was prepared according to a standard recipe. Its main components were rice, tomato, onion, and vegetable oil. Calculated energy values per 100 g of test foods were 294 kJ for aklui and 444 kJ for riz-au-gras.

In the first and second Zè studies, only aklui was used. However, it was prepared by our research team (and not by a local vendor) using a commercially available dried maize product (68 of a dried maize product per liter of boiling water). Per 100 g of cooked aklui, the amount of sugar added was 10 g for the first Zè study and 7.5 g for the second Zè study. Calculated energy values of the test foods were 263 kJ (first Zè study) and 237 kJ (second Zè study).

In the third Zè study, appetite test was performed using only riz-au-gras. The amounts of vegetable oil and raw rice used per 100g riz-au-gras were slightly higher than that used in the Cotonou study. The calculated energy value of the test food was 563 kJ per 100 g. After preparation, a thermo-container was used for keeping test foods warm, about 50° C during the test.

Each test day a group of eight to fifteen children participated in the appetite test. Before the first appetite test day of a child, mothers and caretakers were instructed on the test procedure. From the last meal of the day preceding the appetite test up to the moment the test was performed, the child was not allowed to eat any food, not even breast milk. Each mother and her child were seated in a room. They were separated from the other mother/child couples to avoid interference. Mothers were not allowed to talk to each other during the test. Before the start of each test, mothers were given a brief reminder on how to participate in the test procedure. At start, a known amount of the test food was served in a standard bowl or plate and this amount was the same for all children. Each mother helped her child in such a way that the child was eating *ad libitum*. If children asked for more test food, their plates were filled again with a known amount of food. When the child stopped eating, the amount eaten was recorded as well as the duration of the eating episode. After a 5 minutes break, the child was invited to continue eating. This second eating episode offered a second opportunity to the child to continue eating if he or she was not satisfied after the first episode. Food intake during this second episode was also measured along with eating duration. In Cotonou, the standard procedure included two episodes whereas in the Zè studies, there was a third episode. During the appetite test, each child was continuously observed by an observer whose task was to present the food, to ensure that the child was not verbally encouraged or forced to eat or getting any kind of pressure from the mother or caretaker. The observer recorded the duration of each eating episode, performed the interview on morbidity and made notes on mother's comments or remarks about the child's attitude during the test. Observers were given strict instructions for limiting their influences on child's attitudes during the test.

Based on intake of test food and eating duration, individual food intake per minute was calculated for the first eating episode and for the sum of all episodes, for each day.

Morbidity status. In Zè studies, mothers were interviewed once a week on appetite test days about frequency of specific symptoms of illness throughout the day preceding the test day, and at the test day itself. This was done using a checklist including questions related to presence of diarrhea, fever, coughing and cold. Diarrhea was defined as at least four liquid or semi-liquid stools per day. In the third Zè study, at test day, presence of fever was determined by temperature measurement using a ear thermometer (Braun ThermoScan Instant Thermometer model IRT 1020, ThermoScan Inc., San Diego, CA/USA).

Statistical analyses

Between days, between subjects and within subjects variations in daily energy intake energy intake from breakfast, intakes of test food, duration of eating episodes and intakes per minute were studied by using analysis of variance. For each variable, day and subject were the main sources of variation studied. Since there was no significant day effect, analysis of variance was also performed with only subject as main source of variation and the mean square of the residual variance was used as an estimation of the day-to-day within subject variance. Within subjects day-to-day and between subjects variations were calculated and expressed as coefficients of variation. The relation between appetite results and food records was studied by Pearson's correlation procedure. For each appetite measurement period, between days variability of morbidity status was studied by means of Friedman analysis of variance. Whether presence of fever, diarrhea, cold or coughing had affected intake of the test food at each appetite test day was assessed by comparing test food energy intakes between groups of children with or without fever, diarrhea, cold or coughing using group t-test. Significance levels were set at $p < 0.05$. Data analyses were performed using SPSS Statistical Package for windows.

Results

Daily and breakfast energy intakes

As indicated by Figure 3.1, dietary intakes have been measured in the Cotonou and in the second Zè studies. Mean daily and breakfast energy intakes as calculated from records of weighed food intakes over 3 consecutive days are presented in Table 3.1. For the second Zè study, individual values of the daily energy intakes were plotted against individual values of breakfast energy intakes (Figure 3.2). In both Cotonou and second Zè studies, there were no systematic differences between measurement days. This was true for both the daily energy intake and the breakfast energy intake (as derived from the daily energy intake). Subsequently, the within subject and the between subjects variations in energy intakes were calculated (Table 3.1).

Table 3.1 Daily and breakfast energy intakes

	Mean±SD (MJ)	Within Subject SD (MJ)	Within subject CV (%)	Between subjects SD (MJ)	Between Subjects CV (%)
Cotonou study (n=8)					
24-hour	4.8±2.1	1.1	23	1.9	40
Breakfast	1.0±0.7	0.5	45	0.5	48
2nd Zè study (n=38)					
24-hour	3.8±1.5	1.2	30	1.0	26
Breakfast	0.8±0.4	0.3	43	0.3	34

The breakfast energy intake expressed as percentage of the daily energy intake was 21% for both the Cotonou and second Zè studies. This percentage was independent of absolute values of the daily energy intake.

Appetite test

Table 3.2 presents results of appetite tests in the Cotonou, the first and the second Zè studies. Table 3.3 presents results of appetite tests in the third Zè study. The quantities mentioned concerned the amount of test food eaten, the eating duration and the eating rate for both the first episode and the total of the two or three episodes. For these quantities, there were no systematic differences between measurement days. Subsequently, for each quantity, the within-subject day-to-day and the between-subjects variations were calculated. As shown in Table 3.3, the total energy intake from riz-au-gras was about 11% less with *ad libitum* water intake during the appetite test than the energy intake when water was not provided during the test. This difference did not reach statistical significance.

Energy intake from test foods in relation to daily and breakfast energy intakes

Correlation coefficients between appetite results and food records are shown in Table 3.4. For the second Zè study, individual values of the daily and breakfast energy intakes were plotted against individual values of test food energy intakes (Figures 3.3 and 3.4). With riz-au-gras as test food, there were positive correlation coefficients with the daily and the breakfast energy intakes but the correlations did not reach statistical significance. With aklui as test food, the coefficients were positive and the correlations were significant, in particular in the second Zè study (n=38, $r=0.41$, $p < 0.05$).

Morbidity status

Morbidity data were collected in Zè studies by oral interviews with the mothers. Analysis of these data revealed that within each appetite measurement period, there were no

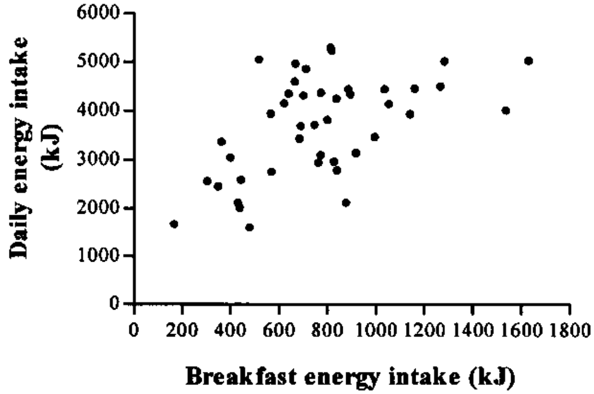

Figure 3.2 Individual values of breakfast energy intake plotted as function of daily energy intake in the second Zè study

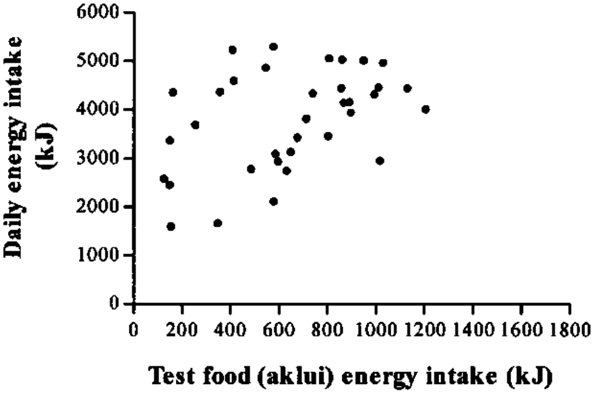

Figure 3.3 Individual values of test food (aklui) energy intake plotted as function of daily energy intake in the second Zè study

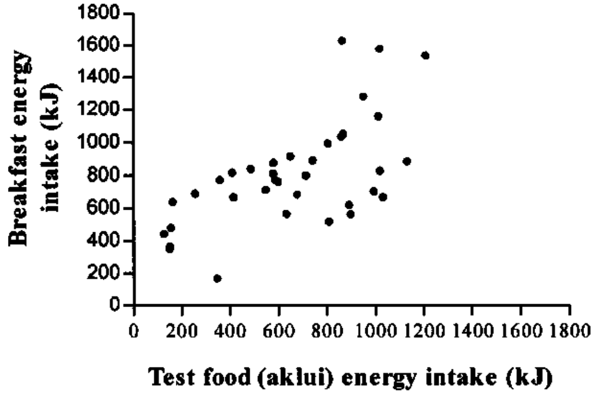

Figure 3.4 Individual values of test food (aklui) energy intake plotted as function of breakfast energy intake in the second Zè study

Table 3.2 Appetite test in the Cotonou, the first and the second Zè studies [1]

	Mean±SD				Within-subject CV (%)				Between-subjects CV(%)			
	Cotonou Rice	Cotonou Aklui	Zè 1 Aklui	Zè 2 Aklui	Cotonou Rice	Cotonou Aklui	Zè 1 Aklui	Zè 2 Aklui	Cotonou Rice	Cotonou Aklui	Zè 1 Aklui	Zè 2 Aklui
Amount eaten												
Episode 1 (g)	134±81	256±129	202±120	184±125	31	26	44	41	55	45	38	55
(MJ)	0.6±0.4	0.7±0.4	0.5±0.3	0.4±0.3								
Total (g)	148±82	285±130	258±153	264±159	25	18	44	36	51	44	34	40
(MJ)	0.7±0.4	0.8±0.4	0.7±0.4	0.6±0.4								
Eating duration (min)												
Episode 1	17±9	8±4	9±5	7±4	32	45	59	46	34	30	19	29
Total	19±11	10±5	12±7	13±6	33	49	48	39	48	5	24	21
Eating rate												
Episode 1 (g/min)	9.1±3.7	35±13	27±19	30±28	32	34	49	55	25	18	51	72
(J/min)	40±16	103±38	72±51	72±66								
Total (g/min)	8.8±3.6	33±15	23±14	23±18	32	42	44	61	26	12	43	42
(J/min)	39±16	97±44	62±38	55±43								

[1] n=8 (Cotonou), n=38 (Zè)

Table 3.3 Appetite test in the third Zè study [1]

	Mean±SD		Within-subject CV(%)		Between-subjects CV(%)	
	Test 1 (with water)	Test 2 (without water)	Test 1 (with water)	Test 2 (without water)	Test 1 (with water)	Test 2 (without water)
Rice intake						
Episode1 (g)	167±100	210±108	35	26	49	45
(MJ)	0.9±0.6	1.2±0.6				
Total (g)	234±117	263±114	26	24	43	37
(MJ)	1.3±0.7	1.5±0.6				
Eating duration						
Episode1 (min)	11±6	13±6	43	29	31	35
Total (min)	18±8	18±7	28	27	33	27
Eating rate						
Episode1 (g/min)	15±6	17±8	21	23	37	44
(kJ /min)	84±34	96±45				
Total (g/min)	13±6	15±8	25	21	38	46
(kJ/min)	73±34	84±45				
Water intake						
Episode1 (g)	48±59	-	73	-	98	-
Total (g)	90±73	-	43	-	70	-

[1] n=25; *ad libitum* water intake was allowed during the appetite test 1 procedure.

systematic differences between days in the frequencies of fever, diarrhea, cold and coughing. When the study sample was split into 2 groups based on the presence of fever, diarrhea, cold or coughing during the test day or the day before the test day, the test food energy intake of children having one of these symptoms was not significantly different from that of their symptom-free counterparts.

Discussion

The objective of the studies described in this paper was to end up with a standardized, reproducible and valid procedure for appetite measurement in young children. Such a procedure might be used as an instrument to evaluate nutrition intervention programmes.

