IMPACT OF VITAMIN A NUTRIFIED SOYBEAN OIL ON THE VITAMIN A STATUS OF A SELECTED COMMUNITY IN SOUTHERN BRAZIL

by

URSULA G. FRADERA

B.Sc. (Human Nutrition), University of Stuttgart-Hohenheim, W-Germany, 1986

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE STUDIES

DIVISION OF HUMAN NUTRITION
SCHOOL OF FAMILY AND NUTRITIONAL SCIENCES

We accept this thesis as conforming

to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

September 1990

In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the head of my department or by his or her representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of Nutritional Sciences

The University of British Columbia
Vancouver, Canada

Date September 25, 1990

ABSTRACT

Previous studies have revealed that marginal vitamin A deficiency poses a common public health problem among poor population groups in Southern Brazil. Nutrification programs in Guatemala (sugar) and in the Philippines (MSG) have resulted in improved vitamin A status, accompanied by positive changes in the anthropometric and general health status. Accordingly, the main purpose of the present pilot study was to evaluate the potential impact of vitamin A nutrified soybean oil on the vitamin A status of a selected community in Southern Brazil. Additional objectives were to assess the vitamin A and general nutritional status of the participants.

The study was conducted in Vila Piratininga, a shanty town located on the periphery of Ribeirao Preto, a typical agricultural town in the sugarcane and coffee region of the state of Sao Paulo. Fifty-seven families were randomly assigned to experimental and control groups and were provided for four months with soybean oil that was either fortified with vitamin A (92,000 IU of retinyl palmitate per one litre can) or unfortified.

To evaluate the impact of nutrification, biochemical and dietary indicators of vitamin A status were assessed before and after the supplementation. Dietary intake data were obtained from the female head of each household using the 24 hour recall method. Additional information on the usual consumption pattern of vitamin A containing foods was collected at the start of the study, using a food frequency questionnaire. Plasma retinol and β-carotene levels of all subjects were analysed. Anthropometric measurements served as additional indicator of general nutritional status. Furthermore, throughout the intervention mothers were questioned about the incidence of diarrhea and respiratory diseases among their children.

Results from the serum vitamin A analysis at baseline confirmed that vitamin A deficiency was a public health problem among this low income population. Forty percent (control group) and

Abstract

59% (experimental group) of preschool children, the most vulnerable age group, had low or deficient serum retinol concentrations. Plasma β-carotene and dietary data showed that the intake of vitamin A and carotenoids from the rice and bean based diet was very low. The consumption of nutrified soybean oil increased the estimated daily vitamin A intake of the experimental group by 500% ($p<0.001$). However, this improved intake was not reflected in plasma retinol levels. Plasma vitamin A values of supplemented individuals dropped significantly ($p<0.001$) during the intervention, indicating that vitamin A nutrified soybean oil was not effective in improving their vitamin A status.

The lack of response to supplementation in this study does not necessarily suggest that soybean oil is not a feasible vehicle for vitamin A nutrification. The fortified oil was well accepted by the participants and used daily for the preparation of meals; eventual losses of vitamin A during cooking and/or storage can be regarded as minimal. Therefore, this negative impact is more likely due to other factors. In populations with marginal vitamin A deficiency, as in Vila Piratininga, the limitations of serum retinol levels as an accurate indicator of vitamin A body reserves should be appreciated. In addition, morbidity data revealed that infections were common among participating children. Infections may have impaired vitamin A absorption and utilization, increased requirements and consequently interfered with a successful nutrification.

This was the first study to use vegetable oil as the carrier for vitamin A. Although this trial did not result in the expected impact on vitamin A status of the present study population, the feasibility of this vehicle should be further studied. In future investigations, the relative dose response test (RDR), which is a more sensitive index of vitamin A body reserves, should be performed on a subsample of the whole study population. Furthermore, concurrent reduction in the magnitude and severity of precipitating or contributary risk factors such as parasitic infestation, diarrheal and respiratory infections could improve the outcome of a nutrification intervention.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEDGEMENTS

I sincerely thank my research supervisor **Dr. Indrajit D. Desai** for initiating this project and assisting me throughout it.

I am extremely grateful to **Dr. Susan Barr** for her endless support and helpful comments and criticisms in thesis draft revision and her invaluable encouragement throughout the completion of this thesis.

I also wish to express my appreciation to my other research advisors, **Dr. Melvin Lee** and **Dr. Nancy Waxler-Morrison**, for their valuable suggestions offered during the preparation of this thesis.

I gratefully thank **Dr. Joseph Leichter** and **Dr. David Kitts** for serving on my committee.

Muito obrigada to the many Brazilian people without whom the project would not have been possible:

Jacob Ferreira for his help and excellent collaboration during the field work in Vila Piratininga.

Dr. J.E. Dutra de Oliveira, Head of the Nutrition Division, University of Sao Paulo Medical School, Ribeirao Preto, for his constant interest and guidance in the project and allowing us to use the various facilities and equipment during the study.

Rosa M. D. Fávaro for her endless patience and invaluable help with various organisational problems.

Maria Rosaria Unamuno for taking the time to help with the blood collection.

The missionary couple directing the Casa de Sao Francisco for initiating the contact to the participating families.

Special thanks to the participants for their patience and excellent cooperation.

I thank **Virginia Green**, UBC Faculty of Arts Computing Centre, for her endless support in the data analysis.

I give my thanks to the University of British Columbia for providing a Graduate Fellowship which was essential for living and travelling expenses to Brazil.

I gratefully acknowledge the technical and financial support for this research project received from: Hoffmann LaRoche, Basle, Switzerland, SANBRA, Brazil, Conselho Nacional de Desinvolvimento Científico e Tecnológico (CNPq), Brazil and National Sciences and Engineering Research Council (NSERC), Canada.

Finally, extra special thanks to my husband Angelo for his enthusiastic help during the field work and his immeasurable support doing this thesis.

I. INTRODUCTION

Vitamin A deficiency is widespread in many developing countries and it is one of the most prevalent and serious of all nutritional deficiency diseases (McLaren 1986). Some 250,000 children become blind or partially blind each year as a result of this nutritional deficiency. The prevalence is difficult to estimate because of lack of data and the high mortality associated with the deficiency. However, it is estimated that worldwide nearly three million children are blind from this cause (Mason et al. 1987).

Although ecological and nutritional factors may negatively influence the efficiency of dietary vitamin A utilization, the main underlying cause in the affected countries has been shown to be insufficient vitamin A intake (WHO 1976). The dietary deficiency starts a series of events: the liver reserves become depleted and this may be characterized by a fall in serum retinol levels; however, clinical signs of vitamin A deficiency are initially absent (WHO 1976). A marked and prolonged deficiency of vitamin A can cause xerophthalmia in various degrees with blindness as a consequence. Xerophthalmia literally means "dry eye" and describes clinical ocular manifestations of hypovitaminosis A (Sommer & West 1987).

Those most at risk are preschool children because of their lower initial hepatic reserve, their relatively high organic needs for growth and their susceptibility to protein energy malnutrition and infection (Roncada 1983).

In Southeast Asia, vitamin A deficiency is known to be the most important and common cause of childhood blindness and morbidity (WHO 1976). Sommer et al. (1983) found that Indonesian children with mild xerophthalmia experienced a mortality rate four to twelve times that of neighboring children with normal eyes and that the excess mortality increased with the severity of xerophthalmia.

I. Introduction

In other less developed areas of the world, such as Latin and South America, blindness due to vitamin A deficiency is not as common, but a subclinical or marginal deficiency has been shown to be prevalent (WHO 1976). Considering the poor socio-economic situation and problems of widespread malnutrition, this marginal vitamin A deficiency should be controlled before ocular lesions and blindness become a serious problem.

In recent years, it has become obvious that vitamin A deficiency is a common public health problem in Brazil, not only in the low income populations of the northeast but also in the expanding sugarcane regions of Southern Brazil. Urbanization in rapidly developing states such as Sao Paulo is introducing significant changes in the dietary habits of this population, including a decreased intake of foods rich in vitamin A and carotenoids. The consumption of abundantly available local foods rich in preformed vitamin A and carotene is inadequate in families of low socio-economic status (Desai et al. 1980).

One of the most effective ways of increasing vitamin A intake is to fortify suitable local foods that are inexpensive and commonly used. In developing countries, the identification of a feasible "universal" vehicle for vitamin A is a major limiting factor. Diets of low socio-economic groups, who are mainly affected by vitamin A deficiency, are simple consisting of only a few predominant staple foods (West & Sommer 1987).

In Guatemala, fortification of sugar with vitamin A was shown to improve the vitamin A status of the population consuming this food item regularly (Arroyave et al. 1979a). Although sugar could be a suitable carrier for vitamin A in Brazil, alternative local nutrification vehicles still need to be explored. For the lipid soluble vitamin A, edible vegetable oils have great potential as a carrier for nutrification. Brazil has a rapidly growing vegetable oil industry most of which is located in the state of Sao Paulo. The most predominant vegetable oil being soybean oil which is inexpensive and used daily by low income Brazilians (Desai et al. 1988b). The "classic" carriers for vitamin A, milk, butter

and margarine, are rarely consumed by these population groups because of their comparatively high price (Desai et al. 1988b) and although these food items are fortified with vitamin A in Brazil, they are only accessible to those who are least at risk for vitamin A deficiency.

In the present study, 57 families of low socio-economic status, living in a shanty town (favela) in Southern Brazil, were provided twice a week for a period of four months with soybean oil that was either vitamin A fortified or unfortified. The study was designed to assess the potential impact of vitamin A nutrified soybean oil on the vitamin A status of this selected community. The outcome of this intervention was evaluated by biochemical responses of control and experimental groups to this form of supplementation. Additional objectives of this project were to assess the vitamin A and general nutritional status of the participants.

For the purpose of this research, it was hypothesized that:

1. There would be no difference in the frequency of consumption of vitamin A from natural food sources between the experimental and control groups.

2. The vitamin A nutrified soybean oil would significantly increase the dietary vitamin A intake of the experimental group.

3. At the end of the intervention, adults and children consuming the fortified oil would have significantly higher serum retinol levels than individuals in the control group.

5. During and at the end of the intervention, children supplemented with vitamin A fortified soybean oil would have a significantly lower morbidity load than those children consuming the placebo oil.

The following assumptions were made:

1. The participants would use the supplied oil regularly.

2. All family members would eat all meals at home.

II. LITERATURE REVIEW

1. VITAMIN A DEFICIENCY IN BRAZIL

In Brazil, the situation with respect to vitamin A deficiency varies from region to region. Hypovitaminosis A and xerophthalmia are known to be a problem in the Northeastern part of Brazil. (WHO 1976). In 1963, the Interdepartmental Committee on Nutrition for National Development (ICNND 1965) conducted a survey of over 5,500 individuals in this region. It was found that 60% of the population had either low or deficient levels of serum vitamin A, which may indicate depletion of vitamin A stores. In the same area, Simmons (1976) observed a high incidence of xerophthalmia, including blindness among preschool children. In Salvador, 30% of children between three weeks and two years old, who had died of various causes, had extremely low liver vitamin A reserves, suggesting inadequate vitamin A nutriture of Brazilian children in this region (Olson 1979).

Subclinical vitamin A deficiency appears to be widespread in the urban and periurban areas in the state of Sao Paulo in Southern Brazil. In 1981, Wilson et al. (1981) examined biochemical and clinical indices of vitamin A status in preschool children. Their results showed serum retinol levels below 10 μg/dl in 39% and below 20 μg/dl in 74% of these preschoolers. According to WHO criteria (1982), vitamin A deficiency represents a public health problem among this population. Roncada and associates (1981, 1984) revealed a similar problem in several communities in Sao Paulo state.

More recent studies were conducted by Fávaro et al. (1986) to assess the vitamin A status of young children from low income families, living in poor periurban neighborhoods of Ribeirao Preto, a typical agricultural town in the sugarcane growing region of the state of Sao Paulo. Findings of these investigations revealed that the intake of vitamin A and its precursors from the rice and bean based diet was low and appeared to influence blood concentrations of vitamin A. With respect to serum retinol, 1.8% of the children had deficient levels (<10 μg/dl) whereas 48.8% had low values

(<20 μg/dl). Most subjects (about 50%) responded positively to a massive dose of 200,000 IU vitamin A, suggesting that those individuals may be at risk of having low liver stores of vitamin A.

It is obvious from these investigations that vitamin A deficiency poses a problem at the public health level in Southern Brazil. Simple and easily implemented preventative programs such as nutrification of foods with vitamin A along with appropriate nutrition education programs are essential to improve the vitamin A status of poor population groups.

2. INTERVENTION PROGRAMS

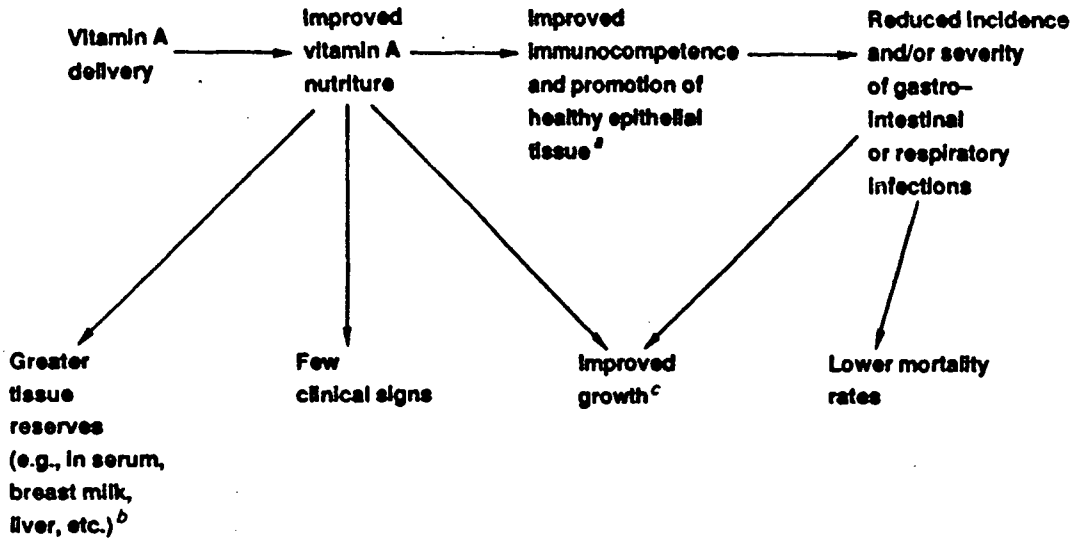
Longterm studies carried out in developing countries such as India, Indonesia and the Philippines indicate that there are three possible alternatives for treatment and prevention of vitamin A deficiency. These are periodic prophylactic doses, such as 200,000 IU every six months; nutrification of a suitable carrier food which is commonly consumed in the region (ie. MSG, sugar); and education programs to promote increased production and utilization of local foods rich in carotenoids such as leafy green and yellow vegetables and fruits. The possible outcomes of vitamin A delivery are summarized in Fig. 1.

Nutrition Education

Nutrition education is considered a more "natural" solution to the problem, since it pursues its objectives through the normal diet, without additives or medicines. However, sociological factors compound the difficulty of substantially changing dietary habits which are deeply ingrained in a person's culture (Solon 1986). In developing countries, many of the carotene-rich vegetables are considered to be food only for the poor or animals. Consequently, dietary changes require a long time and can only be regarded as a longterm goal.

Large Dose Vitamin A Distribution

First suggested in 1964 (McLaren 1964) and soon followed by early pilot field trials, large dose vitamin A distribution has gained wide acceptance as a standard intervention. It was recommended that all children at risk may be given a massive dose of vitamin A every six months. Currently, UNICEF distributes approximately 80 million vitamin A capsules (200,000 IU/capsule) annually (Sommer & West 1987). However, periodic vitamin A distribution is generally viewed as an interim approach until longterm solutions to control hypovitaminosis A can be implemented, since it has no apparent longterm effect on serum vitamin A levels.



[a] Not recommended for inclusion.
[b] Recommended, but assessment may vary by population
[c] Small effects should be expected.

FIG. 1: Possible outcome variables of improved vitamin A nutriture (NRC 1987)

Nutrification

A more permanent strategy includes nutrification or fortification of a common food with vitamin A. Nutrification is defined as the addition of one or more nutrients to one or more foods or food ingredients (Bauernfeind 1983). It can be put into practice quickly, the amount and number of nutrients can be easily changed and the practice can be easily monitored. Nutrification involves no dietary changes by the consumer, a minimal amount of educational effort to get the project under way, and is a socially acceptable method of changing the nutrient intake of a given population. It makes maximum use of local foods, providing one or more of these foods are centrally processed (Bauernfeind 1983).

In industrialized countries, nutrification of foods is very common and its technology is quite advanced. In contrast, in developing countries where the application of this technology is more important, nutrification of foods is much less common (Solon et al. 1985).

A study on the island of Cebu, Philippines (Solon et al. 1978), was conducted to compare the relative effectiveness and cost of different interventions for controlling vitamin A deficiency. The three strategies selected were: a. public health and horticulture intervention; b. vitamin A capsule distribution; and c. the nutrification of small packets of monosodium glutamate with vitamin A.

Each intervention was monitored in four different bairros for two years. Clinical, biochemical and dietary examinations were performed before and after the interventions in all bairros. The MSG fortification was the only intervention that resulted both in a significant reduction in clinical signs of xerophthalmia and a significant rise in serum retinol levels. It was concluded from this evaluation that nutrification of foodstuffs with vitamin A is the most appropriate and cost-effective approach for the control of vitamin A deficiency in developing countries (Solon et al. 1979), including Brazil.

The feasibility and effectiveness of nutrifying MSG with vitamin A was tested for 20 months in three provinces of the Philippines. Socio-economic, dietary, clinical, biochemical and

anthropometric data from a total of 1,800 children were collected. The conclusions from this three-province study were that nutrified MSG (15,000 IU/2.2 g packet of MSG) was well accepted by the consumers and was effective in improving the vitamin A status of the study population (Solon et al. 1985).

More recently, Muhilal and coworkers (1988ab) showed that fortification of commercially marketed MSG with vitamin A had a positive impact on serum retinol levels of young children and on breast milk vitamin A content of lactating women in Indonesia. These improvements in vitamin A indices were accompanied by dramatic changes in other health variables. Xerophthalmia rates declined and at the same time growth, hemoglobin levels and child survival improved.

In Guatemala, sugar containing approximately 10 μg of retinol per gram was used as the dietary vehicle for vitamin A nutrification. Its effectiveness was evaluated by a study conducted in 12 rural communities (Arroyave et al. 1979a). After a two-year supplementation period, dietary, biochemical, clinical and anthropometric data showed significant improvement in the vitamin A status of the study population. These findings indicated that introduction of vitamin A fortified sugar raised per capita intake of vitamin A and resulted in increased blood retinol levels of preschool children. Small pilot studies have confirmed these results in Northeastern Brazil where vitamin A fortified sugar (1,000 IU/100 g) was given to 52 preschool children for a period of 18 months. Blood levels of vitamin A were significantly higher in the supplemented group compared to the control group (Araujo et al. 1978).

3. SELECTION OF DIETARY VEHICLE

Vitamin A fortification of foods such as margarine and dairy products, now practiced in some 30 nations around the world, has provided a strong technological basis for extending vitamin A fortification to numerous other potential food vehicles (Bauernfeind 1983).

II. Literature Review

In determining which of the local foods would be suitable as a carrier for vitamin A, the following criteria should be met (Bauernfeind 1983):

1. The food must be consumed by essentially all of the population, including the "at risk" age groups.

2. The per capita intake of such a food should vary within a relatively narrow range from day to day and from person to person.

3. The nutrient added should be stable under proper conditions of storage and use.

4. Nutrification should not result in detectable alterations of the organoleptic qualities of the food in question (such as flavor, odor, color) to ensure its continuing acceptability by the consumer.

5. The nutrient should be physiologically available from the food.

6. There should be a reasonable assurance against excessive intake to a level of toxicity.

7. It should be economically feasible to nutrify the food on an industrial scale.

As described before, fortified MSG and sugar were successfully used to improve the vitamin A status of populations in the Philippines, Indonesia and Central America. In Brazil, MSG is not commonly consumed and therefore not suitable for vitamin A nutrification. In contrast, sugar is a staple food among low income Brazilians and could serve as a carrier for vitamin A. However, other regional or local nutrification vehicles still need to be explored. Results of preliminary studies suggested that common vegetable oil was a suitable dietary ingredient for the nutrification with vitamin A (Desai et al. 1988a) and fulfilled all the criteria described by Bauernfeind (1983).

The use of soybean oil instead of traditional lard is rapidly gaining popularity among low income families in Brazil (Desai et al. 1980). Commercially extracted and refined soybean oil is locally produced and is less expensive than manufactured vegetable fats such as margarine and shortenings, and animal fats such as lard and butter. The poorest segments of the population with

high risk for vitamin A deficiency mostly purchase this affordable dietary fat and use it daily for cooking and occasionally in salads (Desai et al. 1980, Swann 1979). Since all family members usually eat the same monotonous diet consisting of rice and beans, soybean oil is also consumed regularly by young children, the most vulnerable age group (Desai et al. 1980). Soybean oil reaches the target population of children and mothers with relatively little variation in the per capita consumption, which was estimated to be 35 g per day (Desai et al. 1980, Swann 1979).

The stability and retention of vitamin A palmitate added to soybean oil has been tested during storage and cooking under various conditions (Desai et al. 1988a, Fávaro et al., unpublished data). The results demonstrated that vitamin A palmitate in soybean oil is reasonably stable when stored at room temperature (23-25°C) and most of it was retained during normal cooking procedures. The appearance, taste and color of soybean oil were not noticeably changed with the addition of this golden-yellow oily substance (Fávaro et al., unpublished data, Hoffmann-LaRoche product description).

As shown in animal studies, vitamin A palmitate added to soybean oil possesses full physiological availability. The vitamin A liver reserves of rats increased significantly when they were fed increasing concentrations of vitamin A palmitate in soybean oil (Fávaro et al., unpublished data). Naturally occurring vitamin E in soybean oil enhances its absorption (Bauernfeind 1983). Furthermore, since soybean oil is only used for cooking and not likely to be consumed by itself, there is a reasonable assurance against excessive intakes to reach a level of toxicity (Dr. Desai, personal communication).

Extraction and refining of soybean oil is highly centralized and is controlled by large corporations or cooperatives (Desai et al. 1988b). In the final step of refining, a stable form of vitamin A ester can be easily added and packed in bulk or retail containers protected from light and

oxygen (Bauernfeind 1983). This technological process is simpler than the nutrification of water soluble substances such as MSG and sugar (Bauernfeind 1983).

Compared to sugar, soybean oil provides a nutritional benefit as a rich source of polyunsaturated fatty acids and vitamin E; Vitamin E enhances vitamin A absorption and as an important biological antioxidant, it protects vitamin A from oxidation (Desai et al. 1988b). Sugar, on the other hand, only provides "empty" calories.

4. ASSESSMENT OF VITAMIN A STATUS

Vitamin A status can be classified into four categories: deficient, marginal or low, adequate and normal (Arroyave 1982). Each category can be assessed by different criteria (Arroyave 1982, Olson 1988). Use of a combination of biochemical, dietary and clinical methods strengthens the validity of assessment of vitamin A status (NRC 1987).

Since liver is the major repository of vitamin A in the body (>90%), direct measurement of liver vitamin A concentrations is the best method to evaluate vitamin A status. The only way to obtain such information is by liver biopsy, which is not applicable in field conditions (Arroyave 1982). Other methods used to assess vitamin A status include measurement of serum or plasma vitamin A level, relative dose response test (RDR), serum or plasma β-carotene, breast milk vitamin A content, dietary intake and anthropometric parameters.

Plasma Vitamin A Level

The widely used plasma or serum retinol values reflect vitamin A status only when liver vitamin A stores are severely depleted (< 20 μg/g liver) or excessively high (> 300 μg/g liver). When liver vitamin A concentrations are between these limits, serum retinol concentrations are

homeostatically controlled; levels remain relatively constant and do not reflect total body reserves of vitamin A (Olson 1984).

A variety of extraneous factors may also affect serum retinol concentrations, which are independent of the size of vitamin A stores in the liver. Febrile or liver disease, protein and zinc deficiency, stress in the form of an infection or even in the form of emotional factors may influence the serum vitamin A levels by decreasing mobilization from hepatic stores, increasing cellular need and/or possibly increasing renal loss (Underwood et al. 1974). In the absence of all these factors, low body stores of vitamin A are detected reliably only when the serum concentration is below 10 μg/dl (Olson 1984).

Cutoff points for the various categories of plasma or serum retinol levels are relatively arbitrary. Especially, the setting of an "adequate" or "acceptable" range for serum vitamin A which is universally applicable poses many problems (Arroyave 1982). Nevertheless, according to the International Vitamin A Consultative Group (IVACG) criteria (Arroyave 1982), the serum vitamin A concentrations can be interpreted as follows:

deficient	< 10 μg/dl
low	10-20 μg/dl
adequate	20-30 μg/dl
normal	≥ 30 μg/dl

In populations with marginal vitamin A status, as in Southern Brazil, the prevalence of serum levels below 10 μg/dl and below 20 μg/dl may be very low. There is likely to be a higher prevalence of levels ranging from 20 to 30 μg/dl (NRC 1987). The interpretation of values in this range is very difficult because of the lack of data linking serum concentrations in this range with consistent clinical and/or biochemical evidence of vitamin A inadequacy, especially in children (Lewis et al. 1990).

Relative Dose Response Test (RDR)

Because of the difficulties in interpreting serum vitamin A values, Loerch and coworkers (1979) developed a more functional technique to estimate total body stores of vitamin A, the relative dose response test (RDR). The test is based on the observation that in vitamin A deficiency, retinol binding protein (RBP) accumulates in the liver. Following a test dose of vitamin A, the latter binds to this relative excess of RBP and is subsequently released from the liver. Consequently, in vitamin A depleted individuals, a rapid sustained increase in serum retinol occurs after the ingestion of a small dose of vitamin A.

For the test, a baseline blood sample is taken immediately before the administration of a small oral dose (450 μg) of vitamin A, followed by a second blood sample, five hours later. The RDR (%) is calculated as:

$$\frac{\text{Plasma Vit A at 5 h - plasma Vit A at 0 h}}{\text{Plasma retinol at 5 h}} \times 100$$

This procedure was found to be practical for assessing marginal vitamin A deficiency of Brazilian low income children (Flores et al. 1984). Among this group, a serum vitamin A level of 20 μg/dl or less was invariably associated with a positive RDR test (>20%). Moreover, 86% of children with serum retinol concentrations between 21-29 μg/dl, and 26% of children with serum retinol values 30 to 40 μg/dl were identified as being at risk of inadequate vitamin A status, as indicated by elevated RDR values. Following supplementation with vitamin A, all elevated RDR values reverted to normal. These results suggest that the RDR is a more sensitive index of marginal vitamin A status than is serum vitamin A level.

Plasma β-Carotene

Plasma or serum β-carotene concentrations only reflect recent dietary intakes of the vitamin A presursor. However, in countries where dietary carotenoids provide the main source of vitamin A and where dietary patterns are relatively constant, serum carotenoids may serve as a useful secondary index of vitamin A deficiency (Gibson 1990).

