International Center for Research on Women

NUTRITION OF ADOLESCENT GIRLS
RESEARCH PROGRAM

*Research Report Series*
*No. 2*

# Response of Endogenous Growth Factors to Exercise and Food Supplementation in Stunted Pubertal Girls in Guatemala

ICRW

**International Center for Research on Women**
1717 Massachusetts Avenue NW, Suite 302
Washington, DC 20036

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# Response of Endogenous Growth Factors to Exercise and Food Supplementation in Stunted Pubertal Girls in Guatemala

December 1995

by

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## Contents

## Executive Summary

This project was conceived to explore whether previously stunted, pubertal-age girls were capable of showing an adequate secretion of endogenous growth factors in response to a physiological stimulus (physical exercise), and whether nutritional supplementation during puberty would enhance such response. This was explored applying recent advances in endocrine and laboratory technologies that allow the assessment of human growth hormone (hGH) and insulin-like growth factor I (IGF-I) production through their secretion in urine.

Three hundred sixty girls, 10 and 11 years old, from low-income families, who attended public schools in Guatemala City, were screened for eligibility to participate in the study. Most were currently well nourished. Based on their weight-for-height and body mass index, only 6 and 19% respectively could be classified as mildly undernourished, whereas the same indicators suggested that 5 and 11% respectively were overweight. However, 67% had some degree of stunting and 27% were more than 2 standard deviation units (Z score) below the NCHS median of height-for-age. Their socioeconomic background suggests that a suboptimal nutrition in earlier childhood played a role in this linear growth retardation.

Twenty-four of the girls who met the entry criteria -- early stage of puberty (Tanner Breast Development Stage II), pre-menarcheal, and stunted but not wasted nor overweight -- were selected for the study. A cross-over dietary intervention trial was conducted. Half the girls began receiving energy, protein and micronutrient dietary supplements for three months, followed by three months of a placebo; the cycle was repeated for a total of four 3-month periods of study. Twelve other girls followed a similar protocol, but began without the dietary supplements. This approach was used to account for changes related to the girls' increase in chronological age and maturation, rather than to the dietary intervention.

At the end of each 3-month period, maximal, submaximal and endurance exercise tests were performed on a treadmill at weekly intervals to elicit a human growth hormone response which, in turn, stimulates IGF-I production. These endogenous growth factors were measured in urine and plasma. Other tests and data collected at the three monthly intervals included:

* Submaximal exercise tests to estimate total daily energy expenditure in conjunction with heart rate monitoring and calculations of basal and resting metabolic rates;
* Anthropometric measurements;
* Body composition;
* Maturational stage and menarcheal status;
* Morbidity;
* Food frequency and 24-hour dietary recall; and
* Blood hemoglobin levels.

**Dietary Intake**

Home diets were generally deficient in calcium and riboflavin. Half the girls also had a consistent deficiency in iron intake, a problem expected to increase with menarche. Vitamin C was deficient and energy intake was marginal in some trimesters. All deficiencies were corrected by the supplement except for calcium.

Forty-eight percent of the girls enrolled in the study were anemic at baseline. Circulating hemoglobin (Hb) status improved significantly with supplementation.

**Endogenous Growth Factors**

Stunted Guatemalan girls had normal levels and appropriate responses of hGH to physiologic stimuli. Urinary and plasma levels of hGH were comparable to those of adequately nourished, healthy U.S. children of pubertal age, suggesting that the potential for growth is appropriate in the Guatemalan girls. There was no effect of supplementation on hGH response.

The girls also had normal plasma IGF-I levels, compared with well nourished, healthy U.S. girls. Urinary levels, however, were between 2 and 3 times greater than those obtained from the U.S. controls, suggesting that IGF-I production was higher, its bound fraction was lower, or both. This unexpected result requires further investigation. Dietary supplementation, as used in this study, had no effect on IGF-I levels.

**Anthropometry**

Girls grew significantly in height during each trimester. Height growth velocity in the Guatemalan girls was similar to that expected in the U.S.-based NCHS reference population. As anticipated due to the short duration of the study periods, there were no differences in linear growth in relation to dietary treatment nor evidence of catch-up in height.

Girls gained weight in each trimester of the study, but significantly more during supplementation periods than when they received placebo. This indicated adequate compliance with supplement intake and non-significant substitution of the home diet. Similarly, the girls increased more in BMI and percentage of body fat during the supplementation than the placebo periods. This suggests that they gained more fat than lean mass.

**Physical Activity**

Girls had significantly higher energy expenditure during the second supplemental period compared to both the first supplemental period and the second placebo period. This can be partly explained by the energy expenditure needed to displace the additional weight they had gained. Overall, their dietary energy requirements assessed by total energy expenditure and energy needs for growth, were 66 ± 11 kcal/kg/day. This corresponded to a mean physical activity level of 1.81 times their basal metabolic rate.

The girls led an energetic lifestyle spending, on average, a total of 4½ to 5½ hours per day in activities of moderate intensity and 15-30 minutes in heavy activities. Girls with the best record of compliance with dietary supplement intake ("Complete Compliers") spent more energy in moderate and heavy activities during the supplementation than the placebo periods.

**Conclusions and Recommendations**

Endogenous growth factors were well within normal ranges for this age group and responded adequately to strenuous exercise. Furthermore, stunted girls showed elevated urinary IGF-I levels after exercise. Their urinary hGH excretion, which is a reflection of the integrated pattern of changes in plasma hGH, tended to be higher on the night after

strenuous exercise. These findings suggest not only that the potential for normal growth was present, but that the hormonal capacity for catch-up growth was also present. Height changes were not affected by supplementation. This result had been anticipated due to the short duration of the intervention.

Diets were seriously lacking in calcium and, to a lower degree, in riboflavin and iron. There was also a small seasonal deficiency in dietary intake of vitamin C. During supplementation, all intakes were increased to provide the INCAP-FAO/WHO recommended levels, with the exception of calcium. As lime (i.e., calcium)-treated corn tortillas are a major source of calcium in the diet of Central American and other populations, policy makers must examine the implications of industrial tendencies to manufacture tortillas without prior treatment of corn with lime. The trend to change dietary habits from consumption of corn tortillas to bread must also be considered.

The level of iron in the supplement, 18 mg/d, resulted in an increase in blood hemoglobin concentration, but it was apparently not enough to build iron stores in girls during the rapid growth spurt of puberty. Problems due to iron deficiency become more serious with the onset of menstrual blood losses. Consequently, long-term iron fortification or effective supplementation schemes are recommended.

Diets of these girls from low-income families did not provide adequate amounts of retinol equivalents, except when there was a seasonal intake of fruits with high carotene contents. However, an effective ongoing sugar fortification program allowed satisfying the recommended dietary allowances of vitamin A throughout the year.

Given the positive outcomes of this investigation in terms of an adequate response in secretion of endogenous growth factors, longer term studies should be done, beginning prior to puberty and continuing until after menarche in girls, or mid-adolescence in boys. These will allow determining whether dietary supplementation alone or in combination with adequate exercise conditions during puberty and adolescence will allow catch-up growth to compensate for nutritional growth retardation in early childhood.

# 1. Introduction

Many scientists and health workers believe that pubertal girls who are stunted due to nutritional deficiencies during infancy and early childhood cannot catch-up in height during puberty. However, that conclusion is based on observations without controlled interventions during puberty, when profound and rapid metabolic changes take place in conjunction with increased growth velocity.

This project was conceived to explore whether stunted girls were capable of showing an adequate secretion of endogenous growth factors in response to a physiological stimulus (physical exercise), and whether nutritional supplementation during puberty would enhance such a response. This was explored applying recent advances in endocrine and laboratory technologies that allow the assessment of human growth hormone (hGH) and insulin-like growth factor I (IGF-I) through their secretion in urine.

A cross-over dietary intervention trial was conducted with 24 stunted girls, 10 and 11 years old, from low-income Guatemalan families who were in an early stage of puberty (Tanner's breast developmental stage II). Twelve girls began receiving dietary supplements that provided energy substrates, proteins and micronutrients for three months, followed by three months without supplements; the cycle was repeated for a total of four 3-month periods of study. Twelve other girls followed a similar protocol, but began without the dietary supplements. This approach was used to differentiate between changes related to the girls' increase in chronological age and maturation, and changes due to the dietary intervention.

At the end of each 3-month period, treadmill-based submaximal, endurance, and near-maximal exercise tests were performed at weekly intervals. All urine voided on the day of the exercise test and on the preceding and following days was collected to assess the immediate and delayed secretion of hGH and IGF-I. It is known that an exercise bout of maximal intensity may induce the secretion of growth hormone, and whether they were stimulated by submaximal or prolonged exercise of moderate intensity (endurance) that may be more related to everyday activities was also of interest in this study. The submaximal

exercise test would also allow estimating total daily energy expenditure in conjunction with heart rate monitoring and calculations of basal and resting metabolic rates.

Anthropometric measurements were obtained on admission and at the end of each three-month period. Body composition was also assessed at those time intervals. However, no changes in growth velocity were expected due to the short duration of the intervention and the wide variability of growth velocity during early puberty. Dietary intake was evaluated at baseline and every three months, and the possibility of a substitution effect by the supplements was examined.

Previous studies by our group at INCAP showed that preschool children with mild/moderate weight deficit became more active when their nutritional status improved, and that well-nourished but stunted pubertal boys were more active than boys with adequate weight and height from a better socioeconomic class. It is not clear whether this latter finding was due to differences in past nutritional history (i.e., present stunting) or to differences in lifestyle and demands imposed by the social environment of the two groups of children. Based on this, the effect of the dietary intervention on the physical activity intensity pattern of the girls at the end of each 3-month period was also explored.

Daily interviews with the girls and records kept by them were used to assess health status throughout the study. Blood hemoglobin concentration was measured on admission and every three months to evaluate whether the supplements corrected any deficiencies.

Since the participants in this study interacted frequently with the project personnel and an excellent rapport was established, it was decided to interview the girls' teachers for their subjective appraisal of changes in school performance and overall behavior by the end of the project.

This project was expected to provide information that might indicate the appropriateness of conducting a longer-term longitudinal study on growth modification of stunted adolescent girls over a 3- or 4-year period, from the beginning of puberty until after menarche. Additional preliminary support for such a project was examined in Berkeley through a study to evaluate growth characteristics of immigrant and US-born girls of Latin American origin in the San Francisco Bay Area. Results from that study are included here.

If the outcomes of this study and of a future longer-term project indicate that stunted girls conserve a biological potential for catch-up growth, and that nutritional improvement and other propitious environmental conditions during puberty enhance that response, they will provide strong support for targeted interventions in schools and the community. Furthermore, this will open a whole field of research related to nutrition and growth, and its application in the too often neglected age group of adolescence.

## 2. Background and Significance

### Pubertal Growth

Pubertal growth is defined here as the period of rapid growth between the beginning of accelerated growth, or take-off velocity (TOV), and achievement of adult height. The average duration of the acceleration phase of pubertal growth [from TOV to peak height velocity (PHV)] is 1.5 years; the average duration of the deceleration phase [PHV to menarche (M)] is 1.3 years (1). Total growth from TOV through menarche for well-nourished U.S. girls averages 26.1 $\pm$ 7.75 cm and PHV is 7.8 $\pm$ 1.65 cm/yr (2).