Choice of test food

A key aspect in designing an appetite test is the choice of the test foods. A test food should be culturally appropriate and well preferred by the target group. Mothers were

Table 3.4 Correlation between appetite results and food records

	24-hour energy intake		Energy intake at breakfast	
	Cotonou (n=8)	Zè study 2 (n=38)	Cotonou (n=8)	Zè study 2 (n=38)
Aklui as test food				
Episode1 (MJ)	0.76*	0.45**	0.41	0.48**
Total (MJ)	0.79*	0.41*	0.40	0.52**
Episode1 (MJ/min)	0.82*	0.61**	-0.13	0.43**
Total (MJ/min)	0.93*	0.51**	0.11	0.43*
Rice as test food				
Episode 1(MJ)	0.38	-	0.19	-
Total (MJ)	0.37	-	0.22	-
Episode 1(MJ/min)	0.51	-	0.11	-
Total (MJ/min)	0.51	-	0.17	-

* Correlation is significant at 0.05 level (2 tailed)

** Correlation is significant at 0.01 level (2 tailed)

interviewed on their children's food habits and food preferences. The foods commonly eaten by most of the young children were: (1) a thick maize product, "pâte", eaten with a sauce as side dish (several types of sauces were used in the study area), (2) rice eaten with a tomato sauce, (3) beans eaten with a vegetable oil and "gari" (a flour made from cassava) and (4) "coco", a maize porridge. The first three foods can be considered as solid foods and the maize porridge as a liquid food. The advantage of a liquid test food above a composite solid one might be the level of standardization that can be achieved, e.g. in preparing the test food, offering the test food, and performing quantitative measurements. Therefore, we decided to have at least a liquid test food. However, the solid foods might be better preferred than the liquid one. But the problem with these solid foods is that they are composite foods. Nevertheless, rice can be cooked in the tomato sauce as a single food (riz-au-gras). Therefore, two test foods were chosen: a solid food (riz-au-gras) and a liquid one (aklui). Aklui is a porridge prepared using a commercially available dried maize product. To maintain the composition of these two foods stable throughout a study period, the raw products used were purchased in bulk, stored safely throughout each study period and the preparations were done according to standard recipes. Therefore, we assumed that these two test foods could be cooked in a standardized way. In a small group of children in Cotonou and later in a larger group in Zè, whether appetite test could be performed according to a standardized procedure using both solid and liquid test foods was studied. Figure 3.1 shows the types of test food used in each study.

Test procedure

Setting up the test procedure included: (1) the choice of an appropriate moment of the day for serving the test food, (2) designing an appropriate offering procedure and (3) deciding whether or not water should be supplied during the test, particularly in the case of a solid test food.

To avoid possible disturbing effects due to dietary intakes before the test, it was performed in the morning after an overnight fast, so that the test food was the first food eaten by the children. Mothers were instructed for withholding any food, including breast milk, until they arrived to the measuring center. Mothers' compliance, as assessed every test day through a recall of all foods eaten by the children at home or on the way to the measuring center, was high. This suggests that it is possible to perform an appetite test on these children in the morning after an overnight fast.

The standard offering procedure included two episodes (Cotonou) or three episodes (Zè) with a 5 minutes break in between. The purpose of having more than one episode is to ensure that the children were really satisfied when they stopped eating. It appeared that 70 to 90% of the test food intake were consumed during the first episode. As many children continued eating during the second and even during the third episode, the offering procedure that includes 2 or 3 episodes is the most appropriate one.

Whether or not water should be supplied during the appetite test with the solid test food was examined. In the Cotonou study, the children were not allowed to drink some water during the appetite test. This may have affected intakes of the solid test food as some children may have eaten less riz-au-gras when they were not allowed to drink some water during the test in Cotonou. It is also possible that some children would have eaten less riz-au-gras if they were allowed to drink some water. The unclear correlation between intake of riz-au-gras and the daily energy intakes in the Cotonou study could have been explained by some difficulties in standardizing an offering procedure with a solid test food. It was, therefore, worthwhile assessing the feasibility of water supply in the offering procedure and whether or not it affects intakes of the solid test food. Table 3.3 indicates that intakes of riz-au-gras with *ad libitum* water intake were not significantly different from intakes without water supply. This suggests that it is possible to standardize an offering procedure using a solid test food without a free access to water. Therefore, both solid and liquid foods can be used as appropriate test foods for young children.

Reproducibility of appetite measurements

The within-subject day-to-day variability of the test food energy intake was lower for the total of two or three episodes in comparison to that of the first episode. This suggests that appetite measurement has a better reproducibility for the total intake from 2 or 3 eating episodes than from a unique eating episode. The average within-subject day-to-day variability for the total test food energy intake was 40% for aklui and 25% for riz-au-gras. These values are in line with results of other studies in which food intakes have been measured (Birch *et al*, 1991; Boer *et al*, 1987).

When the study sample was split up based on whether or not a child had fever, diarrhea, cold or coughing on the test day or on the day preceding the test day, the difference in test food intake between 'ill' and 'healthy' children did not reach statistical significance. This suggests that the reproducibility of the appetite test was not affected by the morbidity status of the children.

Validity of appetite measurement

Appetite is usually assessed by the habitual dietary intake. In two of our studies (Cotonou and second Zè studies), we measured the habitual daily energy intake and also derived the habitual breakfast energy intake. The breakfast energy intake represents about 21 % of the daily energy intake, and this is in line with values reported for children in other studies (Ruxton and Kirk, 1997). The within subject day-to-day variability of the daily and the breakfast energy intakes, as shown in Table 3.1 were also in line with values reported in other studies (Boer *et al*, 1987; Pearcey and De Castro, 1997; Shea *et al*, 1992).

As shown in Table 4.3, correlations between energy intake from aklui as test food in one hand and daily energy intake or breakfast energy intake on the other hand were positive and statistically significant. Moreover, test food (aklui) and breakfast energy intakes were comparable. These results suggest that the test food energy intake can be considered as a valid approach for appetite assessment in these young children.

It is remarkable that the within-subject variability of the breakfast energy intake was higher than that of energy intake from aklui (Tables 3.1 and 3.2). This suggests that energy intake from the appetite test using the test procedure has a better reproducibility when compared to the energy intake from the habitual breakfast. Statistical power calculations revealed that if an identical sample size would be used to assess changes in appetite after a nutrition intervention, the appetite test procedure will require three measurement days whereas four measurement days will be needed for assessing the habitual breakfast energy intake. This would mean that assessing the habitual breakfast energy intake is labor-intensive and requires a larger sample in comparison to performing an appetite test. Moreover, using the appetite test rather than the breakfast or the daily energy intake to assess appetite may avoid biases that a limited access to foods at household level might cause in appetite assessment.

Reproducibility of appetite measurements with the standardized offering procedure should be taken into account in calculating statistical power and sample size required to evaluate appetite in nutritional intervention studies. For example, in samples of about 44 children per treatment group, a 20 % change in appetite can be detected with 80% power at 5% significance level, using the standard appetite measurement procedure as described. We conclude that the appetite test can be considered as an appropriate tool in appetite evaluations. The appropriateness of this tool for appetite evaluation will be further examined in small-scale nutritional intervention studies in young children in Zè.

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4

Effects of multivitamin-multimineral supplementation on appetite of young stunted Beninese children

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Abstract

Objective: In the developing world, food intake of under 5 year old children is often insufficient for healthy growth. Reduced appetite due to several factors including micronutrient deficiencies might be an explanation. Therefore, we assessed whether a multivitamin-multimineral supplementation will improve appetite of stunted children in southern Benin.

Subjects and methods: We performed a placebo-controlled intervention study in 17-32 month old children using multivitamin-multimineral supplements (VITALIA-tablets containing 11 vitamins and 8 minerals). Stunted children (height-for-age Z-score < -2) were randomly assigned to either multivitamin-multimineral supplement (n=48) or placebo (n=53) group. Supplementation was daily and supervised throughout 6 weeks. Knee-heel length, length, weight, arm circumference and appetite were assessed three times per child, once per week, before and after supplementation. Growth was additionally assessed 4 months after the intervention. Appetite was assessed using a validated appetite test and knee-heel length was assessed by knemometry. Each test day, morbidity data and mother's report on child's appetite throughout the preceding day were recorded.

Results: Changes in reported appetite by mothers, intake of the test food and knee-heel length were not different between groups. The same was true for growth parameters 4 months after the end of the intervention. Morbidity status was comparable in both groups before as well as after supplementation.

Conclusion: A 6 weeks multivitamin-multimineral supplementation alone failed to improve appetite and growth of stunted 2-year old children.

Introduction

In most developing countries, low food intake may be one of the underlying causes of linear growth retardation in children (Waterlow, 1994). Low food intake is generally associated with low food availability at household level and inappropriate maternal feeding practices. However, loss of appetite resulting from infections, unbalanced and monotonous diets and micronutrient deficiencies may also be an important cause of a low food intake (Brown *et al*, 1995; Golden & Golden, 2000; Golden & Golden, 1991; Golden, 1995; Pereira & Begun, 1987; Piwoz *et al*, 1994; Stephensen *et al*, 1999). Therefore, improvement of appetite in young children could make an important contribution to better nutrition and health status.

There have been intervention studies with deworming or supplementation with a single nutrient or with anthelminthic treatment to improve appetite in school children (Hadju *et al*, 1996; Hadju *et al*, 1998; Latham *et al*, 1990; Lawless *et al*, 1994; Stephenson *et al*, 1993). However, with respect to appetite improvement in younger children, who have the highest energy and nutrients requirements because of their rapid growth, and particularly in the stunted ones, very little has been reported. Also, it has been suggested that most intervention studies had failed because single nutrients were used while several nutrient deficiencies may occur simultaneously in the malnourished children, particularly in the stunted children (Allen, 1994; Golden & Golden, 2000; Golden, 1995; Rosado, 1999).

Improvement of appetite and growth, if possible, would be achieved within a relatively short period after supplementation if an appropriate micronutrient mix is supplied (Golden, 1995; Golden & Golden, 2000). Therefore, we hypothesised that a daily supplementation during a 6-week period with a commercially available multivitamin-multimineral tablet would improve appetite and growth of young stunted Beninese children.

Subjects and methods

Study area

The study was carried out in Zè, a rural area situated about 50 km from Cotonou, the economic capital of the republic of Benin. The main income generating activities in this area are subsistence farming, small-scale food processing and traditional rearing of animals, mainly pigs, chicken and goats. Households in the study area had poor living conditions and a low purchasing power.

Subjects and sampling

A list of children aged 17-32 months living in the study area was obtained from the maternity hospitals in Zè. Body length of about 300 children was measured and children with Z-score height-for-age less than –2 (about 40%) were listed. An oral interview with mothers revealed that various foods were consumed by their children. The most preferred liquid food was "aklui", a fermented maize porridge. One hundred eleven stunted children who liked this

Table 4.1 Baseline characteristics of the children at the beginning of the study

	Multivitamin-multimineral (n=48)	Placebo (n=53)	Overall sample (n=111)
Age (months)	24.0±4.0	23.5±4.1	23.7±4.0
Length (cm)	76.9±3.1	76.1±3.8	76.4±3.5
Weight (kg)	8.7±0.9	8.5±1.2	8.6±1.0
Knee-heel length (mm)	209±13	207±14	208±14
MUAC [a] (cm)	13.5±1.0	13.6±1.0	13.5±1.0
HAZ [b]	–2.79±0.67	-2.95±0.87	-2.87±0.78
WHZ [c]	–1.62±0.75	–1.55±0.74	-1.59±0.74

Mean±SD

[a] MUAC: mid-upper arm circumference; [b] HAZ: height-for-age Z-score; [c] WHZ: weight-for-height Z-score

maize porridge were invited to join the study. Informed consent to participate in the study was obtained from their parents. At recruitment, about half of the children's group was still breast fed. Baseline characteristics of the children are presented in Table 4.1. A full data set was available for 105 children who finished the study. Data of 4 children who had marginal intakes of the test food before and after supplementation were excluded from the final statistical analyses. A full data set for knee-heel measurements was available for 76 children before and after supplementation.

Study design

The study was blind, placebo-controlled and started with 111 subjects. The children were randomly assigned to one of two treatments: (1) multivitamin-multimineral and (2) placebo. Mothers and field workers were not aware of which treatment a child received. The multivitamin-multimineral supplements, VITALIA-tablets, contained 11 vitamins and 8 minerals (Table 4.2) and were manufactured by Dansk Dröge A/S (DK-2635 Ishøj – Denmark). The placebo tablets, SERESTA FORTE, were manufactured by WYETH-LEDERLE (Hoofddorp - The Netherlands). The multivitamin-multimineral supplements or the placebo tablets were given to the children at home, daily throughout 6 weeks by well-trained field observers. Appetite, anthropometric and morbidity assessments were performed before and after supplementation (Figure 4.1). Four months after the intervention study, additional anthropometric measurements were performed.