According to ICNND (1965) guidelines, the serum carotene levels can be classified as follows:

deficient	< 20 μg/dl
low	20-39 μg/dl
acceptable	40-99 μg/dl
high	≥ 100 μg/dl

It should be noted, however, that these cutoff points refer to the total carotenoid concentration in blood rather than to β-carotene specifically.

Breast Milk Vitamin A Content

The concentration of vitamin A in breast milk may also contribute to the assessment of vitamin A nutritional status. In populations with generally inadequate dietary intakes of vitamin A, lactating women secrete milk with a reduced vitamin A content. Since all the retinol present in the milk is taken up from the lactating women's retinol pool, a decreased concentration of vitamin A in the milk reflects both the mother's poor vitamin intake and her inadequate body stores (Arroyave 1982).

The beneficial effect of sugar nutrification on the vitamin A content of breast milk was observed in Guatemalan lactating women. When they consumed fortified sugar (10 μg/g) during their last trimester of pregnancy and the first four months of lactation, the vitamin A levels in breast milk as well as in the blood of both mother and the nursing infant rose (Arroyave et al. 1974). A

similar positive impact was reported from the MSG nutrification program in Indonesia (Muhilal et al. 1988a).

Apart from the influence of the nutritional state of the lactating mother on milk vitamin A values, human milk can also serve as a useful indirect indicator of the vitamin A status of breast-fed infants. In many developing countries, breast-fed children receive insignificant amounts of additional vitamin A in their complementary foods (Arroyave 1982).

The average vitamin A content of breast milk from well-nourished lactating women is around 50 μg/dl (FAO/WHO 1988). This value, together with an estimated daily milk volume of 700 ml has been used to estimate the RNI of vitamin A for infants (FAO/WHO 1988). In many situations, however, both the retinol level and the daily milk volume are much lower. Therefore, it has been proposed that a vitamin A level of 20 μg/dl of milk be taken as the cutoff point below which the vitamin A intake of both mother and breast-fed infant are likely to put them at risk for vitamin A deficiency. A prevalence of 15% or higher of values below 20 μg/dl is considered inadequate from the public health standpoint (Arroyave 1982).

Evaluation of the national vitamin A nutrification program in Guatemala showed that the prevalence of breast milk samples with less than 20 μg/dl fell from 39% to 11% after a two-year supplementation period. The response of the lactating women to the nutrification of sugar may also vary with the length of lactation. However, the results showed a beneficial effect for all lactation periods (0-11 months) (Arroyave et al. 1979a).

Dietary Intake

There are several methods that exist for the collection of food intake data: food records, dietary histories, food frequency questionnaires and 24-hour recall. All of these methods have limitations and vary in their degree of validity and reliability (Stuff et al. 1983).

II. Literature Review

Food records are intended to describe the actual or usual intake of individuals or families (Gibson 1990). They require subjects to weigh, measure or estimate foods consumed during a particular time period, commonly one to seven days. It is necessary to record ingredients in recipes, as well as to account for any plate waste at the conclusion of each meal. The success of this method depends on the interest, cooperation and educational status of the subjects. Furthermore, due to the extra work in recording foods consumed, subjects may alter their usual dietary habits (Smiciklas-Wright & Guthrie 1984).

The dietary history is designed to determine an individual's food intake pattern over a longer period of time. A detailed one-hour interview is conducted which includes questions designed to estimate both the usual amounts and the frequencies of food intake over periods of a year or more (Burke 1947). The major drawback to this method is that it can be very time consuming (Smiciklas-Wright & Guthrie 1984).

The aim of the food frequency questionnaire is to obtain qualitative, descriptive information about usual intakes of foods or classes of foods during a specified time period. Specific combinations of foods can be used as predictors for intakes of certain nutrients; for example, green leafy vegetables and carrots can serve as predictors of carotenoid intakes. The questionnaire can be semiquantitative when subjects are asked to quantify usual portion sizes of food items. This method is rapid with low respondent burden but the accuracy is lower than in other methods (Gibson 1990).

The 24-hour recall may be the most appropriate method to use in nutrition surveys in developing countries because subjects need not to be literate and the method is less time consuming. It usually involves interviewing individuals or families as to their food consumption during the preceeding 24 hours. The method is inexpensive and with low respondent burden so that cooperation is high (Pekkarinen 1970, Gibson 1990). Limitations such as memory and inaccurate estimation of usual intake may be not such a problem in diets which are simple and vary little from day to day.

Problems, however, such as accurately estimating serving size, overestimating portion sizes when intakes are low, or underestimating intake in expectation of help, may occur (Burk & Pao 1980).

Several studies have shown wide variations in vitamin A intake (Nelson 1989), however, the inter and intraindividual variability even for this nutrient should be greatly reduced in a population with a consistent meal pattern.

For groups, there is general agreement that one day's diet, whether obtained by 24-hour recall or food record, can provide valid information on usual nutrient intake (Emmons & Hayes 1973). Flores et al. (1974) carried out a dietary survey among rural Nicaraguan families. The food intake was estimated by three different methods: a three-day daily record, a one-day record with weighing of foods and a 24-hour recall. Comparisons of the methods revealed small but insignificant differences. These findings suggest that among families of low socio-economic status with a relatively monotonous daily diet, the simple 24-hour recall yields similar results as more time consuming and elaborate methods for the collection of food intake data.

Anthropometric Parameters

Vitamin A supplementation may have an effect on growth (Fig. 1). Although it is believed that this effect is less important than other outcomes of an improved vitamin A nutriture, it is recommended that anthropometric measurements should be included, since they are useful indicators of the general nutritional status (NRC 1987).

In two population based trials in Indonesia, children assigned to receive added vitamin A gained more body mass (West et al. 1988) and grew taller , on an average 1 cm annually (Muhilal et al. 1988b), suggesting that vitamin A supplementation may improve growth where endemic deficiency exists. Whether vitamin A's influence on growth is direct or secondary to alterations in the frequency and severity of infections is still unclear (Sommer 1989).

5. MORBIDITY

Vitamin A deficiency has been associated both in animal and in human populations with systemic and gastrointestinal infections. Severe vitamin A deficiency usually does not occur as an isolated problem but is almost invariably accompanied by other nutrient deficiencies. However, earlier studies on the lack of vitamin A (Wolbach et al. 1925, Blackfan et al. 1933) have shown that even mild hypovitaminosis A leads to patchy keratinization of the epithelial lining of the respiratory, genitourinary and gastrointestinal tracts. The epithelial barrier is the first step of the protection mechanisms against infection, and it has been suggested that keratinization leads to bacterial colonization and infections (Sommer et al. 1983). Other studies have also indicated that vitamin A plays an important role in the immune system (Scrimshaw 1966, Nauss 1986). Vitamin A is capable of modifying immune responses, both in vivo and in vitro.

Mainly cross-sectional studies have been carried out to investigate the relationship between vitamin A deficiency and infectious diseases. For example, Arroyave and Calcano (1979b) found that serum retinol levels in Guatemalan subjects were significantly lower during an infectious episode (diarrhea or respiratory tract infections) compared to those after recovery. In the Philippines, children with mild xerophthalmia did not differ from controls in their incidence of diarrhea but more frequently presented with respiratory diseases (Solon et al. 1978). Another study is that of Sommer and coworkers (1984) who examined rural Indonesian children every three months for 18 months. Children with mild xerophthalmia developed respiratory disease at twice the rate of those with normal eyes and diarrhea at three times that rate. Thereby the risk of respiratory disease and diarrhea was more closely associated with vitamin A status than with general nutritional status. Campos et al. (1987) found that an outbreak of chickenpox among preschool Brazilian children accelerated the depletion of liver reserves, as evaluated by the RDR test.

More recently, Bloem and colleagues (1990) investigated the association between mild vitamin A deficiency and the occurrence of diarrhea and respiratory diseases using three different epidemiologic approaches in Northeastern Thailand. The **cross-sectional** analysis showed that children with a history of diarrhea and respiratory disease had lower levels of serum retinol and retinol binding protein. A dose dependent relation between serum vitamin A and incidence of respiratory disease was observed in the three months **follow-up** study. Children with deficient and low levels of serum retinol at the baseline examination were 3.6 times and 2.4 times, respectively, more likely to develop respiratory diseases. The results of the **intervention** trial revealed that supplementation with vitamin A capsules (200,000 IU) reduced the incidence of both diarrhea and respiratory disease for a period of at least two months.

The findings of the study in Thailand indicate that hypovitaminosis A may lead to a higher risk of infections. The data thus suggest that it is important to prevent and treat marginal vitamin A deficiency. This aspect is especially important, considering that Sommer et al. (1983) observed an increased mortality in children with mild vitamin A deficiency. A greater susceptibility to respiratory infections and diarrhea, the two conditions mostly responsible for mortality in preschool children, may have been the cause (Sommer et al. 1984).

6. SUMMARY

In Southern Brazil, vitamin A intakes among families of low socio-economic status are inadequate and marginal vitamin A deficiency represents a public health problem. When evaluating the three supplementation strategies to prevent and treat hypovitaminosis A, food nutrification was identified as the most appropriate and cost-effective intervention. Fortification programs in Guatemala (sugar) and in the Philippines (MSG) have resulted in improved vitamin A status, which was reflected in increased retinol levels in blood and breast milk and a lower prevalence of

xerophthalmia or eye lesions among the study populations. Furthermore, these improvements in vitamin A indices were accompanied by positive changes in the anthropometric and general health status.

In Brazil, MSG is not commonly used and therefore, not feasible for nutrification. Although sugar is a staple food among those population groups most at risk for vitamin deficiency and could be a suitable carrier for vitamin A, alternative local nutrification vehicles still need to be explored. Soybean oil fulfills all the necessary selection criteria. The objective of the present pilot project was to assess the potential impact of vitamin A fortified soybean oil on vitamin A status of a selected community in Ribeirao Preto, Southern Brazil.

III. EXPERIMENTAL DESIGN AND METHODS

1. STUDY LOCATION

The study was conducted in Ribeirao Preto, which is located in the interior of the state of Sao Paulo in Southern Brazil, 310 km by road from the city of Sao Paulo. Ribeirao Preto, with a population of 700,000, is a rapidly growing, typical agricultural town and the centre of a prosperous sugarcane and coffee growing region.

Attracted by the prosperity of that area, in recent years, there has been increased migration of unskilled workers from other states and poorer regions, who, for the most part, end up living in the "favelas" or slums on the periphery of the city.

The favela Vila Piratininga, where the study population was sampled from, consisted of approximately 120 families. Half of the 55 households interviewed said that they decided to come to Ribeirao Preto to earn more money, have a better job and better medical care.

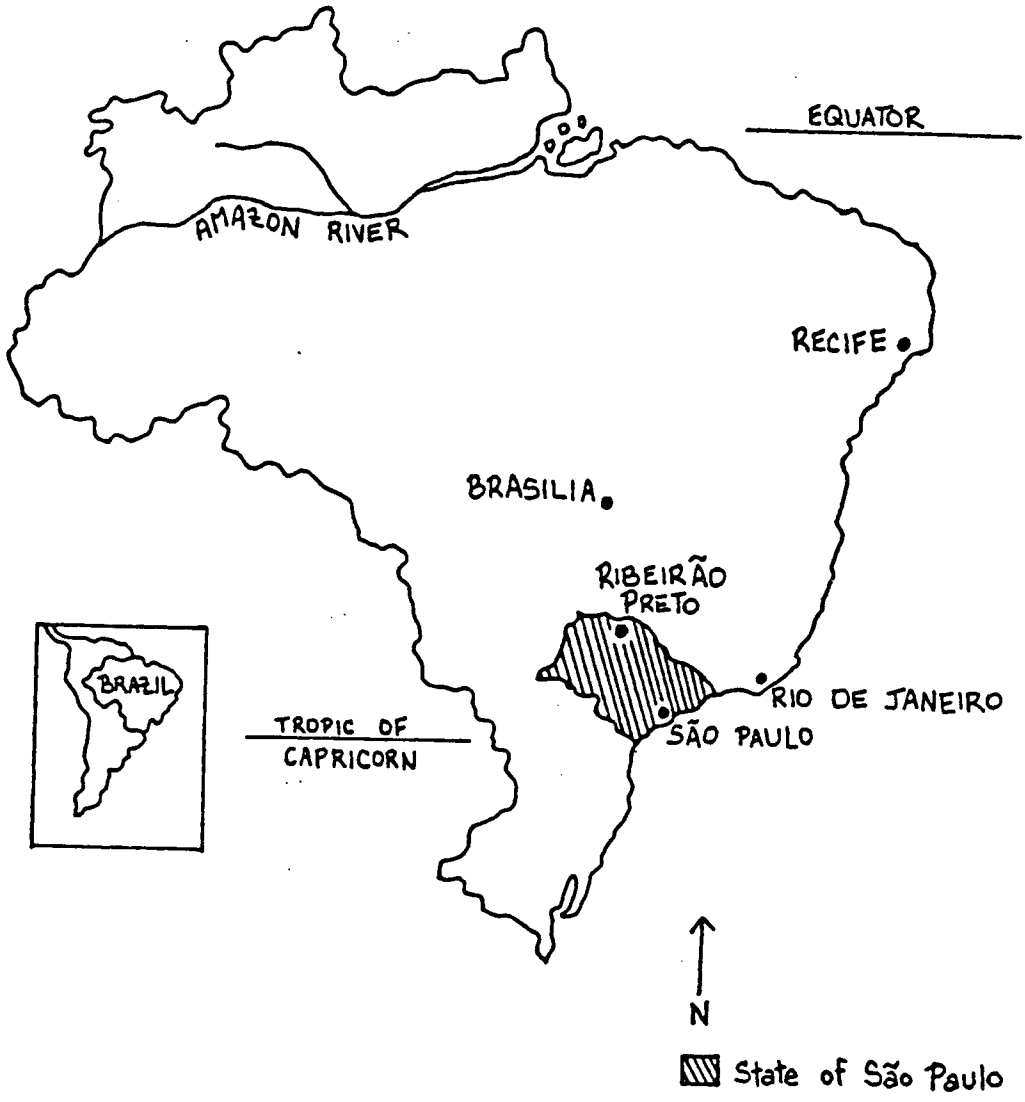


FIG. 2: Map of Brazil

2. STUDY POPULATION

Fifty-seven households of Vila Piratininga were randomly selected for the study. Initial contact was made with the help of a local missionary couple running a daycare centre for the children of Vila Piratininga residents and other favelas. Research personnel were introduced to two "leader families", who subsequently acted as a liaison between the residents and the researchers.

The purpose of the study was explained to the participants and written or oral consent was obtained either from the male or female household head (Appendix 1). Permission to involve children was obtained from the parents. Participants were assured that the information would be kept confidential and that they could withdraw from the study at any time and without any prejudice.

Fifty-five female household heads (two were excluded from the analyses since they were never available) were interviewed in Portuguese by the primary investigator to collect data on the socio-economic background, food habits, health related practices of the family, dietary intake and morbidity of the children (Appendices 3,4,6,10). Blood samples and anthropometric measurements were obtained from almost all family members.

A total of 237 subjects participated in the study and consumed the supplied oil; of these, 61% were children and 39% were adults, an adult being defined as anyone over 18 years of age. Whenever possible, data were collected on all individuals, however, some male subjects (eight in the control and ten in experimental group) were not available for **any** of the measurements and tests (they had to go to work) and for some family members, a complete data set (biochemical parameter, dietary intake, anthropometric measurements) could not be obtained. Only those individuals with a complete data set were included in the analyses (n=171). The average family unit consisted of two adults (range 1-4) and four children (range 1-10) with a total of six family members (range 2-12) in the house.

III. EXPERIMENTAL DESIGN AND METHODS

3. NUTRIFICATION

Thirty of the participating families were randomly assigned to the experimental group. Each family was provided with vitamin A nutrified soybean oil, whereas the remaining 27 households served as the control group and received unfortified soybean oil. Families were not aware which oil they were given. In this single-blind design, the expectations of the researchers could have influenced the results. However, the possibility of such a bias was reduced, since only the Brazilian coworker, who distributed the oil, knew exactly which family was supplemented or not.

Soybean oil was nutrified with vitamin A palmitate (1.7 mIU/g), stabilized with tocopherol, from Hoffman-LaRoche. The nutrification was carried out commercially by "Sanbra", a local oil processing company. The oil was packed in one liter cans, each containing 92,000 IU of retinyl palmitate (100 IU/g). The amount of retinol necessary to achieve adequate intakes has been estimated from the daily consumption of soybean oil in former studies (Desai et al. 1980). The subjects were given detailed oral and written instructions on how to use the oil (Appendix 2).

The soybean oil was distributed twice a week by a Brazilian coworker. Each time, the participants could pick up a full can of oil in exchange for their empty or partially empty can. The cans were marked with name and family number and the oil consumption per family was recorded. The families received oil according to their needs and family size and no restrictions were made in regard to oil consumption. Weekly visits and an excellent personal contact with the subjects assured that each family used only the assigned oil during the study.

4. RESEARCH PROTOCOL

Fig. 3 presents a summary of the research protocol, which was carried out over a period of four months from July to November 1988. The main purpose of this experimental design was to

compare responses of experimental and control group to the vitamin A nutrification of soybean oil, and thereby reveal the extent of its biological effectiveness.

Before the vitamin A supplementation, the baseline vitamin A and nutritional status of the study population was assessed. This included biochemical parameters and anthropometric measurements of all subjects as well as dietary intake data. After the baseline study was completed, the families were provided the specified soybean oil twice a week. At the end of the study, those baseline measurements were repeated once again.

Breast milk samples of lactating mothers as well as morbidity data of children were collected seven times during the supplementation period at 15-17 day intervals.

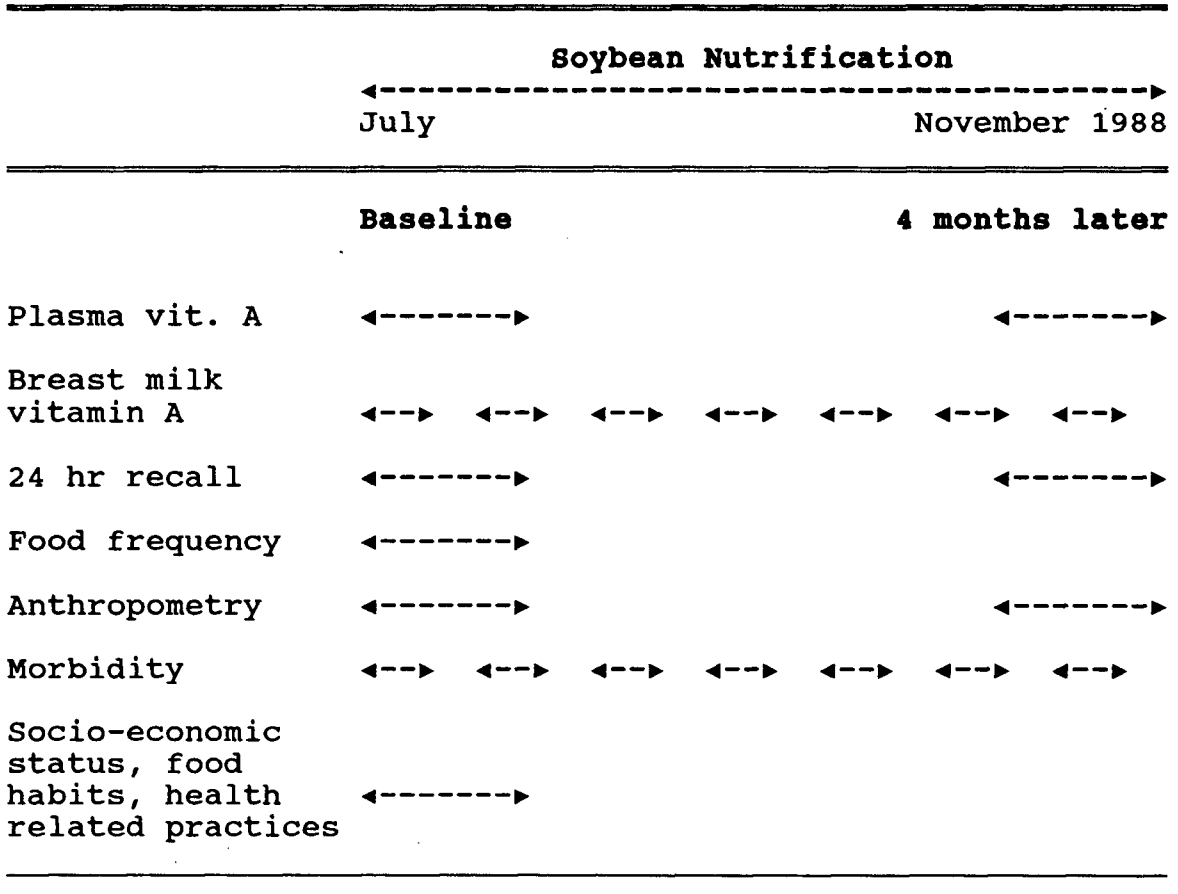


FIG. 3: Research protocol

III. EXPERIMENTAL DESIGN AND METHODS

5. *ASSESSMENT OF VITAMIN A AND NUTRITIONAL STATUS*

Socio-Economic Factors

Socio-economic factors that may have an influence on nutritional status in general were assessed by a questionnaire (Appendix 3) administered to the female head of the family during a visit in each home at the beginning of the study. This information as well as data on food habits and health related practices of the family were expected to provide helpful background in interpreting results of more direct tests of nutritional status.

Dietary Data

24 Hour Recall

Information on dietary intake was obtained using the 24h-recall method (Appendix 4); before and after the four month supplementation period, the matriarchal head of each household was interviewed. For the estimation of the food consumption of children, three boys and five girls (age 7-13 years) were asked to recall their previous day's dietary intake at the beginning of the study. Only eight children were available for the collection of those data.

The questionnaires were presented to the subjects after they had completed the blood collection.

Foods eaten were recorded in Portuguese and quantities were expressed in Brazilian household measures:

> Colher de cha (teaspoon), colher de sopa (table spoon), espumadeira (slotted spoon), concha (ladle), copo (glass), xicara de cha (tea cup) xicara de cafe (coffee cup), prato razo (standard plate), prato fundo (soup plate) (Fig. 4).

FIG. 4: Brazilian household measures

III. EXPERIMENTAL DESIGN AND METHODS

All 24 hour food intake interviews were conducted by one interviewer to maintain the consistency in the interviewing procedure.

Interviews were conducted during week days to determine intake during the week as well as Sunday, a day when food intake tended to vary. All foods recorded for each subject were then coded for food number, food group number and converted into metric portion sizes. Appendix 5 lists all the foods in Brazilian household measures and respective metric conversions that were used for the analyses. The metric portion sizes were derived from tables of "Hospital das Clinicas da Faculdade de Medicina" in Ribeirao Preto (CNPq 1981) and from the "Agricultural Handbook" (Watt & Merrill 1963). If a food was not listed in either of these sources, the average weight, determined by weighing the portion sizes of a typical meal eaten by the participants or weighing various foods sold in the markets of Ribeirao Preto, was used.

For nutrient analysis, the modified food composition table for Brazilian foods developed by Swann (1979) and Waddell (1981) was used, with the addition of 32 foods. Information on the composition of 32 foods not included in the original table was obtained from a local food composition table (ENDEF, 1977). For mixed dishes, Brazilian recipes were gathered from local home makers and the ingredients and quantities of foods were approximated. The mixed dish was then coded in terms of individual ingredient foods.

The available vitamin A from the nutrified soybean oil was estimated from the amount of oil consumed per family and then converted into daily intake per person by dividing it by the number of family members. The participants were aware that the study and the **free** oil delivery would last for four months. Therefore, during the last month of the study, they would pick up as much oil as they were allowed to for storage. For this reason, the per capita oil consumption in a family was averaged over a three-month period only.

III. EXPERIMENTAL DESIGN AND METHODS

Food Frequency

At baseline, in addition to the 24h recall, a food frequency questionnaire (Appendix 6) was presented to the female head of the household. It served to estimate the customary consumption pattern of locally available vitamin A food sources throughout the year. Dietary intake and food frequency data of migrant workers in a suburb of Ribeirao Preto (Desai et al. 1980, Doell 1984) were used to select the food items on this questionnaire.

Biochemical Tests

Blood collection

In the beginning and at the end of the supplementation period, subjects were asked to give blood samples after an overnight fast. Approximately 10 ml of venous blood were drawn using sterilized disposable needles and celluloid centrifuge tubes containing the anticoagulant ethylene diamine tetra-acedic acid (EDTA). The procedure was carried out by a local nurse of the University Hospital in Ribeirao Preto (Fig. 5).

Immediately after the blood collection, the specimens were transferred to a cooler where they were kept refrigerated and protected from light and oxygen until the completion of the day's sampling. Then all the samples were transported to the laboratory of the University of Sao Paulo Medical School in Ribeirao Preto where the plasma was separated by centrifugation at 2500 rpm for 10 minutes. The plasma was transferred to two tubes protected from light (one tube containing 1 ml, the other with the remaining plasma), tightly capped, properly labelled and stored frozen (at -20°C) until ready for analysis.

FIG. 5: Blood collection

Plasma Analysis

After completing each collection period, all the 1 ml plasma samples were sent in dry ice to Hoffmann-La Roche in Basle, Switzerland, for simultaneous analysis of retinol and β-carotene with high pressure liquid chromatography (HPLC).

Aliquots of 200 μl of plasma were diluted with ethanol to denature serum proteins and the retinol and β-carotene were extracted with hexane. After centrifugation, two aliquots of the organic phase were injected onto normal phase HPLC columns, followed by an eluting solvent of suitable polarity. Retinol, which is eluted as a sharp peak within 5 minutes, is detected by a sensitive detector set at 313 nm. The retention time and wavelength for detection of β-carotene are 3.7 minutes and 436 nm, respectively. Retinol and β-carotene were quantitated using peak height ratio or peak area ratio relative to an internal standard (Vuilleumieur et al. 1983).

Breastmilk Vitamin A

The lactating mothers were instructed to express approximately 10 ml of milk, when possible from a full breast, into a clean test tube. These specimens were obtained at 15-17 day intervals. At each collection, there were 9 to 11 lactating women from the study population. The samples were frozen at -20°C and sent in dry ice to Hoffmann-LaRoche in Basle, Switzerland, for vitamin A analysis by HPLC.

Measurements of Anthropometric Status and Growth

At the time of blood collection, the anthropometric measurements of all participants were obtained (Appendix 8), according to standard recommended procedures (Jelliffe 1966). The measurements included height, weight, triceps skinfold (TSF) thickness, mid-upper arm circumference (MUAC) and head circumference (for children under 3 years). Measurements were

conducted by the primary investigator in one of the favela homes (Fig. 6) while the subjects were waiting for or shortly after they had completed the blood sampling. On completion of all tests and measurements, the participants received breakfast.

Weight

To measure weight, a platform beam balance was used. Subjects were weighed before breakfast, without shoes and wearing minimal clothing. Weights of infants and toddlers were determined by subtracting the weight of the mother from the total weight of the mother and child. All weighings were read to the nearest 0.1 kg.