Little is known about accelerated growth during puberty among previously stunted girls undergoing a change in environment. The few reports available refer to growth in stable environments (2-8). Most investigators agree that growth during puberty is similar among more and less developed populations (3,5,6,8), and that the height deficit of adults in developing countries is due to early childhood insults. Some studies, however, suggest an increased accelerated growth during puberty in children of low-income families and a tendency to regain growth lost during prior nutritional insult (3,4).

In a longitudinal study of growth, Johnston, *et al.*(9), found no difference in prepubertal growth of affluent Guatemalan (Hispanic) and European children attending the same school in Guatemala City. However, the European children grew an average of 5 cm more during puberty than did the Guatemalan children. Similar results are seen in the cross-sectional analysis of the Hispanic Health and Nutrition Examination Survey (HHANES), which showed little deficit in height among Hispanic girls compared to the NCHS references for girls of 2-5 or 6-11 years, but a significant height deficit in girls 12-17 years old (10,11). At this latter age, Hispanic girls were on average 4 cm shorter than their non-Hispanic white counterparts. The conclusion drawn was that the adult height deficit seen in Hispanic populations is due to genetically determined reduction in growth during puberty rather than during childhood. This study has several limitations: it was cross-sectional, it did not examine sexual maturation stages, and immigration status was not taken into account. Thus, children who had only recently moved to the U.S. were grouped for analysis with long-term immigrants and with second- and third-generation

Hispanic-Americans. As a result, the growth deficit reported during puberty may be a cohort effect (with adolescent children showing shorter stature due to larger deficits during early childhood) or an artifact due to the recent, shorter immigrants who reduce the mean height of 12-17 year old Hispanic-Americans.

**Hormonal Control of Growth**

Linear growth is regulated by a sequence of hormonal releases instigated prepubertally by growth hormone-releasing hormone (GHRH) and during puberty, in girls, by estradiol (12,13). hGH, IGF-I, sex steroids, insulin, thyroid, and other hormones are directly responsible for the initiation and progress of growth.

Growth hormone plays an integral role in linear growth. Briefly, hGH is released from the anterior pituitary in response to hypothalamic GHRH. hGH acts on the liver and other target tissues (14), which respond by producing IGF-I, also called somatomedin-C. IGF-I stimulates DNA synthesis, cell multiplication, and incorporation of inorganic sulfate into cartilage tissue (15). Plasma concentration of hGH varies throughout the day, as the hormone is released periodically -- specifically during deep sleep, after eating, or following exercise. By contrast, IGF-I concentration in plasma is fairly stable throughout the day (16), and may increase slightly after exercise (Keim, personal communication). IGF-I is hGH-dependent and has a negative feedback system: when plasma IGF-I is high, no hGH is released; and when it is low, GHRH triggers the release of hGH.

Factors other than hGH can affect plasma IGF-I levels. Patients and laboratory animals with prolonged nutrient deficits, particularly of protein, have negligible plasma levels of IGF-I (17,18). Low levels of IGF-I stimulate an increased production of hGH, but if nutrient intake remains low, no commensurate increase in IGF-I will occur. Thus, the malnourished child, especially with kwashiorkor, has elevated hGH levels but nearly absent levels of IGF-I. As a consequence, growth ceases.

Insulin is also important for normal growth (19). The mechanism for its promotion of growth is still under investigation. Recent studies have shown that physiological doses of insulin have a direct effect on longitudinal growth in rats and can enhance incorporation of sulfate at the cartilage growth plate (20). These effects are not mediated by IGF-I. It

has been hypothesized that they may be due to a cross reaction of insulin with the IGF-I receptor (21,22).

These growth-promoting factors can be affected by environmental factors, such as nutritional status and activity levels. It is, therefore, important to measure them when examining the relationship between growth and environment. Few investigators have measured hGH in normal children because the periodicity of hGH release requires 24-hour blood sampling. Recently however, a breakthrough on the measurement of hGH production was achieved: it was shown that overnight urinary excretion of hGH reflects integrated levels of plasma hGH (23). Sukegawa, et. al (23), reported correlations between nocturnal urine and nocturnal plasma hGH of 0.76 ($p<0.001$), between 24-hour urine and 24-hour plasma of 0.77 ($p<0.001$), between nocturnal urine and 24-hour plasma of 0.84 (0.001). This non-invasive technique allows monitoring of the daily average hGH production during the pubertal growth spurt and to explore changes in hGH production in response to physical exercise. The same group reported correlations between 24-hour urinary IGF-1 and plasma levels of 0.78 ($p<0.001$)(24).

**Physical Activity and Growth**

Growth may be optimum when it occurs in a "favorable environment", defined here as an environment where overall health is promoted and safeguarded, and where food intake and physical exercise patterns are adequate.

There is indirect and direct evidence that exercise is an important factor in child growth. There is a seasonal pattern of growth in length, with accelerated growth during months that favor physical exercise (25). Stunted cardiac pediatric patients with low physical activity show catch-up growth in height after cardiac repair that allows greater physical activity, even without increase in dietary intake (26).

In rats with mild/moderate dietary deficits, increased physical activity was associated with an increase in the efficiency of food utilization (i.e., g weight gain/g food consumed) (27,28). Pair-fed weanling rats with restrictions in activity grew less than their exercise-stimulated counterparts, regardless of dietary intake (29). In another study,

well-nourished female hamsters that had just reached a mature, stable size grew further when they exercised, while undernourished hamsters lost this capacity to respond to exercise (30).

Studies in young children during rapid growth while recovering from protein-energy malnutrition, showed that those stimulated to participate in mild/moderate physical exercise grew better in height and lean body mass deposition than those without increased exercise but similar psychosocial stimulation and identical nutrient intake (29,31,32).

In well-nourished populations, physical activity has not been clearly associated with increased linear growth during puberty, but it has enhanced growth in lean body mass (33). Accelerated growth under these conditions might be reflected by increased secretion or enhanced effects of certain growth factors such as hGH, IGF-I and insulin. In these populations, it has been shown that acute, heavy exercise stimulates the release of hGH (34,35). However, it is not known whether milder or submaximal exercise performed regularly or for a prolonged time induces immediate or 24-hour increases in hGH secretion and, consequently, of IGF-I.

## 3. Rationale for the Study

Little is known about the relationship of exercise and growth in children who live under stable and chronic conditions of suboptimal nutrition, leading to stunting or mild degrees of malnutrition. Studies on activity patterns and energy expenditure of such children of preschool age have shown that those with better weight-for-height are more active and that improvement in nutritional status is accompanied by increase in physical activity (36). There is supporting evidence for this in studies on children of school age (37). However, no cause-effect relationship has been clearly established and the influence of exercise under these chronic, suboptimal nutritional conditions has not been studied.

It is not known whether pubertal children who are stunted, yet stable in their nutritional status, will respond to the exercise stimulus. Neither is it known whether an improvement in dietary intake will increase their growth hormone response to the stimulus and, thus, improve growth.

In theory, improved nutrition might enhance the capacity to engage in physical exercise. It would then be expected that, given adequate nutritional intake to cover the energy cost of physical activity and the protein and other nutrient needs for body mass synthesis, as hGH production is stimulated by exercise, IGF-I would increase. Alternatively, exercise may alter sleep depth and increase hGH release at night.

In this study, some of these relationships between nutritional status, dietary intake and endogenous growth factors during puberty among stunted, but presently well nourished, Guatemalan girls were investigated. The hormones hGH and IGF-I were selected because they are both directly related to linear growth and susceptible to everyday influences of diet and exercise.

### General Objectives

The study was undertaken to explore the response potential and the possibility that a short-term (three months) improvement in nutrient intake would increase the capacity of pubertal girls with nutrition-related moderate stunting to produce endogenous growth factors, specifically human growth hormone (hGH) and insulin-like growth factor I (IGF-I).

Other important aspects of the girls' nutritional, behavioral, and developmental conditions studied included their growth velocity, changes in weight and body composition, intensity pattern of daily physical activity, total energy expenditure, habitual dietary energy and nutrient intakes, and changes in physical fitness and sexual development during one year of puberty.

Parents and teachers were interviewed to assess whether the girls modified their overall behavior and school performance during the year of participation in the project and regular interaction with the project personnel.

## Hypotheses and Project Aims

### *Hypotheses*

The primary hypotheses to be tested was: Stunted girls with adequate nutritional intake for their age, based on ideal body weight (NCHS), would have greater immediate and delayed secretion of certain endogenous growth factors (specifically, hGH and IGF-I) in response to physical exercise of different intensities, compared with similar girls who do not receive dietary supplements.

In practical terms, this hypothesis implies that when girls are well nourished, everyday physical activity induces greater responses of endogenous factors that enhance growth. In the case of hGH, this may occur by enhanced nocturnal and/or postprandial production of the hormone.

Two secondary hypotheses were also tested: 1) Higher production of the aforementioned growth factors will be associated with greater growth in height, body mass and body segments because of increased growth velocity; and, 2) Improvement in dietary intake will increase the spontaneous physical activity of pubertal girls with linear growth retardation.

### *Specific Aims*

- To determine if there is a nutrition-mediated interaction between an exercise stimulus and the urinary excretion of endogenous hGH and IGF-I, and if so, how does the response differ with exercise of different intensities.

· To measure over time growth in body mass and body components, including height, weight and body segments, and to determine if growth velocity is associated with the production of endogenous growth factors.

· To determine if increased nutrient intake modifies the pattern of daily physical activity of stunted pubertal girls.

*Additional Explorations*

To test the above-mentioned hypotheses and pursue the stated aims, data were collected to address additional issues regarding the physiology and behavior of pubertal girls, as well as methodological matters. Thus, the following topics were also addressed in this report, although they were not part of the primary objective.

· Determination of total daily energy expenditure and assessment of whether it was influenced by the changes in energy and nutrient intake. This provided information on the energy requirement of girls of this age group.

· Determination of habitual dietary intake and the influence of dietary supplements on the home diet intake ("substitution effect").

· Assessment of physical fitness and its modifications during one year of puberty.

· Evaluation of whether participation in a one year project with regular individual interaction and attention by project personnel influenced the overall behavior and school performance of the girls.

· Correlation of 12- and 24-hour hGh and IGF-I urinary secretion with changes in plasma concentration before and after a maximal exercise test.

## 4. Experimental Design and Methodology

A cross-over dietary intervention trial, with each girl serving as her own control, was used. Twelve girls (Group B) began receiving dietary supplements for a three-month period ("first supplement period", S1) followed by three months with a placebo ("first placebo period", P1). These two treatment schemes were repeated in a 12-month period (periods S2 and P2). The other 12 girls (Group A) were subject to a similar treatment, but they began with the "placebo period" (i.e, P1, S1, P2, S2). This is illustrated in Table 1. The four sequential trimesters will be identified in this report as T1, T2, T3 and T4, regardless of the dietary treatment.