Measurements

Appetite test. The test food consisted of a culturally appropriate food, which was well- accepted by all children included in the study. It was a liquid porridge, "aklui", cooked every morning from a fermented dry maize product. The energy content of the test food (porridge with sugar)

	Weeks 1-3	*Weeks 4-9*	*Weeks10-12*
Appetite test (weekly) **• Aklui** [a]	[□] [c]		[□] [c]
Morbidity reports	[□□] [d]		[□□] [d]
Daily multivitamin-multimineral supplementation		□	
Anthropometry **• Weight** **• Length** **• MUAC** [b] **• Knee-heel length**	[□] [c]		[□] [c]

[a] liquid maize porridge; [b] mid-upper arm circumference

[c] measurements were done once per week for each child

[d] morbidity was assessed the day preceding the test day and the test day itself

Figure 4.1 Design of the first Zè intervention study

was 195 kJ per 100g. After preparation, the test food was stored in thermo-containers at about 50 ° C.

Each test day a group of about seventeen children participated in the appetite test. Before the first appetite test day of a child, his mother or caretaker was instructed on the test procedure. From the last meal of the day preceding the appetite test up to the moment the appetite test was performed, the child was requested not to eat any food, not even breast milk. During the appetite test, each mother and her child were seated in a room. They were separated from the other mother/child couples to avoid interference. Mothers were not allowed to talk to each other during the appetite test. Before the start of each appetite test, the mothers were given a brief reminder on how to offer the food to the child. At start, 300 g of test food was served in a standard bowl. Each mother helped her child in such a way that the child was eating *ad libitum*. If the children asked for more test food, their bowls were filled again with 200 g of food. When the child stopped eating, the amount eaten was recorded, as well as the duration of the eating episode. After a 5 minute break, the child was invited to continue eating. This second eating episode offered a second opportunity to the child to continue eating if he or she was not satisfied after the first episode. Food intake during this second episode was also measured along with the eating duration. The standard procedure included three episodes. During the appetite test, each child was continuously observed by an observer whose task was to measure and to present the food. The observer also ensured that the child was not verbally encouraged or forced to eat or getting any kind of pressure from the mother or caretaker. The observer recorded the duration of each eating episode, performed the interview on morbidity and made notes on the mother's comments or remarks about the child's attitudes during the test. Observers were given strict instructions for limiting their influences on the child's attitude during the test. Based on the intake of test food and eating duration, the individual food intake per minute was calculated for the first eating episode and for the sum of the three episodes, for each test day.

Anthropometry. Body weight was measured to the nearest 0.1 kg using a beam weighing scale (Babies and toddlers scale model 625 T, CMS Weighing equipment Ltd., London, England). Length was measured to the nearest 0.1 cm and mid-upper arm circumference on the left side of the body half way between the tip of the shoulder and the elbow with the subject's arm hanging freely along the body using a flexible tape. Readings were done to the nearest 0.1cm. Each appetite test day, weight was measured for each child after he or she has completed the appetite test and it was corrected later for the amount of test food eaten. This procedure was used in order to limit possible fear from the child, which may affect the appetite test. The knee-heel length was measured using a knemometer (type KNB Serial number 0066, Force Institute, DK-2605 Brondby Denmark) (Michaelsen et al, 1991; Michaelsen, 1994). Each measuring session consists of 3 sets of 5 readings.

Table 4.2 Composition of multivitamin-multimineral supplements (per VITALIA-tablet) [a]

Vitamins		Minerals	
Name	Amount	Name	Amount
Vitamin A	400 µg	Iron	10 mg
Vitamin B1	0.7 mg	Zinc	5 mg
Vitamin B2	0.8 mg	Copper	1 mg
Vitamin B6	0.9 mg	Iodine	70 µg
Vitamin B12	1 µg	Manganese	1 mg
Folic acid	20 µg	Chromium	20 µg
Niacin	9 mg	Selenium	25 µg
Panthothenic acid	3 mg	Molybdenum	50 µg
Vitamin C	40 mg		
Vitamin D	5 µg		
Vitamin E	5 mg		

[a] Each child received 1 VITALIA tablet per day

Weight-for-height and height-for-age Z scores were calculated based on the National Centre for Health Statistics (NCHS) reference data by means of the Epi info software (Centre for Diseases Control, Atlanta, United States).

Morbidity status and reported appetite. Mothers were interviewed on frequency of specific symptoms of illness on the day preceding the test day, and on the test day itself. This was done using a checklist that included questions related to presence of diarrhea, coughing and cold. Diarrhea was defined as three or more liquid or semi-liquid stools per day. Presence of fever was assessed by using an ear thermometer (Braun ThermoScan Instant Thermometer model IRT 1020, ThermoScan Inc., San Diego, CA/USA). Fever was defined as temperature above 38°C. Each mother was also asked to report on her child's appetite on the day preceding the test day. To the question "how did your child eat throughout the day before the test day? ", the mother's answers were the following: (1) my child ate well or (2) my child did not eat well. When the child ate well, his or her appetite was labeled as good. When the child did not eat well, his or her appetite was labeled as not good. Per child, before and after supplementation, frequencies of fever, diarrhea, coughing and cold were estimated as number of days with each specific symptom divided by the number of observation days. Frequency of good appetite as reported by mothers was estimated in the same way, before and after supplementation.

Statistical analyses

Before and after treatment, between subjects and within subjects variances in test food energy intake, intake per minute and eating duration were studied using analysis of variance. For each variable, day and subject were the main sources of variation studied. Variances in appetite measures were expressed as within subjects day-to-day and between subjects coefficients of variation. When there was no systematic day effect on each quantity, mean values were calculated for each subject, before and after supplementation. When there were any, changes in intake of test food, intake per minute, eating duration and anthropometric parameters were compared within study groups using paired t tests and between study groups using independent samples t tests. Frequencies of fever, diarrhea, cold, coughing and good appetite as reported by mothers were compared within each study group by means of McNemar test and between groups by means of Mann Whitney and Wilcoxon Signed Ranks tests. All statistical tests were two-tailed and statistical significance was set at 5%. Data analyses were performed using SPSS statistical package for Windows.

Results

Appetite test

Energy intake from the test food, eating duration and energy intake per minute are presented in Table 4.3. The total energy intake from the test food increased significantly in the placebo group ($p=0.002$) while the increase did not reach statistical significance in the multivitamin-multimineral group. However changes in the total energy intake from the test food, in eating duration and eating rate did not differ between groups. In the multivitamin-multimineral group, eating duration decreased significantly after supplementation ($p=0.03$), while it did not change in the placebo group. Food intake per minute increased significantly in both groups after supplementation ($p < 0.01$).

Variations in the energy intake from the test food, eating duration and eating rate were quite substantial and were split into within and between-subjects variations (Table 4.4). For all these quantities, the within-subject day-to-day coefficients of variation ranged between 30 and 40%. The between-subjects coefficients of variation ranged between 50 and 70% for the total intake, between 40 and 50% for the eating rate and between 40 and 53% for the eating rate.

Anthropometry

Length, weight, arm circumference, knee-heel length, height-for-age and weight-for-height Z-scores are presented in Table 4.5. In each study group, weight increased significantly after the 6-week supplementation period and also 4 months after the study ($p < 0.01$). Length increased in each study group after the 6-week supplementation period, but the increase reached statistical significance only in the placebo group ($p < 0.01$). Four months after the study, length increased significantly in both groups ($p < 0.01$). Arm circumference decreased significantly in each study group after the 6-week supplementation period ($p < 0.05$), but increased significantly again, 4 months after the study ($p < 0.05$). Weight-for-height Z-score increased slightly in the multivitamin-multimineral group after the 6-week supplementation period ($p=0.049$). Four months after the study, it was increased significantly within both groups ($p < 0.01$). Height-for-age Z-score increased slightly only in the multivitamin-multimineral group, 4 months after the study ($p=0.048$). The knee-heel length increased significantly in each study group after the 6-week supplementation period and also 4 months after the end of the study. The observed changes in growth parameters after the 6-week supplementation period and 4 months after the end of the study were not different between study groups.

Morbidity and reported appetite

Morbidity status and appetite as reported by mothers were not different between groups before as well as after supplementation (Table 4.6). The frequency of diarrhea was significantly reduced in both groups after supplementation. It decreased by about half of its value, from 20% to 10% ($p < 0.05$). Good appetite was reported more often in both groups after supplementation than before supplementation. The frequency of good appetite increased from 54% to 72% after supplementation ($p < 0.05$). The frequency of fever stayed low, between 3% and 5% in both groups throughout the study. Within each group, the frequency of coughing did not vary after supplementation. Frequency of cold increased significantly in the placebo group after supplementation ($p < 0.05$).

Table 4.3 Intake of the test food

	Multivitamin-multimineral (n=48)			Placebo (n=53)		
	Before [a]	After [b]	Changes	Before [a]	After [b]	Changes
Intake						
Episode 1 (g)	159±146	178±146	19±99	137±120	180±148	44±96
(kJ)	311±285	348±285	37±193	267±234	352±288	85±188
Total (g)	231±176	258±184	27±124	213±171	270±184	57±129
(kJ)	451±343	504±360	53±242	416±334	526±360	110±251
Eating duration						
Episode 1 (min)	8±5	7±4	-1±4	7±4	7±4	0±4
Total (min)	13±6	11±5	-2±5	13±7	12±6	-1±6
Eating rate						
Episode 1 (g/min)	20±12	25±12	6±8	18±12	25±13	7±9
(kJ/min)	38±24	50±24	12±15	36±23	49±26	14±18
Total (g/min)	18±11	23±12	6±6	16±10	23±13	7±9
(kJ/min)	35±21	45±23	11±11	31±19	45±26	14±18

Mean±SD

[a] Before supplementation; [b] after 6-week supplementation

Discussion

The results of the present study suggest that after the 6-week supplementation, the knee-heel length and the appetite of the children, as reported by their mothers, increased in both groups and that there was no difference in changes in these variables between groups. The same was true for the intake of the test food. If the 6-week multivitamin-multimineral supplementation would have resulted in an improvement of the intake of the test food by 140 kJ, representing about 25% of the average intake after the intervention, we would have detected a significant difference between groups with a power of 80% at a significance level of 5%. Growth performance was also not improved by the intervention. Morbidity status was not different between groups throughout the study period.

It is very unlikely that the lack of improvement in appetite would be related to the supplements given to the children since these supplements contain a wide range of vitamins and minerals in amounts required to fulfil young children's daily needs during a period of rapid growth. It is also very unlikely that the duration of supplementation would not be adequate since improvement of appetite and growth, if possible, would follow the supplementation of the missing nutrients (Golden and Golden, 2000; Golden, 1995).

Table 4.4 Within and between subjects variability of appetite test before and after supplementation

	Within subject CV (%)				Between subjects CV (%)			
	Multivitamin-multimineral (n=48)		Placebo (n=53)		Multivitamin-multimineral (n=48)		Placebo (n=53)	
	Before	After	Before	After	Before	After	Before	After
Intake								
Episode 1	36	37	48	55	85	74	74	61
Total	34	31	40	40	68	64	70	56
Eating duration								
Episode 1	47	38	52	29	54	49	52	44
Total	37	37	38	29	40	43	50	50
Eating rate								
Episode 1	28	26	46	46	54	40	43	44
Total	27	29	38	35	53	41	48	44

Table 4.5 Anthropometric parameters

	Multivitamin-multimineral			Placebo		
	Before [a]	After [b]	Follow-up [c]	Before [a]	After [b]	Follow-up [c]
Length (cm)	77.1±3.0	78.2±2.9	81.1±2.8	76.3±3.8	77.4±3.7	80.5±3.6
Weight (kg)	8.8±1.0	9.1±0.9	10.1±1.1	8.6±1.1	8.9±1.2	9.8±1.9
Knee-heel (mm)	211±13	216±12	218±10	208±14	214±13	215±14
MUAC [d] (cm)	13.5±1.0	13.3±0.9	13.8±1.1	13.5±1.0	13.3±1.1	13.9±1.1
HAZ [e]	-2.76±0.66	-2.77±0.71	-2.61±0.75	-2.91±0.85	-2.92±0.86	-2.74±0.78
WHZ [f]	-1.55±0.79	-1.46±0.78	-1.02±0.85	-1.58±0.67	-1.50±0.68	-0.75±1.71

Mean±SD

[a] Before supplementation; [b] after 6-week supplementation ; [c] Follow-up 4 months after intervention

[d] MUAC: mid-upper arm circumference; [e] HAZ: height-for-age Z-score; [f] WHZ: weight-for-height Z-score

Moreover, the lack of improvement of appetite may not be explained by a confounding effect of morbidity since morbidity status was similar in both supplemented and control groups, before as well as after supplementation. Therefore, we can assume that the multivitamin-multimineral supplementation did not improve appetite and growth of these young stunted children. Due to lack of similar studies in young children in less developed countries, our results could not be compared. As the type and amount of supplement, the duration of supplementation and the morbidity status of the children could not explain the lack of improvement of appetite, other factors might had played an important role. It may be that, although a child swallowed

Table 4.6 Frequencies of morbidity signs or good appetite through each 3-week period (%)

	Before supplementation		After supplementation	
	Multivitamin-multimineral (n=48)	Placebo (n=53)	Multivitamin-multimineral (n=48)	Placebo (n=53)
Diarrhea	22	17	11[a]	8[a]
Fever	3	3	5	5
Cold	35	24[b]	38	35[a]
Coughing	36	25[b]	35	25
Reported good appetite	54	54	73[a]	72[a]

[a] Significantly different from pre-treatment ($p < 0.05$, Wilcoxon Signed Rank test)

[b] Significantly different from intervention group ($p < 0.05$, Mann-Whitney U test)

the tablets in the presence of an observer, there was a competition between his body and intestinal parasites for the utilization of the ingested vitamins and minerals. Possible pathways through which helminth infections may alter human nutrition have been suggested (Stephenson, 1987; Solomons, 1993). An alternative hypothesis is that most of the children were iron deficient and/or anemic and therefore needed higher iron supplies. If that would be the case, the multivitamin-multimineral supplements that contained 10 mg of elemental iron would not provide sufficient iron to improve the children's iron status. In case of iron deficiency anemia, the daily dose of iron needed is higher, about 3 mg elemental iron per kg of body weight, that means in the present situation a daily supplementation of at least 30 mg of elemental iron per child (DeMaeyer *et al*, 1989). Therefore, an additional amount of iron should be supplied.