Height

The height of subjects greater than 100 cm was determined using a platform beam balance equipped with a vertical measuring rod. Participants were measured to the nearest 0.1 cm without shoes. Crown to heel length of infants and children less than 100 cm was determined by means of a recumbent length board.

Mid-Upper Arm Circumference (MUAC)

MUAC was measured with a nonstretchable but flexible, plasticized tape. The tape was placed around the freely hanging left arm at a point halfway between the acromion and olecranon. Measurements were read to the nearest mm.

FIG. 6: Collection of anthropometric data

Triceps Skinfold Thickness (TSF)

TSF thickness was determined using a Lange skinfold caliper which exerted a uniform pressure of 10 g/mm^2. The measurements were taken to the nearest mm on the fully relaxed left arm and at the site used for the measurement of MUAM.

Mid-Upper Arm Muscle Circumference (MUAMC)

Using the values obtained for MUAC and TSF, the MUAMC was calculated using the following formula (Jelliffe 1966):

$$MUAMC = MUAC - \pi \times TSF$$

Head Circumference

The head circumference was measured to the nearest mm for children below three years of age. A tape was placed firmly around the frontal bones, passing the tape around the head above the ears and over the maximal prominence at the back of the head.

All anthropometric measurements were compared to both international (NCHS 1979, 1987, Frisancho 1981) and Brazilian standards (Marcondes 1979, 1983) where possible. The general level of nutrition was assessed on the basis of weight for age, height for age, and weight for height for children aged between 0 and 18 years during the study period.

Morbidity

Morbidity data on diarrhea and respiratory disease were collected by asking each mother about the health of her children during the preceeding two weeks (ie. Did your child(ren) have diarrhea in the last two weeks? How often?).

A protocol was designed to report these data (Appendix 10). The diagnosis of diarrhea or respiratory diseases such as coughing, hoarseness, sore throat and running nose was left up to the mother's judgement. The morbidity load was totalled for children in each group. These data served as additional information on the general health and vitamin A status of the participating children.

6. STATISTICAL ANALYSES

The 1988 version of BMDP, a statistical software package for biomedical sciences was used for the analyses of the biochemical parameters. All the other statistical analyses were performed with SYSTAT, system for statistics on the PC, second edition (1989).

Dietary Data

24 Hour Recall

Dietary food recalls were analysed for energy and the following nutrients: protein, lipids, carbohydrates, calcium, iron, thiamin, riboflavin, niacin, vitamin A, C, E, using a computer program developed by a statistician at the University of British Columbia. Special emphasis was put on the assessment of the vitamin A intake from natural food sources **only** and from natural food sources **plus** the nutrified oil.

III. EXPERIMENTAL DESIGN AND METHODS

Mean daily nutrient intakes of women in the experimental and control groups before and after the four- month nutrification period were compared to FAO/WHO recommended daily intakes (Beaton & Patwardhan 1976, FAO/WHO 1988). Since the interest focused on the **qualitative** aspects of the women's diet, nutrient density per 1000 kcal was chosen to investigate any changes in nutrient intakes during the study period. For this purpose, an analysis of variance (ANOVA) for a 2 (treatment) x 2 (time period) factorial experiment with repeated measures (RM) on the second factor was performed.

Food Frequency

Foods on the food frequency questionnaire (Appendix 6) were grouped according to vitamin A content per 100 g (Appendix 7). The consumption pattern of vitamin A containing food sources was compared between experimental and control groups using a Chi Square test.

Biochemical Parameters: Vitamin A and β-Carotene

To determine changes in plasma retinol and β-carotene during the nutrification period, a 3 way ANOVA for a 2 (treatment) x 3 (agegroup) x 2 (time period) factorial experiment with RM on the third factor was applied. When applicable, post-hoc comparisons were applied to determine statistical differences between pairs of means. The Bonferroni method was used to maintain the overall error rate at $p<0.05$. Missing values were omitted from the calculations.

Breastmilk Vitamin A

Collected breastmilk samples were not available for data analysis, since Hoffmann-LaRoche was not able to analyse the specimens.

III. EXPERIMENTAL DESIGN AND METHODS

Anthropometric Measurements

A 3 way ANOVA for 2 (treatment) x 3 (agegroup) x 2 (time period) factorial experiment with RM on the third factor was used to analyse the anthropometric parameters. Ten pregnant women were omitted from the analysis.

Morbidity

The incidence of morbidity among children in both groups was analysed using a Chi Square test.

IV. RESULTS

1. DESCRIPTION OF VILA PIRATININGA COMMUNITY

Vila Piratininga, one of many favelas in the outskirts of Ribeirao Preto, is located approximately 8 km or 35 minutes by bus from the city centre. Approximately 120 families occupy a relatively small area of land which belongs to the City. The houses are clustered together along unpaved streets, which are noted for their ruts and rocks. Attempts have been made to fence in some yards with rickety fences, however, children and domestic animals seem for the most part, free to roam the streets. Sounds of children playing or crying add to the clamor of the sound of radios blaring in the background. The red soil (terra roxa) in the area appears fertile, though little vegetation is grown. There are very few trees to provide shade from the hot sun. In a dry period, the area is extremely dusty and in a wet period, extremely muddy.

In general, the people of Vila Piratininga were very friendly and cooperative. Fifty-five out of 57 participating families completed the study. One family moved away during the four month study period and the other family was not willing to participate in the second blood collection. Those data were omitted from the analyses.

Socio-Economic Characteristics

Socio-economic and demographic characteristics of the study population are summarized in Table 1. No differences between control and experimental group were found, therefore, data of both groups have been pooled.

RESULTS

The ages of female household heads ranged from 16-86 years with a mean of 31 years; the men's age ranged from 18-67 years with a mean of 32 years. Children of all ages (range 1 month to 23 years) with a mean age of 6.6 years (male) and 6.4 years (female), respectively, were represented in the study population.

Literacy rate and level of education among participants were equally low in men and women. Most subjects (64% and 60%, respectively) did not report more than five years of schooling, and this may have included repeated grades. Reasons most frequently cited by mothers for children's poor school attendance were that the children disliked school or that they were working.

The majority of households consisted of one wage earner, mainly the male household head, supporting an average of six people. Most women stayed at home to look after their (many) children; those who were employed were generally single mothers or had older children.

TABLE 1
Socio-economic and demographic characteristics of the study population[1]

	Household heads		Children	
	Male (n=38)	Female (n=56)	Male (n=68)	Female (n=75)
Age (years)[2]	32±9.3	31±11.3	6.6±5.6	6.4±4.3
Ability to read & write[3]	59%	53%	52%	52%
Formal education				
None	13%	20%	-	-
< 1-5 years	64%	60%	-	-
Children currently attending school[4]			47%	47%
Employment Rate	98%	18%	2%	6%
Occupation				
Agricultural worker	37%	7%	1%	-
Night Guard	10%	-	-	-
Construction worker	25%	-	1%	-
Domestic	4%	11%	-	-
Other[5]	20%	-	3%	-

[1] Data on control and experimental groups have been pooled.
[2] Mean ± SD
[3] Of all schoolaged boys and girls.
[4] Boys and girls together.
[5] Garbage collector, carpenter, bus-ticket controller, blacksmith, painter, mechanic's assistant, bottle collector, street vendor.

RESULTS

Men, women and children worked primarily in construction or as agricultural labourers (cutting sugarcane); the earnings varied widely, depending upon the individual's motivation and quantity of work performed. For most occupations, wages were below Brazilian minimum wage guidelines equivalent to US $41.36 per month. At the time of the interview, 67% of the families had a **total monthly income** equivalent to US $79 or less. Fifty-two percent had a monthly **per capita income** equivalent to US $13.16 or lower.

Living Conditions

Table 2 describes the living conditions in Vila Piratininga, which were generally poor and unhygienic.

Forty percent of all participants had lived five years or more in this bairro. Most families (85%) lived in one or two-room houses that they constructed themselves. Roofs were made of corrugated iron, walls consisted of wood or concrete blocks and floors were either packed earth or concrete. Natural lighting and ventilation in those homes was often poor due to the lack of windows. Fig. 7 illustrates a typical Vila Piratininga housing condition.

TABLE 2
Typical living conditions of study population

Conditions	%
Housing:	
Own house	84
Rented house	17
Number of rooms	1.5
Electricity	73
Running water	88
Sanitation:	
Toilet in house	30
Sewage or gutter system	none
Cooking Facilities:	
Gas[1]	80
Firewood	20
Domestic Equipment:	
Radio	54
TV	52
Tape recorder	16
Pressure cooker	70
Refrigerator	38
Electric fan	16
Iron	48
Electric blender	34
None of the above	16

[1] Some families use gas and firewood

FIG. 7: Typical Vila Piratininga housing

RESULTS

Seventy-three percent of all households had electricity and 88% had running water in their homes; more than half of the families owned a TV or radio and a great deal of leisure time was spent watching soap operas and listening to music.

No sewage system existed in Vila Piratininga, so sewage and other waste water flowed into the streets. Only 30% of the families had a toilet in their house, whereas 44% used open gutters and backyards for toilet facilities.

A common street scene is shown in Fig. 8, where children played barefoot next to garbage and effluent.

A large percentage of the households (63%) disposed of garbage openly on the street or yard. Another factor contributing to an unhygienic environment was that 59% of the families owned dogs, cats, birds, chicken, ducks or horses.

FIG. 8: Common street scene in Vila Piratininga

RESULTS

Food Habits and Practices

Most families (80%) bought food, usually once a week, at local grocery stores (Table 3). These stores had limited stock selection and prices were generally higher than in the supermarkets in downtown Ribeirao Preto. Fresh produce was rarely sold. Few families travelled to the city centre to do their grocery shopping because they did not own motorcycles or horses for transportation. Most depended on bicycles or the bus to go to Ribeirao Preto; bus travel, however, was costly.

Nine percent of the participants depended solely on food donations, mainly by church and other charity organizations. Almost half of the households (45%) received coupons from the government for one litre or more of milk/day, depending upon the number of small children in the family.

Twenty-three percent of the respondents reported growing vegetables and/or having fruit trees in their backyards; lettuce, tomatoes, onions, cabbage, manioc, bananas, guava (goiaba) and papaya were the most commonly grown foods. Only a few families (18%) raised animals, mostly chickens for household consumption.

Meals were usually prepared by women using cooking facilities inside and outside the house with gas or firewood (or a combination of both) as cooking fuel. Most left-overs would be kept for the next meal or fed to animals.

TABLE 3
Food habits and practices

	RESULTS
	%
Purchase of groceries:	
Grocery store	80
Market	11
Donation	9
Garden in house:	29
Vegetables and fruits planted:	
Lettuce	14
Tomatoes	9
Onions	13
Cabbage	7
Guava	7
Banana	5
Animals raised for eating:	18
Food assistance received:	
Milk (1 litre/day)	45
From church	9
None	46
Food storage in:	
Open cupboards[1]	32
Closed cupboards[1]	63
Fridge	38
Left-over meals:	
Keeping for next meal	73
Giving to animals	13
Throw away	4

[1] Some families kept food in cupboard and fridge

RESULTS

The women in Vila Piratininga recognized their poor socio-economic situation and wanted to improve their financial conditions and living environment. Evidence for this was gathered from responses on how they would use money if they won the lottery. Most of the respondents indicated that they would build better houses and deposit money in the bank. More than one-third (35%) of the women thought food intakes were inadequate and stated they would buy larger quantities of food, especially more meat, fish and fruit.

The majority of women enjoyed living in Vila Piratininga, however, several improvements for the shanty town such as installation of a sewage system, paved streets and garbage collection were suggested.

In summary, considering the above described poor socio-economic characteristics, unhygienic living conditions and food related practices of Vila Piratininga residents, nutritional problems could be anticipated in this population.

2. ASSESSMENT OF VITAMIN A AND NUTRITIONAL STATUS

Dietary Assessment

Food Frequency

Female household heads were asked to indicate the frequency of consumption of several foods containing various amounts of vitamin A (Appendix 6). Those food items were then grouped according to their vitamin A content (Appendix 7) and the frequency of consumption of those food groups was compared between experimental and control group. The results are reported in Table 4.

The consumption pattern of all food groups was the same in both groups, except for foodgroup 4. Here, 22.9% of the respondents in the experimental group indicated that they sometimes consumed foods like peas, mango, chicken, red potatoes, cheese, squash and eggs, however, only 13.1% of women in the control group did so. This difference was of borderline significance ($p=0.048$).

Foods low in vitamin A were generally consumed on a daily basis whereas food items with a higher vitamin A content were infrequently or never eaten. Insufficient income was the reason most often cited by the women for not eating certain foods. There was no significant difference between the two treatment groups in the frequency of consumption of all 35 food items.

TABLE 4
Frequency of consumption of certain foods in control (n=24) and experimental (n=30) groups[1]

		Daily	Sometimes	Never
Foodgroup 1 0-50 µg[2]	Control	50.3	15.5	43.3
	Experim.	46.0	19.4	34.6
Foodgroup 2 51-100 µg[2]	Control	53.3	20.0	26.7
	Experim.	61.1	16.7	22.2
Foodgroup 3 101-200 µg[2]	Control	38.7	20.0	41.3
	Experim.	33.3	22.2	44.4
Foodgroup 4 201-356 µg[2]	Control	31.4	13.1*	55.4
	Experim.	29.1	22.9*	48.1
Foodgroup 5 525-790 µg[2]	Control	5.3	14.7	80.0
	Experim.	14.4	14.4	71.1
Foodgroup 6 >1,000 µg[2]	Control	24.0	18.7	57.3
	Experim.	24.4	23.3	52.2
All foods	Control	39.7	16.0	44.3
	Experim.	38.3	20.0	41.7

[1] All values expressed as percent of total responses in the respective group.

[2] Vitamin A content expressed per 100 g of food (see Appendix 7)

* p=0.048

RESULTS

24 hr Recall: Vitamin A Intake and Changes During Study Period

Mean daily vitamin A intakes of both groups at the start (time 1) and at the end (time 2) of the supplementation period are reported in Tables 5 and 6 and presented graphically in Fig. 9. The absolute values were compared to FAO/WHO recommended intakes (as indicated by the dotted line in Fig. 9). One subject with an unusually high vitamin A intake was omitted from the data analysis.

As shown in Table 5, at time 1, women in the control and experimental groups had similar, extremely low vitamin A intakes (249 μg±244, 217 μg±361, respectively; mean±SD) which were approximately half of that recommended. At time 2, the daily vitamin A consumption of the control group was slightly lower (182 μg±136), whereas the total vitamin A intake of the experimental group had increased to 320% of that recommended because of the use of nutrified oil (1631 μg± 973).

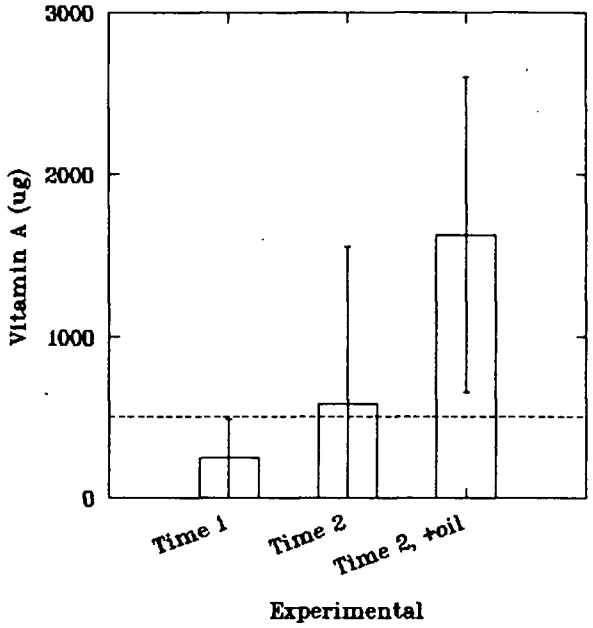
The changes in vitamin A intake/1000 kcal with and without the contribution of nutrified oil were investigated separately. Here, the nutrient density was chosen, since the main focus was on the qualitative aspects of the diet. Table 6 presents the results.

TABLE 5
Estimated mean daily energy and nutrient intakes of women in control (n=24) and experimental groups (n=30)

Nutrient	Time	Daily Intake						FAO Recommended Daily Intake[1] (RNI)
		Control Group			Experimental Group			
		Mean	± SD	%RNI	Mean	± SD	%RNI	
Vitamin A (μg)	**1**	**217**	**± 361**	**43**	**249**	**± 244**	**49**	**510[3]**
	2	**182**	**± 136**	**36**	**1,631**	**± 973[2]**	**320**	
Calories (kcal)	1	1,084	± 423	49	1,203	± 520	55	2,200
	2	1,099	± 433*	50	1,367	± 478	62	
Protein (g)	1	30	± 19	73	32	± 21	79	41[4]
	2	22	± 11**	54	34	± 18	83	
Calcium (mg)	1	171	± 129	43	204	± 128	51	400-500
	2	147	± 116	37	209	± 152	52	
Iron (mg)	1	5.3	± 3.2	28	5.3	± 3.0	28	19[5]
	2	3.9	± 1.7***	21	6.0	± 2.6	32	
Thiamin (mg)	1	0.4	± 0.2	43	0.5	± 0.4	50	0.9
	2	0.3	± 0.1*	32	0.5	± 0.3	58	
Riboflavin (mg)	1	0.5	± 0.5	38	0.5	± 0.3	38	1.3
	2	0.3	± 0.2*	24	0.5	± 0.3	38	
Niacin (mg)	1	5.8	± 5.6	40	6.0	± 5.8	41	14.5
	2	3.3	± 1.5***	23	6.4	± 4.1	44	
Vitamin C (mg)	1	16	± 19*	52	51	± 77	171	30
	2	13	± 29**	45	41	± 51	136	
Vitamin E (mg)	1	4.1	± 1.9	69	4.9	± 2.4	81	6[6]
	2	6.0	± 2.7	100	6.6	± 1.7	110	

[1] FAO/WHO, Beaton and Patwardhan, 1976.
[2] Vitamin A intake from natural sources plus fortified oil.
[3] FAO/WHO, Vitamin A requirements, 1988.
[4] Based on a mixed cereal-legume diet with small amounts of animal source foods.
[5] If proportion of energy derived from animal sources or soybean is 10-25%.
[6] Based on Canadian Recommended Daily Nutrient Intake, 1990.

Compared to experimental group, nutrient intake was significantly different:
***$p<0.001$ **$p<0.01$ *$p<0.05$



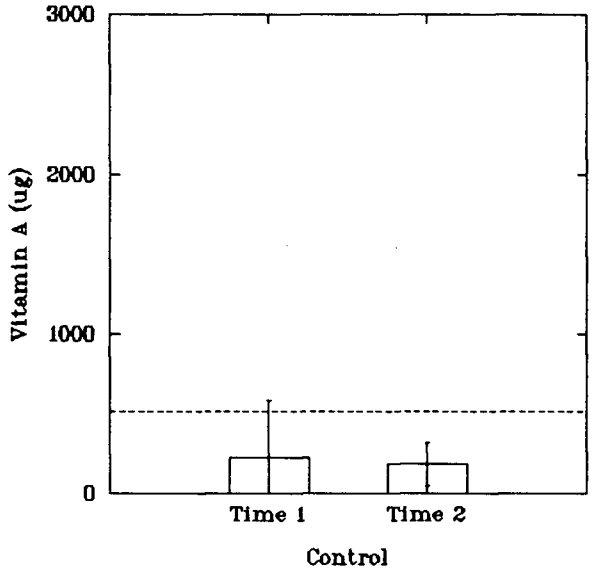


FIG. 9: Daily intake of vitamin A, expressed as mean ± SD

TABLE 6
Results of the repeated measures ANOVA on the changes of

1. Vitamin A intake (per 1000 kcal) from natural vitamin A sources only over 4 months

	Mean Intake		Treatment(T) effect F(p)	Time Period(TP) effect F(p)	T x TP F(p)
	Time 1	Time 2			
Control	197 μg	167 μg	4.11(0.048)	N.S.	N.S.
Experi-mental	208 μg	402 μg			

2. Vitamin A intake (per 1000 kcal) from natural vitamin A sources plus nutrified oil over 4 months

	Mean Intake		Treatment(T) effect F(p)	Time Period(TP) effect F(p)	T x TP F(p)
	Time 1	Time 2			
Control	197 μg	167 μg	55.1(<0.001)	48.5(<0.001)	54.4 (<0.001)
Experi-mental	208 μg	1248μg			

RESULTS

The two (treatment) x two (time period) ANOVA with repeated measures on the second factor indicated that there was no time period (TP) effect and no treatment (T) x TP interaction for the intake of natural vitamin A sources only. The main treatment effect was of borderline significance ($p=0.048$) suggesting that the experimental group had a slightly higher intake of vitamin A foods (305 μg) than the control group (182 μg), when averaged over time periods (Table 6).

The soybean nutrification increased the daily vitamin A intake of the experimental group by an average of 1096 μg ± 512 (Mean ± SD) (Fig. 9). This resulted, when considering the combined intake of vitamin A containing foods and nutrified oil, in significant overall changes in the vitamin A intake during the study period.

Averaged over time period, individuals of the experimental group had a significantly higher vitamin A intake/1000 kcal (728 μg) than those of the control group (182 μg).

Averaged over treatment, the nutrification of oil with vitamin A was associated with a significant increase in the vitamin A consumption; it improved from 203 μg/1000 kcal at baseline to 768 μg/1000 kcal at the end of the supplementation period.

The significant interaction of T x TP indicates that the changes in mean vitamin A intake/1000 kcal of the two treatment groups were different over the study period. At baseline, control and experimental groups had similar vitamin A densities in the diet (197 μg vs. 208 μg). Four months later, however, women of the experimental group achieved a considerably higher vitamin A intake (1248 μg/1000 kcal), whereas those of the control group consumed a slightly smaller amount of vitamin A (167 μg/1000 kcal) compared to the baseline intake.

RESULTS

Level of Nutrification

To ensure the level of fortification (100 IU/g of oil), aliquots of oil from the cans containing the fortified soybean oil were taken several times during the intervention and analysed for their vitamin A content. The analysis indicated an average concentration 92.9 ± 3.5 IU of vitamin A per gram of oil (Table 7).

TABLE 7
Vitamin A content in samples of soybean oil

Sample	Vitamin A content (IU/g oil)
1	94.9
2	97.3
3	94.7
4	92.0
5	90.4
6	86.6
7	91.7
8	91.2
9	97.2
Mean ± SD	92.9 ± 3.5

RESULTS

24 hr Recall: Adequacy of Women's Nutrient Intake

The diet of all the respondents was generally quite monotonous. Breakfast usually consisted of white bread with coffee or milk, while lunch and supper usually consisted of rice and beans supplemented occasionally with meat, eggs or vegetables. Table 5 presents the mean daily nutrient intakes of women in the control and experimental group, respectively, at baseline and four months after the study period. Absolute values as well as % of FAO/WHO recommended intakes are shown.

At baseline, both groups had similar, considerably low intakes of most nutrients. The only nutrients for which intakes were **above** two-thirds of those recommended were protein and vitamin E. The mean vitamin C intake of the experimental group was over 100% of the FAO recommended intake and significantly higher than in the control group.

At time 2, a generally higher mean nutrient intake was observed in the experimental group, whereas women of the control group tended to consume less of many nutrients. However, mean intakes of most nutrients still remained substantially below the FAO recommended intakes. Only the vitamin C consumption of the experimental group and the vitamin E intake of either group were more than adequate. Mean protein intake of the supplemented women was above two-thirds of that recommended. The nutrients which women consistently did not meet one-third of FAO levels was iron.

At the same time, mean intakes of calories, protein, iron, thiamin, niacin and vitamin C were significantly higher in the experimental group.

Distribution of women's energy intake from macro nutrients in both groups (Table 8) seemed satisfactory at time 1. Approximately half of the total energy intake originated from complex carbohydrates, with only a small amount being derived from simple sugars. Diets in general were high in polyunsaturated fatty acids and low in saturated fatty acids. However, during the four month supplementation period, the fat intake of all women (per 1000 kcal and in absolute terms)

increased significantly (53 g at time 1 and 70 g at time 2, when averaged over group; F=11.2, p=0.002), which in turn shifted the proportion of protein and carbohydrates downward. This tended to occur to a greater extent in the control group than in the experimental group.

24 hr Recall: Changes in Nutrient Intakes

Averaged over treatment group, the iron intake/1000 kcal decreased and the vitamin E intake increased significantly over the four-month study period (Table 9). A significant interaction of T x TP for iron intake indicates that those changes over time were different for control and supplemented groups. At baseline, both groups had similar iron intakes/1000 kcal (4.6 mg and 4.4 mg, respectively). During the supplementation period, the iron intake of the experimental group remained constant at 4.6 mg, whereas that of nonsupplemented women decreased to 3.4 mg/1000 kcal.

Significant treatment effects were noted for thiamin and vitamin C. Averaged over time periods, supplemented subjects had significantly greater intakes of thiamin and vitamin C/1000 kcal (0.37 mg of thiamin, 40 mg of vitamin C) than the control group (0.3 mg and 14 mg, respectively). The changes in thiamin intake need further interpretation as there was a significant interaction of T x TP. Over four months, thiamin density in the diet of the experimental group remained stable (0.36 mg vs. 0.37 mg), whereas in the control group it dropped from 0.35 mg to 0.25 mg.

There was no overall difference between treatment groups in the intake of all other nutrients.

TABLE 8
Distribution of energy intake from macro nutrients

		Energy intake (% of total)		
		Women		Children
	Time	Control	Experimental	Combined
Protein	1	11.6	11.2	17.3
	2	8.4	10.4	
Carbohy-drates	1	52.3	48.9	46.5
	2	42.4	44.9	
Fat	1	40.3	43.1	39.2
	2	53.0	49.3	

TABLE 9
Results of repeated measures ANOVA on the changes in nutrient intake over 4 months

Nutrient	Time	Mean Intake /1000 kcal		Effect of		
		Control	Experi-mental	Treat-ment (T)	Time period (TP)	TxTP
Protein (g)	1	27	26	-	-	-
	2	20	24			
Calcium(mg)	1	152	184	-	-	-
	2	123	147			
Iron (mg)	1	5	4	-	*	*
	2	3	4			
Thiamin(mg)	1	0.4	0.4	*	-	*
	2	0.3	0.4			
Riboflavin (mg)	1	0.4	0.4	-	-	-
	2	0.3	0.4			
Niacin (mg)	1	5.2	4.6	-	-	-
	2	3.0	4.6			
Vit. C (mg)	1	16	46	**	-	-
	2	11	33			
Vit. E (mg)	1	3.7	4.1	-	***	-
	2	5.6	5.1			

* $p<0.05$ ** $p<0.01$ *** $p<0.001$

RESULTS

Oil Consumption During Study Period

The oil consumption of both groups increased significantly during the four month supplementation period (Table 10) ($F=22.5$, $p<0.001$). At the beginning of the study, women of control and experimental groups consumed 21 g and 25 g, respectively, whereas at the end of the intervention the per capita intake was 39 g and 35 g, respectively.