As Table 2 shows, information was obtained at the onset of the study (basal data), and at the end of each trimester. This included the performance of three types of exercise on a treadmill at weekly intervals. All urine voided was collected on the day before, the day of, and the day after each exercise test. Venous blood was also collected shortly before and two minutes after the maximal exercise test.

Other tests and measurements done at the end of each trimester included anthropometric measurements, assessment of body composition by various methods, calculation of dietary intake, measurement of total daily energy expenditure, assessment of physical activity pattern, evaluation of physical fitness, appraisal of breast development (Tanner sexual maturation index), and measurement of blood hemoglobin concentration. Health status was monitored every day through interviews from Monday through Friday, and by records kept by the girls on weekends, vacations, and absences from school.

Initial or "basal" information was obtained prior to beginning the first supplementation period (S1). For girls in Group B, this was done in most instances at the end of the first trimester with placebo (P1), as no dietary intervention had yet been involved. For variables that required estimates of change (either in absolute terms or as velocity), information was also obtained in Group B at the beginning of P1 (Table 2).

The third trimester of the study (T3) included the first two months of the 3-month school vacation. Compliance with supplement intake (S2) by Group B in that trimester was monitored through home visits by a member of the research team, and the tests scheduled

for the end of the trimester were done providing special transportation to INCAP laboratories.

For Group A, who received the placebo in T3 (Tables 1 and 2), that trimester finished two months before the fourth experimental trimester (i.e., T4) started. Thus, prior to beginning T4, dietary information and anthropometric and body measurements were obtained in Group A to evaluate the changes that occurred in the last supplement period (S2).

**Recruitment and Selection of Girls**

Sample size calculations were based on an expected 4-fold increase in plasma hGH with exercise, a coefficient of variation of 55% (based on reported levels of urinary hGH in normal, short children), and the assumptions that there would be: a) no order (or carry over) effect, and b) an intra-child correlation of repeated measures of hGH of 0.6. Using repeated-measures analysis of variance, a type I error of 0.05 and a type II error of 0.20, group mean differences of 81% or more could be detected with a sample size of 20 girls. To compensate for a possible drop-out rate of 20%, 24 girls were recruited.

As stated in the introduction, height growth velocity is directly related to maturational stage (2). Without longitudinal height measurements, it is not possible to determine where a child is along this pubertal path. It is possible, however, to estimate it by assessing Tanner maturational stages. Most girls in Tanner Breast Stage 1 (no breast development) have not yet started puberty. Tanner Stage 2 (budding) and Stage 3 (enlargement) are associated with girls in the early and late acceleration phase, respectively. Tanner Stages 4 (enlargement; nipple/areola development) and 5 (adult size and shape) are associated with reaching menarche and adult height, respectively. Admission criteria were:

(a) Development Status: Tanner's breast developmental stage II/pre-menarche.

Because of the short duration of the study, it was important to recruit girls who were in the early acceleration phase of puberty. Thus only girls in Tanner Breast Stage 2 and who had not yet had their first menstrual period were recruited.

(b) Age: 10 y to 11 y 11 m.

Kaplowitz and Martorell (38) reported the average age at menarche of Guatemalan girls to be 13.5 years. Given that average duration between TOV and M is 2.8 years, girls between the ages of 10 years and 11 years+11 months were recruited to optimize the number of girls surveyed who were already in the accelerated growth phase of puberty, but who had not yet reached menarche.

(c) Anthropometric Status: Height-for-age 1.5 or more standard deviations below the median of the NCHS reference data and weight-for-age within ± 1.2 standard deviations of the NCHS median.

To measure improvements in height due to the dietary intervention without the confounding factor of catch-up growth from current malnutrition, only stunted but not wasted girls were included in the study.

(d) Socio-Economic Status: Low.

Low socioeconomic conditions were chosen because, in the Guatemalan milieu, they are often associated with suboptimal nutrition in infancy and early childhood that leads to nutritional stunting.

(e) Good health and no physical or mental impediments to perform the tests and follow instructions.

(f) Willingness of the girl and her parents/caretakers to participate in the study.

(g) Willingness of school authorities to collaborate.

(h) Regular school attendance and no plans to move to another school district in the following 12 months.

Three hundred and sixty girls between 10 years and 11 years plus 11 months old were screened in 10 public schools to select the 24 participants. Thirty-six of them (10%) were excluded because they had already reached menarche (Among them, 4 had breast development at Tanner stage 1, 16 at Tanner stage 2, and 16 at Tanner stage 3). Of the remaining 324 girls, 93 were excluded due to breast development at Tanner stage 1, and 17 at stage 3.

Among the 214 girls still remaining, only 94 (44%) had a height-for-age 1.5 standard deviation (Z score) or more below the NCHS median. Twenty-seven of the 94 girls were

excluded for having a weight-for-height greater than +1 Z or lower than -1.2 Z in relation to the NCHS median. This left only 67 potential candidates for the study, or 19% of the 360 girls initially screened for menstrual history, breast development, height and weight.

Group and individual meetings were held with the potential candidates and their parents to obtain further information, explain the procedures, and request their consent to participate. Several girls were excluded because their parents were planning to move to a distant place within the next year, they lived too far from school to allow longitudinal follow-up with the project's resources, their parents refused permission, or findings during physical examination and clinical history precluded their admission. In addition, girls from 6 schools were excluded because the small number of qualified candidates in each school and the distance between them were practical limitations for the study.

Fifteen girls from two schools that were 7 blocks apart, and 17 girls from two other schools that were 3 blocks apart were the final candidates. Eight of these girls with the highest weights-for-height were excluded, in order to work with twelve girls from each pair of schools, in accordance to the sample size previously established. They are called Groups A and B in this report. Their mean ages, weights and heights are shown in Table 3.

**Dietary Supplements**

During the "supplementation" periods (S1, S2), each girl received the high-energy/high-protein cookies, orange-flavored beverages and vitamin/mineral tablets described in Table 4[1]. Cookies and a beverage were selected to reduce the risk of substitution effect on the home diet, as they were seen by the girls more as "snacks" than as "food".

Every day each girl received four 28-gram cookies (eight more on Fridays to take home for the weekend), one 200-ml cup of the beverage (on Fridays, also two polyethylene

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[1] The multivitamin and mineral tablets were kindly prepared and donated by UNIPHARM, S.A., who modified their commercial product (Vimex Forte™) to increase its zinc content according to our specifications. UNIPHARM also contributed with the preparation of the placebo tablets described below. The cookies, prepared at INCAP, were similar to those used by Guatemala's Ministry of Education in their school nutrition program. The orange-flavored beverage was made from a powdered commercial product, sweetened with vitamin A-fortified sugar, according to Guatemalan law.

bags with the dehydrated components for the weekend) and one vitamin/mineral tablet (two more on Fridays for the weekend). From Monday through Friday, the girls drank the beverage, ingested the tablet and ate one or two cookies under direct supervision of a field worker who visited them every day at school and periodically at home during vacations. Additional supplements were given to take home on school holidays. Additional cookies and powdered ingredients for the beverage were also given to take home for other children who lived in the same house. This was done, both for ethical reasons in low-income households, and to reduce the risk that the study subjects would share the supplements instead of ingesting them.

It was not possible to manufacture an identical placebo for the cookies. Therefore, during the placebo periods (P1, P2) each girl received a coated lactose/starch tablet identical to the vitamin/mineral tablet, plus a non-coated white lactose/starch tablet instead of the cookies and beverage. These were distributed in the same manner and with the same frequency as the nutrient supplements.

The field worker asked each girl every day about the number of cookies eaten the previous day. On Monday the questions included the cookie, tablet (vitamin or placebo) and beverage consumption over the weekend. This information was compared with the daily records that the girls kept -- which also included the hours of bedtime and symptoms of illness -- and with the data from the 24-hour dietary recall surveys, in order to assess compliance with supplement intake.

**Exercise Tests**

At the end of each three-month period, the girls were taken three times at weekly intervals to INCAP's Work Physiology laboratory for maximal, submaximal and prolonged ("endurance") exercise tests on a treadmill. Resting heart rate (telemetry) and energy expenditure (open-circuit indirect calorimetry) were measured lying down, sitting and standing, before each test. Heart rate was monitored continuously to ensure that the girls were exercising at the required intensity level.

The maximal test was done by increasing the inclination and/or speed of the treadmill at 2-minute intervals until the girl decided to stop. This usually required 7 or 8

graded work increments and the final heart rate was between 195 and 210 beats/minute. Oxygen consumption ($VO_2$) was determined during the last 30 seconds with each workload.

The submaximal test involved walking for 4½ minutes at each of five increasing workloads (exhaled air was collected during the last minute to determine oxygen consumption). The heaviest workload was around 70% of maximal effort (i.e., 70% of each girl's $HR_{max}$, $VO_{2\ max}$).

The endurance test consisted of walking three times for 30 minutes at a workload with a heart rate corresponding to 55-60% of $VO_2$ max, alternating with 30 minutes of rest (watching television, reading, writing, or playing table games).

**Urine Collection**

Urine was collected one day before (Day 1), one day after (Day 3), and on the day of each exercise test (Day 2). Day and nighttime urine was collected in separate bottles, all of which were kept in an icebox. On Day 2, urine was also collected separately immediately before the exercise test and for 6 hours after the test. Urine voided in school or outside the home, was collected in a container that the girl kept in a shoulder bag. The container was placed in the icebox as soon as the girl returned home. Urine containers were collected every day and transported under refrigeration to INCAP for measuring, aliquoting and freezing at -70°C until analyzed.

**Blood Collection**

Ten mL of venous blood were obtained shortly before and two minutes after the maximal exercise test. Plasma was stored frozen at -70° C until analyzed.

**Endogenous Growth Factors**

Growth factor responses were explored by measuring hGH and IGF-I in plasma and urine. Plasma hGH was measured before and at the end of maximal exercise tests, and IGF-I was measured in the pre-exercise blood sample. Both hGH and IGF-I were measured in the urine voided throughout the day and night prior to each exercise test (D1, N1); on the day of the exercise test, in a pre-exercise sample (Pre-ex), and in all urine voided during

less than 6 hours after the test (LT6), more than 6 hours for the remainder of the day (GT6), and during the night (N2); and, in the urine voided throughout the day or night of the following day (D3 and N3).

The rationale for this sampling scheme was as follows: (a) hGH is released periodically as a response to diverse physiological stimuli during the day, such as exercise of certain intensity, and more so during REM sleep; thus, normally more hGH is secreted at night; (b) hGH response to exercise is usually detected by measuring the hormone concentration in plasma before and immediately after the exercise; (c) an "integrated, time-related" hGH response can be assessed by measuring urinary hGH excretion during specific collection periods; if the exercise tests would modify the sleep pattern, changes in hGH excretion would be expected on N2; and (d) IGF-I is produced in response to hGH secretion after a lag period of several hours, and it has a longer biological half-life. Thus, IGF-I concentration in a single blood sample represents the amount of hormone that is acting on its target tissues, whereas its content in a timed urine collection (e.g., 12 hours) represents an "integrated, time-related" response to the stimulus that caused its production, mediated by hGH secretion.