Because of the living conditions of the children, they might indeed have specific health problems i.e. a high burden of intestinal helminths, a chronic malarial infection, iron deficiency and/or iron deficiency anemia. Iron deficiency and/or iron deficiency anemia may be caused by an insufficient intake or assimilation of iron from the habitual diet, by blood losses as a result of recurrent helminth infections or by a combination of these factors. In fact, it had been reported that helminth infections, iron deficiency and iron deficiency anemia are problems of a public health importance in Southern Bénin (Hercberg *et al*, 1986; UNICEF, 1996). Moreover, evidence of the presence of helminth infections in Southern Benin is supported by a study performed recently in the area by our research group. The study revealed the presence in young children of helminth infections mainly by *Ascaris lumbricoides* (53%), *Trichuris trichiura* (51%) and hookworms (12%) (chapter 2). The contribution of parasitic infections to decreased food intake, loss of appetite and iron deficiency has been well documented (Lawless *et al*, 1994; Solomons, 1993; Stephenson, 1987; Stephensen 1999; Stoltzfus *et al*, 1997; Tshikuka *et* *al*, 1997). To tackle the recurrent helminth infections in the study area, deworming treatment was used as a routine practice by health workers in Zè. However, due to the low attendance to health centers, helminth infections might still be highly prevalent in the area. As a result of recurrent helminth infections and poor diet, iron deficiency and iron deficiency anemia might still be serious health problems in the study area. This situation was confirmed by a screening for anemia in a group of stunted children in Zè. It appeared that 63 % of these children had hemoglobin concentration less than 110 g/L. Therefore, it is likely that a large number of children in our sample were iron deficient and/or have iron deficiency anemia. It is also likely that the multivitamin-multimineral supplementation did not have any effect on their appetite and growth because most of the children were iron deficient or anemic during the intervention. Therefore, to achieve a better impact on appetite in these children, it might be necessary, in addition to the multivitamin-multimineral supplementation, to provide these children with an anthelminthic treatment and with an additional iron treatment. A substantial reduction of the competition for nutrients and of nutrient losses is expected after deworming. Additional iron supplies may result in a quick improvement of the children's iron status.

We conclude that a 6-week multivitamin-multimineral supplementation alone failed to improve appetite and linear growth of young stunted Beninese children. We hypothesise that beneficial effects of the intervention were prevented by the burden of helminth infection and iron deficiency. Therefore, deworming treatment and additional iron treatment may increase the effect of the intervention.

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5

Effect of multivitamin-multimineral and iron supplementation on appetite of young stunted and anemic Beninese children

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Abstract

Objective: In developing countries, low food intake is often reported in under 5 year old children. Reduced appetite may be a contributing factor. We investigated whether a combination of multivitamin-multimineral and additional iron treatment can improve appetite and growth of 18-30 month old stunted and anemic Beninese children.

Subjects and methods: The study was placebo-controlled using VITALIA-tablets (11 vitamins + 10 minerals) and ferrous fumarate tablets (66 mg iron). Hundred fifty stunted (height-for-age Z-score < -2) and anemic children (Hb < 110 g/L) were randomly assigned to one of four treatments: 1. multivitamin-multimineral + iron, 2. multivitamin-multimineral + placebo, 3. placebo + placebo and 4. placebo + iron. Daily supplementation was supervised throughout 6 weeks. Appetite, knee-heel length, dietary intakes and morbidity were assessed before and after supplementation. Length, weight, arm circumference and hemoglobin concentration were assessed before, just after supplementation and 4 months after the intervention. Appetite was assessed by means of a validated appetite test using a test food, riz-au-gras, eaten *ad libitum* after an overnight fast. Dietary intakes were assessed during 3 consecutive days (not overlapping with the appetite test days) by means of the observed weighed record method.

Results: Energy intake from the habitual breakfast was significantly correlated with that from the test food (n=38, r=0.49, p=0.002). There was no difference in changes in appetite and growth performance between groups. The habitual diet of the children was monotonous and provided little amounts of animal products. Morbidity status of the children was comparable in all study groups, before as well as after supplementation.

Conclusion: The 6-week multivitamin-multimineral supplementation with additional iron treatment failed to improve appetite and growth of the children.

Introduction

In most developing countries, especially in poor areas, the high prevalence of stunting in young children is often ascribed to inadequate dietary intakes, infections and the mother-infant interaction (Waterlow, 1994). Reduced appetite due to several factors may play an important role in this phenomenon (Brown *et al*, 1995; Golden & Golden, 2000; Golden, 1995; Waterlow, 1994). Therefore, an improvement of appetite in stunted children, if possible, may contribute to an improvement of their linear growth.

In previous studies carried out in Zè, a rural area in south of the republic of Benin, we developed a tool for appetite evaluation in young children and investigated whether a 6-week multivitamin-multimineral supplementation can improve appetite and growth of young stunted children. However, increases of appetite and growth after supplementation were not different between the supplemented and the control groups. Further investigations in the study area revealed that 63% of young stunted children between 18 and 30 months of age were anemic (Hb < 110 g/L). Previous studies in southern Benin (Hercberg *et al*, 1986; UNICEF, 1996) and findings of our own research group suggested that iron deficiency and parasitic infections may be the main causes of anemia in young children in the south of Benin. We found in a large area in south of Benin that preschool children were infected mainly by *Ascaris lumbricoides* (53%), *Trichuris trichiura* (51%) and hookworms (12%). Given that the environmental and living conditions are comparable in rural areas in south of Benin, the situation described above may also apply for the Zè area. Therefore, we hypothesised that the lack of improvement of appetite is associated with a high burden of helminth infections and iron deficiency. In the present intervention study, we investigated whether a combination of multivitamin-multimineral and iron supplementation can improve appetite and growth of stunted and anemic Beninese children. Before and during the study, intestinal parasites were controlled by means of deworming treatments.

Subjects and methods

Study area

The study was carried out in Zè, a rural area situated about 50 km from Cotonou, the economic capital of the republic of Benin. The main income generating activities in this area are subsistence farming, small scale food processing and traditional rearing of animals, mainly pigs, chicken and goats. Most households in the study area have poor living conditions and a low purchasing power.

Subjects and sampling

The present intervention study was carried out in young stunted and anemic children. The recruitment period lasted 6 months followed immediately by the baseline measurements. A list of young children aged 18-30 months old living in the study area was obtained from the maternity hospitals. Length was measured and all children with height-for-age Z-score below –2 (about 48%) were listed and their hemoglobin concentration was measured. Stunted children with hemoglobin concentration below 110 g/L (63% of the stunted children) were selected. The children were invited to join the study only if their mother had certified that they like 'riz-au-gras', which was used as the test food. At the beginning, 154 stunted and anemic children participated in the study. Informed consent to participate in the study was obtained from the parents. Due to loss to follow-up, a complete data set was available for 150 children. At the age of 2 years, about half of these children were still breast fed. The baseline characteristics of the children are presented in Table 5.1.

Study design

The study was blind and placebo-controlled. The children were randomly assigned to one of four treatments: 1. multivitamin-multimineral + iron, 2. multivitamin-multimineral + placebo, 3. placebo + placebo and 4. placebo + iron. The multivitamin-multimineral supplements (VITALIA-tablets) contain 11 vitamins and 10 minerals (Table 5.2) and were manufactured by Dansk Dröge A/S (DK-2635 Ishøj – Denmark). The iron tablets were manufactured by Pharmaquick (Cotonou, Republic of Benin). The daily dose was 66 mg of elemental iron in the form of one tablet of ferrous fumarate. The placebo tablets, SERESTA FORTE Placebo, were manufactured by WYETH-LEDERLE (Hoofddorp, The Netherlands). The tablets were given to the children at home, daily throughout 6 weeks by well-trained field observers. One week before the study and during the last week of supplementation, all children received a deworming treatment. Each child was treated with 600 mg of mebendazole (200 mg per day for 3 consecutive days). Appetite, dietary intake, weight, length, knee-heel length, mid-upper arm circumference, morbidity and the mother's report on child's appetite were assessed before and

Table 5.1 Baseline characteristic of the children

	17-22 months (n=69)	22-26 months (n=44)	26-32 months (n=37)	Overall (n=150)
Age (months)	18.8±2.0	24.0±1.1	27.9±1.0	22.6±4.1
Length (cm)	73.9±2.6	77.8±2.6	79.7±2.5	76.5±3.6
Weight (kg)	8.2±0.9	9.2±0.9	9.5±1.2	8.8±1.2
HAZ [a]	-2.72±0.66	-2.56±0.72	-2.55±0.69	-2.63±0.68
WHZ [b]	-1.51±0.89	-1.44±0.80	-1.31±0.84	-1.44±0.85
MUAC [c] (cm)	13.0±0.9	13.4±1.1	13.4±1.2	13.2±1.1
Knee-heel length (mm)	203±10	217±8	222±10	213±13
Hb (g/L)	95±10	96±10	96±10	95±10

Mean ± SD

[a] HAZ: height-for-age Z-score; [b] WHZ: weight-for-height Z-score; [c] MUAC: mid-upper arm circumference

Table 5.2. Composition of multivitamin-multimineral supplements (per VITALIA-tablet) [a]

Vitamins		Minerals	
Name	Amount	Name	Amount
Vitamin A	400 µg	Iron	8 mg
Vitamin B1	0.7 mg	Zinc	5 mg
Vitamin B2	0.8 mg	Copper	0.4 mg
Vitamin B6	0.8 mg	Iodine	70 µg
Vitamin B12	1 µg	Manganese	1 mg
Folic acid	75 µg	Chromium	20 µg
Niacin	9 mg	Selenium	25 µg
Panthothenic acid	3 mg	Molybdenum	50 µg
Vitamin C	40 mg	Calcium	100 mg
Vitamin D	10 µg	Magnesium	21.5 mg
Vitamin E	5 mg		

[a] Each child received 1 VITALIA tablet per day

after supplementation. Hemoglobin concentration was assessed at recruitment, and in the first and in the third weeks after the supplementation period (Figure 5.1). Four months after the intervention, additional anthropometric and hemoglobin measurements were performed.

Measurements

Dietary intakes. The habitual food intake of the children was assessed during 3 consecutive days by means of the observed weighed record method (Cameron and van Staveren, 1988). From the weighed observed records, the daily energy intake, the breakfast energy intake, the protein and iron intakes were calculated for each child using a computer program, Komeet 2.1 (Scholte, 1995a), based on a nutrient database created with Vbs edit 1.0 (Scholte, 1995b). The database was made using data on local foods commonly eaten in the study area and existing food composition tables for foods commonly eaten in Africa (Platt, 1979; West *et al*, 1988; Woot, 1970). Breakfast was defined as the first meal eaten each day between 7 and 10 a.m.

Appetite test. The test food chosen was riz-au-gras, which is rice cooked in tomato sauce. For the choice of the test food, children's food habits and preferences were taken into account. It is a culturally appropriate food, well accepted by most young children in the study area. It was prepared and supplied in a standardized way. Its main components were rice, tomato and vegetable oil. The calculated energy value of riz-au-gras was 550 kJ per 100 g. A thermo-container was used for keeping the test food warm, about 50° C, during the appetite test.