TABLE 10
Oil consumption during the study period

	Oil consumption (g/day)		Effect of		
	Time 1 (Mean ± SD)	Time 2 (Mean ± SD)	Treat-ment (TP)	Time period (TP)	TxTP
Control	21 ± 12	39 ± 22	-	***	-
Experimental	25 ± 17	35 ± 16			

*** $p<0.001$

RESULTS

24 hr Recall: Adequacy of Children's Nutrient Intake

Daily nutrient intakes of eight Vila Piratininga children (mean age 11 years) and comparison with international standards are reported in Table 11. One outlier for niacin was excluded from the calculations. Data from both groups and sexes were pooled.

Children's estimated daily nutrient intakes appeared more satisfactory than those reported by women. The mean intake was **greater** than two-thirds of the recommended intakes for all nutrients, except for thiamin. Distribution of children's energy intake from macro nutrients was similar to that of adults (Table 8), although only the proportion of protein in the children's diet was greater.

TABLE 11
Comparison of estimated mean daily energy and nutrient intakes of children (n=8) with International Standards[1]

		Daily intake	
Nutrient	Recommended daily intake (RNI)	Mean ± SD	% of RNI
Vitamin A (μg)	**500[2]**	**343.6 ± 344**	**69**
Calories(kcal)	2,275[3]	2,458 ± 761	108
Protein (g)	42[3,4]	101 ± 46	240
Calcium (mg)	600 - 700	491 ± 259	70 - 82
Iron (mg)	7[5]	9.7 ± 4.2	139
Thiamin (mg)	1	0.65 ± 0.2	65
Riboflavin(mg)	1.5[3]	1.33 ± 0.67	89
Niacin (mg)	16.4	11.24 ± 5.7	69
Vitamin C (mg)	20	23 ± 28	115
Vitamin E (mg)	7.5[6]	8.1 ± 5.7	108

[1] FAO/WHO, Beaton and Patwardhan, 1976.
[2] FAO/WHO, vitamin A requirements, 1988.
[3] Average recommended intake for boys and girls, age 10-12 years.
[4] Based on a mixed cereal-legume diet with small amounts of animal protein.
[5] If proportion of energy derived from animal sources or soybean is 10-25%.
[6] Based on Canadian Recommended Daily Nutrient Intakes, 1990.

RESULTS

Biochemical Parameters

Plasma Retinol Level

Fig. 10 presents the distribution of subjects from control and experimental groups in four different categories of plasma retinol at baseline and after supplementation.

At time 1, only 1% of participants in the control group and 4% in the experimental group could be classified as deficient (<10 μg/dl); 20% and 24%, respectively, had low plasma retinol levels (<20 μg/dl). Values of most individuals in both groups were in the adequate or normal range (> 20 μg/dl).

Four months later, the proportion of subjects from the control group in each category had not changed very much; there were a few more cases with low retinol values (23%) but no deficient individuals were observed. Paradoxically, retinol levels in the supplemented group had shifted toward lower values, thus increasing the number of subjects in the deficient (5%) and low (37%) categories.

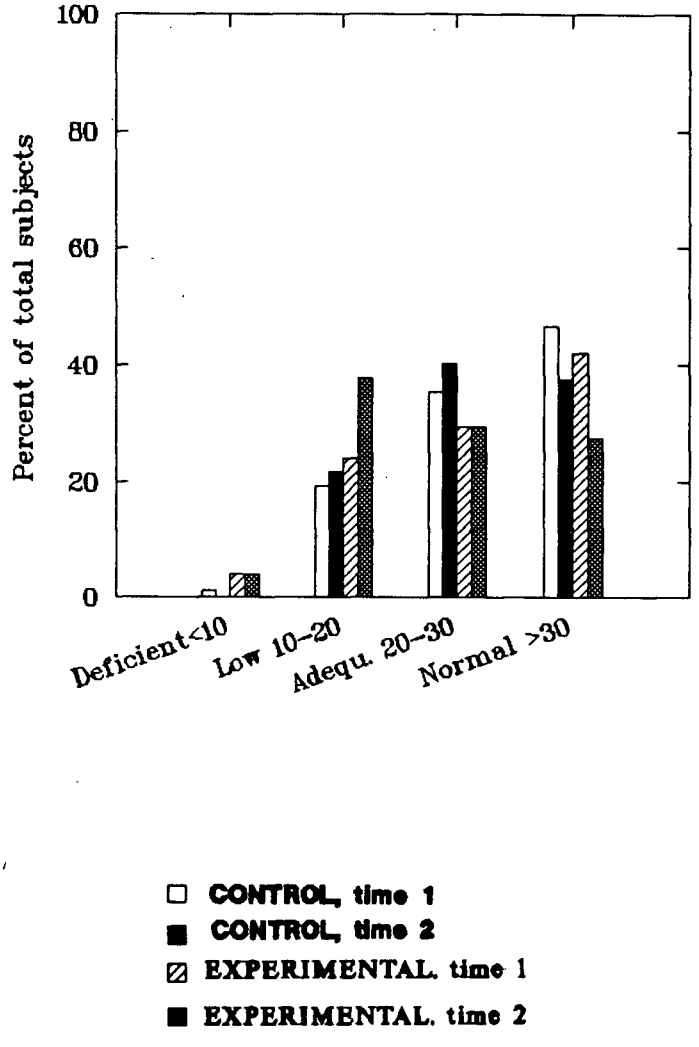


FIG. 10: Distribution of plasma vitamin A levels (μg/dl) during the study period

RESULTS

Because preschool children constitute the high risk group, the distribution of their plasma retinol values is shown in a separate graph (Fig. 11). Before the intervention started, 4.5% and 9% in control and experimental groups, respectively, had deficient vitamin A concentrations; and 36% and 50 %, respectively, belonged to the low category. It is noticeable that considerably more children of the control group compared to the experimental group (50% vs. 26%) had vitamin A concentrations between 20 μg and 30 μg/dl. Few preschoolers were observed with values above 30 μg/dl.

At the end of the study period, the number of control subjects in each category had changed little; preschoolers with deficient levels were no longer found. In contrast, vitamin A values of the experimental group shifted toward lower retinol levels, thus increasing the percentage of low (62%) and deficient (12%) subjects.

The mean plasma retinol values according to treatment, age and time are reported in Table 12. In general, plasma retinol increased with age. Over time, values of the control group remained more or less stable in all age categories, whereas vitamin A levels of all individuals in the experimental group decreased slightly.

The 2 (treatment) x 3 (age category) x 2 (time) ANOVA with repeated measures on the 3rd factor indicates that there was no significant treatment effect ($F=0.33$, $p=0.57$). This indicates that subjects from both groups had the same plasma retinol levels, when averaged over time and age (30 μg in control group vs. 27 μg in experimental group).

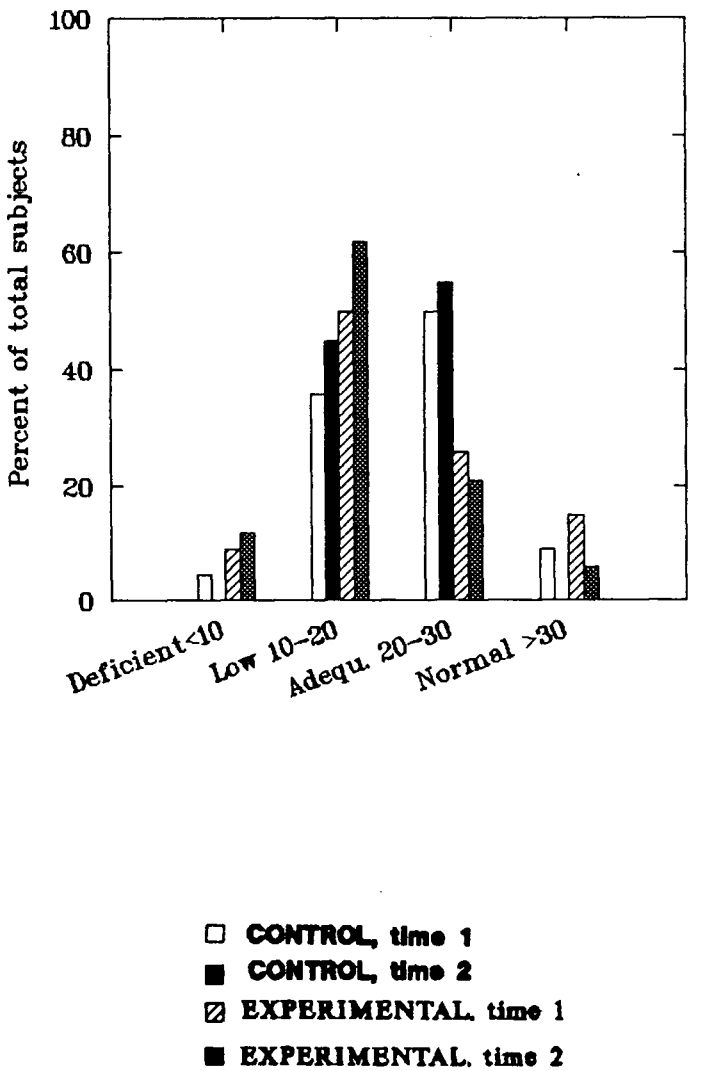


FIG. 11: Distribution of plasma vitamin A levels (μg/dl) among preschool children

TABLE 12
Results from 3 way ANOVA on plasma retinol level in control and experimental groups

	Age (years)	Plasma retinol level (μg/dl) Time 1 (Mean ± SD)	Time 2 (Mean ± SD)
Control Group	0 - 6 (n=22)	22 ± 6	21 ± 5
	6 -18 (n=23)	25 ± 8	25 ± 9
	> 18 (n=32)	39 ± 12	38 ± 11
Experimental Group	0 - 6 (n=36)	20 ± 9	17 ± 6
	6 -18 (n=28)	31 ± 14	26 ± 8
	> 18 (n=30)	38 ± 14	33 ± 13

3-Way ANOVA Summary

Effect	F (p)
Treatment (T)	N.S.
Age category (A)	51.1 (<.001)
Time Period (TP)	14.5 (<.001)
T x A	N.S.
T x TP	6.1 (0.015)
A x TP	N.S.
T x A x TP	N.S.

RESULTS

Averaged over treatment and time, significantly different retinol levels were observed in the three age categories. The post-hoc contrasts showed that adults had higher plasma vitamin A values (37 μg/dl) than children aged 0-6 years (19.6 μg/dl) and 6-18 years (26.9 μg/dl).

Averaged over treatment and age, mean plasma retinol levels were significantly higher at baseline (29.4 μg/dl) than four months later (27 μg/dl). The significant interaction of T x TP indicates, however, that these changes over time were different for the two groups. While plasma vitamin A values of the control group were stable (30 μg/dl vs.29 μg/dl) throughout the study period, those of the experimental group dropped from 29 μg/dl to 25 μg/dl.

Plasma β-Carotene Level

As shown in Fig. 12, the majority of individuals from the two groups had plasma β-carotene levels in the deficient range (<20 μg/dl) at time 1. Although this distribution shifted toward slightly higher values in both participating groups at time 2, most subjects could still be classified as deficient, indicating inadequate intake of provitamin A.

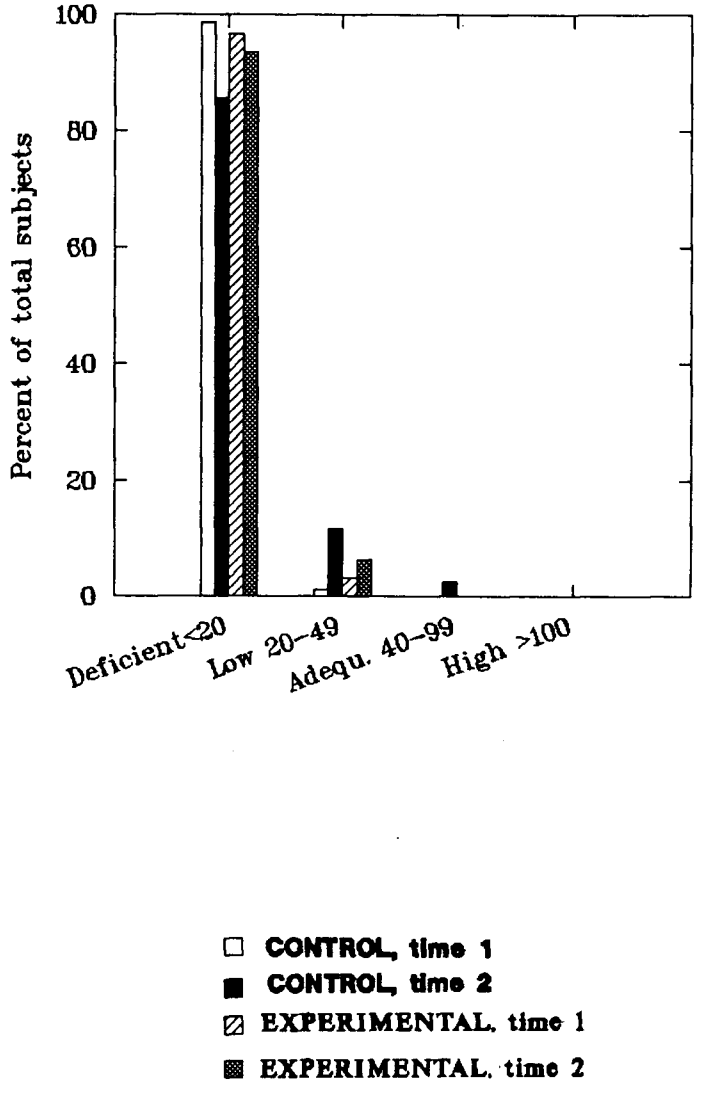


FIG. 12: Distribution of plasma β-carotene levels

RESULTS

Results from the 3 way ANOVA on plasma β-carotene levels are reported in Table 13. The significant treatment effect indicates that participants of the control group had a significantly higher mean plasma β-carotene level (10.8 μg/dl) than supplemented subjects (8.6 μg/dl), when averaged over age and time.

Similar to plasma retinol, an age effect was found. Averaged over time and treatment, mean plasma β-carotene levels were significantly different for subjects in the three age categories. Post hoc comparisons showed that children in preschool age had lower plasma β-carotene values (7 μg/dl) than older individuals (12 μg/dl and 11 μg/dl, respectively).

The significant main effect of time period reflects the increase in mean plasma β-carotene values from time 1 to time 2. Averaged over age category and treatment, a significant overall improvement in serum β-carotene values from baseline (7.8 μg/dl) to the end of the study perid (11.5 μg/dl) was revealed.

The significant interaction of T x TP shows, however, that the improvement was greater in the control (+ 5 μg/dl) than in the experimental group (+ 2.5 μg/dl). Furthermore, with regard to the various age categories, the greatest increase in plasma β-carotene levels over time was noted among 6-18 year old subjects (+ 5.9 μg/dl). Those individuals below 6 and above 18 years showed smaller but similar increases (+ 2.2 μg/dl and + 2.5 μg/dl, respectively). These changes were indicated by the significant A x TP interaction.

TABLE 13
Results from 3 way ANOVA on plasma ß-carotene levels in control and experimental groups

	Age (years)	Plasma ß carotene level (µg/dl) Time 1 (Mean ± SD)	Time 2 (Mean ± SD)
Control Group	0 - 6 (n=22)	6 ± 4	10 ± 5
	6 -18 (n=23)	8 ± 4	17 ± 13
	> 18 (n=32)	10 ± 5	13 ± 6
Experimental Group	0 - 6 (n=36)	5 ± 4	6 ± 5
	6 -18 (n=28)	9 ± 6	14 ± 7
	> 18 (n=30)	9 ± 7	11 ± 7

3-Way ANOVA Summary

Effect	F (p)
Treatment (T)	4.1 (0.043)
Age category (A)	14.3 (<0.001)
Time Period (TP)	53.1 (<0.001)
T x A	N.S.
T x TP	7.5 (0.007)
A x TP	7.3 (<0.001)
T x A x TP	N.S.

RESULTS

Anthropometric Assessment

Anthropometric Status of Adults

Mean values (average of time 1 and time 2) of weight, height, mid-upper arm circumference (MUAC), triceps skinfold (TSF) thickness and mid-upper arm muscle circumference (MUAMC) for male and female adults in control and experimental group are shown in Table 14. There was no significant change in any of those parameters over time, therefore, the measurements at the start and at the end of the study were averaged.

Anthropometric measurements were compared to National Centre for Health Statistics (NCHS) standards (HANES I and II data) (NCHS 1979, 1987), since local Brazilian standards were not available. MUAMC percentiles were not reported in the HANES II survey and the data from HANES I survey were used (Frisancho 1981).

Results indicated that the mean values of most anthropometric measurements for adults in the study population were **below** the NCHS's median. Only measurements for TSF thickness of men in the control group and for MUAMC of females in the experimental group as well as weight for height index of female participants from the control group were **above** the North American 50th percentile.

No great differences in the percentile values between control and experimental group were observed.

Weight, MUAC, MUAMC and weight for height percentiles seemed to be generally lower in men than in women, indicating a slightly better nutritional status of female subjects. MUAC and MUAMC measurements for men of both groups were below the 5th percentile, implying that some men probably were malnourished and with little or no protein reserves. In contrast, male individuals appeared to have relatively greater fat stores as indicated by higher TSF percentile values.

TABLE 14
Anthropometric measurements of adults in the study population

	Sex	N	Control (Mean ± SD)[1]	NCHS %ile	N	Experimental (Mean ± SD)[1]	NCHS %ile
Weight (kg)[2]	M	10	66.4 ± 11.8	18th	10	58.5 ± 7.2	<5th
	F	22	54.6 ± 10.1	26th	20	53.3 ± 7.4	22nd
Height (cm)[2]	M	10	170.5± 10.3	17th	10	167.0 ± 7.4	8th
	F	22	154.0± 6.9	7th	20	155.5 ± 5.8	12th
MUAC (cm)[2]	M	6	27.0 ± 2.1	<5th	10	26.7 ± 2.1	<5th
	F	22	26.4 ± 3.7	27th	19	25.8 ± 1.7	21st
TSF (mm)[2]	M	6	13.1 ± 11.2	58th	10	9.8 ± 7.7	39th
	F	22	15.4 ± 7	21st	19	15.8 ± 4.7	22nd
MUAMC (cm)[3]	M	6	22.9 ± 1.7	<5th	10	23.7 ± 2	<5th
	F	22	21.6 ± 2.4	56th	19	20.8 ± 0.9	42nd
Weight for Height[3]	M	10		16th	10		11th
	F	22		58th	22		41st

[1] Mean ± SD; values were averaged over time.
[2] NCHS, Anthropometric Reference Data (HANES II), 1987.
[3] NCHS, Anthropometric Reference Data (HANES I), 1979

RESULTS

Anthropometric Status of Children

Among preschool children, significant increases in weight and height were observed during the intervention period. When averaged over group and sex, mean weight increased from 12.4 kg at baseline to 13 kg four months later ($F=28.7$, $p<0.001$) and mean height increased from 87.1 cm to 89.7 cm, respectively ($F=170.0$, $p<0.001$). However, there was no significant difference in the growth rate between control and experimental groups.

Comparison of children's anthropometric measurements with NCHS standards is presented in Table 15. NCHS standards were derived from US (HANES II) data; weight for height index was not available from this survey and that of HANES I was used. Where possible, these measurements were also compared to Brazilian standards (Marcondes 1979, 1983) (Table 16). Marcondes' standards for Brazil were derived from data obtained primarily from low socio-economic populations. Comparing Marcondes' percentiles with those of NCHS, lower values were generally observed. Marcondes' standards were only available for the age of 0-12 years.

Results are reported in Table 15 and 16. Individual values of subjects, age 0-18 years, from control and experimental group were plotted graphically, using NCHS growth charts (Appendices 9A-9G).

TABLE 15
Comparison of children's anthropometric measurements with NCHS standards

	Group	Sex	Percentiles ≤5th (%)	>5th≤50th (%)	>50th≤95th (%)	>95th (%)
Weight	C	M	27	65	8	-
		F	16	56	25	3
	E	M	41	30	27	3
		F	41	38	21	-
Height	C	M	38	42	19	-
		F	31	47	19	3
	E	M	32	49	19	-
		F	38	46	13	3
Weight for Height	C	M	25	29	42	4
		F	-	32	50	18
	E	M	9	36	52	3
		F	9	45	40	6
MUAC	C	M	24	64	12	-
		F	21	58	13	3
	E	M	31	46	3	3
		F	33	51	13	3
TSF	C	M	8	80	8	4
		F	6	60	33	-
	E	M	17	60	23	-
		F	21	62	18	-
MUAMC	C	M	48	32	16	4
		F	52	30	9	9
	E	M	51	20	9	20
		F	51	26	21	3
Headcir-cumference	C	M	11	44	22	22
		F	20	40	40	-
	E	M	30	20	50	-
		F	21	50	21	7

TABLE 16
Comparison of children's anthropometric measurements with Brazilian standards

			Percentiles			
	Group	Sex	≤5th (%)	>5th≤50th (%)	>50th≤95th (%)	>95th (%)
Weight	Control	M	17	74	9	-
		F	4	68	20	8
	Experim.	M	32	42	23	3
		F	28	61	8	3
Height	Control	M	17	65	13	4
		F	21	54	17	13
	Experim.	M	29	39	26	6
		F	25	53	22	-
Headcir-cumference	Control	M	11	22	44	22
		F	20	40	40	-
	Experim.	M	20	20	40	20
		F	14	57	14	14

RESULTS

1. Weight for Age

For the majority of boys in control and experimental groups (92% and 71%, respectively), weights for age were close to or below NCHS's median (50th percentile) (Table 14, Appendix 9A), implying that a considerable number of boys were underweight for their age. Furthermore, 27% of the boys in the control group and 41% in the experimental group even had weights for age below the 5th NCHS percentile. The same was found for girls; 72% of the control group and 79% of the experimental group weighed less than the NCHS's median. Fewer girls in the control group (16%) than in the experimental group (41%) were found with a weight for age below the 5th NCHS percentile.

Comparing weights for age to Brazilian standards (Table 16), generally fewer boys and girls of both groups had weights below the 5th percentile. For the majority of children weights for age were above Marcondes' 5th and below or close to the 50th percentile.

Although weight for age results indicated some children were underweight, weight for age index does not consider children who, because of a small frame size, may have normal weights. The percentage of children who may be actually classified as underweight might be less than indicated by the weight for age standard.

2. Height for Age

When height or length for age was compared to NCHS standards (Table 15, Appendix 9B), 38% of boys in the experimental group and 32% of the control group had height for age less than the NCHS's 5th percentile. Approximately the same percentage of girls (31% in control group and 38% in experimental group) had heights for age below the 5th NCHS percentile. A smaller number of subjects was found with heights below the Brazilian 5th percentile (Table 16).

Since height for age is an indicator of past nutritional state, these results indicated that stunting seemed to exist in a substantial number of those children. Poor nutrition was probably directly or indirectly involved in stunted growth.

3. Weight for Height

Weight for height or length index considers the weight of subjects in terms of body frame size, so individuals with a small frame but normal weight will not be classified as underweight or malnourished. This index, therefore, is more accurate than weight for age or height for age in describing the present nutritional state of children.

By comparing weight for height of children from both groups with North American standards (Table 15, Appendix 9C), it was confirmed that fewer boys (25% in control group, 9% in experimental group) and girls (none in control group, 9% in experimental group) had height for weight values below the NCHS's 5th percentile.

Furthermore, when weight for height index was used instead of weight for age, the number of cases below the NCHS's median was decreased by 32-56% and more individuals were found above the 50th percentile. No Brazilian standards were available for comparison. Although some children were stunted in growth, the majority appeared to have satisfactory weights for body size.

4. MUAC, TSF Thickness and MUAMC

For these three parameters no Brazilian standards were available for comparison.

MUAC has been used to identify subjects who are malnourished. Results of this study indicated that the majority of boys (88% in control and 77% in experimental group) and girls (79% and 84%, respectively) had values below NCHS's median values. Almost one quarter of children in

the control group and one-third in the experimental group seemed to be malnourished as their MUAC measurements were below the 5th percentile (Table 15, Appendix 9D).

TSF thickness measurements are used as an indication of energy reserves in the form of fat. TSF values less than NCHS's 50th percentile were observed in 88% of boys in the control and 77% in the experimental group; 66% and 83% of the girls, respectively, showed similar low energy stores. More children in the experimental group (17% of the boys and 21% of the girls) than in the control group (8% and 6%, respectively) were found with TSF thickness measurements below the 5th percentile (Table 15, Appendix 9F).

Children's MUAMC values were calculated from TSF thickness and MUAC in order to determine adequate muscle development, which gives an indication of the amount of protein reserve. When MUAMC values were compared to North American standards, most values for boys and girls of both groups fell below the 50th percentile (boys: 80% in control and 71% in experimental group; girls: 82% and 77%, respectively). Half of the individuals in the two groups had values near or below the 5th percentile, implying that muscle development was poor (Table 15, Appendix 9E).

5. Head Circumference

Head growth occurs most rapidly during the first years of life. Therefore, the use of head circumference measurements in children is a sensitive indicator of current malnutrition.

Head circumference of children less than three years of age was compared to North American (Table 15, Appendix 9G) and Brazilian standards (Table 16). More girls (60% in control and 71 % in experimental group) than boys (55% and 50%, respectively) had values below the NCHS's median. Eleven percent of boys in the control and 30% in the experimental group and 20% of girls in both groups had measurements even below the NCHS's 5th percentile.

When these measurements were compared to the Brazilian standards, only the number of boys with head circumference values below the 50th percentile decreased; the same percentage of girls was found below the median.

These results indicate that some young children were probably not growing adequately due to poor nutrition.

Morbidity

Since a decrease in the incidence or severity of diarrhea and respiratory disease is among the potential outcomes of vitamin A supplementation (Fig. 1), morbidity data of the participating children were collected.

The frequency of diarrhea and respiratory disease among children in control and experimental groups is presented in Table 17. Boys and girls of both groups had the same incidence of diarrhea, fever, coughing and sore throat. A significantly higher number of children in the experimental group experienced a running nose during the time of supplementation. No difference was found in the total morbidity load of children in the control and experimental groups.

These findings suggest that both groups were comparable in nutritional, biochemical and health status before the intervention.

TABLE 17
Frequency of diarrhea and respiratory disease among children in the control and experimental groups

	Frequency[1]	
Incidence over 4-month period	Control	Experimental
Fever[2]	15.5	16.8
Diarrhea[2]	16.3	14.6
Coughing[2]	33.3	39.1
Sore Throat[2]	18.1	14.8
Running Nose[2]	42.4	51.6**
Total Morbidity Load[2]	25.1	27.4

[1] All values expressed as percent of total responses in the respective group

[2] Incidence of all symptoms combined over the 4 months period

** $p<0.01$, control group vs. experimental group

V. DISCUSSION

In the present study, 57 families of low socio-economic status living in Vila Piratininga, a favela in Southern Brazil, were provided for four months with soybean oil that was either vitamin A fortified or unfortified. The purpose of the study was to assess the impact of vitamin A nutrified vegetable oil on the vitamin A status of this community. The impact was evaluated by the biochemical responses of control and experimental groups to this form of supplementation program.