It is important to note that the techniques used in this investigation to measure urinary excretion of hGH and IGF-I have only recently been developed and that this study is among the first to measure the "integrated time related" response to exercise[2]. Hormone concentrations have so far been analyzed only in the urine samples associated with the maximal exercise tests, due to financial constraints. All other urine aliquots remain stored in an ultralow temperature freezer at -70° C, for future analysis and evaluation.

**Anthropometry and Body Composition**

Body weight, sitting and standing height, calf length, skinfold thicknesses (biceps, triceps, sub-scapular, supra-iliac, and calf) and abdominal, leg, and arm circumferences were measured on admission and every 3 months. Weight was measured to the nearest 20 g,

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[2] This part of the study was done thanks to the collaboration and technical support of Genentech, Inc., San Francisco, CA, and especially of Dr. Abby Celniker, Dr. Denise Harrison and Mr. Wes Webb.

wearing a hospital gown. Height was measured to the nearest 1.0 mm. Skinfolds were measured to the nearest 0.1 mm with Lange skinfold calipers. Circumferences were measured to the nearest mm with a flexible tape.

Body fat was calculated from anthropometric measurements, using formulas derived from Guatemalan girls of similar body build (40). These use weight, weight/height$^2$, Body Mass Index (BMI), and triceps skinfold.

Body composition was also measured by densitometry through underwater weighing and measurement of residual lung volume by helium dilution. Technical problems did not permit measurements on admission. They were done at the end of every trimester in all but three girls who did not want to go through this test.

Body bioelectrical impedance (BEI) was measured at baseline and every 3 months thereafter (RJL Systems, analyzer model BIA-101). Age and sex specific equations were used to estimate body composition (39).

**Sexual Maturation**

Tanner Breast Stages were determined by visual inspection of the breasts. A trained female field worker obtained the girls' anthropometric measurements while the girls wore loose-fitting, sleeveless hospital gowns. In the process, the field worker discretely glanced at the girls' breasts to assess the level of development.

**Dietary Intake**

An experienced nutritionist (M.M.R.) evaluated individual dietary intakes on admission to the study and every three months. Twenty-four hour recall and 30-day semi-quantitative food frequency intake interviews were performed. Plastic models and containers were used to assess portion size. The 24-hour recall interviews were done on two non-consecutive week-days and on a Sunday, within a 10-14 day period. The data of the three days were combined and assumed to represent the girls' habitual intake for the trimester. The items in the food frequency questionnaire were selected after preliminary work with these and other girls and boys of comparable socioeconomic conditions in Guatemala City.

The Latin American Food Composition table prepared and updated at INCAP was used to determine energy and nutrient intake, after analyzing the recipes and applying appropriate conversion factors of cooked to raw ingredients. Assessment of retinol intake included the mean content of this vitamin in fortified sugar, as mandated by Guatemalan law. Niacin intake was calculated as niacin equivalents, including the niacin derived from tryptophan in dietary protein, which was assumed to be 1.1%.

**Total Daily Energy Expenditure and Activity Patterns**

Total daily energy expenditure (TDEE) and patterns of activity intensity were assessed by minute-by-minute heart rate recording on two different week-days and one Sunday within 10-14 days. The girls wore a light-weight (45 g) heart beat transmitter attached to a belt strapped around the chest, and a receiver that resembled a wrist watch (Polar, Inc., Finland). The data for each day's heart rates were transferred to a computer. Computer programming developed by our group was used to convert the information into energy expenditure and to evaluate the time allocated to activities requiring different intensities of physical effort.

Energy expenditure while awake and active was calculated from the heart rates and the heart rate-$VO_2$ relationship established for each girl during the submaximal exercise test. Resting $VO_2$ (RMR) was used to estimate energy expenditure in sedentary activities. Basal metabolic rates (BMR) calculated with Schofield's equations (40a) and corrected according to our measurements of resting metabolic rate, were used to calculate energy expenditure while sleeping.

The pattern of physical activity was established for each girl from her minute-by-minute energy expenditure, using the following classification:

| | |
|---|---|
| Heavy: | > 50% $VO_2$ max |
| Moderate: | 31 - 50% $VO_2$ max |
| Light: | Between RMR and 30% $VO_2$ max |
| Sedentary: | RMR |
| Sleep: | BMR |

**Physical Fitness**

Physical fitness was evaluated from physiological indices of oxygen consumption and heart rate during the performance of the exercise tests. The indices included: maximal oxygen consumption ($VO_2$ max), regression coefficient (slope) of oxygen consumption on heart rate, oxygen consumption at specific heart rates ($VO_2$-150 and $VO_2$-170), and heart rate after one minute of recovery from maximal exercise.

**Attained Growth of Adolescent Immigrant and US-Born Latino Girls**

As a complement to the research in Guatemala, a cross-sectional study in the United States was conducted. Children born and raised in an environment which includes access to sufficient amounts of nutritious foods, adequate sanitary conditions and reduced exposure to infectious diseases have a greater chance of growing to their genetic potential than children raised in less optimal environments. To determine whether place of birth and maturation affected attained height, weight, and body composition measures, US born and immigrant Latino girls age 8-12 years were recruited from 8 schools in Oakland, California, to participate in the Adolescent Growth Project. Informed consent was attained from the girls and their parents.

Anthropometrics were measured using a standard protocol by standardized personnel. Total body bio-electrical impedance (BEI) was measured using the RJL Systems analyzer (model BIA-101). Total body fat and fat free mass were calculated from BEI and anthropometric measurements using age and sex specific equations from Durenberg, Kusters and Smit (1990).

A trained, older female Latino field worker discretely evaluated breast development for each girl as the anthropometric measurements were being taken. Each girl was categorized into Tanner Stages based on breast development: Tanner Stage 1 (infantile breasts), Tanner Stage 2 (budding), or Tanner Stage 3 and higher (increase in size to adult breast size and shape). Date of menarche was also obtained.

Statistical analysis was carried out using SPSSx. Analysis of covariance was used to compare anthropometric measures between the immigrant and US born girls while controlling for age and development stage.

# 5. Results

## Participants in the Screening Process

Of the 360 girls screened in 10 public schools, 11, 46 and 44% were in the third, fourth and fifth grades, respectively. Fifty-nine percent of them lived with both parents, 28% only with their mother (with or without a stepfather), 3% only with their father (with or without a stepmother), and 10% with other relatives or caretakers, most often with one or two grandparents. Table 5 shows the parents' occupations in ascending order of education requirements, which is frequently associated with income level. Table 6 shows the nutritional indexes used to assess the girls' nutritional status. Six percent had low weight that could be classified as "mild malnutrition" based on the NCHS weight-for-height reference data, or on U.S. data of body mass index. None had moderate or severe malnutrition (< -2 Z weight-for-height or < 3rd centile BMI). In contrast, 67% had some degree of stunting and 27% were more than 2 standard deviation units below the NCHS median of height-for-age. Data on weight-for-age are shown in Table 6 to illustrate that they can be grossly misleading in assessing current prevalence of malnutrition among school-age children. Based on the BMI or weight-for-height reference data, either 11% or 5% of the girls were overweight, respectively, 2% or 3% importantly so (> 94th centile BMI or > + 2.9 Z weight-for-height).

Among the 216 girls who had not reached their 11th birthday, breast development according to Tanner's scale was at levels I, II and III in 37%, 60% and 3% of the girls, respectively. Six percent had reached menarche. The corresponding proportions among the 144 girls between 11 years and 11 years + 11 months old, were 12%, 70% and 18% with breast development at Tanner stage I, II and III, respectively. Seventeen percent had reached menarche.

It is difficult to make a distinction among ethnic groups due to the different degrees of mixture between persons of Mayan Indian and other (usually Caucasian) races. A subjective appraisal of the facial, skin and hair characteristics of the 360 girls who were screened showed predominant Mayan indian features in 34%, Caucasian in 4% and a wide spectrum of probable Mayan-Caucasian mixture in 62%.

## Results of the Intervention Study

### *Dropouts and Compliance*

Of the 24 girls who were recruited for the study, 2 girls did not complete the study. The following results are based on 22 girls who completed the study unless otherwise noted.

Several artifacts could influence the study outcome, such as low compliance with the supplement intake, marked decrease in home diet intake, or illness of several weeks' duration. Results from each of the 24 girls who started in the study were carefully evaluated to select those without such artifacts. A new set of statistical analyses was run on only those girls with no artifacts and are referred to as "Complete Compliers".

Compliance with the dietary supplements was measured through direct observation by a field worker and self-reporting by the girls. During the school-year, 55 ± 5% of the vitamin/mineral tablets and orange-flavored beverage, and 18 ± 4% of the cookies were eaten under direct supervision of the project's field worker.

The following were excluded from the analyses done on Complete Compliers. In the first semester (with either the sequence Placebo 1-Supplement 1, or Supplement 1-Placebo 1) were excluded one girl who reported a supplement intake of 65% and gained less weight than during the trimester with placebo, four girls who reported adequate supplement intakes but gained less weight than with placebo, and two other girls who had an infectious illness that lasted 3 weeks. This left 17 girls (9 from Group A and 8 from Group B) with satisfactory data. These girls are referred to as Group OK1.

Excluded in the second semester (with the sequence Placebo 2-Supplement 2 or Supplement 2-Placebo 2) were two girls who dropped out of the study, five girls who reported adequate supplement intakes, but gained less weight than during the corresponding placebo period, and one girl who fractured a femur and had limited mobility for about 2 months. This left 16 girls (9 from Group A and 7 from Group B) with satisfactory data. They are referred to as Group OK2.

Combining the two semesters, only 12 of those girls had complete, satisfactory, data throughout the study. They are referred to as Group OK. All variables were analyzed with the data from the Complete Compliers -- Groups OK1, OK2 and OK -- for the first semester, second semester or whole study, respectively.

*Endogenous Growth Factors*

The results presented here must be considered preliminary because they are the product of an initial analysis of variance of the data. Final analysis must await multivariate analyses to adjust for health and anthropometric status, as well as current or changes in maturation level.

**Plasma hGH.** Pre-exercise plasma hGH levels (pg/mL) were within normal limits for all subjects, and post-exercise increases in hGH reflected a clear and normal response (Figure A). This occurred independent of the dietary intervention (supplement or placebo) and whether placebo or supplement preceded the current dietary intervention (no carry-over effect). An interesting finding was that the exercise response was significantly higher in the tests performed in the second semester of the study, that is when the girls were 6 months further in to their pubertal maturation. Multivariate analysis taking into consideration maturation rates (stable Tanner 2 or progression to Tanner 3 or to menarche) will be performed in the future.

**Urinary hGH.** Urinary hGH was expressed as average picograms of hGH excreted per minute per 12 hour period. D2 values were the mean of the sum of LT6 and GT6 samples. There was no clear pattern on the peak urinary excretion between both of these collection periods; in some instances LT6 samples were higher than GT6 and vice versa.