	Week 1	Weeks 2-4	Weeks 5-9	Week 10	Weeks 11-13
Deworming	[□□□][a]			[□□□][a]	
Appetite test • **Riz-au-gras**		□			□
Morbidity assessment		□□			□□
Dietary study		[□□□][b]			[□□□][b]
Daily supplementation [c]			□	□	
Anthropometry • **Weight** • **Length** • **MUAC** [d] • **Knee-heel length**		□			□
Hemoglobin assessment	[□] [e]				□

[a] During 3 consecutive days

[b] Once before and after supplementation during 3 consecutive days not overlapping with test days

[c] Multivitamin-multimineral, iron or placebo supplementation

[d] Mid-upper arm circumference

[e] During screening 1 to 2 weeks before baseline appetite measurement

Figure 5.2 Study design

Each test day, a group of about 25 children participated in the appetite test. Before the first appetite test day of a child, the mother was instructed on the test procedure. From the last meal of the day preceding the appetite test up to the moment the test was performed, the child was requested not to eat any food, not even breast milk. During the appetite test, each mother and her child were seated in a room. They were separated from the other mother-child couples to avoid interference. Mothers were not allowed to talk to each other during the test. Before the start of the test, mothers were given a brief reminder on how to participate in the offering procedure. At start, 250 g of test food was served in a standard plate. Then, each mother helped her child in such a way that the child was eating *ad libitum*. When a child asked for more test food, his or her plate was filled again with another 250 g of food. When a child stopped eating, the amount of food eaten was recorded as well as the duration of the eating episode. After a 5-minute break, the child was invited to continue eating. This second eating episode offered to the child the opportunity to continue eating if he or she was not satisfied after the first episode. Food intake during this second episode was also measured along with eating duration. The standard offering procedure included three episodes. During the appetite test, each child was continuously observed by an observer whose task was to measure and present the food. The observer also ensured that the child was not verbally encouraged to eat or forced or getting any kind of pressure from the mother or caretaker. The observer recorded the duration of each eating episode, made notes on mother's remarks about the child's attitudes during the test and performed the interview on morbidity and mother's report on child's appetite. Observers were given strict instructions for limiting their influences on child's attitudes during the test. Based on the intake of the test food and the eating duration, the individual food intake per minute was calculated for the first eating episode and for the sum of the three episodes, for each test day.

Anthropometry. Body weight was measured to the nearest 0.1 kg using a beam scale (Babies and toddlers scale model 625 T, CMS Weighing equipment Ltd., London, England). Length was measured to the nearest 0.1 cm and mid-upper arm circumference was measured on the left side of the body half way between the tip of the shoulder and the elbow with the subject's arm hanging freely along the body using a flexible tape. Readings were done to the nearest 0.1 cm. At appetite test days, weight was measured for each child after he or she has completed the appetite test and it was corrected later for the amount of test food eaten. This procedure was used in order to limit possible fear from the child, which may affect the appetite test. The knee-heel length was measured using a knemometer (type KNB Serial number 0066, Force Institute, DK-2605 Brondby Denmark) (Michaelsen et al, 1991; Michaelsen, 1994). Each measuring session consists of 3 sets of 5 readings.

Weight-for-height and height-for-age Z-scores were calculated based on the National Centre for Health Statistics (NCHS) reference data and by means of the ANTHRO software (Centre for Diseases Control, Atlanta, United States).

Hemoglobin concentration. Hemoglobin concentration (g/L) was measured using a finger prick blood sampling technique and a photometry analysis method: the HemoCue device (Laifer, Kuller and Hill, 1990).

Morbidity status and reported appetite. Mothers were interviewed about the frequency of specific symptoms of illness at the day preceding the test day, and at the test day itself. This was done by means of a checklist that included questions related to presence of diarrhea, coughing and cold. Diarrhea was defined as three or more liquid or semi-liquid stools per day. Presence of fever was assessed by using an ear thermometer (Braun ThermoScan Instant Thermometer model IRT 1020, ThermoScan Inc., San Diego, CA/USA). Fever was defined as temperature above 38°C. Each mother was asked to report on her child's appetite at the day preceding the test day. To the question "how did your child eat throughout the day before the test day?", the mother's answers were the following: (1) my child ate well or (2) my child did not eat well. When the child ate well, his or her appetite was labeled as good. When the child did not eat well, his or her appetite was labeled as not good. Per child, before and after supplementation, frequencies of fever, diarrhea, coughing and cold were estimated as the number of days with each specific symptom divided by the number of observation days. Frequency of good appetite as reported by mothers was estimated in the same way, before and after supplementation.

Statistical analyses

Before and after supplementation the energy intake from the test food, eating duration, intake per minute, habitual daily energy intake and breakfast energy intakes were studied by means of analysis of variance. For each variable, day and subject were the main sources of variance studied. Variances of appetite measures and daily energy intakes were expressed as within subject day-to-day and between subjects coefficients of variation. When there was no systematic day effect on each quantity, mean values were calculated for each subject, before and after supplementation. Changes in the energy intake from the test food, intake per minute, eating duration, habitual daily energy intake and energy intake from the habitual breakfast, height-for-age and weight-for-height Z-scores, arm circumference, knee-heel length and hemoglobin concentration were compared between treatment groups using Oneway analysis of variance. Associations between the energy intake from the test food and energy intakes from habitual daily and breakfast were studied by means of the Pearson's correlation procedure. Before as well as after supplementation, frequencies of fever, diarrhea, cold, coughing and good appetite as reported by mothers were compared within each study group by means of Mann Whitney test and between groups by means of Wilcoxon Signed Ranks tests. All statistical tests were two-tailed and statistical significance was set at 5%. Data analyses were performed using SPSS statistical package for Windows.

Results

Appetite test

Intake of the test food, eating duration and eating rate before and after supplementation are shown in Table 5.3. The within-subject day-to-day variations of energy intake from the test food, expressed as coefficient of variations fluctuated between 33-39% before supplementation and 26-39% after supplementation (Table 5.4). The between-subjects variations fluctuated between 40-64% before and 38-50% after supplementation. The total energy intake from the test

Table 5.3 Appetite before and after treatment

Study groups	Total test food intake (kJ)		Eating duration (min)		Eating rate (kJ/min)	
	Before [a]	After [b]	Before	After	Before	After
Multivitamin-multimineral + iron (n=39)	988±519	1275±691	22±11	22±9	47±18	59±27
Multivitamin-multimineral + placebo (n= 37)	1037±616	1264±593	21±8	24±9	48±22	55±24
Placebo + placebo (n=39)	782±580	1070±647	17±10	19±8	45±24	57±29
Iron + placebo (n=35)	983±605	1198±650	18±8	20±8	57±29	64±32

Mean ±SD

[a] Before supplementation; [b] after 6-week supplementation

Table 5.4 Variations in total intake of the test food

Study groups	Within-subject CV(%)		Between-subject CV (%)	
	Before [a]	After [b]	Before	After
Multivitamin-multimineral + iron (n=39)	34	27	40	47
Multivitamin-multimineral + placebo (n=37)	33	26	50	40
Placebo + placebo (n=39)	39	34	64	50
Iron + placebo (n=35)	35	39	52	38

[a] Before supplementation; [b] after 6-week supplementation

food increased significantly in all treatment groups after supplementation ($p < 0.05$). However, there was no significant difference between groups.

Dietary intakes

The habitual energy, protein and iron intakes were mainly provided by plant foods. The mean values of daily and breakfast energy intakes are shown in Table 5.5. Before as well as after supplementation, there was no significant differences between groups with respect to the daily energy intake, breakfast energy intake, protein and iron intakes. Within each group, the habitual daily and breakfast energy intakes did not change significantly after supplementation. Mean values for the overall sample were 3.6±1.0 MJ for the daily energy intake, 23±11 g for protein intake and 6.2±2.5 mg for iron intake. The breakfast energy intake was significantly associated with energy intake from the test food before supplementation (n=38, r=0.49, p=0.002) as well as after supplementation (n=38, r=0.42, p=0.008). Correlations between energy intake from the test

Table 5.5. Habitual daily and breakfast intakes

Treatment groups	Daily intake (MJ)		Breakfast intake (MJ)	
	Before [a]	After [b]	Before	After
Multivitamin-multimineral + iron (n=11)	3.4±1.0	3.3±0.7	0.9±0.2	0.8±0.3
Multivitamin-multimineral + placebo (n=10)	4.0±1.4	3.7±1.1	1.1±0.2	0.9±0.3
Placebo + placebo (n=9)	3.4±1.9	3.4±0.9	0.8±0.3	0.9±0.3
Placebo + iron (n=8)	3.6±1.4	4.1±0.7	0.8±0.4	1.1±0.3

Mean ±SD

[a] Before supplementation; [b] after 6-week supplementation

food and habitual daily energy intake were positive but less pronounced before supplementation (n=38, r=0.22, p=0.18) as well as after supplementation(n=38, r=0.26, p=0.12).

Anthropometry

Length, weight, arm circumference, knee-heel length, height-for-age and weight-for-height Z-scores during the intervention are shown in Table 5.6. Height-for-age and weight-for - height Z-scores, arm circumference and knee-heel length increased in a similar way in all groups. The improvement of weight-for-height Z-scores was statistically significant in the group of children who received a combination of multivitamin-multimineral and iron supplements and also in the group of children who received only placebos ($p < 0.05$). Knee-heel length increased significantly and in a similar way in all study groups after supplementation ($p < 0.001$). Additional anthropometric measurements performed 4 months after the intervention revealed no significant difference in changes between study groups.

Hemoglobin concentration

In the first week after supplementation, hemoglobin concentration increased by 3 g/L in the children who received both multivitamin-multimineral and iron tablets and by 5 g/L in those who only received iron supplements. The increase was statistically significant in the later group (p=0.007). Hemoglobin concentration decreased by 4 g/L in the children who only received placebos and by 1 g/L in those who received multivitamin-multimineral and placebo. These changes were not statistically significant. Changes in hemoglobin concentration in the first week after supplementation were significantly higher in the two groups of children who received iron supplements than in the other two groups (p=0.027). In the third week after supplementation, hemoglobin concentrations in all groups were similar to concentrations before supplementation. Hemoglobin concentration measured 4 months after the intervention was similar in all groups, with a mean value of 100±14 g/L.

Table 5.6 Anthropometric parameters

	Treatment groups			
	Multivitamin-multimineral + Iron (n=39)	Multivitamin-multimineral + Placebo (n=37)	Placebo + placebo (n=39)	Placebo + iron (n=35)
Before supplementation				
Weight (kg)	8.9±1.2	8.8±1.3	8.8±0.9	8.8±1.2
Length (cm)	76.8±3.1	76.8±4.4	76.3±3.1	76.7±3.5
MUAC (cm)	13.2±1.1	13.1±1.1	13.3±0.97	13.2±1.2
WHZ	-1.33±0.84	-1.49±0.86	-1.43±0.79	-1.47±0.78
HAZ	-2.66±0.65	-2.73±0.86	-2.43±0.56	-2.66±0.57
Knee-heel length (mm)	214±10	218±13	212±11	213±13
After supplementation				
Weight (kg)	9.3±1.2	9.1±1.3	9.2±0.9	9.1±1.3
Length (cm)	78.2±3.1	78.2±4.2	77.7±3.1	78.1±3.5
MUAC (cm)	13.2±1.1	13.1±1.1	13.4±0.87	13.2±1.2
WHZ	-1.26±0.83	-1.44±0.84	-1.28±0.67	-1.45±0.85
HAZ	-2.61±0.70	-2.67±0.82	-2.43±0.60	-2.63±0.58
Knee-heel length (mm)	219±10	223±13	218±11	218±13

Mean±SD; WHZ: weight-for-height Z-score; HAZ: height-for-age Z-score; MUAC: mid-upper arm circumference

Morbidity and reported appetite

Frequencies of fever, diarrhea, cold and coughing were comparable in all groups before as well as after supplementation. Children who were reported by their mothers to have a good appetite had higher energy intake from the test food in comparison to their counterparts who did not have a good appetite ($p < 0.05$), before as well as after supplementation.

Discussion

The objective of the present study was to investigate whether a combination of multivitamin-multimineral and iron supplementation can improve appetite and growth of young stunted and anemic Beninese children. In a previous study, a 6-week supplementation with multivitamin-multimineral alone did not improve appetite and growth of young stunted children and we hypothesised that the lack of effect might be caused by the presence of intestinal helminths and iron deficiency in the children. Therefore, in the present study, we provided an additional iron treatment and all the children received a deworming treatment before and during the study.

As expected, hemoglobin concentrations measured in the first week after the supplementation period were significantly increased in the children who received iron supplements. This means that anemia in the children was mainly due to iron deficiency and that iron supplementation alone was sufficient for improving their iron status.

Our data suggest that none of the differences in changes between groups in the energy intakes from the test food, after the intervention, were statistically significant. If the 6-week micronutrient supplementation had resulted in an improvement of the energy intake from the test food by at least 300 kJ, representing approximately 25 % of their mean intake, we would have detected a significant difference between groups with a power of at least 80 % at a significance level of 5%. Therefore, the intervention did not result in a meaningful improvement of appetite. Likewise, the growth performance of the children was not affected by the intervention.