Major findings were that:

1. vitamin A deficiency appeared to be a public health problem among this low income population;

2. the consumption of the nutrified oil increased the vitamin A intake of the experimental group by 500%; and

3. the plasma retinol levels of participants receiving vitamin A dropped significantly during the intervention period, indicating that the nutrification of soybean oil was not effective in improving the vitamin A status of the study population.

In the following discussion, the major findings of this project will be addressed under the following sections: 1. Socio-economic considerations; 2. General health status; 3. General vitamin A and nutritional status at baseline; and 4. Impact of nutrification of soybean oil with vitamin A on vitamin A intake, plasma vitamin A level, β-carotene level and nutrient intake.

V. DISCUSSION

1. SOCIO-ECONOMIC CONSIDERATIONS

Vitamin A deficiency is a major nutritional problem in less industrialized parts of the world (Sommer & West, 1987). Increased vitamin A intake usually occurs with economic development, as vitamin A availability appears to be closely associated with, for example Gross National Product (DeMaeyer 1986). Although Brazil is considered the most industrialized of developing nations, the rapid economic growth during the 1960's and 1970's has initiated a massive rural-urban migration (particularly in the state of Sao Paulo), has increased regional disparity and has widened the gap between rich and poor even more (Hewlett, 1980). Economic benefits did not "trickle down" to the low socio-economic classes as expected. Instead Brazil's massive foreign debt load of more than US $140 billion and an inflation rate of over 1,000% in 1988 have resulted in currency devaluation, severe wage increase restrictions and high unemployment (Veja, 1989). These stringent economic measures have compounded the problems of low socio-economic groups, such as the study population, providing less available income.

In Vila Piratininga, the income of the participants was extremely low. Although the employment rate was high, literacy rate and years of schooling among favela residents were low. Consequently, all of the wage earners had jobs as unskilled workers with incomes below the minimum wage providing barely enough money to feed the whole family. Children are still regarded as important contributors to family income. This is probably also one of the reasons why less than half of the school-aged boys and girls among the study population were attending school. With a poor educational background in later life, these children will most likely have similar jobs and socio-economic status as their parents, perpetuating the cycle.

Previous studies in Brazil reported that low income is a major factor which severely limits people's ability to obtain an adequate diet (Jansen et al. 1977, Campino 1986). This factor was also apparent among the study population. Stores in Vila Piratininga offered little selection of fruits and

vegetables, and when fresh produce was available, it was relatively expensive. The time and expense of travelling to the city centre, where a greater variety of food was available, precluded families from purchasing these items even if they were affordable. Hence, most people relied on cheap staples like white rice, beans, white bread, soybean oil, coffee and sugar to make up their diet, which provide little or no vitamin A.

2. GENERAL HEALTH STATUS

The poor living conditions observed in this and other studies of Brazilian favela residents (Swann 1979, Waddell 1981, Doell 1984) may have contributed to poor general health status of the population. In Vila Piratininga, housing was found to be crowded and dirty, and sewage facilities were non existent. As will be described, this unhygienic environment likely affects nutritional and vitamin A status, especially in children. Previous studies (Angeleli 1978, Desai et al. 1980) in similar populations have shown that many adults and children suffered from parasitic infestation. Although not tested for in the present study, several participating mothers commented on this problem among their children. The existence of health problems such as bronchial infections and parasitic infestation was not surprising given the crowded and unsanitary conditions these people lived in.

Morbidity data revealed that diarrhea and particularly respiratory diseases were a chronic problem among all participating children. In the presence of respiratory tract and other infections, vitamin A absorption is impaired, although the mechanism is unclear (WHO 1982). Plasma vitamin A concentrations may be affected by a variety of extraneous factors, which are independent of the size of vitamin A stores in the liver. Infections and parasitic infestation are known to lower plasma retinol levels (Gibson 1990). Hence, the high incidence of respiratory disease coupled with parasitic infestation are factors which probably also greatly aggravate any nutritional deficiencies existing among participating children.

V. DISCUSSION

In summary, socio-economic and ecological assessment yielded evidence that impoverished living conditions, low incomes and the presence of infections were factors that the participants, especially children, had to contend with in their daily lives. These factors synergistically influenced and aggravated any existing nutritional problems, and may have had a particular impact on vitamin A status by impairing vitamin A absorption and utilization and increasing vitamin A requirements.

3. GENERAL VITAMIN A AND NUTRITIONAL STATUS AT BASELINE

Dietary Assessment

A diet consisting primarily of rice, beans and coffee with sugar, as consumed by Vila Piratininga residents, would not be expected to contain sufficient vitamin A and all other nutrients in adequate amounts (Swann 1979, Waddell 1981, Doell 1984). Although a wide variety of fruits and vegetables with high carotenoid content was abundantly available all year around in Ribeirao Preto, only a few participating families included these foods in their diet. The majority of female household heads in both groups indicated in the food frequency questionnaire that they rarely or never consumed mangoes, peas, carrots, spinach, wild chicory (rucula) or similar vitamin A precursor containing foods. These food items were relatively expensive and most participants could not afford them.

With the limitations of the 24-hour recall method in mind (Chapter II), results of baseline dietary assessment of the study population will be discussed. Only the female household heads were questioned about their food intake; however, information obtained on the qualitative aspects of the diet can be generalized to the whole population, since due to financial limitations, all family members usually ate the same foods.

V. DISCUSSION

In general, average daily intakes of most nutrients were below the FAO/WHO recommended intakes. However, failure to consume the recommended amounts cannot necessarily be interpreted as a dietary deficiency (Smiciklas-Wright & Guthrie 1984). Many studies have selected two-thirds of the recommended levels (RNI) as the cut-off point for adequacy or inadequacy. Beaton (1975), on the other hand, suggested that intakes below the recommended amounts or below two-thirds of the recommended intakes indicate a greater probability of deficiency risk rather than inadequacy. For the purpose of this study, two-thirds of FAO/WHO recommended intakes was chosen as a rough indicator of low intake.

Energy and Nutrient Intake of Women at Baseline

At the beginning of the study, mean daily intake of the control group was the same as that of the experimental group for all nutrients examined, with the exception of vitamin C.

Energy intakes and overall quantities of food consumed have frequently been reported to be low among Brazilian populations of low socio-economic status (ICNND 1965, Shrimpton 1975, Doell 1984). The low mean energy intake of subjects in this study, which was approximately half of the FAO recommendations (Beaton & Patwardhan 1976), suggests that overall quantities of food consumed were probably low. Seventy-nine percent and 73% of control and experimental group members, respectively, did not meet two-thirds of FAO energy requirements. Considering, however, that the 24-hour recall method may slightly underestimate calorie intake (Gibson 1990) and that those surveyed may have under-reported in expectation of help, the apparent caloric deficiency may be less severe (Burk & Pao 1980). This assumption is supported by the anthropometric data and furthermore, the physical appearance of most women did not evoke the impression of malnutrition.

Energy intake is one of the factors that must be considered in determining the risk of protein deficiency. If energy intake is low, protein will be utilized for energy (Gibson 1990). Protein

status is an important consideration with regard to vitamin A status and vitamin A utilization, since a limited supply of protein substrate decreases the synthesis of retinol binding protein (RBP). This in turn will impair the hepatic release of vitamin A, resulting in decreased serum retinol levels (Gibson 1990).

In this study, **mean** daily protein intake was above the cutoff point and therefore, appeared to be adequate. According to Shrimpton (1975), the Brazilian rice and beans diet would meet both protein and energy requirements, if it were eaten in sufficient quantity. Thus, inadequate dietary protein did not appear to be a cause of the low serum retinol levels.

Among the other nutrients analysed, most severely lacking were vitamin A (which will be discussed separately later), calcium, iron, thiamin, riboflavin and niacin. Sixty-seven to one hundred percent of participants in both groups had intakes below two-thirds of FAO/WHO recommendations of the above nutrients.

Diet recalls of this study showed that the consumption of dairy products was very limited among adults, often no more than a few ounces of milk taken in coffee once or twice a day. Hence, subjects appear to be at some risk for calcium deficiency, especially during pregnancy and lactation when requirements are increased.

Iron was primarily obtained from non-heme sources such as beans and rice and may not have been readily available to the body. Therefore, mean estimated amounts of iron absorbed may be very low compared to FAO/WHO requirements.

Polished rice and white bread supplied a substantial amount of energy in the subjects' diet. Flour and cereals, however, are not enriched in Brazil after refining (Desai et al. 1980) and considerable amounts of B-vitamins may be lost during the commercial refining process.

V. DISCUSSION

Although the mean daily intake of vitamin C in the experimental group was 171% of the FAO/WHO RNI, this figure obscures the fact that while a few individuals consumed large amounts of vitamin C, over 60% had intakes less than two-thirds of the recommended intake.

Of all nutrients, mean daily vitamin E intake was closest to meeting FAO/WHO recommendations. It was mostly derived from rich sources such as oils and legumes.

This study thus confirms previous findings among low income Brazilian population groups, where low or deficient intakes of the same nutrients and minerals were found. The levels of calcium, iron, vitamin A and B vitamins described by Doell (1984) were similar to the amounts observed in the present study. Deficient calcium, iron, riboflavin, niacin, vitamin A and C levels in the diet of migrant workers were reported by Swann (1979) carrying out another study in Ribeirao Preto. A survey in the south of Sao Paulo state (Miguel & Bon 1974) found that intakes of nearly all nutrients were deficient. Calcium, riboflavin, vitamin A and vitamin C intakes were particularly low, although deficiencies were not as severe as observed in this study. Hence, the same nutrients have been repeatedly reported as those most lacking in the diet.

In summary, the very monotonous diet with a limited consumption of dairy products, fruits, vegetables and animal protein sources and the resultant low intakes of many nutrients should put the study population at high risk for multiple nutrient deficiencies.

Estimated Nutrient Intakes of Children at Baseline

The 24-hour recall with eight boys and girls of the study population served as an approximate estimation of children's intakes. It should be mentioned, however, that the eight children questioned in our study were not randomly selected. They may not have been representative of the entire study population, since only those who were willing and able to were interviewed.

V. DISCUSSION

When their mean nutrient intakes were compared to women's intakes, the children's diet seemed to be more satisfactory, with most nutrients meeting the FAO/WHO requirements. Only the intake of thiamin was below two-thirds of the dietary standard. A possible explanation for the observed differences may be that children were eager to talk about what they had eaten and were more likely to answer truthfully than adults (Frank et al. 1977), who may have under-reported their daily intakes in the expectation of help. It is also possible that in order to leave more food available for their children, mothers ate smaller meals.

A very high mean daily protein intake of almost two and half times the FAO/WHO requirement was found. This protein was mainly derived from milk, since families with more than three small children received free coupons for one litre of milk per day (or more, depending on the number of children) from the government. Milk in Brazil is fortified with vitamin A, containing an average of 42 μg/100g and 3.7% fat (Jacob Ferreira, USP, personal communication).

Protein and energy intakes of Brazilian children in other studies (Waddell 1981, Doell 1984, Desai et al. 1980) were reported to be adequate, but the protein level was never as high as in the present study. With regard to other nutrients, the overall quality of food consumed by children in this study appeared to be better than that of previously surveyed children, where evidence of deficient nutrient intakes was found.

Vitamin A Intake at Baseline

Estimated mean daily vitamin A intakes from natural food sources were very low in both groups (Table 5), with 77% to 92% of women in control and experimental groups (Fig. 9) not meeting two-thirds of FAO/WHO recommended intakes (FAO/WHO, 1988). Although the general food consumption may have been under-reported, vitamin A was one of the nutrients most severely lacking in their diet. This was expected because of infrequent use of vitamin A-rich animal foods

such as organ meats, milk and milk products and margarine fortified with vitamin A. Dietary vitamin A was mainly derived from plant foods but the intake of rich carotene sources was limited to small amounts of tomatoes, wild chicoree leaves and occasional use of squash, spinach or carrots. Among the plant carotenoids, β-carotene has the highest biologic activity and is the most abundant in food (Takahashi 1984).

It is important to consider that the values used to compute vitamin A intake from the 24-hour recalls actually may overestimate its availability. Recent developments in high pressure liquid chromatography methods have shown that the food analysis techniques from which reported vitamin A values were derived, generally overestimate vitamin A levels in many fruits and vegetables (Roidt 1988).

The amounts of vitamin A consumed by participants of this study were considerably lower than those described by Doell (1984) but similar to the levels reported by Swann (1979) among Brazilian migrant workers living in the peripheral favelas of Ribeirao Preto. Roncada (1972, 1981, 1984), surveying various towns of Sao Paulo state, also found deficient vitamin A intakes among low socio-economic groups. He observed that just over 30% of the vitamin A activity came from the actual vitamin and almost 50% from the precursor β-carotene.

Vitamin A was the nutrient most severely lacking in the children's diet. Similar results were reported by Fávaro et al. (1986) among poor Ribeirao Preto children. She found that the intake of vitamin A appeared to be adequate for children two to four years of age but then deteriorated to insufficient levels with increasing age.

Thus, considering that the consumption of vitamin A rich foods among Vila Piratininga residents ocurred infrequently, vitamin A liver reserves were likely low or exhausted.

V. DISCUSSION

Anthropometric Assessment

Anthropometric Status of Adults

According to anthropometric data, male adults tended to be more underweight than their female counterparts. In comparison to North American standards (NCHS 1979), mean weights for heights of male subjects were at the 16th and 11th percentiles for control and experimental groups, respectively. Jelliffe (1966) stated that low body weights for heights are principally a reflection of body thinness due to subnormal amounts of subcutaneous fat and muscle, the result either of poor development or tissue wasting or a combination of both. Considering that mean values for mid-upper-arm circumference (MUAC) (<5th percentile) and mid-upper-arm muscle circumference (MUAMC) (<5th percentile) measurements were extremely low, but values for triceps skinfold (TSF) thickness were close to average, it can be concluded that body thinness was primarily due to subnormal amounts of muscle rather than fat.

In contrast, weights for heights among female subjects of both groups were close to the NCHS's median, suggesting a more satisfactory nutritional status. These findings, however, are not in agreement with dietary data indicating insufficient energy intake. As already mentioned earlier, women could have under-reported their food consumption in the 24-hour recall interview.

Energy reserves of women, as indicated by mean absolute values for TSF thickness, were generally higher than in men but when compared to NCHS standards, percentiles were lower. The close to average MUAMC values of both groups suggest that female subjects had more adequate protein reserves than men did.

The observed differences between men and women in weight for height could be explained by the higher level of activity in male subjects. They were doing long hours of physical labor while women remained at home, leading a more sedentary lifestyle.

V. DISCUSSION

Anthropometric Status in Children

It has been proposed that improved growth may be a possible outcome of vitamin A supplementation (NRC 1987). The duration of this study, however, was likely too short to detect any differential effects on growth of supplemented and unsupplemented children. West and coworkers (1988) suggested a supplementation period of more than twelve months may be required to detect an effect on linear growth of marginally deficient children.

Poor growth was evident in Vila Piratininga children when comparisons were made to either Brazilian (Marcondes 1979, 1983) or North American (NCHS 1979, 1987, Frisancho 1981) standards, although smaller proportions were classified as undernourished (<5th percentile) when anthropometric measurements were compared to the local Brazilian reference. While the use of local anthropometric reference standards is desirable, it should be noted that Brazilian standards for height, weight and head circumference were derived from a study of children of low socio-economic status (Marcondes 1971). Jelliffe (1966) has cautioned that the use of such standards in developing countries is deceiving because they provide a picture of development achieved by children who are probably nutritionally compromised. Standards ideally should be developed using healthy subjects from upper socio-economic groups who have had the opportunity to consume adequate diets (Jelliffe 1966). The WHO has recommended the NCHS reference growth data as an international standard (Gibson 1990).

Comparisons with NCHS standards suggest that many subjects of this investigation were underweight for their age. According to the weights for heights, however, the nutritional status of most boys and girls appeared to be more satisfactory and fewer children could be considered acutely malnourished. But this does not necessarily indicate that optimal growth was achieved, since the heights for age of the children reflect that some stunting had occurred. Stunting has been described as the slowing of skeletal growth and stature (Gibson 1990). This condition, which results from

extended periods of inadequate food intake and increased morbidity, was found in over one-third of the boys and girls. The prevalence of stunting is the highest during the second or third year of life (WHO 1986). Vitamin A deficiency, which is also most prevalent among preschool children, may play a role in this process, since growth failure is one of its early signs (West 1988, Gibson 1990).

Arroyave and colleagues (1979a) observed that vitamin A deficient Guatemalan children had lower heights for age than children with an adequate vitamin A status. A field trial in Indonesia demonstrated increased linear growth in children receiving vitamin A-fortified monosodium glutamate (MSG) (Muhilal et al. 1988b). Study conditions in Vila Piratininga, however, do not rule out the possibility that the observed linear growth stunting among participating children is caused by multiple nutritional deficiencies and repeated infections rather than by vitamin A deficiency alone.

A large number of children had TSF thickness, MUAC and MUAMC values below the 5th percentile. These findings indicate that undernutrition was severe enough to prevent accumulation of fat stores and to cause muscle wasting. The very low protein stores among half of the participating boys and girls are not consistent with the dietary data. A possible reason may be that children who were willing to provide dietary data, were not representative of the study population.

Chronic malnutrition during the first few months of life, or intrauterine growth retardation, may decrease the number of brain cells and result in an abnormally low head circumference (Gibson 1990). Low values of many Vila Piratininga children indicate that some nutritional deprivation was or is occurring.

The results of this study suggest that both groups were comparable in anthropometric status. It appears that children had adequate weights for heights; however, undernutrition seemed to be manifested in low body weights for age, stunted growth and low fat and protein reserves.

V. DISCUSSION

Biochemical Vitamin A Status

Plasma Retinol

Plasma or serum vitamin A, because of the homeostatic control exerted by the liver, is not a good general indicator of total body stores. Nonetheless, low serum vitamin A values (< 20 μg/dl) in a **population**, in which habitual intakes of vitamin A are low, are suggestive of a depleted state, with or without the presence of clinical signs of deficiency (Arroyave, 1982). When the marginal vitamin A status of Brazilian children was assessed using a more sensitive indicator, the relative dose response test (RDR), a serum vitamin A level below 20 μg/dl was invariably associated with a positive response (Flores et al. 1984).

In the present study, 21% of subjects in the control group and 28% in the experimental group had low (<20 μg/dl) or deficient (<10 μg/dl) plasma retinol concentrations (Fig. 10) indicating marginal or partially depleted body reserves of vitamin A. Although preschool children are usually the most vulnerable group, in populations like this one, where the diet is generally poor in vitamin A, other subgroups may also be deficient. The observed low vitamin A intakes could put pregnant and lactating women with increased vitamin A requirements at risk. As a consequence, newborns have poor liver reserves, the breast milk has a low vitamin A content and breast-fed infants have a poor vitamin A nutriture. Such an inadequate vitamin A status in early life was revealed by the low mean serum vitamin A concentrations (19.6 μg/dl) of young children.

The significantly higher mean plasma retinol levels (37 μg/dl) (Table 10) in adults compared to preschool and schoolage children suggested that vitamin A deficiency was not as great a problem among the adult population. According to the guidelines of the International Vitamin A Consultative Group (IVACG) (Arroyave 1982), a daily dietary vitamin A intake of at least 400 μg is necessary to maintain an adequate vitamin A status. The results of the present study, however,

suggest that normal plasma retinol levels may be maintained with lower intakes of vitamin A (217 μg and 249 μg among women in control and experimental groups, respectively). It is possible that adult subjects can utilize the available vitamin A with high efficiency due to a process of adaptation to relatively low intakes (Bates et al. 1983).

Mean retinol levels of six to eighteen year olds were in the adequate range. This was expected since in this age group, growth rate has decreased, infectious diseases are less frequent and the diet has become a bit more varied (McLaren 1986).

When the prevalence of plasma or serum vitamin A levels below 10 μg/dl in preschool children is greater than 5%, or when more than 15% have plasma levels below 20 μg/dl, WHO and the IVACG (WHO 1982, Arroyave 1982) consider that there is a deficiency problem at the public health level. The findings of this study revealed that 4% of the preschool age children in the control group and 9% in the experimental group had deficient vitamin A levels (<10 μg/dl) and 41% and 59%, respectively, had low plasma retinol concentrations (<20 μg/dl) (Fig. 11). Consequently, the preschool children in this study population could definitely be regarded as at risk with respect to vitamin A deficiency. Although not examined in this study, this condition may or may not have included clinical evidence of deficiency such as xerophthalmia.

Previous investigations have yielded similar results (Fávaro et al. 1986, Roncada et al. 1984, 1981 and 1978, Wilson et al. 1981). Plasma vitamin A levels and clinical examinations in these studies showed that hypovitaminosis A poses a widespread public health problem among Brazilian population groups of low socio-economic status in the state of Sao Paulo, Southern Brazil.

Plasma β-Carotene

A fairly reliable indicator of the nutritional intake of provitamin A is the plasma level of β-carotene. Over 90% of the population examined had deficient plasma β-carotene concentrations (<20

μg/dl) (ICNND 1965) (Fig. 12). This reflects recent low dietary intakes of carotene-rich green, orange and yellow vegetables and fruits, and was predicted by the results of the 24-hour recall and food frequency questionnaire. When such low plasma β-carotene levels are found in association with low plasma retinol concentrations, the evidence for inadequate vitamin A nutriture is strong (Arroyave 1982).

Data of the current baseline assessment confirmed findings of other Brazilian surveys (Fávaro et al. 1986, Roncada et al. 1984) where similar insufficient vitamin A intakes and low serum vitamin A and β-carotene values were reported.

4. IMPACT OF NUTRIFICATION OF SOYBEAN OIL WITH VITAMIN A

Vitamin A Intake

Dietary vitamin A intake from natural food sources did not change significantly in either group throughout the study period . However, the nutrified soybean oil increased the dietary vitamin A intake of the experimental group significantly (Table 6). The fortified oil added an average of 1096 μg of vitamin A to the subjects' daily diet and tripled the retinol equivalents provided by natural food sources. This total vitamin A intake is equivalent to more than three times the RNI suggested by the FAO/WHO for adult women (FAO/WHO 1988).

The dietary intake of preschool children (the high risk target group) was not assessed in this study. Because the financial situation of the families did not allow them to buy special foods for children, they usually ate smaller quantities of the same foods as their mothers or they were breast-fed. Either way, these young children likely received additional vitamin A supplied through the oil, although smaller amounts than adults and older siblings.

V. DISCUSSION

Plasma Vitamin A Level

The increased total vitamin A intake of all family members in the experimental group was not reflected in the plasma retinol concentrations. In contrast to the expected increase, mean plasma vitamin A levels of the experimental group dropped significantly during the intervention period in all age groups (Table 12). Although the overall decrease of plasma retinol values from baseline (29 μg/dl) to four months later (25 μg/dl) was **statistically** significant, such a small change, as long as values fall in the normal range, may not be regarded as **nutritionally** significant (Olson 1984).

As already indicated earlier, preschoolers were the most vulnerable agegroup and also consumed the lowest amounts of oil among the family. This is the reason why the plasma retinol levels of this agegroup can be regarded as a good indicator for evaluating the impact of a nutrification program (Arroyave et al. 1979a). Considering the findings of the present study, the nutrification of soybean oil with retinyl palmitate was not effective in improving the vitamin A status of the high risk target population. In actuality, four months after introduction of fortified oil, more subjects in the experimental group, principally preschool age children, had deficient and low plasma retinol values than at baseline (Fig. 10 and 11). In contrast, values in the control group remained unchanged suggesting that the observed changes were not related to extraneous factors such as seasonality.

It can be speculated why the vitamin A status of supplemented individuals did not improve during the intervention, although the observed drop in their serum vitamin A levels cannot be readily explained. Possible reasons for the failure of the nutrification program to raise serum retinol levels include: length of the study period; use of soybean oil as the fortification vehicle; serum retinol as an indicator of vitamin A status; concurrent infection; general nutritional status; nutrient interrelationships; estimated oil intake and vitamin A analyses. Evidence for each of these factors will be discussed below.

V. DISCUSSION

Length of Study Period

The impact of the additional dietary vitamin A could have been limited by the comparatively short duration of the present intervention (four months). Previous fortification studies in Brazil (Araujo et al. 1978), Guatemala (Arroyave et al. 1979a), the Philippines (Solon et al. 1979) and Indonesia (Muhilal et al. 1988ab) used a supplementation period ranging from eleven months to two years. These studies demonstrated an improvement in vitamin A status, indicating that the vitamin A supplement reached the neediest segments of the population. Olson (1987) suggested that the full recovery of the complex homeostatic system for the control of plasma vitamin A values is slow in depleted subjects and usually requires either a long period of moderate doses or single large doses. It appears, however, that the length of the study period may have had only a minor influence on the outcome, since the serum vitamin A levels of preschool Indonesian children improved significantly five months after introduction of an additional 210 μg of vitamin A to their daily diet through fortified MSG (Muhilal et al. 1988a).

Use of Soybean Oil as a Fortification Vehicle

The foods successfully used in previous trials were sugar and MSG. In the present pilot study, the feasibility of soybean oil as a vehicle for nutrification was tested. In Brazil, margarine is commercially fortified with vitamin A, however, participants could not afford to buy this kind of dietary fat, since it is twice the price of soybean oil (Desai et al. 1988b).

To ensure the desired level of supplementation, aliquots of the commercially nutrified oil were taken throughout the intervention and analysed for their vitamin A content. Results of this analysis showed the oil contained an average of 93 IU/g, very close to the intended level of 100 IU/g.

It might have been possible that the amount of vitamin A actually absorbed was considerably lower than was determined by the 24-hour recall and the per capita oil consumption.

V. DISCUSSION

Since the main use of the supplied soybean oil was for frying and cooking of food, a considerable amount of vitamin A in the oil may have been destroyed by heat, oxidation and/or exposure to light (Bauernfeind 1983). Furthermore, considering the hot weather (38-45°C) and that many families did not have a fridge to store the left-overs, the retention of vitamin A in foods cooked with fortified oil could have been substantially decreased under field conditions.

Recent findings (Fávaro et al., unpublished data), however, do not support these assumptions. Vitamin A palmitate added to soybean oil (200 IU/g oil) was entirely stable for up to six months in open metal cans stored in light at room temperature. The retention of vitamin A at nine months of storage under similar conditions was 50% of the original whereas practically all of the vitamin A (99%) was retained when stored in sealed containers. Various trials involving cooking of rice and beans, the staple foods of the present study population, with vitamin A fortified soybean oil, indicated that retention of vitamin A in cooked rice was 99% and in beans 90% when cooked under pressure for one hour. Fifty-eight percent of vitamin A was still retained after use of nutrified soybean oil for four repeated fryings of potatoes in the same oil. Only after twelve repeated fryings was most of the vitamin A destroyed.

These results demonstrate that vitamin A palmitate added to soybean oil remains stable during commonly used storage and cooking procedures, suggesting that the failure to raise serum retinol levels of supplemented subjects cannot be explained by the choice of fortification vehicle. Therefore, the lack of response to supplementation is likely due to other factors.