As expected, mean night values were higher than their corresponding mean day values (N1>D1, N2>D2, N3>D3; Figure B). However, the inter-subject variability was high, making the mean differences between corresponding night and day samples non-significant. Also, D1, D2, and D3, as well as N1, N2, and N3 did not differ from each other. Total D samples, however, were lower than total N samples.

Results were no different whether the collections correspond to the periods of placebo or supplement, whether the samples belonged to the first or second semester, or if they preceded or followed a period of supplementation or placebo (no carry-over effect). The urinary hGH values were within the range of values observed in normal U.S. controls.

**Plasma IGF-I.** Plasma IGF-I values (ng/mL) were within normal limits and were no different from each other independent of the nutritional intervention or period (1st or 2nd semester). There was no evidence of carry-over effect. A significant interaction between period and type of intervention was detected: plasma IGF-I values were higher in the supplemented group in the second semester than in the first, while they were lower in the placebo group in the second semester than in the first (Figure C). The separation of effects (intervention and sequence), however, failed to reach significance for either of these components.

**Urinary IGF-I.** Urinary IGF-I was expressed as average nanograms per minute per 12-hour period. So far only the samples corresponding to the pre-exercise day and night (D1 and N1) and that for the night of the exercise (N2) have been analyzed. They were similarly independent of the intervention, period or sequence of interventions, i.e., no carry-over effect (Figure D).

Urinary IGF-I levels were between 2 and 3 times higher among both the intervention and placebo groups, and during the first and second semesters than those obtained in the normal U.S. controls. This was an unexpected and very significant finding, suggesting that IGF-I production was higher or its bound fraction was lower or both. Analysis of IGF-I binding protein-3 (BP3) is proceeding at Genentech. These results may shed light on the mechanistic aspects of the higher IGF-I excretion in the face of normal levels in plasma.

**Conclusions on the four endogenous growth factors.** These preliminary results can be summarized as follows:

a) hGh production and its physiologic regulation does not seem to be impaired in stunted, currently well nourished pubertal girls, based on the hormone concentration in plasma prior to and in response to maximal exercise.

b) Nutritional supplementation, as applied in this study, did not augment the observed normal response of circulating hGH to maximal exercise.

c) Although the combined 3-day excretions of urinary hGH were higher during daytime than at night, the difference was not so clear on a daily basis. This could be due

to the fact that the girls in this study were quite active, averaging TDEE of 65 kcal/kg/day, a physical activity level of 1.94 times basal metabolic rate, 28 minutes/day in heavy activities and 342 minutes/day in moderate activities. Consequently, they were subject to important physiologic stimulation for hGH production during daytime.

d) The IGF-I resting plasma levels were within normal values and, therefore, do not seem to be an obstacle for normal pubertal growth.

e) The elevated urinary IGF-I excretion values in relation to those of "normal controls" (i.e., well nourished girls in the U.S.) are additional proof that IGF-I production is not deficient. Moreover, the high urinary IGF-I with normal plasma concentrations suggests that hormone production may be increased. This is in contrast with the depressed levels of IGF-I expected in plasma and urine of children with moderate/severe energy malnutrition.

f) These results support the concept of a possibility for normal pubertal growth among stunted girls similar to those in this study, and they do not dismiss the potentiality for catch-up growth during puberty and adolescence. On the contrary, the high levels of urinary IGF-I are consistent with the concept that endogenous growth factors are produced in amounts that may sustain accelerated catch-up growth.

*Linear Growth*

Mean height increased gradually during the study, from 132 ± 4 cm at the beginning to 140 ± 4 cm fifteen months later. This increment coincided with that expected for U.S. girls according to the NCHS reference data. As anticipated, dietary supplementation did not influence the velocity of linear growth among the whole set of girls nor among the Complete Compliers (Table 7). Maturity was not a major issue in assessing height at the beginning of the study, since all girls were selected to be in Tanner's breast developmental stage II. By the end of the study there were differences in maturation among the girls, 41% of whom remained in Tanner stage II, 36% progressed to stage III or IV without menarche, and 23% reaching menarche. Both age and maturational stage were significant predictors of height at the end of the study ($p < 0.05$).

Height growth velocity was 18.8 ± 7.0 mm during the first trimester, after which it decreased and remained fairly constant during the following three trimesters (ranging from 16.6 ± 4.9 to 16.0 ± 5.4 mm in 90 days). The difference in growth velocity between the first and succeeding trimesters is more evident among the group of Complete Compliers, whether the analysis is done in both semesters with Group OK (n=12), or looking at Group OK1 (n=17) in the first semester and Group OK2 (n=16) in the second. As Figure E shows, there was a significant negative regression coefficient of growth velocity over time (p=0.02), which suggests that the girls had already reached or surpassed their peak growth velocity during the first trimester of study. If the four Complete Compliers who reached menarche are excluded, the regression coefficient decreases in statistical significance (p=0.061). Growth velocity expected from the NCHS median for this age group is 16.0 mm in 90 days.

Tables 8 and 9 show that linear growth involved both the upper and lower segments of the body (sitting height and calf length, respectively). The absence of a consistent tendency in the growth velocity of body segments with time (trimesters in the study) and dietary treatment indicates that this growth took place in spurts.

*Growth in Body Mass*

The girls gained weight as they grew older in each trimester, from an average of 27.9 ± 2.7 kg on admission to 33.7 ± 4.3 kg at the end of the study. Weight growth velocity was significantly higher during the supplementation periods (Table 10) indicating that the girls were, in general, consuming the supplements without a major substitution effect on their home diets. As would be expected, Complete Compliers gained more weight during the supplementation period than the group as a whole.

Body Mass Index (BMI) on admission was 16.0 ± 1.1 and it increased gradually to 17.3 ± 2.0 by the end of the study. Table 11 shows that the changes in BMI followed the same pattern as those in body weight, and that the increment associated with the dietary supplements was greater among the Complete Compliers. Table 12 shows that the changes in body fat, both in absolute terms and as a proportion of total body weight, followed the same pattern as body weight and BMI, suggesting that the increase in weight was mostly body fat.

*Physical Activity and Physical Fitness*

At the beginning of the study, mean total daily energy expenditure (TDEE) of all the girls was 1867 ± 338 kcal/day, 64 ± 11 kcal/kg/day or 1.72 ± 0.29 times basal metabolic rate (XBMR). At the end of the study it was 2170 ± 441 kcal/day, 65 ± 11 kcal/kg/day or 1.90 ± 0.31 XBMR. The disappearance of the difference when TDEE is expressed per unit of body weight indicates that the increase in absolute terms was related to the girls' increase in body mass and the energy cost of displacing the larger weight.

As Table 13 shows, TDEE was significantly higher during the second supplementation period. An analysis of physical activity levels (i.e., TDEE as a multiple of basal metabolic rate) gave similar results. In addition to the increase in body weight compared with the first semester of the study, as discussed above, this could be partly because measurements of energy expenditure corresponding to S2 were done in half the girls (Group B) during the month of December, when Guatemalan children are on vacation and participating in traditional pre-Christmas festivities.

Table 14 shows the time allocated by the whole set of girls to activities requiring different levels of physical effort varied by trimester and dietary treatment. There was a significant increase in light activity from the beginning to the end of the study (191 vs. 291 minutes/day, $p<0.01$) with a corresponding decrease in sedentary activity (435 vs. 299 minutes/day, $p<0.01$).

Although there was no consistent trend of changes in physical effort pattern in relation to dietary intervention, analysis of the data of the group of Complete Compliers suggests that the increase in TDEE described above was the result of spending more time in moderate activities during S2 than P2 (341 ± 144 vs 264 ± 109 minutes/day, $p<0.05$), and a similar tendency in relation to heavy activities (27 ± 24 vs 16 ± 12 minutes/day, $p=0.064$).

There was a gradual increase in total $VO_{2\ max}$ in each trimester of the study, from 1279 ± 169 at the beginning to 1458 ± 187 ml $O_2$/min at the end ($p<0.01$), but the differences disappeared when expressed per unit of body weight (43.1 ± 3.7 and 42.9 ± 3.9 ml $O_2$/min/kg at the beginning and end, respectively). However, other indicators of physical fitness that are not necessarily influenced by body weight, such as the regression coefficient of heart rate on $VO_2$ and the oxygen uptake at specific heart rates, also increased with time

($p<0.01$). This indicates that fitness improved with age, since there were no changes in activities that may have induced a training effect. Table 15 shows that the observed changes in physical fitness were not related to the dietary treatment.

*Dietary Factors*

As mentioned above, some girls admitted a low compliance in supplement intake, and weight changes or other circumstances shed doubts about others. Therefore, nutrient intakes, with and without the supplements, were analyzed separately for the girls described as "Complete Compliers". Although they are not identical to the data from the 22 participants (Tables 16-19), the group differences are of no nutritional consequence and the interpretation of the results is unaffected. Therefore, the results of the whole set of participants will be used in the discussion and interpretation of the study.

Taken as a group, compliance with supplement intake was satisfactory in the whole set of girls (Table 16). Dietary surveys suggested that the home diet of girls in Group B provided 20-30% more energy and nutrients than that of Group A throughout the study. In general, living conditions were also somewhat better among Group B. However, the comparison of body weights and the similarity in TDEE were not consistent with the apparently greater dietary intake of Group B.

Regardless of those differences, home diets in both groups did not satisfy the recommended daily allowances of calcium (1,000 mg) and riboflavin (1.0 mg); (Table 17). Group A also had a deficit in the recommended intake of iron (12 mg/d before menarche) and, in some trimesters, of vitamin C (45 mg/d), and their dietary energy intake was marginal. Retinol equivalents in the diet were below recommended intakes, but the existence of a fortification program of sugar in Guatemala, prevented a dietary deficit of vitamin A (Table 17). All dietary deficiencies detected were corrected with the supplements, except for calcium (Tables 18 and 19).

When supplements were given, there was a small decrease in energy intake from the home diet (substitution effect). This was not significant for the whole set of girls, as shown in Table 17 and Figure F, but Group B showed a substitution of approximately 200 kcal/day, which was only about 30% of the additional energy provided by the supplements.

Since the home diet of these girls apparently provided 200-400 kcal/day more than that of Group A, the additional energy provided by the supplements represented a larger surplus for Group B. Consequently, the substitution effect may have been a mechanism to modulate energy intake and avoid excessive weight gain.

The variability between trimesters in the contents of some micronutrients in the home diets (especially vitamins A and C) may be partly explained by the intake of seasonal fruits rich in those vitamins, such as mango, papaya and pineapple. This will be further explored by ongoing analysis of interviews on food frequency intake that were done at the same intervals as the 24 hour recall interviews.

*Hematology*

Forty-eight percent of the girls enrolled in the study were anemic at baseline based on the FAO/WHO cutoff of hemoglobin levels of < 12.5 g/dl at the elevation of Guatemala City. Mean hemoglobin and hematocrit levels during each of the intervention periods is shown in Table 20. Although dietary surveys indicated that girls in Group A had a lower intake of iron and, in some trimesters, of vitamin C, prevalence of anemia was similar in both Groups.