It is unlikely that the lack of improvement of appetite and linear growth would be related to the targets of the intervention. Indeed, young children having height-for-age Z-scores less than –2 and blood hemoglobin less than 110 g/L were participating to the study. Most of the children showed a pronounced trend to wasting. They were all likely to have multiple micronutrient deficiencies and reduced appetite. Therefore a positive appetite and growth response to the intervention was expected. It is also unlikely that the morbidity status of the children would be a confounding factor during the study. In fact, helminth infection was under control. Frequencies of fever and diarrhea stayed low during the study period and frequencies of cold and coughing were not different between groups throughout the study. It is also unlikely that the dose of each micronutrient present in the multivitamin-multimineral supplement would be insufficient. On the contrary, for all vitamins and minerals present, the recommended daily allowances for proper growth were fulfilled. The additional iron was also supplied in sufficient amount (66 mg elemental iron per day), in agreement with the current recommended doses for the treatment of iron deficiency anemia (Hallberg *et al*, 2000; DeMayer *et al*, 1989). The lack of improvement of appetite could not be related to the method used for appetite measurement. In fact, the appetite test procedure has been validated in the previous studies in young children in the same environment. The within-subject day-to-day variation of the energy intake from the test food is in line with variations commonly reported in dietary studies (Boer *et al,* 1987; Pearcey and De Castro, 1997; Shea *et al,* 1992). In addition, the energy intake from the test food was significantly associated with the energy intake from the habitual breakfast of the children as assessed during the same period. Therefore, the appetite test provided a valid estimate of the children's habitual energy intake. The results of the dietary assessment suggested that the children's habitual energy intake was insufficient, covering only 75% of the recommended energy intakes for children of the same age (WHO, 1985). The quality of their usual diet may also be inadequate, as suggested by its monotony and the marginal amounts of animal foods consumed. This means that the overall dietary intakes of the children were not adequate and therefore, most of them might have not only a multiple micronutrient deficiency but also a chronic energy-protein deficiency. This finding is in line with the results of our previous dietary studies in young children in Southern Benin. It also supports the general agreement that the level of the dietary intakes of young children in developing countries is less than the current recommendations (Brown, 1997; Hautvast *et al*, 1998). If the children had a chronic energy-protein deficiency in addition to multiple micronutrient deficiencies, it might be that micronutrient supplementation alone would not be enough to generate a significant improvement of their nutritional status. Consequently, their appetite and linear growth might not improve meaningfully until these deficiencies are eliminated. Therefore, supplementation of energy and high quality protein may be needed as well.

A second factor, that may explain the lack of improvement of appetite and growth, is the duration of the supplementation. Because the suspected multiple micronutrient deficiencies in the children may have been present for a long period, the size of body stores and tissue concentrations of several micronutrients could be marginal. Consequently, the metabolic pathways that depend upon these micronutrients might be severely compromised and therefore, a longer supplementation period would be required to replete micronutrient stores and permit an optimum body functioning.

We conclude that a 6-week multivitamin-multimineral and iron supplementation improved iron status but was not sufficient to improve appetite and growth of young stunted and anemic Beninese children. These children have probably been continuously exposed to a poor quality diet and possibly also have a chronic energy-protein deficiency. Therefore, we suggest that to achieve a substantial improvement of appetite and growth in these children, supplementation of a combination of energy-protein-micronutrients for a sufficiently long period might be necessary.

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6

General discussion

The main objective of the research presented in this thesis was to assess whether micronutrient supplementation improves appetite and linear growth of stunted young Beninese children. To achieve this objective, we have performed three micronutrient supplementation studies. These studies were preceded by a methodological study to set up a tool for appetite measurement in young children.

The methodological study revealed that an appetite test consisting of measuring the *ad libitum* intake of a culturally appropriate and well-accepted test food after an overnight fast is a valid tool in children provided a standardised procedure is used. The first intervention study, a 3-month iron supplementation in combination with deworming, resulted in a significant increase of hemoglobin level by 5 g/L. This increase was still observed 7 months after the intervention. However, there was no improvement of growth performance. The two other interventions, 6-week multivitamin-multimineral supplementation alone or in combination with an additional iron treatment did not result in an improvement of appetite or growth performance but hemoglobin level was significantly improved by 5 g/L in the children who received iron supplements.

In paragraph 6.1, some methodological aspects of appetite measurement in young children will be discussed in more detail. Subsequently, the effects of micronutrient supplementation on hemoglobin level (paragraph 6.2) will be discussed. The lack of effects of micronutrient supplementation on linear growth (paragraph 6.3) and appetite (paragraph 6.4) will also be considered. Finally, taking into account the considerations in paragraphs 6.1 to 6.4, suggestions will be made for desirable future studies (paragraph 6.5).

6.1. Methodological aspects of appetite measurement using the appetite test procedure

Independent of the type of test foods, the average within-subject day-to-day variation in the test food intake in our studies was 40%. This variation is quite high and occurred in spite of an appropriate choice of the test food and the use of a standardized offering procedure. However, it is in line with the observed variations in dietary intakes in other studies (Boer *et al,* 1987; de Graaf, 2000; Pearcey and de Castro, 1997; Shea *et al,* 1992). It means that a single measurement obtained by this method may not give a valid estimation of a child's appetite. Therefore, several replications should be performed and the average value of the test food consumption should be calculated. For practical reasons we decided to limit the number of replications to three and to use the average of three consecutive replications to estimate a child's appetite. It also means that large samples are needed to study possible intervention effects on appetite. For example, in our studies, we were interested in detecting differences in appetite between groups of 25%. Such an improvement seems to be feasible if we consider the decrease in food intakes that is commonly observed in children living in comparable environments. Indeed, according to data from community-based studies in several developing countries, the decrease in food intake due to various infections in young children may fluctuate between 15% and 33% and even more in case of severe infections (Brown *et al*, 1990; Pereira and Begum, 1987; WHO, 1998). It should be recognised that even if appetite is measured in triplicate, to be able to detect a difference of 25% with 80% power at 5% significance levels, the minimum number of subjects needed per study group would be 50 subjects. If one would be interested in a smaller difference even, a larger number is needed.

The observed high value for the within-subject day-to-day variation was probably not related to the appetite test method *per se*, but to the variations in factors which may affect a child's appetite. In our studies, three possible sources of variation of a child's appetite are: (1) the level of physical activity of the child prior to appetite measurement, (2) the food intake of the child at home during the day or days prior to the measurement day, and (3) the health status of the child at the measurement day or at the day prior to the measurement day. By performing appetite measurements early in the morning, we assume that the effect of differences in the level of physical activity of the child will be negligible. According to the measurement procedure, a child should not have consumed any food prior to the appetite test. Although possible effects of a child's food intake during the preceding day might still exist, we consider further standardization as inappropriate. Of course, a child may have fever, diarrhea or any other health problem at the appetite test day or during the preceding day, but it is difficult to exclude such events. However, sometimes it is possible to reduce the size of the problem. For example in our study area, because infections by intestinal parasites were expected to be high, we attempted to reduce the possible disturbing effects of these infections on the child's morbidity status and appetite by means of deworming.

6.2. Effects of micronutrient supplementation on hemoglobin level

Most of the children were anemic at recruitment, with hemoglobin levels below 100 g/L. In these children, iron supplementation resulted in a positive hemoglobin response. This means that these children were suffering from iron deficiency anemia and that they were iron deficient (Hallberg *et al*, 2000). For that reason, it was not really necessary to include in our studies more indicators of iron status. The multivitamin-multimineral supplementation alone did not result in an improvement of their hemoglobin level. A possible explanation is that the iron content of the supplement (8 mg) or the duration of the multivitamin-multimineral supplementation was not appropriate to improve the iron status of the children. These results demonstrated that, although a multiple micronutrient deficiency was suspected in the children, iron supplementation alone was enough to improve their hemoglobin concentration. Therefore, the suggestion made in previous studies that hemoglobin synthesis might be limited by multiple micronutrient deficiencies (Black *et al*, 1994; Rosado *et al*, 1997; Allen *et al*, 2000) is not supported by our findings.

6.3. Micronutrient supplementation and linear growth

In the first intervention study, iron supplementation was given daily during 3 months. There have been studies on iron supplementation with a comparable duration that have resulted in improved linear growth (Chwang *et al*, 1988; Lawless *et al*, 1994; Angeles *et al*, 1993). This suggests that the lack of improvement of linear growth in our study cannot be simply ascribed to the relatively short duration of supplementation. Based on power calculations (power 80% and significant level 5%), at least 35 subjects were required per study group to test the hypothesis that iron supplementation improves linear growth rate of preschool children by 20%. Therefore, a lack of significant effect on linear growth means that we can safely exclude the possibility that improvement of growth rate by 20% or more had occurred.

A child of 46 months of age with a height similar to the median of the NCHS reference (WHO, 1983) is expected to grow on average 1.9 cm over a 3-month period. In our study, the average increase of height was 1.9 cm (100% of the median growth rate) in the children in the iron groups whereas it was 2.3 cm and 2.1 cm (121% and 110% of the median growth rate) in the groups who did not receive iron. However, linear growth performance was not significantly different between the study groups. Contrary to our findings, a significant difference in growth rate was observed in Indonesian children studied by Angeles *et al* (1993). Indeed, over the 2-month intervention period, the linear growth rate of the supplemented children was 86% higher than that of their counterparts in the control group. Moreover, the mean height-for-age Z score changed from -2.33 ± 0.83 to -1.96 ± 0.73 (difference of 0.37 ± 0.41) in the iron group and from -2.18 ± 0.96 to -2.10 ± 0.92 (difference of 0.07 ± 0.23) in the control group, reflecting a marked improvement of linear growth. It is conceivable that the children involved in our study are in some aspects different from the children involved in the studies in which a positive linear growth response to iron treatment was reported. Possible differences might be the prevalence of iron deficiency anemia, level of protein-energy deficiency and presence and severity of other micronutrient deficiencies. For example, contrary to the children in our study, the Indonesian preschool children were also wasted.

It has also been suggested that linear growth faltering may be caused by deficiency of several micronutrients (Golden and Golden, 2000; Golden, 1995; Allen, 1994; Rosado *et al*, 1999). If that was the case in the children in our study, a multi-micronutrient supplementation might be needed to achieve an improvement of linear growth. Therefore, we have hypothesised that a multivitamin-multimineral supplementation improves the short-term linear growth of stunted 2-year old children. This hypothesis has been tested in two 6-week multivitamin-multimineral supplementation studies. Our findings suggest that linear growth had not improved over the 6-week supplementation period. In fact none of differences observed between groups reached statistical significance. Even by measuring the children's knee-heel length using knemometry, a method for measuring short-term growth velocity with high accuracy (Michaelsen *et al*, 1991; Michaelsen, 1994), we did not find any improvement of linear growth. However, we cannot exclude the possibility that the duration of the multivitamin-multimineral supplementation had been too short to stimulate linear growth in these children. Moreover, most of the children were energy-protein deficient and this may have contributed to the lack of effects on linear growth.

6.4. Micronutrient supplementation and appetite

It has been suggested that a decreased appetite caused by micronutrient deficiency may be recovered if the limiting micronutrient would be provided (Golden and Golden, 2000; Golden, 1995). Therefore, we hypothesised that a 6-week multivitamin-multimineral supplementation would improve the appetite of stunted 2-year old children. Over the intervention period, no improvement of appetite has been observed. In fact, none of the differences in the test food intake observed between groups reached statistical significance. The study has sufficient power (80% at 5% significant level) to detect an increase of the test food intake by approximately 25%. Therefore, we may safely assume that, in our children, appetite improvement by 25% or more had not occurred over the 6-week supplementation periods.

Because the supplements given to the children contained several micronutrients which may be lacking in the diet of stunted children, the lack of improvement of appetite could be related to the fact that the duration of the supplementation was not sufficiently long to permit a meaningful improvement of the micronutrient status of the children. An alternative explanation is that micronutrient supplementation alone may be insufficient to increase appetite. It might be that the energy and protein levels of the diets of these children are inappropriate for the multivitamin-multimineral supplementation to be effective. Energy, protein and amino acids requirements for children in healthy and diseased states have been discussed extensively (Waterlow, 1992; WHO, 1985; Garlick and Reeds, 2000). Based on the present knowledge in nutrition, there is sufficient evidence that proper body functions and optimal health require a balanced energy, protein and micronutrient supply. Therefore it is conceivable that for our children appropriate supplements of energy and good quality protein may also be required.