Plasma Retinol as an Indicator of Vitamin A Status

The limitations of plasma retinol levels as an accurate indicator of vitamin A body reserves should be appreciated. Plasma or serum retinol values reflect vitamin A status only when liver reserves are severely depleted (<20 μg/dl) or excessively high (>300 μg/dl) (Olson 1984). Between

these limits, plasma retinol levels remain relatively constant and do not reflect possible changes in total body reserves of vitamin A (Olson 1984). This factor becomes important when the response to vitamin A supplementation in a population with marginal vitamin A status, as observed in Vila Piratininga residents, is evaluated.

In the present study, the additional vitamin A in the diet provided by the nutrified soybean oil did not lead to an increase in plasma retinol levels. A similar lack of impact of vitamin A supplementation was reported in some previous studies. In Indonesia, Kusin and colleagues (Sommer & West 1987) found that a single 300,000 IU supplement had no demonstrable effect on serum retinol measured after three months (18 μg/dl compared to 16 μg/dl at baseline) and six months (16 μg/dl), among one to five year old children with marginal vitamin A status. Similarly, a study among preschool children in Thailand (Sommer & West 1987) demonstrated no effect associated with a 100,000 IU supplement throughout the five months of observation. In Brazil, Araujo et al. (1978a) also reported no difference from baseline serum retinol levels four weeks after a 200,000 IU dose was administered to poor children consuming a low vitamin A diet.

The results of these studies, including the intervention in Vila Piratininga, suggest that in populations with marginal vitamin A deficiency, a more sensitive functional test is needed to evaluate a possible improvement of total vitamin A liver reserves. The only available method, the RDR, proved to be impractical under field conditions.

Concurrent Infection

The synergism between vitamin A deficiency and infection is widely recognized. In the presence of common gastro-intestinal infections from giardia, ascaris, salmonella and other entero-pathogenic organisms, vitamin A absorption is significantly impaired (Sommer & West 1987). In addition, during common respiratory infections, the absorption of physiologic doses of vitamin A

decreases from 100% to about 75% (Sommer & West 1987). In the present trial, respiratory disease was a chronic problem among participating children. Morbidity data showed that symptoms like coughing and running nose were most prevalent. Combined with diarrhea or parasitic infestation (although the latter was not assessed here), these conditions could have counteracted significant effects of the supplementation. Furthermore; increased metabolic losses (Sommer & West 1987) and tissue requirements for vitamin A during these infectious episodes (Thurnham 1989) may have prevented vitamin A accumulation in the liver and consequently, an increase of plasma retinol levels. In a study in Brazil, Araujo et al. (1978b) demonstrated that environmental factors can be important in the response to vitamin A administration. Although children in his control group did not receive vitamin A fortified sugar, their vitamin A status improved due to the treatment of gastro-intestinal parasites. During a sugar fortification program in Guatemala (Arroyave et al. 1979a), the presence of morbidity resulted in a significant decrease of serum retinol, whereby three or more symptoms in children produced the most notable decreases.

The fact that morbidity symptoms generally occurred with the same frequency in both groups but significantly more children in the experimental group experienced a running nose may have contributed to the outcome of this study.

General nutritional status

In addition, marginal protein and energy status of a few subjects, as indicated by anthropometric measurements, may have impaired vitamin A utilization by affecting its intestinal absorption and release from the liver (Mejía 1986), thus weakening the overall impact of soybean nutrification.

V. DISCUSSION

Nutrient Interrelationships

Zinc seems to be necessary for the formation of proteins essential for vitamin A function such as retinol binding protein (RBP) and opsin (Olson 1988). Although the zinc intake was not assessed in this study, it is likely that the monotonous rice and beans diet did not provide adequate amounts of zinc, which is mainly found in meat products (Health & Welfare Canada 1990). A lack of zinc may have impaired the synthesis of RBP and consequently depressed plasma retinol levels.

So far, only physiological reasons for the ineffectiveness of vitamin A nutrified soybean oil in improving the vitamin A status of the study population have been discussed; however, possible methodological errors also need to be considered.

Estimated Oil Intake

The additional vitamin A intake was estimated from the per capita oil consumption and could have possibly been overestimated, since toward the end of the intervention, participants, realizing the upcoming end of the free oil supply, tended to pick up more cans than they did previously. To avoid such an overestimation, only the average oil consumption per family during the first three months of the study was used to calculate vitamin A intake through the supplied oil.

Furthermore, it is possible that young children simply did not eat large enough portions of the meals prepared with vitamin A nutrified oil to have an effect on their vitamin A status. For this reason, the level of nutrification (an average of 93 IU $\approx$ 28 μg RE/g of soybean oil; Table 7) may not have been high enough to provoke an increase of serum retinol concentrations among this age group. This possibility, however, seems unlikely for the following reasons. In Guatemala, sugar was nutrified with 10 μg RE per gram and contributed a daily average of 330 μg RE to the dietary vitamin A intake of preschool children (Arroyave et al. 1979). In Indonesia, the consumption of fortified MSG

provided children with an additional 210 μg RE per day (Muhilal et al. 1988ab). Dietary data of preschool children in Vila Piratininga were not obtained, however, a previous study in Ribeirao Preto (Doell 1984) indicated that this age group consumed approximately two-thirds of the daily energy intake of adults. Based on this estimate and an average per capita oil consumption of 35 g/day in the experimental group (Table 10; two-thirds = 23 g), fortified soybean oil added **approximately** 644 μg RE to the dietary vitamin A intake of preschool children in the present study. Although the above figure is only a rough estimate, young children seemed to have consumed higher or at least similar additional amounts of vitamin A than their counterparts in previous successful nutrification programs. It appears that the level of supplementation in the present pilot project was not a major contributory factor to the negative outcome.

It should be mentioned that some preschool children went to a nearby daycare centre where they received breakfast, lunch and supper. Obviously, they did not consume any or very little of the vitamin A provided by the fortified oil used in the household. This factor could have also contributed to the negative outcome.

Vitamin A Analyses

Although the intra-assay coefficient of variation of the plasma vitamin A analyses was less than 5% (Dr. Schalch, Hoffmann LaRoche, personal communication), methodological problems with the analysis may have been another source of error. The specimen of the second blood collection arrived thawed at Hoffman-LaRoche in Switzerland and consequently, some vitamin A could have been destroyed. Unfortunately, the stability of retinol in blood samples has not been thoroughly studied and the conditions in which destruction occurs are not well established (Flores et al. 1983). Therefore, to assess whether or not this factor had an impact, 100 randomly selected plasma samples

were sent again for re-analysis. Although some values of retinol were higher in the duplicate analysis, the overall results of the statistical analyses did not change.

These findings thus indicate that vitamin A nutrified soybean oil was not effective in improving plasma retinol levels of this marginally deficient study population. This negative outcome, however, does not necessarily indicate that vegetable oils are not a feasible carrier for vitamin A nutrification. Vitamin A palmitate added to soybean oil appears to be relatively stable under various storage and cooking conditions. Therefore, duration of the intervention, the use of plasma retinol levels as an indicator of vitamin A status and various extraneous factors may possibly explain the lack of impact. The reasons why vitamin A status appeared to deteriorate slightly in the group receiving the fortified oil cannot be elucidated.

Plasma β-Carotene Level

Examination of plasma β-carotene concentrations revealed significant increases in both groups over time, yet the majority of individuals could still be classified as deficient. Because the total vitamin A intake from natural food sources remained unchanged during the four months, as indicated by the results of the dietary assessment, it is unlikely that the increase was due to greater intakes of β-carotene. This observed increase of plasma β-carotene levels may have resulted from a significantly higher amount of fat in the daily diet of the families. It has been demonstrated that supplements of fat can facilitate and increase the absorption of β-carotene considerably (Jayarajan et al. 1980, Dimitrov et al. 1988, Bloem et al. 1989). The greater fat intake could be mainly attributed to the increased consumption of oil (23 g/day at baseline to 37 g/day four months later; Table 10). This was not surprising, considering that soybean oil was provided free of charge to the participants.

V. DISCUSSION

Nutrient Intake

Despite the fact that more calories were supplied through the additional fat, the daily energy intake of women in either group did not increase. The observed shift in macronutrient intake (from carbohydrate and protein to fat) may explain the observed decrease in iron intake and the higher vitamin E density in the women's diet throughout the study period. The significantly greater intakes of thiamin and vitamin C among the experimental group can primarily be attributed to the slightly higher energy consumption of the supplemented subjects compared to control subjects at the end of the intervention.

These dietary results show that the free distribution of soybean oil did not improve the overall caloric intake of the study population. The shift in macronutrient intake towards fat appeared to compromise the already marginal intakes of some nutrients.

5. *LIMITATIONS OF THE STUDY*

1. Dietary intake of preschool children was not assessed, yet this group was assumed to be at greatest risk of deficiency. Because of financial constraints, families could not afford to purchase special foods for their children. Hence, they usually ate smaller quantities of the same foods as their mothers. Previous studies in Ribeirao Preto had already provided evidence for this dietary habit and it was decided that the intake of the female household heads would provide sufficient information on the vitamin A intake of this population group.

2. Oil was provided free of charge to the participating families and it was expected that this free delivery would increase the oil intake of the study population. Such an increase, however, would not interfere with the objectives of this pilot study and the level of supplementation was low enough to prevent a possible toxicity.

3. The impact of this nutrification program on the vitamin A status was evaluated by the subjects' plasma retinol levels. However, because of the homeostatic control exerted by the liver, plasma vitamin A concentration is not a reliable indicator of total vitamin A body reserves unless the latter are virtually depleted or excessive. To evaluate improvement or subtle changes of the marginal vitamin A status of this study population, a more sensitive and functional index would have been necessary. Unfortunately, the only alternative methods, such as the RDR test or direct measurement of liver vitamin A stores by biopsy, were too invasive or impractical under study conditions.

4. Study conditions did not provide a perfectly controlled environment. Although the compliance and cooperation of the participants were excellent, the actual use of the oil could not be supervised in each family. Superstition and skepticism may have prevented the consumption in a few families.

VI. CONCLUSIONS

Dietary and biochemical assessment of the study population at baseline revealed that a problem of marginal vitamin A deficiency existed. As plasma retinol levels indicated, preschoolers were most affected.

Vitamin A deficiency was further complicated by general malnutrition and various other micronutrient deficiencies that could be expected from the simple, monotonous rice and beans diet. Parasitic infestation and infections were common among participants; these extraneous factors impair vitamin A absorption and utilization and increase the requirements.

The findings of this pilot study show that vitamin A nutrified soybean oil increased the estimated daily vitamin A intake of subjects in the experimental group. This higher intake was not reflected in the plasma retinol concentrations. In contrast to the expected increase, plasma vitamin A levels of supplemented individuals dropped slightly, indicating that the vitamin A nutrification of soybean oil was not effective in improving their vitamin A status.

A number of factors may have contributed to the observed lack of improvement in vitamin A status; however, no reason was found to explain the decline of plasma retinol levels during the intervention.

The lack of response to supplementation in this study does not necessarily suggest that nutrification of soybean oil is not a feasible way to improve the vitamin A status of low income Brazilians. The fortified oil was well accepted by the participants and used daily for the preparation of meals; eventual losses of vitamin A during cooking and/or storage can be regarded as minimal. Consequently, it is more likely that other factors rather than the choice of fortification vehicle may have interfered with a successful outcome of the nutrification. The duration of supplementation may have been too short to observe an increase in plasma retinol. In addition, the presence of infections could have depressed serum retinol concentrations independently of liver vitamin A stores.

VI. Conclusions

In this study, plasma retinol levels served as the indicator for an effective intervention. However, especially in populations with marginal vitamin A status, as in Vila Piratininga, plasma vitamin A values are not good indicators of total body reserves. Vitamin A may actually have accumulated in the liver of subjects receiving the fortified oil without a corresponding rise in their plasma vitamin A values. Therefore, to evaluate improvement or subtle changes of a pre-pathological vitamin A status, more sensitive and functional indices are needed. At present, the only available method is the RDR but unfortunately, the application of this test was not feasible under field conditions. The most reliable assessment of the vitamin A status would be obtained by vitamin A analysis of liver samples from biopsy material. For obvious reasons, this is not applicable in a field trial.

Studies in Indonesia, the Phillipines and Central America have shown that food fortification can offer a medium and long-term solution for improving vitamin A nutritional status in chronically deficient populations. Once a suitable carrier is found, vitamin A nutrification appears to be the least costly and most effective of the three intervention strategies (massive oral dose distribution, food fortification and nutrition education). This form of supplementation can also provide a simple way to increase the vitamin A intake among population groups of low socio-economic status in Southern Brazil, where severe hypovitaminosis A with ocular lesions does not seem to be as much a problem as marginal vitamin A deficiency.

This was the first study to use vegetable oil as the carrier for vitamin A. Although this trial did not result in the expected impact on the vitamin A status of the present study population, the feasibility of this vehicle should be further studied. Besides rice, beans and sugar, soybean oil is one of the predominant staple foods and the only dietary fat used by low income Brazilians. So far, it is not technologically feasible to nutrify rice or beans. Compared to sugar, soybean oil provides the

nutritional benefit as a rich source of polyunsaturated fatty acids and vitamin E; vitamin E enhances vitamin A absorption and as a biological antioxidant, it protects vitamin A from oxidation.

Future investigations should be carried out to elucidate whether vitamin A nutrified cooking oils can be a suitable vehicle for improving the vitamin A status of low socio-economic groups in Southern Brazil. Before more sensitive measures of the vitamin A status are developed, the RDR test should be performed on a subsample of the whole study population. In addition, concurrent reduction in the magnitude and severity of precipitating or contributary risk factors such as parasitic infestation, diarrheal and respiratory infections could improve the impact of a nutrification intervention.

Multiple benefits are associated with an improved vitamin A status, including better growth, and lower morbidity and mortality due to a lower susceptibility to infections. Accordingly, effective and easily implementable programs to treat and prevent even marginal vitamin A deficiency need to be further investigated.

BIBLIOGRAPHY

Angeleli, W. 1978. Programa de suplementaçao alimentar para grupos prioritários de áreas de dificil acesso na regiao de Ribeirao Preto. Research report, University of Sao Paulo, Ribeirao Preto, Brazil.

Araujo R.L., E.L. Borges, J. Silva, P.C. Val, and E.C. Vieira. 1978a. Effect of massive dose supplementation on vitamin A status of young children in Brazil. Proceedings of XI. International Congress of Nutrition, Rio de Janeiro, Brazil.

Araujo, R.L., E.L. Borges, J.D.B. Silva, R.D. Palhares, and E.C. Vieira. 1978b. Effect of the intake of vitamin A fortified sugar by preschool children. *Nutr. Rep. Int.* Vol. 18, No. 4: 429-436.

Arroyave, G., I. Beghin, and M. Flores. 1974. Efectos del consumo de azúcar fortificada con retinol, por la madre embarazada y lactante cuya dieta habitual es baja en vitamina A - Estudio de la madre y el niño. *Arch. Latinoam. Nutr.* 24: 485-512.

Arroyave, G., J.R. Aguilar, M. Flores, and M.A. Guzmán. 1979a. Evaluation of sugar fortification with vitamin A at the national level. Pan American Health Organization Scientific Publication 384, Washington, D.C.

Arroyave, G., and M. Calcano. 1979b. Descenso de los niveles séricos de retinol y su proteina de enlace (RPB) durante las infecciones. *Arch. Latinoam. Nutr.* 29: 233-260.

Arroyave, G., C.O. Chichester, and H. Flores. 1982. Biochemical methodology for the assessment of vitamin A status. International Vitamin A Consultative Group. Washington, D.C., The Nutrition Foundation.

Bates, C.J. 1983. Vitamin A in pregnancy and lactation. *Proc. Nutr. Soc.* 42: 65-79.

Bauernfeind, J.C. 1983. Vitamin A: Technology and applications. *World Rev. Nutr. Diet.* 41: 110-199.

Beaton, G.H. 1975. Evaluation of nutrient intake: new statistical approaches. *Arch. Latinoam. Nutr.* 25: 31-46.

Beaton, G.H. and V.N. Patwardhan. 1976. Physiological and practical considerations of nutrient function and requirements. *In*: Nutrition in preventive medicine, Beaton, G.H. and J.M. Bengoa (eds.). Geneva: WHO.

Blackfan, K.D. and S.B. Wolbach. 1933. Vitamin A deficiency in infants: a clinical and pathological trial. *J. Paediatr.* 3: 679-706.

Bloem, M.W., M. Wedel, R.J. Egger, A.J. Speek, J. Schrijver, S. Saowakontha, and W.H.P. Schreurs. 1989. Iron metabolism and vitamin A deficiency in children in Northeast Thailand. *Am. J. Clin. Nutr.* 50: 332-338.

Bloem, M.W., M. Wedel, R.J. Egger, A.J. Speek, J. Schrijver, S. Saowakontha, and W.H.P. Schreurs. 1990. Mild vitamin A deficiency and risk of respiratory tract diseases and diarrhea in preschool and school children in Northeastern Thailand. *Am. J. Epidemiol.* 131: 332-339.

Burke, B.S. 1947. The dietary history as a tool in research. *J. Am. Diet. Assoc.* 23: 1041-1046.

Burk, M.C. and E.M. Pao. 1980. Analysis of food consumption survey data for developing countries. FAO, Rome.

Campino, A.C.C. 1986. Aspectos sócio-econômicos da desnutriçao no Brasil. *Rev. Saúde Públ.* 20 (1): 83-101.

Campos, F.A.C.S, H. Flores, and B.A. Underwood. 1987. Effect of an infection on vitamin A status of children as measured by the RDR. *Am. J. Clin. Nutr.* 46: 91-94.

CNPq, Conselho Nacional de Desinvolvimento Científico e Tecnológico. 1981. Bóias frias - uma realidade Brasileira. Academia de Ciências do Estado de Sao Paulo. Publicaçao ACIESP No. 30.

DeMaeyer, E.M. 1986. Xerophthalmia and blindness of nutritional origin in the third world. *Children in the Tropics,* No. 165, International Children's Centre, Paris.

Desai, I.D., M.L. Garcia Tavares, B.S. Dutra de Oliveira, A. Douglas, F.A.M. Duarte, and J.E. Dutra de Oliveira. 1980. Food habits and nutritional status of agricultural workers in Southern Brazil. *Am. J. Clin. Nutr.* 33: 702-714.

Desai, I.D., R.M. Duarte Fávaro, H. Vannucchi, and J.E. Dutra de Oliveira. 1988a. Studies on Fortification of vegetable oils with vitamin A in Southern Brazil. *FASEB Journal* 2: A1204 (Abstract).

Desai, I.D., H. Bhagavan, R. Salkeld, and J.E. Dutra de Oliveira. 1988b. Vitamin E content of crude and refined vegetable oils in Southern Brazil. *J. Food Comp. Analysis* 1: 231-238.

Dimitrov, N.V., C. Meyer, D.E. Ullrey, W. Chenoweth, A. Michelakis, W. Malone, C. Boone, and C. Fink. 1988. Bioavailability of β-carotene in humans. *Am. J. Clin. Nutr.* 48: 298-304.

Dickson, W.J. (ed.), M.B. Brown, L. Engelman, M.A. Hill, and R.I. Jennrich. 1988. BMDP Statistical Software Manual, Volume 1, University of California Press, Berkeley.

Doell, A.M. 1984. Nutritional needs assessment of rural agricultural migrants of Southern Brazil: designing, implementing and evaluating a nutrition education program. MSc Thesis, UBC, Vancouver, Canada.

Emmons, L. and M. Hayes. 1973. Accuracy of 24 hr recalls of young children. *J. Am. Diet. Assoc.* 62: 409-416.

ENDEF. 1977. Tabelas de composiçao dos alimentos. Secretaria de Planejamento da Presidência da República. Fundaçao Instituto Brasileiro de Geografia e Estatística. Rio de Janeiro.

FAO/WHO. 1988. Requirements of vitamin A, iron, folate and vitamin B 12. Food and Nutrition Series # 23. Rome.

Fávaro, R.M.D., N.V. de Souza, S.M. Batistal, M.G.C. Ferriani, I.D. Desai, and J.E. Dutra de Oliveira. 1986. Vitamin A status of young children in Southern Brazil. *Am. J. Clin. Nutr.* 43: 852-858.

Fávaro, R.M.D., J.F. Ferreira, I.D. Desai, and J.E. Dutra de Oliveira. 1990. Studies on fortification of vegetable oils with vitamin A in Brazil: stability during cooking and storage. Unpublished data.

Ferreira, J. 1988. University of Sao Paulo, Medical School Ribeirao Preto, personal communication.

Flores, M., M.T. Menchú, and M. Guzmán. 1973. Evaluación de familias u preescolares mediante la aplicación de diferentes métodos y técnicas - Area rural de Nicaragua. *Arch. Latinoam. Nutr.* 23: 325-344.

Flores, H., C.R.C. Araujo, F.A.C.S. Campos, and B.A. Underwood. 1983. Importance of the early diagnosis of vitamin A deficiency at the epidemiological level. *Int. J. Vitam. Nutr. Res.* 24 (suppl.): 23-34.

Flores, H., F. Campos, C.R.C. Araujo, and B.A. Underwood. 1984. Assessment of marginal vitamin A deficiency in Brazilian children using the RDR procedure. *Am. J. Clin. Nutr.* 40: 1281-1289.

Frank, G.C., G.S. Berenson, P.E. Schilling, and M.C. Moore. 1977. Adapting the 24 hr recall for epidemiologic studies of school children. *J. Am. Diet. Assoc.* 71: 26-31.

Frisancho, R.A. 1981. New norms of upper limb fat and muscle areas for assessment of nutritional status. *Am. J. Clin. Nutr.* 34: 2540-2545.

Gibson, R.S. 1990. Principles of nutritional assessment. New York, Oxford, Oxford University Press.

Health and Welfare Canada. 1990. Nutrition Recommendations. The Report of the Scientific Review Committee, Ottawa.

Hewlett, S.A. 1980. The cruel dilemmas of development: twentieth-century Brazil. New York, Basic Books Inc. Publishers.

ICNND (Interdepartmental Committee on Nutrition for National Development). 1965. Nutrition survey: Northeast Brazil, March-May 1963. Washington: United States Government Printing Office.

Jansen, G.R., N.B. Jansen, C.T. Shigetomi, and J.M. Harper. 1977. The effect of income and geographic region on the nutritional value of diets in Brazil. *Am. J. Clin. Nutr.* 30: 955-964.

Jayarajan, P., V. Reddy, and M. Mohanram. 1980. Effect of dietary fat on absorption of β-carotene from green leafy vegetables in children. *Indian J. Med. Res.* 71: 53-56.

Jelliffe, D.B. 1966. The assessment of the nutritional status of the community. WHO Monograph Series No. 53, Geneva, Switzerland.

Lewis, C.J., M.A. McDowell, C.T. Sempos, K.C. Lewis, and E.A. Yetley. 1990. Relationship between age and serum vitamin A in children aged 4-11 y. *Am. J. Clin. Nutr.* 52: 353-360.

Loerch, J.D., B.A. Underwood, and K.C. Lewis. 1979. Response of plasma levels of vitamin A to a dose of vitamin A as an indicator of hepatic vitamin A reserves in rats. *J. Nutr.* 109: 778-786.

McLaren, D.S. 1964. Xerophthalmia: A neglected problem. *Nutr. Rev.* 22: 289-291.

McLaren, D.S. 1986. Pathogenesis of vitamin A deficiency. *In*: Vitamin A deficiency and its control, J.C. Bauernfeind (ed.), Academic Press Inc. London.

Marcondes, E., E.S. Berquo, J. Yunes, J. Luongo, J. de Souza Martins, M.A.S. Zacchi, M.S.F. Levy, and R. Hegg. 1971. Estudo antropometrico de crianças Brasileiras de zero a doze anos de idade. Anais Nestle: Fasciculo No. 84.

Marcondes, E. 1979. Desvio-padrao vs. percentil. *Pediat. (Sao Paulo)* 1: 148-158.

Marcondes, E. 1983. Normas para a caracteriçao dos perímetros cefálico e torácico. *Pediatr. (Sao Paulo)* 5: 249-255.

Mason, J.B., S.J. Eastman, and M. Lotfi. 1987. Introduction and policy implications. *In*: Delivery of oral doses of vitamin A to prevent vitamin A deficiency and nutritional blindness, K.P. West Jr. and A. Sommer. United Nations, Nutrition Policy Discussion Paper No.2.

Mejía, L.A. 1986. Vitamin A nutrient interrelationships. *In*: Vitamin A deficiency and its control. J.C. Bauernfeind (ed.). Academic Press Inc. London.

Miguel, M. and A.M.X. Bon. 1974. Resultados do inquérito alimentar realizado nas cidades de Apiaí, Ribeira e Barra do Chapéu (Sao Paulo, Brasil). *Rev. Saúde Públ.* 8: 75-86.

Muhilal, A. Murdiana, I. Azis, S. Saidin, A.B. Jahari, and D. Karyadi. 1988a. Vitamin A-fortified monosodium glutamate and vitamin A status: a controlled field trial. *Am. J. Clin. Nutr.* 48: 1265-1270.

Muhilal, D. Permeisih, Y.R. Idjradinata, Muherdiyantiningsih, and D. Karyadi, 1988b. Vitamin A-fortified monosodium glutamate and health, growth, and survival of children: a controlled field trial. *Am. J. Clin. Nutr.* 48: 1271-1276.

Nauss, K.M. 1986. Influence of vitamin A status on the immune system. *In*: Vitamin A deficiency and its control, J.C. Bauernfeind (ed.), Academic Press Inc. London.

Bibliography

NCHS (National Centre for Health Statistics). 1979. Weight by height and age for adults 18-74 years: United States 1971-74. Data from the National Health Survey. Series II, No. 208. US Department of Health, Education and Welfare, Hyattsville Md.

NCHS (National Centre for Health Statistics). 1987. Anthropometric reference data and prevalence of overweight. United States, 1976-80. Data from the National Health Survey. Series II, No. 238. US Department of Health, Education and Welfare, Hyattsville Md.

NRC (National Research Council). 1987. Subcommittee on vitamin A deficiency, prevention and control. Vitamin A supplementation: methodologies for field trials. National Academy Press, Washington, D.C.

Olson, J.A. 1979. Liver vitamin A reserves of neonates, preschool children and adults dying of various causes in Salvador, Brazil. *Arch. Latinoam. Nutr.* 29: 521-545.

Olson, J.A. 1984. Serum levels of vitamin A and carotenoids as reflectors of nutritional status. *JNCI* Vol. 73, No. 6:1439-1444.

Olson, J.A. 1987. Recommended dietary intakes (RDI) of vitamin A in humans. *Am. J. Clin. Nutr.* 45: 704-716.

Olson, J.A. 1988. Vitamin A, retinoids and carotenoids. *In*: Modern Nutrition in Health and Disease. M.E. Shils and V.R. Young (eds.), 7th edition, Lea & Febiger, Philadelphia.

Pekkarinen, M. 1970. Methodology in the collection of food consumption data. *World Rev. Nutr. Diet.* 12: 145-172.

Roncada, M.J. 1972. Hipovitaminose A: niveis séricos de vitamina A e carotena em populaçoes litorâneas do estado de Sao Paulo, Brasil. *Rev. Saúde Públ.* 6: 3-18.