Mean hemoglobin status at the end of the study had improved ($p<0.05$). When hemoglobin status was compared to the immediate previous period, improvement due to the supplement became clear. During placebo periods, 29% of the girls showed improvement, 35% showed no change, and 35% had lower hemoglobin levels. The corresponding percentages during supplement periods were 53% improvement, 31% no change, and 16% reduction in hemoglobin levels.

*Sexual Maturation*

After excluding the two girls who dropped out of the study and the one with incomplete data due to fracture of the femur, during the 14 months of the study 12 girls progressed in breast development from Tanner stage II to stage III or IV. Four of them had menarche during this time. Most changes took place in the second semester. In the first

semester only three girls progressed from Tanner stage II to III, and one of them had menarche.

The effect of sexual maturation on each study variable was explored in the second semester using a two-way analysis of variance (Sexual maturation X Treatment), with Treatment as a repeated measure (Supplement and Placebo). The girls were divided in three groups according to sexual maturation: 1) no change; 2) progress in breast development without menarche; and, 3) progress in breast development and menarche. After excluding the girls with unsatisfactory supplement intakes, the three groups were left with 7, 6 and 3 girls, respectively. Analyses were done among the three groups or between group 1 and a combination of groups 2 and 3, due to the small number of girls with menarche.

Except for the apparent influence of menarche on the decrease in height growth velocity previously mentioned, there did not seem to be any other effects of sex maturation on the outcomes of this study. This is primarily due to the lack of variability in sexual maturation among the girls.

### *Social Factors*

**Living conditions.** Table 21 shows that only 10 of the 24 girls lived at home with both their father and mother. One girl, who lived with her father and stepmother, abandoned the study after approximately 6 months, because she didn't get along with her stepmother and moved to another city with her paternal grandmother. The other girls lived only with their mother or father, or with grandparents. Tables 22 to 24 show the characteristics of the girls' housing and living environment. Most girls in Group A had worse living conditions than girls in Group B. Three girls from Group A lived in shacks with tin walls and earth floor adjoining a Municipal garbage dump. As Table 24 shows, all households with electricity (21 of 22) had television sets, and all except one had radios and audio cassette recorders/players. This indicates the importance of mass media as an instrument for health, nutrition, and overall education.

**Relations of the project personnel with the girls, parents and teachers.** An excellent rapport was established between the girls and the research personnel. The girls felt at ease in the laboratory and enjoyed the attention they received. They also enjoyed the visits and conversations with the field worker who visited them daily at school, or every other day at home during school vacations. Parents and teachers were kept informed about the outcomes of the study. Home visits made evident the interest of the project personnel in the girls' well being. Talks on nutrition and health were given in the schools, at the request of principals and teachers. As a result of all this, only one of the 24 girls dropped out of the study, and most girls and their parents/caretakers expressed their appreciation. For example, at the end of the study several girls gave presents to the members of the research team and hand made "Thank you" cards "for having taken care of us," and one of the girls' parents sent a gift to the project physician on his birthday.

**Appraisal of project benefits.** At the end of the project, the girls' schoolroom teachers were interviewed and asked to fill out a questionnaire appraising the girls' school performance, overall behavior and physical appearance during the year of the study. The general consensus was that participation in the project had positive effects. Most teachers said that they would be happy if the project continued or if similar projects were done with other girls in their schools.

Table 25 summarizes their subjective evaluation of physical, behavioral, and school performance changes in 21 girls who participated in the study throughout the year (no information was obtained on the girl who dropped out of the study nor on the two girls who moved away from the school district). School teachers reported that, in general, girls became more confident and self-assured. They considered that about 75% of the girls became more sociable and participated more in class, commenting that the girls became "less shy", "more communicative" and "more active," or were "talking more with her classmates."

Teachers also commented that the girls' attitudes towards school improved in about 85% of the cases, and about 60% of the girls improved in their school assignments and obtained better grades. Only 4 girls showed a decrease in school performance, and the

teachers associated this with problems at home. Classmates who did not participate in the project seemed somewhat envious or particularly curious at the beginning, but these feelings disappeared shortly thereafter.

The teachers felt that 19 girls (90%) had "very good relations" with the project's personnel, 1 (5%) had "good relations," and no comment was obtained about 1 girl whose new teacher couldn't appraise what had transpired during the preceding months.

Among the 14 teachers surveyed, only one felt that the girls lost important school time due to the project's demands, mainly when laboratory tests were conducted every three-months. However, all teachers had a positive impression about the project and made very good comments. Among the most frequent:

-- "The program should be applied to more students"

-- "It is beneficial for the girls"

-- "It helped their nutrition ....It gave good results"

-- "The girls appreciated the attention and affection they received from the project's personnel....some girls do not have this at home."

In summary, the research team's approach resulted in cooperation and good relations at the school, and it also had an unanticipated effect of improving the girls' self-confidence.

**Results from Immigrant and U.S.-Born Latino Girls in California**

A total of 123 immigrants and 135 US-born Latinos were included in this study. Immigrant and US-born girls had similar age distributions (Table 26). Eighty-one percent of the immigrants were from Mexico -- the remainder were from Guatemala, Nicaragua, Honduras, and El Salvador.

Fifty percent of the immigrant girls had been in the US for 3 years or less. 11% of the girls had been in the U.S. for 7 years or more (Table 27). No differences were seen in attained height by time in the US, nor was any interaction detected between time in the US and age.

Figures G and H compare weight and height, respectively, to NCHS reference data and are not adjusted for maturation because the NCHS data are not adjusted. US born Latino girls tended to be heavier at every age than the NCHS median; this trend reached

statistical significance for girls 10 years of age only (Figure G). No differences in height were seen at any age of the U.S. born girls compared to the NCHS median (Figure H). Similarly, height of 8-11 year old immigrants was not statistically different than the NCHS median, but 12 year old immigrants were 4 cm shorter than the NCHS median. This deficit is similar to that reported for 12-17 year old Hispanic-Americans in the HHANES study (10).

After adjusting for age and maturation using analysis of covariance, significant differences were seen between the two groups for all anthropometric measurements examined. Because of this and because rate of growth and body composition are very different in pre-menarcheal and post-menarcheal girls, anthropometrics were compared separately for both groups by menarcheal status.

Adjusted weight by menarcheal status was compared between immigrant and US born girls in Figure I. Maturation was defined pre-menarche as Tanner Stage I-III and post-menarche as time since menarche. Pre-menarche, US-born girls were 4.24 kg heavier than immigrant girls. Place of birth (U.S. vs. elsewhere) was significant at the 0.002 level. Both age ($p=0.006$) and maturation ($p\leq 0.001$) were important covariables pre-menarche. The difference between the two groups was larger post-menarche (6.69 kg, $p=0.058$) after adjusting for maturation and age. As would be expected, neither age ($p=0.692$) nor maturation ($p=0.490$) were important covariates post-menarche.

Similar patterns were seen for Body Mass Index (Figure J) and body composition (Figures K and L). BMI was significantly higher in US-born girls both pre- and post-menarche (1.32 $kg/m^2$ and 3.29 $kg/m^2$, respectively; Figure J). Only maturation pre-menarche was an important covariate ($p\leq 0.001$). Pre-menarche, US-born girls had 3.21 kg more fat mass than immigrants (Figure K). Post-menarche the difference was 4.12 kg. The differences in fat mass were significant by place of birth (POB) pre-menarche. That POB was not significant post-menarche was probably due to small sample sizes. Again, only maturation pre-menarche was a significant covariate. Figure L shows significant differences in fat free mass (FFM) by POB pre-menarche (delta=3.00 kg, $p\leq 0.001$), but no differences in FFM post-menarche (delta=2.72 kg, $p=0.186$). Age and maturation were important covariates pre-menarche, but neither were important post-menarche.

While adjusted height was significantly lower for immigrant Latino girls who had not yet reached menarche compared to US born pre-menarcheal girls (-2.64 cm), there was no difference in height for those girls who had reached menarche (0.69cm; Figure M).

*Conclusions of the California Study*

Place of birth, used here as a proxy for environment, does effect attained height, weight, and body composition. Clearly, US-born girls weighed more and had more fat mass than immigrant girls. This was true for girls both pre- and post-menarche after adjusting for both maturation and age. Interestingly, place of birth (whether immigrant or US-born Latino) was an important determinant for height before menarche but not after, once height had been adjusted for maturation and age. Because the data were collected cross-sectionally, whether or not the immigrant girls will experience reduced growth following menarche as has been seen in other immigrant studies could not be predicted (41).

This study suggests that the difference in height between Latinos in the U.S. and the U.S. NCHS population is due to an "immigrant" factor rather than solely to a genetic factor, as has been suggested in other studies (9,10). This may have implications on the possibility of catch-up growth during puberty. Longitudinal studies are needed to give conclusive answers to this question.

# 6. Discussion and Recommendations

## Interpretation and Discussion of Major Findings

This study was designed to explore whether the potential to produce endogenous growth factors, such as hGH and IGF-I, is preserved in nutritionally stunted pubertal girls, and whether a short-term improvement of their diets with wide spectrum supplementation would increase the production of those endogenous growth factors in response to an exercise stimulus. If that were the case, and especially if the response was enhanced by the dietary intervention, catch-up growth in puberty and adolescence may be physiologically possible.

### *Hormones*

Plasma levels of hGH and IGF-I were within normal limits and the response of the former to maximal exercise was highly significant. Urinary excretion of hGH was also normal, but excretion of IGF-I was higher than that of well-nourished, not stunted girls. This could be due to either: a) increased production of IGF-I; b) high urinary excretion of IGF-I secondary to a low binding of the hormone by IGF-I binding protein-3 (BP3), making it more filterable by the kidney; or, c) a combination of increased production and decreased binding.

Whichever mechanisms are involved, their cause is a matter of speculation, such as: excessive production of IGF-I due to unsuppressed feed-back regulation by hGH in chronic energy deficiency; the possibility that a low dietary calcium intake induced over-production of IGF-I; or that IGF-I receptors may have down-regulated over the years of chronic undernutrition. In any event, the findings in this investigation suggest that, not only was the biological potential for normal growth present in the stunted girls, but they may also have the hormonal capacity for catch-up growth.

### *Height*

It was anticipated that the short periods with and without dietary supplementation would not allow significant differences in linear growth velocity. However, the girls maintained a normal growth velocity throughout the study. This is in agreement with other

studies that did not involve any intervention during puberty or adolescence (3, 5, 6, 8), and coincides with our findings of endocrine functions, thereby supporting the thesis that biological potential for normal linear growth, and probably for catch-up growth, is preserved.

*Weight and Body Composition*

The supplements had a clear impact on weight gain. In relation to their age, the Guatemalan girls in the study reduced their mean weight deficit, compared with the median of the NCHS reference data, from 12.4 kg at the beginning, to 10.5 kg at the end of the study. This response, and the data from the periodic dietary surveys, indicate that the supplementation program improved energy and nutrient intakes. While there was an increase in the proportion of body fat, mean BMI and weight-for-height remained within normal range. Given that weight improvement precedes height improvement, at least in recovering malnourished children (42-44), and that the habitual dietary intakes of the girls in this study were deficient in calcium, iron, riboflavin, sometimes vitamin C, and possibly other micronutrients, such as zinc, the advantages of a dietary intervention like that used in this investigation, outweighs the risk of producing obesity in this population group.