6.5. Suggestions for future studies

We cannot exclude the possibility that the duration of the multivitamin-multimineral supplementation has been too short to stimulate appetite and growth performance of stunted children. In addition, the energy and protein intakes of the children might have been insufficient to cause the multivitamin-multimineral supplementation to be effective. Therefore, a future study might focus on the effect of a combined energy-protein-micronutrient supplementation on appetite and growth performance of young stunted Beninese children. The duration of that study should be sufficiently long to correct the energy-protein-micronutrients deficiency.

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Summary

Linear growth retardation (stunting) still has a high prevalence rate in developing countries (ACC/SCN, 2000). In the republic of Benin reported prevalence rates range from 25% to 40%. In malnourished children it is quite common to observe a combination of multiple micronutrient deficiency, loss of appetite and growth retardation. However, possible interactions between these parameters are not yet adequately mapped.

The research presented in this thesis was performed to examine whether micronutrient supplementation may improve appetite and growth in young stunted children. We have performed three micronutrient supplementation studies on stunted children in southern Benin and we have measured effects on appetite and growth performance. In order to be able to assess appetite, an appropriate measuring procedure for appetite had to be developed.

For measuring appetite in humans there is no standard, but the habitual daily energy intake is usually considered to be the most appropriate proxy for appetite. However, the common methods to assess habitual daily energy intake are quite invasive and time consuming, and therefore perhaps not appropriate for a quick assessment of young children's appetite. To develop an appropriate appetite measurement tool which can be applied in young Beninese children, we have carried out four trials on a total of 109 children, 18-30 months of age (Chapter 3). *Ad libitum* intakes of test foods (a maize porridge or rice) served after an overnight fast according to standardized offering procedures were measured on three days. The reproducibility (as coefficient of variation) of the appetite test as calculated from the triplicate measurements was 40% for the maize porridge ('aklui') and 25% for rice ('riz-au-gras'). Habitual breakfast and daily food intake were measured during three consecutive days. Energy intake from the maize porridge was positively and significantly associated with the daily energy intake ($n=38$, $r=0.41$, $p < 0.05$) and with the energy intake from breakfast ($r=0.52$, $p < 0.01$). Correlations for the rice test food were less pronounced and non-significant. We conclude that the *ad libitum* energy intake from a culturally appropriate and well-accepted food as maize porridge can be considered a valid estimate of appetite, provided a standardized procedure is used. If the appetite test is applied in intervention studies it may be necessary to perform the test in duplicate or triplicate (like we did) to perform statistical tests with sufficient power.

The first intervention study was carried out to assess the impact of iron supplementation alone or in combination with a deworming treatment on the growth performance of 3-5 year old children (Chapter 2). A total of 177 children were selected from low-income households in a rural area in southern Benin and were allocated to one of four treatments: iron (60 mg elemental iron/day) + albendazole (200 mg/day for 3 consecutive days, at start of study and after one month), iron + albendazole-placebo, albendazole + iron-placebo or placebos. The group of children can be considered as stunted (58% had height-for-age Z-score < -2) and anemic (76% had Hb < 110 g/L), but not as wasted (2% had weight-for-height Z-score < -2). After three months of intervention the blood hemoglobin level in the iron-treated children was significantly increased by 5 g/L ($p=0.032$) when compared with the levels of the placebo children. There

were no significant effects of iron and deworming treatment on growth performance. As expected, *Ascaris lumbricoides* and hookworm infections decreased significantly in albendazole-treated subjects ($p < 0.05$). A food consumption survey, performed after the supplementation period was finished, revealed that the overall diet of the children should be considered as marginal. Therefore, we hypothesised that the absence of positive intervention effects on growth performance might be due to a multiple micronutrient deficiency.

Therefore, we have studied the effect of a multivitamin-multimineral supplementation on appetite and growth (Chapter 4). A placebo-controlled study was performed in which 101 stunted children (height-for-age Z-score < -2), aged 17-32 months, received either a multivitamin-multimineral supplement (VITALIA-tablets, containing 11 vitamins and 8 minerals) or a placebo. The tablets were given daily to the children for 6 weeks. Knee-heel length, length, weight, arm circumference and appetite were assessed three times before and three times after supplementation. Growth was also assessed 4 months after the intervention. Appetite was assessed using the appetite test procedure as described in Chapter 3 and knee-heel length was assessed using knemometry. Each test day, morbidity data and the mother's report on the child's appetite throughout the preceding day were recorded. We hypothesised that a 6-week supplementation would improve appetite of the children by 25%. However, no significant effects on appetite and growth could be detected. We hypothesised that this lack of effect in the present study might be associated with the high burden of helminth infections and the high level of iron deficiency. Perhaps, the iron content of the multivitamin-multimineral supplement had not been sufficient to compensate the iron deficiency or beneficial effects can only be obtained when the worm load is under control.

Therefore, the aim of the third intervention study was to assess whether a combination of a multivitamin-multimineral supplement and an iron supplement would improve appetite and growth of 18-30 months old stunted and anemic Beninese children, provided that their wormload would be under control (Chapter 5). Hundred and fifty stunted (height-for-age Z-score < -2) and anemic children (Hb < 110 g/L) were selected and randomly assigned to one of four treatments: multivitamin-multimineral + iron, multivitamin-multimineral + placebo, placebo + placebo, and placebo + iron. Supplements were provided daily for 6 weeks and intake of tablets was supervised. The multivitamin-multimineral supplements were VITALIA-tablets (11 vitamins + 10 minerals) and iron was given as ferrous fumarate tablets (66 mg iron/tablet). A week before supplementation and during the last week of supplementation, all children received a deworming treatment. Each child was treated with 600 mg of mebendazole (200 mg per day during 3 consecutive days). Appetite, knee-heel length, dietary intake and morbidity status were assessed before and after supplementation. Length, weight, arm circumference and hemoglobin were assessed before, just after supplementation and 4 months after the intervention. Appetite was assessed by the validated appetite test using 'riz-au-gras' as test food. Dietary intakes were assessed during 3 consecutive days by means of the observed weighed record

method. As expected, the iron-supplemented groups did show a significant improvement of blood hemoglobin level, but supplementation with iron or the multivitamin-multimineral supplement did not improve appetite or growth performance, despite the deworming treatment. The lack of improvement is difficult to explain. One of the explanations might be that micronutrients are only effective if the diet contains enough protein and energy. The dietary data indeed suggest that the overall quality of the children's diet was not appropriate and that most of the children suffer from a chronic protein-energy malnutrition. An other explanation might be that the duration of supplementation (6 weeks) had been too short to observe a meaningful improvement of appetite and growth. We suggest that future studies on appetite and growth performance should use a combined energy-protein-micronutrient supplement and that the supplementation duration should be sufficiently long for correcting the energy-protein-micronutrient deficiency.

Résumé

Le retard de la croissance linéaire est toujours fortement prédominant dans les pays en voie de développement (ACC/SCN, 2000). En république du Bénin, entre 25% et 40% des enfants d'âge préscolaire sont concernés par ce phénomène. Chez les enfants sous-alimentés, il est assez courant d'observer une coexistence d'un déficit en plusieurs micronutriments, de la perte d'appétit et du retard de croissance physique. Cependant, une possible interaction entre ces paramètres n'est pas encore établie de manière appropriée. Les travaux de recherche présentés dans cette thèse examinent si la supplémentation en micronutrients pourrait améliorer l'appétit et la croissance physique chez des jeunes enfants ayant un retard de croissance linéaire. Nous avons réalisé au sud du Bénin trois études de supplémentation en micronutriments sur des jeunes enfants ayant un retard de croissance linéaire et nous avons mesuré les effets sur l'appétit et la croissance physique. Afin d'estimer l'appétit une procédure appropriée de mesure doit être développée.

Pour mesurer l'appétit chez les êtres humains, il n'y a aucune méthode standard mais l'apport énergétique journalier est souvent considéré comme l'estimation la mieux appropriée de l'appétit. Cependant, les méthodes courantes employées pour estimer l'apport énergétique journalier sont assez encombrantes et demandent trop de temps et donc ne sont pas appropriées pour une estimation rapide de l'appétit chez de jeunes enfants. Pour mettre au point une procédure appropriée de mesure de l'appétit de jeunes enfants nous avons effectué quatre études sur un total de 109 enfants Béninois âgés de 18 à 30 mois (chapitre 3). La consommation *ad libitum* d'aliments test (une bouillie de maïs ou bien du riz) servis le matin à jeun suivant des procédures standards a été mesurée sur trois jours. La reproducibilité (exprimée en terme de coefficient de variation) du test d'appétit telle que calculée à partir des trois mesures effectuées était de 40% pour la bouillie de maïs ('aklui') et de 25% pour le riz ('riz-au-gras'). La consommation alimentaire habituelle au petit déjeuner et la consommation alimentaire totale journalière étaient mesurés pendant trois jours consécutifs. L'apport énergétique de la bouillie de maïs était en corrélation positive et significative avec l'apport énergétique journalière ($n=38$, $r=0.41$, $p < 0.05$) et avec la consommation énergétique au petit déjeuner ($r=0.52$, $p < 0.01$). Les corrélations avec la consommation du riz étaient moins prononcées et pas significatives. Nous concluons que la consommation énergétique *ad libitum* d'un aliment culturellement approprié et bien accepté par les enfants tel que la bouillie de maïs peut être considérée comme étant une valide estimation de leur appétit, pourvu qu'une procédure standard soit utilisée. Si le test d'appétit est utilisé dans des études d'intervention il pourrait être indispensable de le pratiquer en deux ou en trois répétitions (comme cela a été le cas dans nos études) afin de faire des tests statistiques appropriés.

La première étude d'intervention a été réalisée en vue d'estimer l'impact de la supplémentation en fer seule ou en combinaison avec le déparasitage sur la croissance des enfants de 3 à 5 ans (Chapitre 2). Au total 177 enfants étaient sélectionnés dans des ménages à faible revenu dans une région rurale au sud du Bénin et assignés de manière aléatoire à l'un des

quatre traitements suivants: fer (60 mg de fer élément par jour) + albendazole (200 mg par jour sur 3 jours consécutifs, au début de l'étude et après un mois), fer + albendazole-placebo, fer-placebo + albendazole ou placebos. Les enfants peuvent être considérés comme ayant un retard de croissance linéaire (58 % d'entre eux avaient un indice taille pour âge inférieur à –2) et anémiés (76 % avaient un taux d'hémoglobine inférieur a 110 g/L), mais pas émaciés (2 % avaient un indice poids pour taille inférieur à –2). Après 3 mois d'intervention, le taux d'hémoglobine des enfants ayant reçu des suppléments de fer avait augmenté de manière significative de 5 g/L ($p=0.032$) comparativement aux taux observés chez les enfants non supplémentés. Le traitement par le fer et le déparasitage n'avaient pas eu d'impact significatif sur la croissance physique des enfants. Comme espéré, le taux des infections causées par *Ascaris lumbricoides* et par les ankylostomes a diminué de manière significative chez les enfants déparasités ($p < 0.05$). Une enquête de consommation alimentaire effectuée après la période de supplémentation a révélé que de manière générale, le régime alimentaire des enfants pourrait être considéré comme étant marginal. Par conséquent nous avons émis l'hypothèse que l'absence d'impact positif de l'intervention sur la croissance des enfants pourrait être due à la présence d'une carence en plusieurs micronutriments.

Par conséquent, nous avons étudié l'effet de la supplémentation de multivitamine-multiminéral sur l'appétit et la croissance physique des enfants (Chapitre 4). Une étude contrôlée a été réalisée dans laquelle 101 enfants âgés de 17 à 32 mois et ayant un retard de croissance linéaire (indice taille pour âge inférieur à –2) ont reçu soit un supplément de multivitamine-multiminéral (un comprimé de VITALIA composé de 11 vitamines et de 8 minéraux) ou un comprimé de placebo. La supplémentation a été faite quotidiennement pendant 6 semaines. La longueur genou - talon, la taille, le poids, la circonférence de bras et l'appétit ont été mesurés trois fois avant et 3 fois après la période de supplémentation. Les mesures anthropométriques ont été répétées 4 mois après l'intervention. L'appétit a été mesuré en utilisant le test d'appétit décrit au chapitre 3 et la longueur genou – talon a été mesurée en utilisant un knemomètre. Chaque jour de mesure de l'appétit, des données sur la morbidité chez les enfants et l'opinion de chaque mère sur l'appétit de son enfant au cours de la journée précédant celle du test d'appétit ont été recueillies par interview. Nous avons formulé l'hypothèse qu'une combinaison de vitamines et de minéraux administrée quotidiennement aux enfants pendant 6 semaines augmentera leur appétit de 25%. Cependant, aucun effet significatif sur l'appétit ou sur la croissance physique n'a été détecté. Nous avons donc suggéré que le manque d'effet dans la présente étude pourrait être lié à une charge élevée en parasites intestinaux et à un fort taux de carence en fer. Il se pourrait que la teneur en fer des suppléments de multivitamine-multiminéral soit insuffisante pour combattre la carence en fer et peut-être des effets bénéfiques ne peuvent être obtenus que si la charge parasitaire est réduite.