Roncada, M.J., D. Wilson, A.L. Netto, O.B. Netto, A.C. Kalil, M.F. Nunes, and E.T. Okani. 1978. Hipovitaminose A em filhos de migrantes nacionais trânsito pela capital de Sao Paulo, Brasil: estudo clínico-bioquímico. *Rev. Saúde Públ.* 12: 345-350.

Roncada, M.J., D. Wilson, R.N. Mazzilli, and Y.R. Gandra. 1981. Hipovitaminose A em comunidades do estado de Sao Paulo, Brasil. *Rev. Saúde Públ.* 15: 338-349.

Roncada, M.J. 1983. Hypovitaminosis A: a public health problem. *In*: Vitamins in medicine. A. Hanck (ed.). Hans Huber Publishers.

Roncada, M.J., D. Wilson, E.T. Okani, and S. Amino. 1984. Prevalência de hipovitaminose A em pré-escolares de município da área metropolitana de Sao Paulo, Brasil. *Rev. Saúde Públ.* 18: 218-224.

Shavelson, R.J. 1988. Statistical reasoning for the behavioral sciences, 2nd edition. Needham, Massachusetts: Allyn & Bacon Inc.

Shrimpton, R. 1975. Nutrition and development in Brazil. MSc Thesis, University of London, England.

Scrimshaw, N.S. 1966. Synergistic and antagonistic interactions of nutrition and infection. *Fed. Proc.* 25: 1679-1681.

Simmons, W.K. 1976. Xerophthalmia and blindness in Northeast Brazil. *Am. J. Clin. Nutr.* 29: 116-122.

Smiciklas-Wright, H. and H.A. Guthrie, 1984. Dietary Methodologies: their uses, analyses, interpretations, and implications. *In:* Nutrition Assessment. M.D. Simko (ed.). Aspen System Corp., Maryland.

Solon, F.S., B.M. Popkin, T.L. Fernandez, and M.C. Latham. 1978. Vitamin A deficiency in the Philippines: a study of xerophthalmia in Cebu. *Am. J. Clin. Nutr.* 31: 360-368.

Solon, F.S., T.L. Fernandez, M.C. Latham, and B.M. Popkin. 1979. An evaluation of strategies to control vitamin A deficiency in the Philippines. *Am. J. Clin. Nutr.* 32: 1445-1453.

Solon, F.S., M.C. Latham, R. Gurriec, R. Florentino, D.F. Williamson, and J. Aguilar. 1985. Fortification of MSG with vitamin A: the Philippine experience. *Food Techn.* Nov. 1985: 71-77.

Solon, M.A. 1986. Control of vitamin A deficiency by education and the public health approach. *In:* Vitamin A deficiency and its control. J.C. Bauernfeind (ed.). Academic Press Inc. London.

Sommer, A., I. Tarwotjo, G. Hussani, and D. Susanto. 1983. Increased mortality in children with mild vitamin A deficiency. *Lancet* 2: 585-588.

Sommer, A., J. Katz, and I. Tarwotjo. 1984. Increased risk of respiratory disease and diarrhea in children with preexisting mild vitamin A deficiency. *Am. J. Clin. Nutr.* 40: 1090-1095.

Sommer, A. 1989. New imperatives for an old vitamin (A). *J. Nutr.* 119: 96-100.

Stuff, J.E., C. Garza, E.O. Smith, B.L. Nichols, and C.M. Montandan. 1983. A comparison of dietary methods in nutritional studies. *Am. J. Clin. Nutr.* 37: 300-306.

Swann, M.A. 1979. Nutritional assessment of agricultural migrant workers in Southern Brazil. MSc Thesis, UBC, Vancouver, Canada.

Takahashi, T. 1984. Vitamin A in new food composition tables from Japan. *JNCI* Vol 73, No. 6: 1405-1407.

Thurnham, D.I. 1989. Vitamin A deficiency and its role in infection. *Transactions of the Royal Society of Trop. Med. Hygiene* 83: 721-723.

Underwood, B.A. 1974. The determination of vitamin A and some aspects of its distribution, mobilization and transport in health and disease. *World Rev. Nutr. Diet.* 19: 123-172.

Veja. May 10, 1989. No. 18:126-127.

Vuilleumier, J.P., Keller H.E., D. Gysel, and F. Hunicker. 1983. Clinical chemical methods for the routine assessment of the vitamin A status in human populations. Part I: the fat-soluble vitamins A and E and β-carotene. *Inter. J. Vit. Nutr. Res.* 53: 265-272.

Waddell, C. 1981. The nutritional status and physical work performance of children of migrant agricultural workers in Southern Brazil. MSc Thesis, UBC, Vancouver, Canada.

Watt, B.J. and A.L. Merrill, 1963. Composition of foods, raw, processed, prepared. Agriculture Handbook No. 8. Washington: Agricultural Research Services, United States Department of Agriculture.

West Jr., K.P. and A. Sommer. 1987. Delivery of oral doses of vitamin A to prevent vitamin A deficiency and nutritional blindness. United Nations, Nutrition Policy Discussion Paper No. 2, FAO, Rome.

West Jr., K.P., E. Djunaedi, A. Pandji, Kusdiono, I. Tarwotjo, A. Sommer, and Aceh Study Group. 1988. Vitamin A supplementation and growth: a randomized community trial. *Am. J. Clin. Nutr.* 48: 1257-1264.

WHO. 1976. Vitamin A deficiency and xerophthalmia. WHO Technical Report Series No. 590. Geneva.

WHO. 1982. Control of vitamin A deficiency and xerophthalmia. WHO Technical Report Series No. 672. Geneva.

Wilkinson, L. 1989. SYSTAT: The system for statistics. Evanston, Illinois: SYSTAT, Inc.

Wilson, D., M.J. Roncada, A.L. Netto, and O.B. Netto. 1981. Hipovitaminose A em pré-escolares internados em uma instituiçao na capital do estado de Sao Paulo, Brasil. *Rev. Saúde Públ.* 15: 395-400.

Wolbach, S.B. and P.R. Howe. 1925. Tissue changes following deprivation of fat-soluble vitamin A. *J. Exp. Med.* 42: 753-777.

APPENDIX 1: CONSENT FORM

Consent Form for Participant

I,_____have had the study explained to me and have consented to participate. I understand that I can refuse to answer any question and can withdraw from the study at any time.

Verbal response: Yes/No

or

Signature:_____

Date:_____

APPENDIX 2: INSTRUCTIONS ON HOW TO USE THE SOYBEAN OIL

Dear participant,

your participation in the project of oil fortification is very important.

We would like to ask for your collaboration in the following:

1. please dont't share the oil with neighbors or friends;

2. we will furnish enough oil for your needs; please don't use another oil, only use the oil supplied by us;

3. use this oil for cooking, frying and for preparing salads;

4. please don't throw the empty can away because in exchange for it you'll receive another full bottle.

The success of this project depends on you. We thank you very much for your participation.

APPENDIX 3: DEMOGRAPHIC/SOCIO-ECONOMIC/FOOD HABITS AND HEALTH RELATED PRACTICES QUESTIONNAIRE

Appendix 3: Demographic/Socio-Economic/Food Habits and Health Related Practices Questionnaire

Code:
Name: Mother:
Father:
Date:

1. Name all persons who eat and sleep in this house every day.

	Name	Sex 1=M 2=F	Relationship to household head	Age	Date of birth	Currently in school	If no, why not?	Years of schooling	Ability to read/write
01									
02									
03									
04									
05									
06									
07									
08									
09									
10									
11									
12									

2. How long have you lived at this address?

3. Did you vote in the last election? Yes No Why?
   Father
   Mother
   Children

4. Where did you live before moving to this house?
   Municipality
   City
   State

5. Why did you decide to move to Vila Piratininga?
   1) to earn more money
   2) to get a better job
   3) better health care
   4) to join friends, parents, family
   5) other (specify)

6. What kind of work did (father, mother, children) do this past month?
   Father
   Mother
   Children

7. How much did (father, mother, children) get paid the last time and was this money for a day, week or months?

	Amount	Time Interval
Father		
Mother		
Children		

8. Did (father, mother, children) receive any bonus? If yes, what and how much?

	Yes	No	Amount
Father			
Mother			
Children			

9. Are you now:
   pregnant
   lactating
   pregnant and lactating
   none of the above

10. Religion
    Catholic
    Spiritual
    Protestant
    None
    Other (specify)

Appendix 3: Demographic/Socio-Economic/Food Habits and Health

Related Practices Questionnaire

HOME ENVIRONMENT

11. House: Rented Owned Other (specify)
Number of rooms in house:
Number of windows in house:
Type of windows: open (with shutters) screened other (specify)
Construction of house (type of material):
Roof
Walls
Floor
Electricity: Yes No
Source of water: Running water in the house
From a well
Village well
Buy water from a vendor
Other (specify)
Sewage disposal: Open gutter
Closed gutters
Other (specify)
Garbage disposal: Public collection
Garbage tins
Open yard
Burn the garbage
What toilet facilities are most often used by your family?
Village latrine
Toilet in house
Outside latrine
Gutters
Open fields
Other (specify)
Where do you do your cooking?
Outside the house
Inside the house
Other (specify)
What kind of cooking fuel do you use?
Wood Gas Charcoal Other (specify)
Which of the following items do you have?
TV Radio Sewing machine Cassette player
Record player Iron Pressure cooker
Fan Mixer Blender Fridge
Do you have any animals/pets? How many?
Dog Cat Birds Pig
Chicken Other (specify)
How are these animals fed?
From meals
Whatever food they can find
Other (specify)

FOOD HABITS

12. In the past week, where did you get your food for the family?
Local store
Local market
Home garden/raised animals
Donations
Other (specify)

13. Do you have a home garden? Yes No
If yes, what foods are grown?

14. What kind of food assistance do you receive?
Food coupons
Money
Free food gifts
Other (specify)

15. How do you store or keep your food?
Open cupboards
Closed cupboards
Fridge
Freezer
Other

16. Do you preserve any foods? Yes No
If yes, what method(s) are used?
Freezer Smoked Dried Canned Other (specify)

17. What do you do with left-over cooked foods?

18. Who in your family shops for food?
Father
Mother
Children
Other (specify)

19. How many times was food purchased in the previous week?
Every day
Several times a week
Once a week
None
What type of transportation was used for shopping?
Walking Bicycle Bus Other (specify)

Appendix 3: Demographic/Socio-Economic/Food Habits and Health Related Practices Questionnaire

20. What foods were purchased last week?
    How much money was spent for these foods?
    When was the last time someone bought alcoholic beverages? Quantity: Price:

21. Who prepares the meals for the family?
    - Father
    - Mother
    - Children
    - Other (specify)

22. Who in the family decides what children will eat?
    - Father
    - Mother
    - Grandmother
    - Older children
    - Doctor
    - Other (specify)

23. Before moving to Vila Piratininga, were there any foods you ate that you now don't eat here?
    - Yes No
    - If yes, name the foods:
    - Why are you not eating these foods now?
      - Not available
      - More expensive here
      - Other (specify)

24. Are there any foods you eat now in Vila Piratininga that you did not eat in your previous place of residence?
    - Yes No
    - If yes, name the foods:
    - Why?
      - Were not available at previous home
      - Price is cheaper here
      - Other (specify)

25. If you won the lottery, what three things would you do or buy?
26. If you were given extra money to spend on food, what would you buy?

27. What changes would you like to see in:
    - Vila Piratininga:
    - Your home:

28. What are some things you like about living in Vila Piratininga? Why?

29. What are some things you don't like about living in Vila Piratininga? Why?

HEALTH RELATED PRACTICES

30. What are some things you like to do when you are not working?
    - Walking
    - Cinema
    - Cycling
    - Visit the park
    - Visit the circus
    - Watch TV
    - Listen to the radio
    - Read the newspaper
    - Visit friends/family
    - Watch soccer match
    - Go to the bar
    - Dancing
    - Other (specify)

31. Smoking habits? (cigarettes)

	Father		Mother		Children	
	Yes	No	Yes	No	Yes	No
Less than 5						
6 - 10						
11- 20						
More than 20						
Don't know						

If you don't smoke, why not?

APPENDIX 4: 24 HOUR RECALL

24 - HOUR FOOD RECALL

Code:
Date:
Name:
Age:

Please state the kind and amount of all foods and beverages you ate/drank in the past 24 hours starting yesterday morning when you first got up.

Time of day	Foods (Description, Preparation Method	Quantity Household units	Metric
After getting up in the morning			
Mid morning			
Mid day (lunch)			
Mid afternoon			
Late afternoon/ early evening (supper)			
Night			

APPENDIX 5: CONVERSION OF BRAZILIAN HOUSEHOLD MEASURES INTO METRIC UNITS

Item	Household measure	Metric unit
Rice, cooked	1 tablespoon	30 g
	1 small ladle	46 g
	1 big ladle	110 g
Beans, cooked	1 tablespoon	25 g
	1 small ladle	80 g
	1 big ladle	160 g
Maccaroni, cooked	1 tablespoon	22 g
	1 big ladle	110 g
Sugar	1 tablespoon	15 g
	1 teaspoon	10 g
Coffee	1 coffee cup	50 ml=42g[1]
	1 glass	150 ml
Coffee, prepared	1 coffee cup	42 g + 10 g sugar
	1 glass	126 g + 30 g sugar
Milk	1 coffee cup	49 g
	1 glass	148 g
Yoghurt	1 cup	200 g
Fruit flavored drink	1 glass	148 g
Coca Cola, Fanta	1 bottle	302 g
White bread	1 roll	50 g
Soyabean oil	1 coffee cup	46 g
	1 glass	138 g
Butter	1 table spoon	10 g
Lettuce	1 medium leaf	5 g
	1 portion	25 g
	1 large portion	50 g
Egg	1 unit	50 g
Chicken, cooked	1 drumstick	65 g
	1 thigh	75 g
	1 small piece	25 g
raw	1 unit	1500 g
Hot dog	1 unit	60 g
Potato, cooked	2 units, medium size	100 g
Potato, fried	1 medium portion	40 g

[1] Without sugar

Appendix 5: Conversion of brazilian household measures into metric units

Item	Household measure	Metric unit
Meat (beef), cooked	1 piece, medium size	80 g
Ham	1 thin slice	15 g
Cheese	1 medium slice	30 g
Freshwater fish (fried)	1 unit	200 g
Sardines	1 can	92 g
Noodle soup	1 big ladle	150 g
Soup (powder)	1 soup plate	196 g
Cookie	1 unit	5 g
Lollipop	1 unit	10 g
Sponge cake	1 square	44 g
Farofa (fried manioc flour)	1 tablespoon	15 g
Wild chicoree (rúcula), spinach	1 bunch	400 g
Watercrest	1 bunch	500 g
Onion	1 unit	30 g
Carrot	1 unit	80 g
Tomato	1 unit	100 g
Tomato paste	1 can	60 g
Chayote (chuchu)	1 unit	275 g
Squash (orange)	1 unit	1900 g
Okra	1 unit	110 g
Avocado	1 unit	900 g
Eggplant	1 unit	550 g
	2 medium slices, cooked	80 g
Corn, yellow	1 unit	350 g
Red beet	1 unit	50 g
Peas	1 small can	250 g
Pepper	1 unit	85 g
Apple	1 unit, small	115 g
Banana	1 unit	100 g
Mandarine, tangerine	1 unit	116 g
Orange, with skin	1 unit	161 g
Mango	1 unit, medium size	350 g
Papaya	1 unit, medium size	1500 g

APPENDIX 6: FOOD FREQUENCY QUESTIONNAIRE

Code:
Date:
Name:

Information supplied by the female head of the household regarding the frequency of consumption of vitamin A containing food sources.

Food item	Frequency					Serving/ Unit	Metric Size	RE
	Day	Week	Month	Sometimes	Never			
Dairy Products								
Cow's milk								
Powdered milk								
Cheese								
Yoghurt								
Icecream								
Eggs								
Meats/Poultry/ Fish								
Beef								
Pork								
Sausage								
Chicken								
Fish								
Organ Meats								
Liver								
Brain								
Legumes								
Kidney beans								
Peas								
Cereals								
Corn (yellow)								
Vegetables								
Chicory								
Manioc leaves								
Red beet leaves								
Lettuce								
Carrots								
Onions								
Squash								
Chayote								
Red potatoes								
Yellow potatoes								
Tomato								
Spinach								
Wild chicory								
Avocado								
Fruits								
Banana								
Mango								
Tangerine								
Orange								
Papaya								

APPENDIX 7: FOODS OF FOOD FREQUENCY QUESTIONNAIRE, GROUPED ACCORDING TO VITAMIN A CONTENT

	Vitamin A content/100g[1]
Foodgroup 1 (0-50 µg RE/100 g)	
Pork	/
Brain	/
Beef	2
Sausage	2
Kidney beans	2
Chayote	2
Onions	5
Powdered Milk	6
Yellow potatoes	22
Milk	30
Papaya	37
Tangerine	40
Orange	40
Yoghurt	42
Fish	44
Banana	50
Foodgroup 2 (51-100 µg RE/100 g)	
Avocado	87
Chicory	99
Lettuce	99
Foodgroup 3 (101-200 µg RE/100 g)	
Corn (yellow)	121
Icecream	132
Tomato	180
Foodgroup 4 (201-356 µg RE/100 g)	
Peas	207
Mango	210
Chicken	221
Red potatoes	300
Cheese	330
Squash	350
Eggs	356
Foodgroup 5 (525-790 µg RE/100 g)	
Red beet leaves	525
Spinach	585
Wild chicory	790
Foodgroup 6 (> 1,000 µg RE/100 g)	
Carrots	1,100
Manioc leaves	1,960
Liver	8,551

[1] All values derived from Brazilian food composition table.

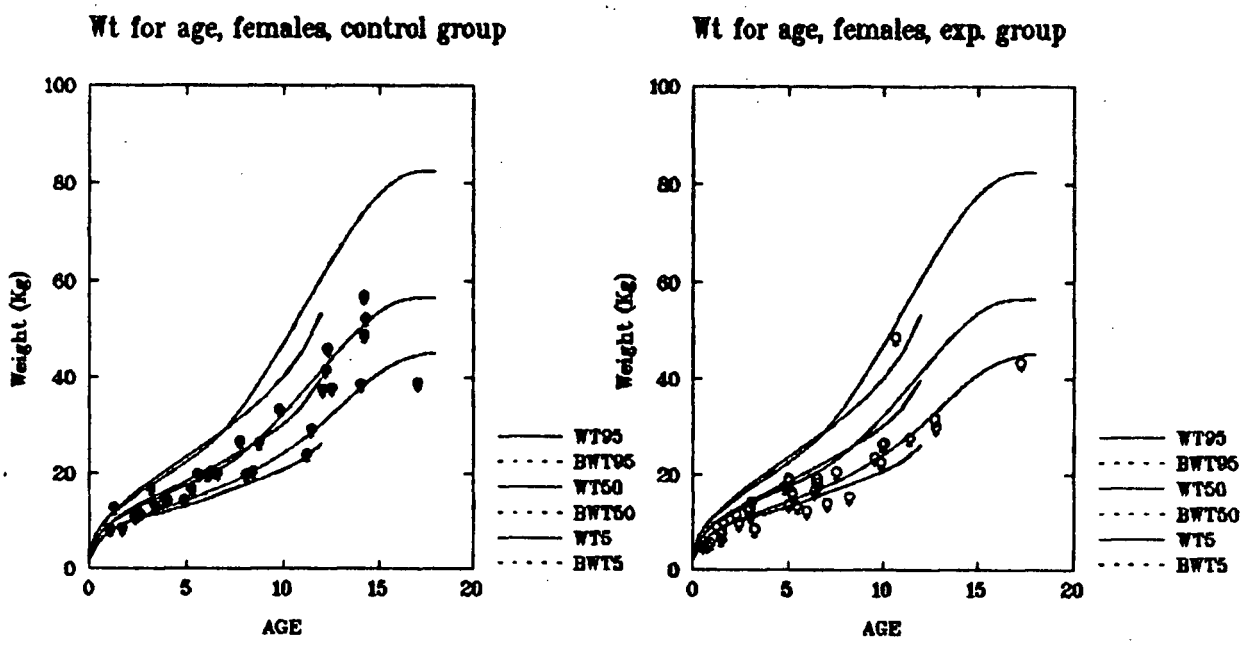
APPENDIX 8: PROTOCOL FOR ANTHROPOMETRIC MEASUREMENTS

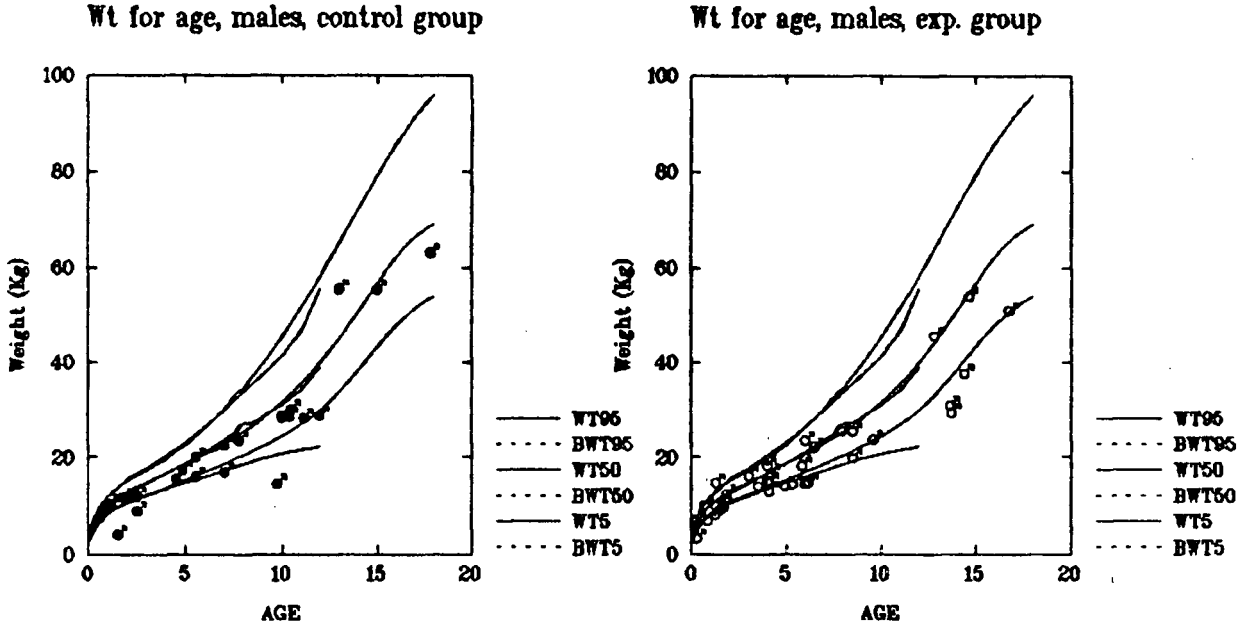
Code:
Family: Mother:
Father:
Date:

Name	Sex 1=M 2=F	Age	Weight (kg)	Height (cm)	MUAC (cm)	TSF (mm)	Headcirc. (cm)
01							
02							
03							
04							
05							
06							
07							
08							
09							
10							
11							
12							