*Anemia and Iron Status*

The hemoglobin levels and the high prevalence of anemia (48%) in this group of girls confirmed our previous findings (45). An interesting and somewhat unexpected result was the hematological response to the supplement and the subsequent fall in hemoglobin levels during the following three months with placebo. This alternating effect on hemoglobin concentration suggests that the level of iron supplementation in the present study (18 mg/day for three months) was insufficient to maintain adequate iron status.

*Early vs. Delayed Menarche: Effects on Height*

Height and maturation are intricately related. In normal, healthy populations, girls who begin puberty at an early age (early maturers) do so with a shorter stature than late maturers. Early maturers tend to reach peak height velocity (PHV) earlier than late

maturers, and although they may have PHV of greater magnitude, they may be shorter at menarche than late maturers.

Delayed menarche, which allows more time for growth prior to the beginning of menses, is often cited as the reason for similar total pubertal growth in healthy and undernourished populations (3,5,6,8). However, some studies have shown that early maturers gain almost twice as much height after menarche than late maturers (7.9 cm vs. 4.4 cm), resulting in no differences in adult height (46). Longitudinal studies have also shown that increased body fat is directly related to early onset of puberty (46-48), but it is not related to timing of menarche nor to final adult height (46,47) in healthy, well-nourished populations.

Through the screening of 360 low-income urban school girls in Guatemala City, the present study showed that while 27% of the girls between 10 and 12 years of age were clearly stunted for their age, their sexual maturation -- evaluated by breast development and menarche -- was accelerated, rather than delayed, compared with healthy, well-nourished populations. Twenty-three percent of these girls reached menarche before 12 years of age.

Of the 24 girls recruited for the study, 5 (21%) had menarche by the end of the project. This may be an underestimation of the total percentage of girls reaching menarche by 12 years, since not all 24 girls were studied until their twelfth birthday. These results are not different from Latino immigrants in the U.S., 24% of whom had menarche by 12 years, while only 14% of Latino girls born in the U.S. had reached menarche by that age.

The phenomena of reduced age at menarche with increased economic development (e.g., better nutrition, reduced exposure to infectious diseases, etc.) is well documented. However, in the past, the reduction in age at menarche has been measured over generations. Whether or not improved nutrition during puberty can have the same impact on reducing menarcheal age, as improved nutrition and health from gestation through childhood and into puberty, cannot be addressed in this study.

The cross-sectional study on immigrant Latino girls in Oakland, California, (49) indicated that post-menarcheal immigrant girls attained the same heights as US-born Latino girls, both being very close to the median of the NCHS reference population, in spite of having an earlier menarche than reported for healthy non-Hispanic whites. It cannot be

predicted whether these girls' post-menarcheal growth will continue to allow them to attain adult heights similar to those of the NCHS reference, or if their post-menarcheal growth rates will falter as reported by Proos et al. (41) among Indian girls adopted by Swedish families. It is clear from the studies presented here that it is useful to control for sexual maturational stage when relating height to age in pubertal age children.

*Energy Expenditure and Balance*

Among the whole set of girls, total daily energy expenditure plus a daily allowance of 2 kcal/kg for growth was 67 kcal/kg/day (66 kcal/kg/day for Complete Compliers), with a coefficient of variation (CV) of 17%. The reported dietary energy intakes were 51 ± 12 and 65 ± 14 kcal/kg/day during the placebo and supplementation periods, respectively, with CV of 24 and 22%. The apparent negative energy balance with placebo and the apparent energy equilibrium with supplementation, however, do not coincide with the observed weight gains. This discrepancy could be due to an underestimation of energy intake, an overestimation of expenditure, or a combination of both. An underestimation of intake seems a more likely explanation than the overestimation of expenditure because: a) dietary intakes are often under-reported in this age group (50); b) the validity of the heart-rate method to estimate TDEE has been established by comparing it with the use of doubly labelled water and with total body indirect calorimetry (50); c) suspiciously high or low values (i.e., > 2 standard deviations above or below the group mean) were obtained in only 6% of the TDEE measurements, but in 19% of the dietary surveys; d) coefficients of variation were greater for the estimates of intake than for expenditure, even when suspiciously high or low values are excluded from analysis; and e) repeated measurements at three-month intervals gave similar results for TDEE per unit of body weight or BMR, which seems consistent with absence of a marked change in the girls' lifestyle and physical activity.

*Pattern of Physical Activity*

The evaluation of minute-by-minute heart rate gave clues about the physical effort imposed by the girls' lifestyle. In general, they were quite energetic for urban dwellers,

spending, on average, a total of 4½ to 5½ hours per day in activities of moderate intensity and between 16 and 27 minutes in activities of heavy intensity. Dietary supplementation coincided with a tendency to spend more time doing things that required moderate and heavy effort, rather than light activities. Unfortunately, it was not possible to identify the specific chores performed with and without dietary supplementation.

### *Physical Fitness*

The girls participating in this study had a normal capacity for endurance exercise and maximal aerobic capacity ($VO_2Max$), given their weight and lean body mass. As described above, they led an active life with periods of moderate and heavy exertion on a daily basis. Thus, their fitness and work capacity may exceed those of girls in more affluent environments. This is an area that deserves further exploration. Their improvement in physical fitness with age also merits further study.

## Policy and Program Recommendations

The girls in the metabolic study were chronically consuming diets low in calcium, riboflavin and, in many instances, iron. Other micronutrients, such as zinc and copper, may also have been deficient in their diet. This alone may be implicated in stunting (51). Methods to improve micronutrient intakes must be explored and implemented. An example of successful feasibility is the finding that the dietary deficiency of vitamin A in these girls, who represent a vast segment of the Guatemalan population, was controlled by an ongoing program of sugar fortification with retinol.

Forty-eight percent of non-menstruating girls in the metabolic study were anemic. Methods of improving iron intake must be implemented. This recommendation is supported by the positive, although transient, hemoglobin response observed in this study when 18 mg of iron were given daily for three months. Either permanent fortification or a long-term supplementation program targeted to adolescent girls -- and probably also to adolescent boys and women of reproductive age -- seem necessary to fight anemia and other consequences of iron deficiency.

Calcium intake was low among the intervention girls and was not increased to recommended levels with the supplementation designed for this study. This finding is important because low calcium intake may be contributing to limited growth in children and it may also have long-term effects leading to osteoporosis later in life. In a country that traditionally has been considered a high calcium consumer because of the widespread use of lime-treated corn in the manufacture of corn tortillas, this finding points to changes in dietary habits whereby urban children substitute bread for tortillas. Furthermore, there is a tendency to use corn without lime treatment in the commercial production of tortillas in urban environments. This is a clear need for regulation in the food industry to avoid nutrient deficiencies.

**Future Research Recommendations**

This preliminary research in a previously unexplored aspect of pubertal growth has provided evidence that favorable endocrine conditions exist for catch-up growth in nutritionally stunted girls, and it has generated several questions that may be answered effectively with further research. Investigations along this line will provide leads into possible interventions to alleviate growth stunting in pubertal girls. One such investigation could be the assessment of a program of physical activity designed to increase hGH production, associated with a nutritional supplementation similar to that reported here.

The supplementation period in the present study was probably too short to detect catch-up in linear growth. Given its positive outcomes, i.e., normal levels of endogenous growth factors and the observed normal growth, longer term supplementation studies should be conducted, starting prior to the onset of puberty and continuing for at least one year after menarche in girls or until mid-adolescence in boys. These 4-5 year studies must be undertaken to understand growth patterns and the related biological mechanisms during puberty, and to determine whether dietary supplementation, alone or in association with adequate conditions of physical exercise, will allow catch-up growth during puberty to compensate for nutritional growth retardation (stunting) in early childhood.

An increase in linear growth, or a reduction of stunting, during adolescence will probably be accompanied by an increase in the intrapelvic diameter of women. This, in

turn, may reduce the risk of complications during childbirth. Although the technology to assess changes in intrapelvic diameter by non-invasive means remains a challenge, this deserves investigation in a longitudinal study.

The fact that the age of menarche is not delayed in stunted girls may contribute to the short stature of adult women from low socioeconomic strata in Guatemala and similar countries. A balance must be found between accelerated growth and accelerated or early maturation; the former leads to improved adult stature with all its benefits, and the latter diminishes the duration of time for growth, therefore counteracting the attainment of taller adult height. At present, there is no clear answer to the vital question of whether the end result is beneficial if both events occur as a result of better nutrition and other stimuli for pubertal growth. A well controlled longitudinal study with experimental and control groups can provide an answer.

It is possible that specific micronutrients (e.g., calcium or iron) and not growth hormone or IGF-I are limiting factors in growth. Calcium deficiency has been associated with stunting in young children (51). Calcium is consumed in suboptimal quantities by this population and it is likely that it is absorbed suboptimally, due to the nature of the diet. The status of bone mineralization of the girls was not assessed in the present metabolic study, but should be in future studies.

**Final Comments**

This study, in its current stage of analysis, must be seen as a pilot exercise that has succeeded in establishing several facts pertinent to the physical growth, body composition, pubertal development, food intake, energy expenditure, spontaneous activity pattern, exercise performance, endogenous growth factors in plasma and urine, and social characteristics of low-income 10-12 year old school girls in Guatemala City. This ambitious study was undertaken with the understanding that new research avenues were being travelled, and these demanded a series of methodological developments.

An excellent rapport was established with the girls, their families, and school authorities. This contributed to the high compliance in nutrient intake, performance of physiological tests and other research activities, with a low drop-out rate, and an invitation

to return to the schools and home environments of these and similar girls to perform more studies of this kind. The girls, their families, and the school authorities appreciated the increased level of attention and care that the study personnel provided, and they believe that several benefits were derived from this interaction, ranging from school performance to health aspects and overall social and physical well-being.

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## Lists of Tables and Figures

### Tables

**Figures**

**Table 1: Sequence of trimesters when supplements or placebo was administered**

| | Trimesters | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| **Group A** | Placebo (P1) | Supplement (S1) | Placebo (P2) | Supplement (S2) |
| **Group B** | Supplement (S1) | Placebo (P1) | Supplement (S2) | Placebo (P2) |

**Table 2: Experimental design periods when measurements and information were obtained.**

| | Basal | | 1* | | 2 | | 3 | | Beginning of Trimester 4** | 4 | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | A*** | B | A | B | A | B | A | B | A | A | B |
| Anthropometry | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Body composition | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Sexual maturation | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Dietary intake | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Urinary hormone secretion | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | ✓ | ✓ |
| Total daily energy expenditure | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | ✓ | ✓ |
| Physical activity pattern | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | ✓ | ✓ |
| Physical fitness | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | ✓ | ✓ |
| Hemoglobin concentration | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | ✓ | ✓ |
| Morbidity | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | ✓ | ✓ |

* 1, 2, 3, 4 = sequential trimesters in the study.