Par conséquent, le but de la troisième étude d'intervention était d'examiner si la combinaison d'un supplément de multivitamine-multiminéral et d'un supplément de fer

pourrait améliorer l'appétit et la croissance physique d'enfants Béninois de 18 à 30 mois ayant un retard de croissance linéaire et anémiés, si leur charge parasitaire est contrôlée (Chapitre 5). Au total 150 enfants ayant un retard de croissance linéaire (indice taille pour âge inférieur à – 2) et anémiés (taux d'hémoglobine inférieur à 110 g/L) ont été sélectionnés et assignés au hasard à l'un des quatre traitements suivants : multivitamine-multiminéral + fer, multivitamine-multiminéral + placebo, placebo + placebo et placebo + fer. Les suppléments ont été distribués quotidiennement pendant 6 semaines. Au domicile de chaque enfant, un enquêteur donne les comprimés et s'assure qu'ils sont avalés par l'enfant. Les suppléments de multivitamine-multiminéral sont des comprimés de VITALIA (11 vitamines et 10 minéraux par comprimé). Les suppléments de fer sont sous forme de fumarate ferreux (66 mg de fer élément par comprimé). Tous les enfants ont été déparasités une semaine avant le début de l'étude, et au cours de la dernière semaine de la période de supplémentation. Le déparasitage avait été effectué en administrant par enfant 600 mg de mébendazole (à raison de 200 mg par enfant par jour durant 3 jours consécutifs). L'appétit, la longueur genou – talon, la consommation alimentaire et la morbidité ont été évalués au début et à la fin de l'intervention. La taille, le poids, la circonférence de bras et le taux d'hémoglobine ont été mesurés avant, aussitôt après et 4 mois après l'intervention. L'appétit a été mesuré en utilisant le test d'appétit déjà validé et avec le 'riz-au-gras' comme aliment test. La consommation alimentaire a été mesurée pendant 3 jours consécutifs par la méthode d'observation et de pesée de tous les aliments consommés par l'enfant au cours d'une journée. Comme espéré, le taux d'hémoglobine a augmenté dans les groupes d'enfants ayant reçu des suppléments de fer, mais la supplémentation en fer ou en multivitamine-multiminéral n'a amélioré ni l'appétit , ni la croissance physique des enfants, malgré le déparasitage. Le manque d'amélioration est difficile à expliquer. Une des explications pourrait être que les suppléments de micronutriment ne peuvent être efficaces que si l'alimentation des enfants contient des quantités suffisantes de protéine et d'énergie. L'enquête de consommation alimentaire a en effet révélé que le régime alimentaire des enfants n'est pas approprié et que la majorité des enfants souffrirait d'une malnutrition protéino-énergétique chronique. Une autre explication pourrait être que la durée de la supplémentation (6 semaines) a été relativement courte pour générer une amélioration significative de l'appétit et de la croissance physique. Nous suggérons que pour les études futures sur l'appétit et de la croissance physique des jeunes enfants, une combinaison energie-protéine-micronutriment soit utilisée en tant que supplément et que la durée de la supplémentation soit suffisamment longue pour permettre de corriger le déficit en énergie, protéine et micronutriments.

Samenvatting

Het achterblijven van de lengtegroei bij jonge kinderen, in het Engels 'stunting' genoemd, komt in ontwikkelingslanden nog steeds veel voor. In de republiek Benin, West-Afrika, worden prevalentiecijfers gevonden van 25% tot 40%. In ondervoede kinderen wordt het achterblijven van de groei vaak aangetroffen in combinatie met tekorten aan micronutriënten en met verlies aan eetlust. Over mogelijke interacties tussen deze factoren is echter nog onvoldoende bekend.

In het in dit proefschrift beschreven onderzoek is onderzocht of bij jonge Beninese kinderen met een groeiachterstand door suppletie met micronutriënten de eetlust en de groei verbeterd kunnen worden. In totaal zijn drie suppletiestudies met micronutriënten uitgevoerd. Hierbij zijn effecten op eetlust en groei bestudeerd. Teneinde eetlust als onderzoeksgrootheid te kunnen gebruiken moest eerst een onderzoeksmethode voor eetlustmeting bij deze kinderen ontwikkeld worden.

Er is geen 'gouden' standaardmethode beschikbaar om bij de mens eetlust te meten. De gemiddelde dagelijkse voedsel- of energie-inneming wordt gewoonlijk als de beste benadering beschouwd. De gangbare methoden om de dagelijkse inneming vast te stellen zijn in het algemeen voor de proefpersonen vrij ingrijpend en tijdrovend, en daarom wat minder geschikt voor eetlustmeting bij jonge kinderen. Daarom is middels een viertal experimenten bij in totaal 109 jonge Beninese kinderen van 18 tot 24 maanden oud, een eetlusttest ontwikkeld (Chapter 3). De kinderen kregen 's morgens vroeg in nuchtere toestand een testvoedsel (een maispap of een rijstgerecht) voorgeschoteld waarvan zij naar believen (ad libitum) konden eten. De wijze van aanbieding en de meetomstandigheden waren strikt gestandaardiseerd. Uit de metingen die op drie dagen plaatsvonden werd de reproduceerbaarheid (uitgedrukt als variatiecoëfficiënt) van de eetlusttest berekend en deze bedroeg 40% voor de maispap (aklui) en 25% voor het rijstgerecht (riz-au-gras). Het ontbijt en de 24uurs-voedselinneming werd over drie opeenvolgende dagen gemeten. De energie-inneming via de maispap was positief geassocieerd met de 24uurs-energie-inneming ($n=38$, $r=0.41$, $p < 0.05$) en met de energie-inneming uit het ontbijt ($r=0.52$, $p < 0.01$). De correlaties met het rijstgerecht waren minder duidelijk en statistisch niet-significant. We concluderen dat de ad libitum inneming van een cultureel geaccepteerd en geschikt testvoedsel als de maispap een valide schatting kan geven van de eetlust, mits de aanbiedingsprocedure en de meetomstandigheden goed gestandaardiseerd zijn. Verder bevelen we aan om in interventiestudies de eetlusttest in twee- of drievoud uit te voeren om statistische toetsen mogelijk te maken met voldoend onderscheidingsvermogen en zodanig dat het aantal kinderen in de studies toch werkbaar blijft.

De eerste interventiestudie werd uitgevoerd om het effect van ijzersuppletie al dan niet in combinatie met een ontwormingsbehandeling op de groei van 3 tot 5 jaar oude kinderen te bestuderen (Chapter 2). In totaal werden 177 kinderen geselecteerd uit laag-inkomen gezinnen wonend in een ruraal gebied in het zuiden van Benin. De kinderen kregen één van de volgende vier behandelingen toegewezen: ijzer (60 mg ijzer per dag) + albendazole (200 mg per dag gedurende drie opeenvolgende dagen, bij aanvang van studie en bij één maand na aanvang); ijzer + placebo voor albendazole; albendazole + placebo voor ijzer; placebo's voor zowel albendazole als ijzer. De kinderen hadden groeiachterstand (58% had een lengte-voor-leeftijd Z-score van <-2) en waren anaemisch (76% had een Hb < 110g/L). De kinderen waren echter niet mager ('wasted') (slechts 2% had een gewicht-voor-lengte Z-score van < -2). Na drie maanden van interventie was het hemoglobinegehalte in de met ijzer gesuppleerde groepen significant toegenomen met 5 g/L (p=0.032). Zoals verwacht namen de *Ascaris lumbricoides* en de mijnworminfecties significant af in de kinderen die albendazole-behandeling gekregen hadden (p < 0.05). Beide behandelingen hadden echter geen effect op de groei. Voedselconsumptieonderzoek bij deze kinderen leerde dat de voeding als marginaal bestempeld moet worden. We veronderstelden daarom dat de afwezigheid van een positief effect op de groei wellicht kwam door een gelijktijdig tekort aan meerdere micronutriënten.

Vandaar dat vervolgens het effect van een multivitamine-multimineraal supplement bestudeerd werd op eetlust en groei (Chapter 4) bij 101 kinderen van 17 tot 32 maanden oud en met een groeiachterstand (lengte-voor-leeftijd Z-score van < -2). De kinderen kregen òf een *multivitamine-multimineraal supplement (*VITALIA *tabletten met* 11 *vitamines en 8 mineralen)* òf een placebo. De tabletten werden dagelijks gedurende 6 weken verstrekt. De lengte, de knie-hiel lengte, het gewicht, de bovenarmomtrek en de eetlust werden driemaal vóór en driemaal nà suppletie gemeten. Op iedere meetdag werden bij de moeder ook morbiditeitsgegevens van het kind over de voorgaande dag alsmede een subjectieve inschatting van de eetlust van het kind nagevraagd. Een verbetering in eetlust van 25% zou in deze studie vrijwel zeker opgemerkt zijn. Echter, we vonden geen significante effecten op eetlust of groei. We veronderstelden dat de afwezigheid van positieve effecten wellicht kwam door de hoge wormbelasting en het grote ijzertekort. Wellicht dat het ijzergehalte in de gebruikte tabletten niet toereikend geweest is.

Het doel van de derde studie was daarom vast te stellen of een combinatie van een multivitamine-multimineraal supplement en een ijzersupplement de eetlust en groei van 18 tot 30 maanden oude anaemische Beninese kinderen met groeiachterstand zou verbeteren, indien de wormbelasting teruggebracht wordt (Chapter 5). In totaal werden150 anaemische kinderen (Hb < 110 g/L) met groeiachterstand (lengte-voor-leeftijd Z-score < -2) ad random aan één van vier behandelingen toegewezen: multivitamine-multimineraal + ijzer; multivitamine-multimineraal + placebo voor ijzer; placebo + ijzer; placebo's voor zowel multivitamine-multimineral als ijzer. De supplementen werden dagelijks gedurende 6 weken verstrekt. De multivitamine-multimineraal tabletten waren weer VITALIA tabletten (nu met 11 vitamines en 10 mineralen) en het ijzer werd verstrekt als ijzerfumaraat tabletten (66 mg ijzer per tablet). Een week voor de suppletieperiode alsook gedurende de laatste week van de suppletie kregen alle kinderen een ontwormingsbehandeling. Ieder kind werd behandeld met 600 mg mebendazole (200 mg per dag gedurende drie opeenvolgende dagen). Eetlust, knie-hiel lengte, voedselinneming en morbiditeitsstatus werden vastgesteld vóór en nà suppletie. Lengte, gewicht, bovenarmomtrek en hemoglobinegehalte werden niet alleen vóór en direct na afloop van suppletie gemeten, maar ook 4 maanden na afloop van de interventie. Bij de eetlusttest werd nu riz-au-gras als testvoedsel gebruikt. Zoals verwacht lieten de met ijzer gesuppleerde groepen weer een verbetering zien van het bloedhemoglobinegehalte, maar zowel ijzer als het multivitamine-multimineraal-tablet gaven geen verbetering van eetlust of van groei, ondanks de ontwormingsbehandeling. De uitkomsten van de studie zijn niet eenvoudig te verklaren. Misschien dat de gesuppleerde micronutriënten alleen effectief kunnen zijn als de dagelijkse voeding genoeg eiwit en energie bevat. De voedselconsumptiegegevens lieten inderdaad zien dat de meeste kinderen een chronische eiwit-energie ondervoeding kennen. Een andere mogelijke verklaring is dat de duur van de suppletie niet lang genoeg geweest is om effect te kunnen hebben.

Aanbevolen wordt om in toekomstige studies naar eetlust en groei gebruik te maken van een energie-eiwit-micronutirent supplement en het supplement gedurende een langere tijd te laten gebruiken.

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Romain

About the author

Romain Anselme Marc Dossa was born on 27 April 1964 in Porto-Novo, in the Republic of Benin. He attended primary and secondary school in Porto-Novo at "Ecole Primaire Publique de Davié" and "Collège d'Enseignement Moyen Général de Davié" respectively. He graduated from the secondary school in 1982 and joined the army for one year from 1982 to 1983. He joined the Faculty of Agricultural Sciences of the National University of Benin and graduated in 1988 as *Ingénieur Agronome: Option Nutrition et Sciences Alimentaires*. From 1989 to 1991, he worked as a research assistant in a local Non-Governmental Organisation involved in mother and child nutrition, in rural areas of the republic of Benin. In 1992, he joined the research team of the Department of Nutrition and Food Science of the Faculty of Agricultural Sciences of the National University of Benin (DNSA/FSA/UNB) and served as a junior staff member.

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In April 1996 he started the PhD-project presented in this thesis. The data have been collected in Benin from August 1996 to August 1999. The preparatory and final phases have been undertaken at the Division of Human Nutrition and Epidemiology of Wageningen University.

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