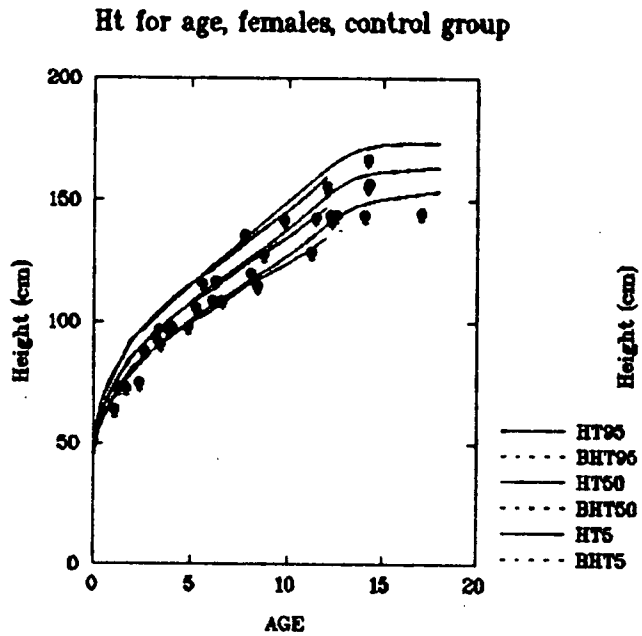
APPENDIX 9: GRAPHICAL PRESENTATION OF CHILDREN'S ANTHROPOMETRIC MEASUREMENTS

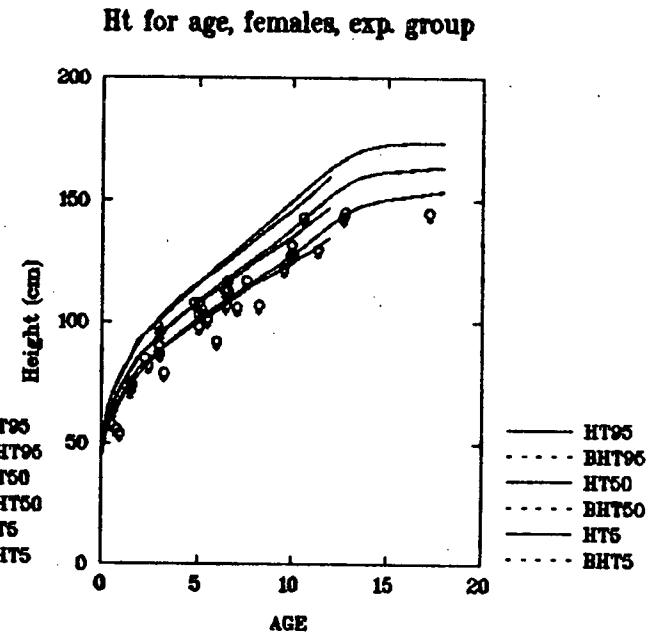
APPENDIX 9A: WEIGHT FOR AGE

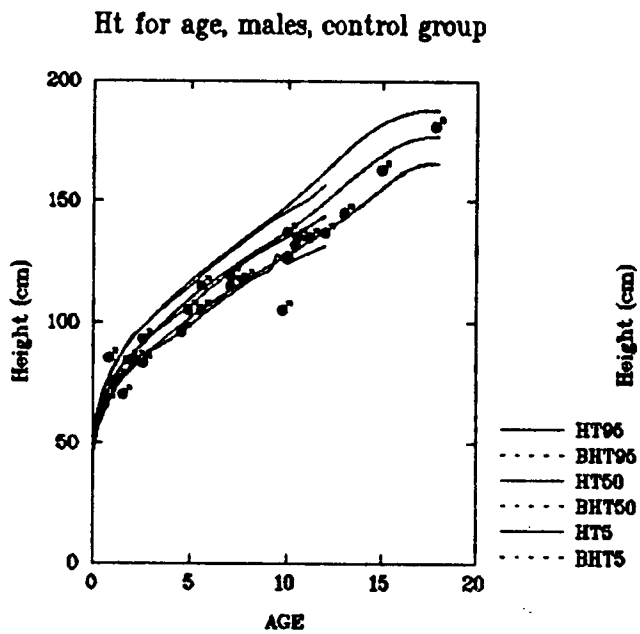


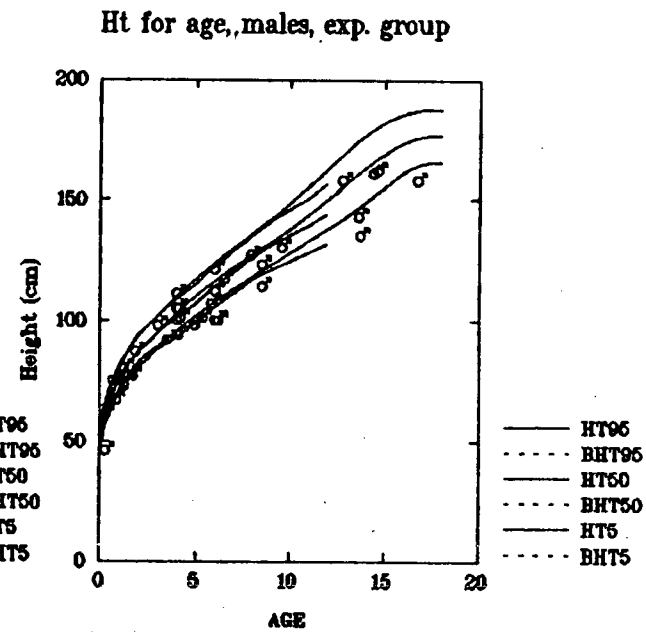


APPENDIX 9B: HEIGHT FOR AGE



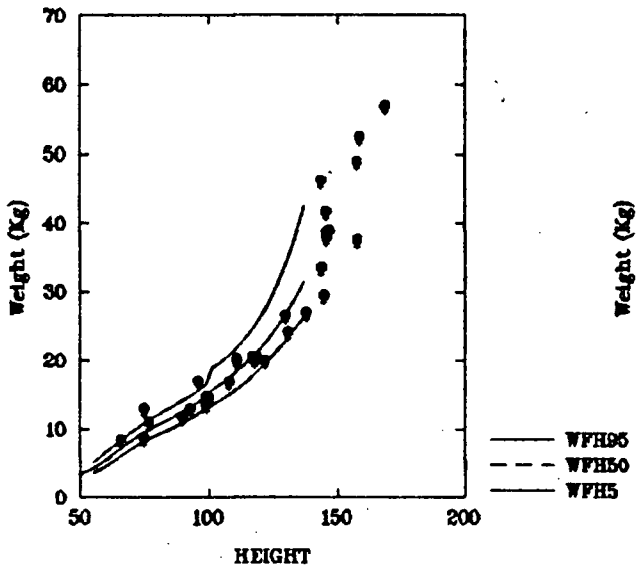




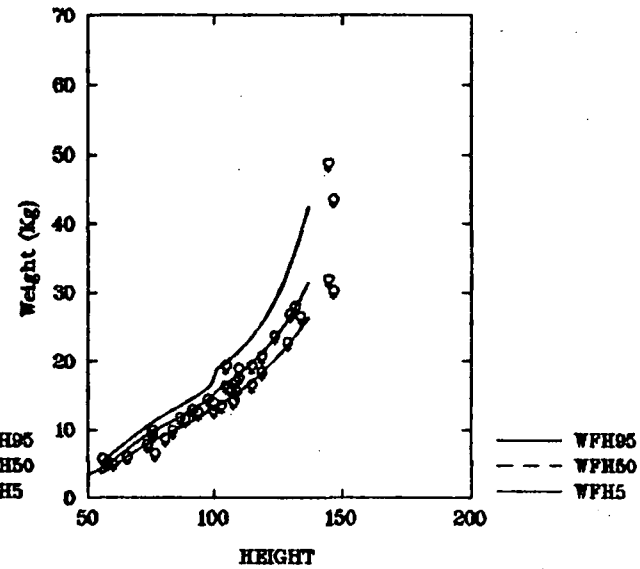


APPENDIX 9C: WEIGHT FOR HEIGHT

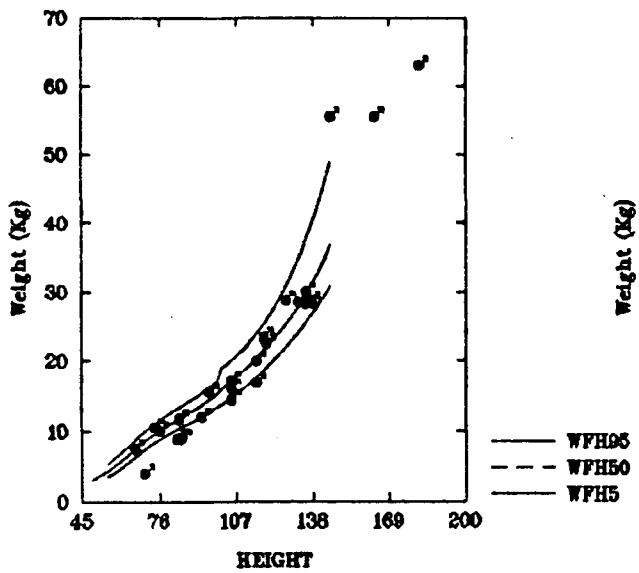
Wt for ht in females of control group



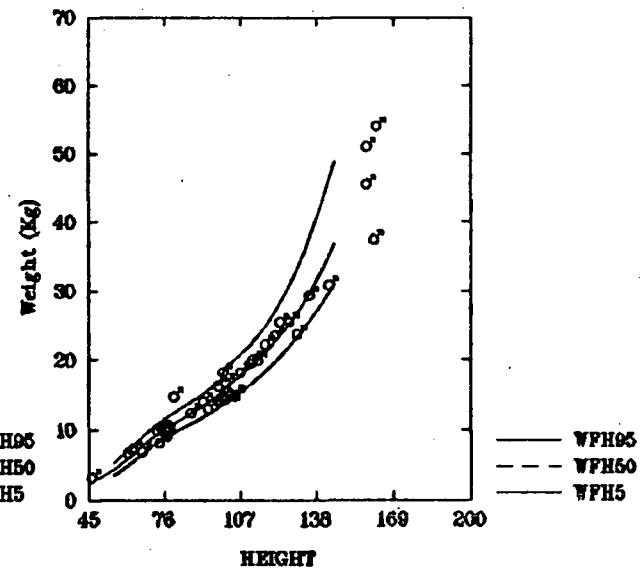
Wt for ht in females of exp. group



Wt for ht in males of control group

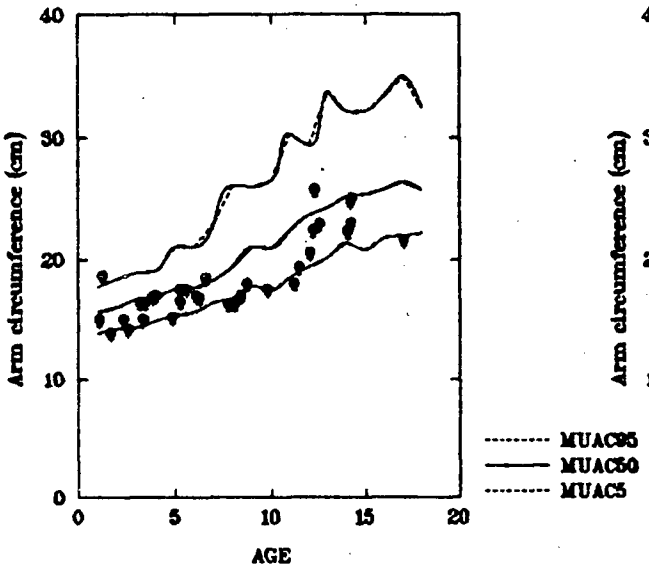


Wt for ht in males of exp. group

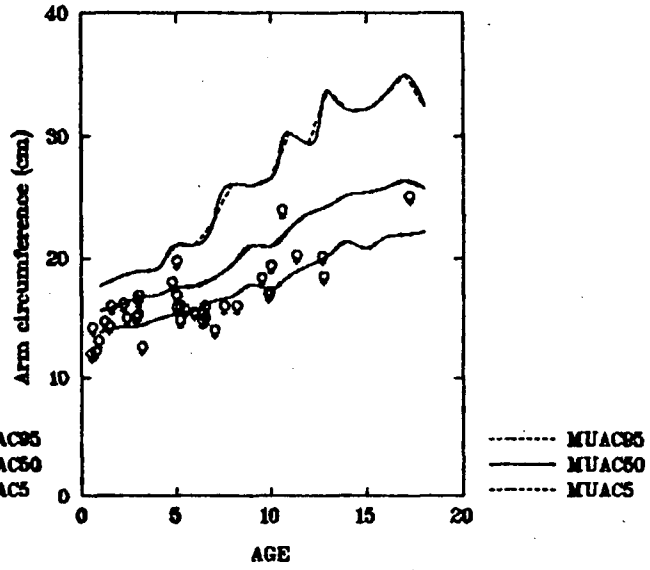


APPENDIX 9D: MID-UPPER-ARM CIRCUMFERENCE (MUAC)

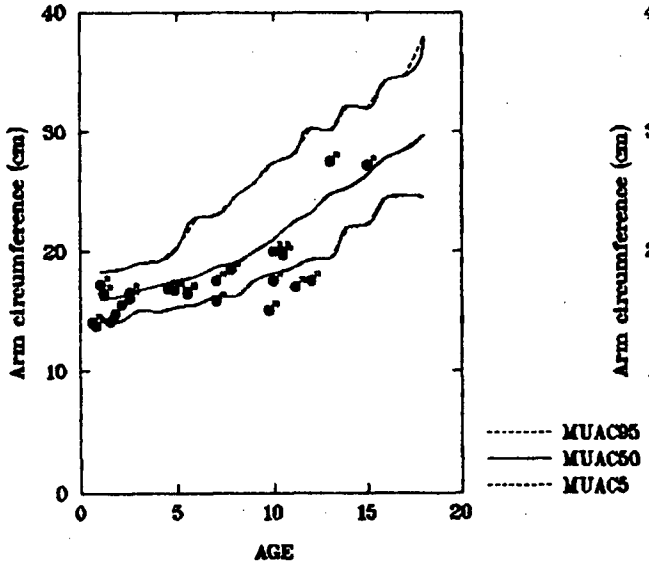
MUAC (NCHS), females, control group



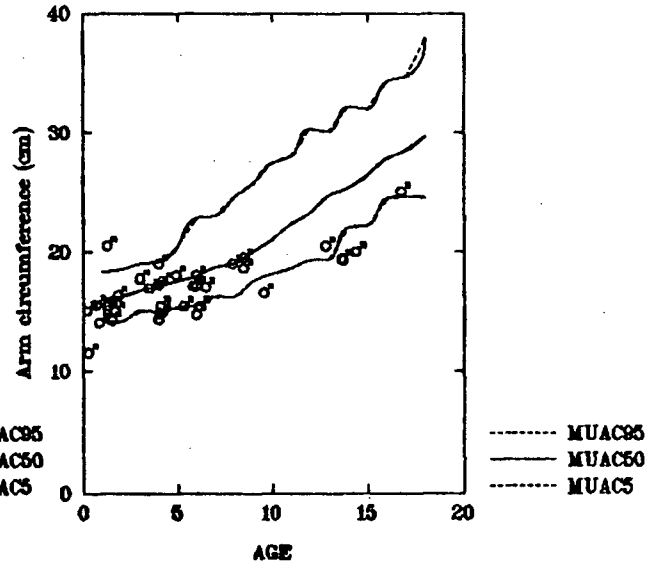
MUAC (NCHS), females, exp. group



MUAC (NCHS), males, control group

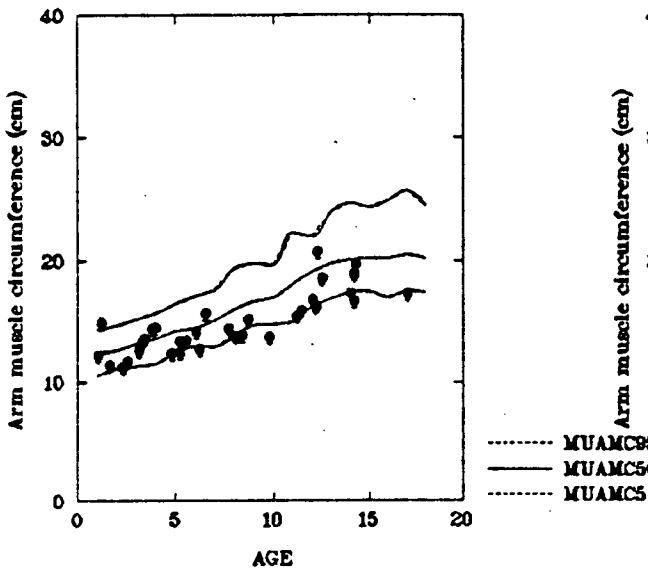


MUAC (NCHS), males, exp. group

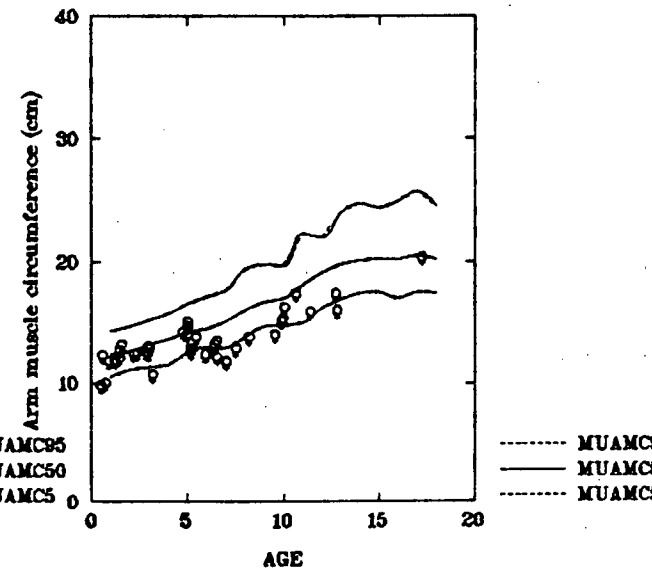


APPENDIX 9E: MID-UPPER-ARM MUSCLE CIRCUMFERENCE

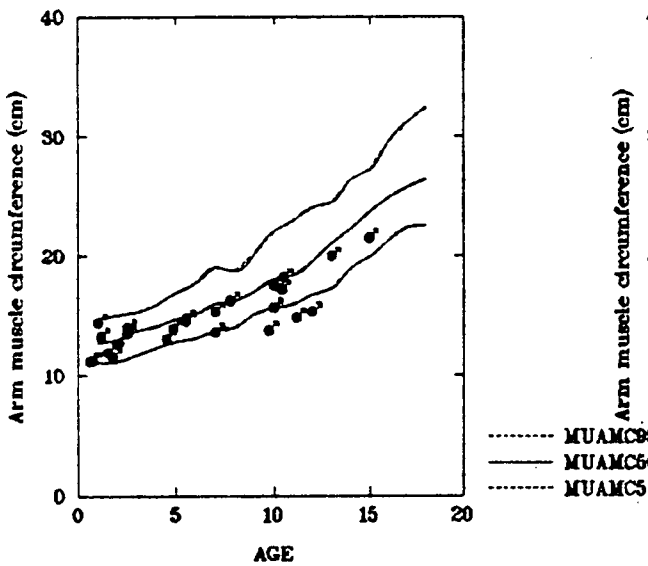
MUAMC (NCHS), females, contr. group



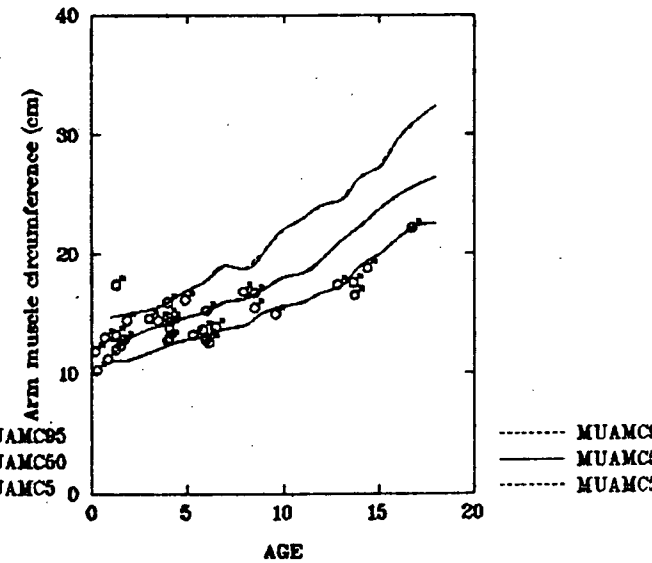
MUAMC (NCHS), females, exp. group



MUAMC (NCHS), males, control group

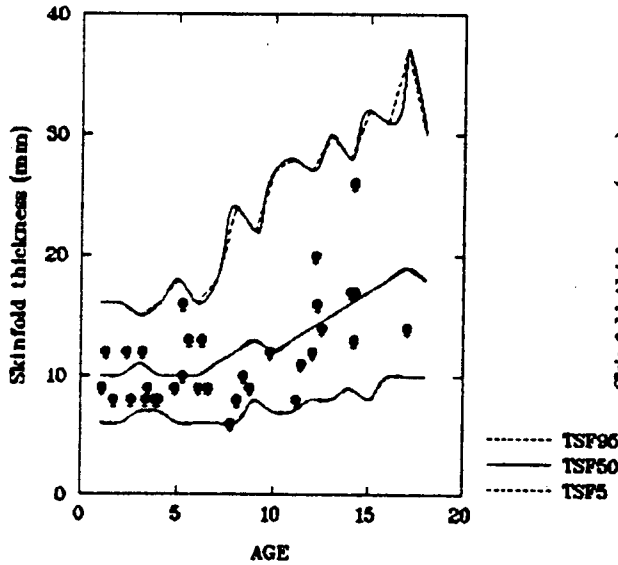


MUAMC (NCHS), males, exp. group

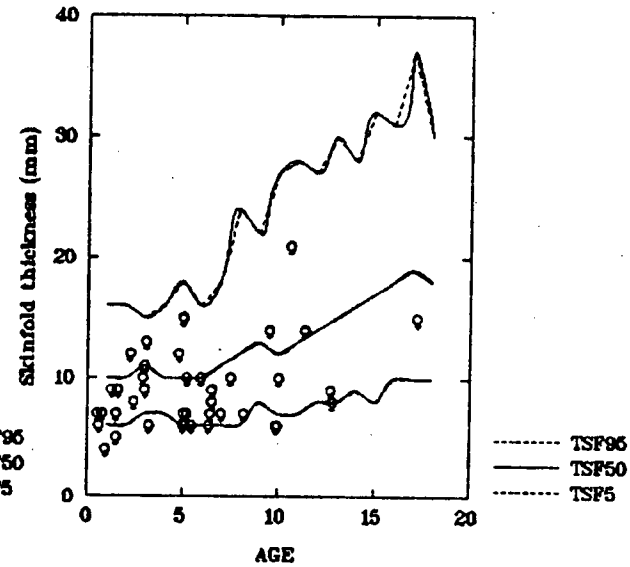


APPENDIX 9F: TSF THICKNESS

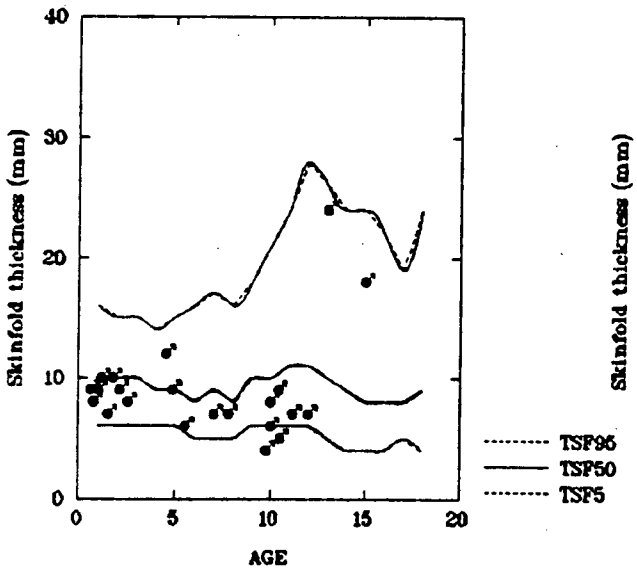
TSF (NCHS), females, control group



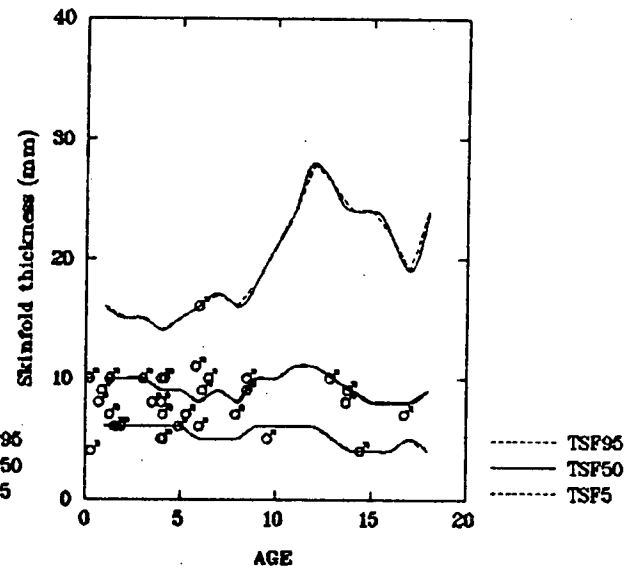
TSF (NCHS), females, exp. group



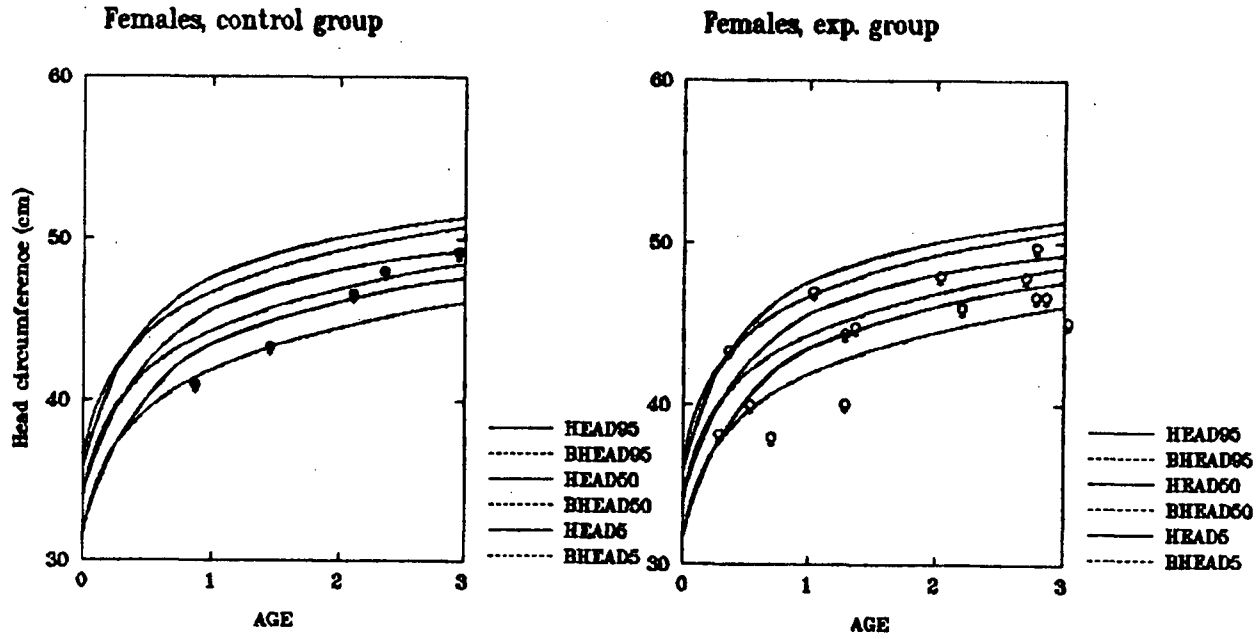
TSF (NCHS), males, control group

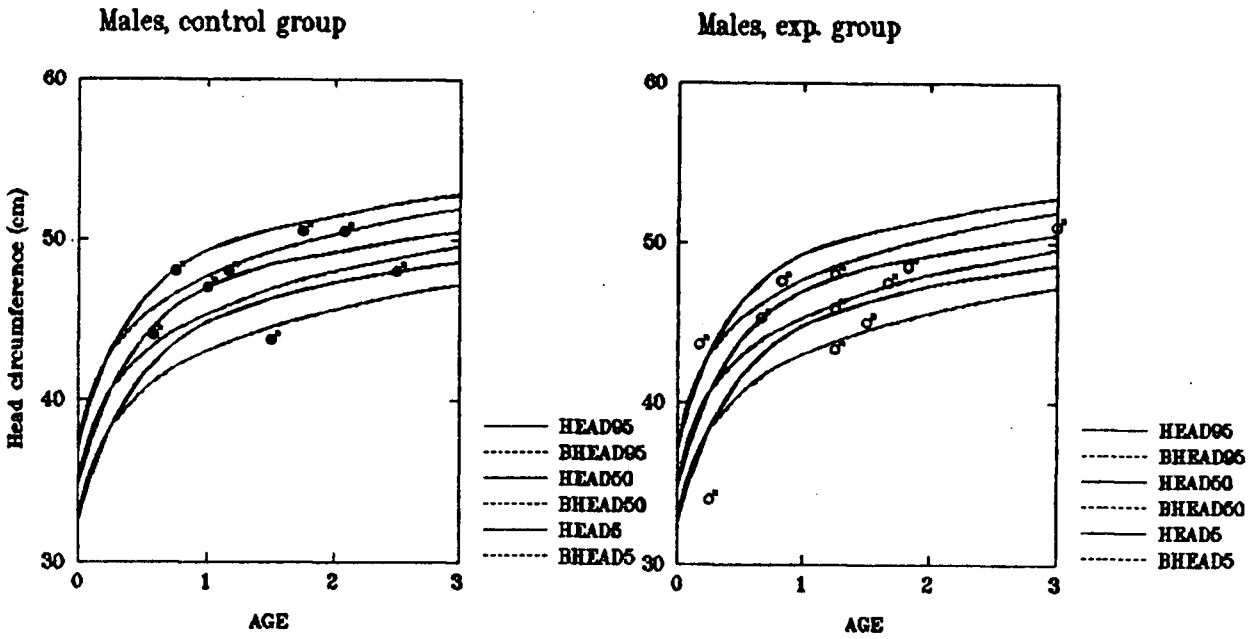


TSF (NCHS), males, exp. group



APPENDIX 9G: HEAD CIRCUMFERENCE





APPENDIX 10: PROTOCOL FOR MORBIDITY DATA

Code:

Family:

Date:

Symptoms	Name (Children)	Sex	Age
Diarrhea			
Fever			
Coughing			
Sore throat			
Running nose			
Other (specify)			