** For Group A, Trimester 3 finished two months before Trimester 4 started. Anthropometric and dietary information were obtained at the beginning of Trimester 4.

*** A, B = girls who began the study with either placebo or diet supplements.

**Table 3: Characteristics of girls on recruitment ***

| | Group A | Group B | Both groups |
|---|---|---|---|
| Age (m) | 134 ± 6# 125 - 143 | 133 ± 7 120 - 143 | 133 ± 6 120 - 143 |
| Weight (kg) | 27.1 ± 2.4 | 27.8 ± 2.6 | 27.5 ± 2.5 |
| Height (cm) | 131.0 ± 2.9 | 131.7 ± 4.4 | 131.4 ± 3.7 |
| Wt-for-Ht (z) | -0.2 ± 0.6 | -0.1 ± 0.5 | -0.2 ± 0.5 |
| Ht-for-age (z) | -2.2 ± 0.5 | -2.0 ± 0.3 | -2.1 ± 0.4 |
| BMI ($kg/m^2$) | 15.8 ± 1.1 | 16.0 ± 0.9 | 15.9 ± 1.0 |
| sitting height (cm) | 70.4 ± 1.5 | 70.2 ± 2.5 | 70.3 ± 2.0 |
| calf length (cm) | 34.0 ± 1.0 | 33.8 ± 1.4 | 33.9 ± 1.2 |
| % body fat | 16.9 ± 2.8** | 17.5 ± 2.2 | 17.2 ± 2.5 |

* The study of Group B began one month after recruitment, and basal information was obtained at that time, which was not identical to the values at the time of recruitment.

** Based on weight, weight/height$^2$ and triceps skinfold.

# Mean ± standard deviation. Range also shown for age.

**Table 4:** **Nutrient composition of supplements**

| **COOKIE** [vegetable flour mix (50% wheat, 35% corn, 15% soy), sugar (fortified with vitamin A), vegetable oil] | | **ORANGE-FLAVORED BEVERAGE** (sweetened with vitamin-A fortified sugar) | |
|---|---|---|---|
| Each 28 g cookie: | | Each 200 ml: | |
| Energy | 140 kcal | Energy | 152 kcal |
| Protein | 2.2 g | Vitamin A (retinol equivalents) | 28 mcg |
| Vegetable oil | 5.6 g | Vitamin C | 70 mcg |
| Vitamin A (retinol equivalents) | 52 mcg | | |

| **VITAMIN-MINERAL TABLET** | | | |
|---|---|---|---|
| Vitamin A | 5000 IU | Fe (fumarate) | 18 mg |
| Vitamin C | 100 mg | Zn (oxide) | 10 mg |
| Vitamin D | 400 IU | Ca (dibasic fosfate) | 20 mg |
| Vitamin E | 5 IU | P (dibasic fosfate) | 10 mg |
| Vitamin B1 | 10 mg | F (Ca fluoride) | 2.5 mg |
| Vitamin B2 | 10 mg | I (K iodate) | 150 mcg |
| Vitamin B6 | 5 mg | Mg (oxide) | 10 mg |
| Vitamin B12 | 15 mcg | Mn (sulfate) | 5 mg |
| Nicotinamide | 20 mg | Cu (sulfate) | 1 mg |
| Folic acid | 0.5 mg | Li (chloride) | 20 mcg |
| Pantothenic acid | 10 mg | | |

**Table 5: Parental occupation of girls interviewed during the screening procedure**

| FATHERS | | MOTHERS | |
|---|---|---|---|
| **Occupation** | **%** | **Occupation** | **%** |
| No salaried work or occasional jobs | 4 | No salaried work or occasional jobs | 39 |
| Street or market vendor, mason, janitor, etc. | 25 | Street or market vendor, waitress, house maid, etc. | 35 |
| Carpenter, mechanic, messenger, driver, store clerk, etc | 31 | Seamstress, cashier, store clerk, etc. | 8 |
| Teacher, policeman, TV technician, etc. | 3 | Teacher, secretary, nurse, etc. | 2 |
| Self-employed, own business | 3 | Self-employed, own business | 2 |
| University professional | 1 | | |
| Does not know | 26 | Does not know | 10 |
| Deceased | 7 | Deceased | 2 |

**Table 6: Anthropometric nutritional indices of girls measured during the screening procedures**

| Z SCORES (NCHS) From To | Wt-for-Ht n = 248 | Ht-for-Age n = 360 | Wt-for-Age n= 360 |
|---|---|---|---|
| ≤3.1 | -- | 3 | -- |
| -3.0 -2.1 | -- | 24 | 6 |
| -2.0 -1.1 | 6 | 40 | 42 |
| -1.0 +1.0 | 80 | 32 | 48 |
| +1.1 +2.0 | 9 | 1 | 4 |
| +2.1 +2.9 | 3 | -- | 0 |
| ≥+3.0 | 2 | -- | -- |

| Centile | Body mass index n = 360 |
|---|---|
| ≤ 5 | 6 |
| 6 - 15 | 13 |
| 16 - 84 | 70 |
| 85 - 94 | 8 |
| ≥ 95 | 3 |

**Table 7: Change in Height (mm/90 days) for all Girls and Complete Compliers**

| Intervention Period | All Girls (Groups A & B) | Complete Compliers (OK1) | Complete Compliers (OK2) |
|---|---|---|---|
| Placebo 1 | 18.1 ± 6.5 (24)* | 18.2 ± 7.2 (17) | NA |
| Placebo 2 | 17.2 ± 5.7 (22) | NA | 16.9 ± 5.0 (16) |
| Supplement 1 | 17.3 ± 5.8 (24) | 19.1 ± 5.5 (17) | NA |
| Supplement 2 | 15.3 ± 5.2 (22) | NA | 14.9 ± 3.8 (16) |

* Mean ± Standard Deviation (number of girls).

**Table 8: Change in Sitting Height (mm/90 days) for all Girls and Complete Compliers**

| Intervention Period | All Girls (Groups A & B) | Complete Compliers (OK1) | Complete Compliers (OK2) |
|---|---|---|---|
| Placebo 1 | 5.7 ± 4.3 (23)* | 4.9 ± 3.2 (17)** | NA |
| Placebo 2 | 9.0 ± 5.3 (22) | NA | 8.4 ± 4.8 (16) |
| Supplement 1 | 7.2 ± 5.8 (24) | 9.1 ± 5.9 (17) | NA |
| Supplement 2 | 8.3 ± 5.7 (22) | NA | 8.2 ± 6.0 (16) |

Trimesters in the study

| | |
|---|---|
| Trimester 1 | 7.0 ± 5.3 (23) |
| Trimester 2 | 6.0 ± 5.0 (24) |
| Trimester 3 | 8.8 ± 4.9 (22) |
| Trimester 4 | 8.5 ± 6.1 (22) |

* Mean ± Standard Deviation (number of girls).
** Placebo 1 differs from Supplement 1, paired *t* test, $p<0.05$

**Table 9: Change in Calf Length (mm/90 days) for all Girls and Complete Compliers**

| Intervention | All Girls (Groups A & B) | Complete Compliers (OK1) | Complete Compliers (OK2) |
|---|---|---|---|
| Placebo 1 | 4.3 ± 3.1 (24)* | 4.2 ± 0.30 (17) | NA |
| Placebo 2 | 4.1 ± 2.9 (22) | NA | 4.3 ± 3.1 (16) |
| Supplement 1 | 3.4 ± 3.3 (24) | 2.5 ± 3.0 (17) | NA |
| Supplement 2 | 4.0 ± 2.5 (22) | NA | 4.0 ± 2.7 (16) |

Trimesters in the study

| | |
|---|---|
| Trimester 1 | 2.3 ± 2.6 (24) |
| Trimester 2 | 5.4 ± 3.0 (24) |
| Trimester 3 | 4.8 ± 2.8 (22) |
| Trimester 4 | 3.3 ± 2.3 (22) |

* Mean ± Standard Deviation (number of girls).

**Table 10: Change in Weight (kg/90 days) for all Girls and Complete Compliers**

| Intervention | All Girls (Groups A & B) | Complete Compliers (OK1) | Complete Compliers (OK2) |
|---|---|---|---|
| Placebo 1 | 1.02 ± 1.04 (24)*+ | 0.85 ± 0.70 (17)+ | NA |
| Placebo 2 | 0.96 ± 0.74 (22)++ | NA | 0.87 ± 0.82 (16)++ |
| Supplement 1 | 1.79 ± 1.11 (24) | 2.14 ± 1.00 (17) | NA |
| Supplement 2 | 1.77 ± 1.31 (22) | NA | 2.11 ± 1.34 (16) |

* Mean ± Standard Deviation (number of girls).
\+ Placebo 1 is lower than Supplement 1, $p < 0.01$.
++ Placebo 2 is lower than Supplement 2, $p < 0.01$.

**Table 11: Change in Body Mass Index ($kg/m^2$/90 days) for all Girls and Complete Compliers**

| Intervention Period | All Girls (Groups A & B) | Complete Compliers (OK1) | Complete Compliers (OK2) |
|---|---|---|---|
| Placebo 1 | 0.14 ± 0.58 (24)* + | 0.04 ± 0.41 (17) + | NA |
| Placebo 2 | 0.10 ± 0.36 (22) + + | NA | 0.06 ± 0.38 (16) + + |
| Supplement 1 | 0.56 ± 0.51 (24) | 0.71 ± 0.47 (17) | NA |
| Supplement 2 | 0.55 ± 0.69 (22) | NA | 0.74 ± 0.69 (16) |

* Mean ± Standard Deviation (number of girls).
\+ Placebo 1 is lower than Supplement 1, $p < 0.01$
\+ + Placebo 2 is lower than Supplement 2, $p < 0.01$.

**Table 12: Change in Percent Body Fat ** (%/90 days) for all Girls and Complete Compliers**

| Intervention Period | All Girls (Groups A & B) | Complete Compliers (OK1) | Complete Compliers (OK2) |
|---|---|---|---|
| Placebo 1 | 0.21 ± 1.35 (24)* + | 0.00 ± 0.87 (17) + + + | NA |
| Placebo 2 | 0.05 ± 0.70 (22) + + | NA | 0.03 ± 0.78 (16) + + + + |
| Supplement 1 | 1.11 ± 1.08 (24) | 1.42 ± 0.95 (17) | NA |
| Supplement 2 | 0.84 ± 1.18 (22) | NA | 1.21 ± 1.09 (16) |

* Mean ± Standard Deviation (number of girls)
** Calculated from weight, weight/height$^2$ and triceps skinfold
\+ Placebo 1 is lower than Supplement 1, $p < 0.001$.
\+ + Placebo 2 is lower than Supplement 2, $p < 0.001$.
\+ + + Placebo 1 is lower than Supplement 1, $p < 0.01$.
\+ + + + Placebo 2 is lower than Supplement 2, $P < 0.01$.

**Table 13:** **Three Day Mean Total Daily Energy Expenditure, expressed as kcal/day (or *MJ/day)*, for all Girls and Complete Compliers**

| Intervention Period | All Girls (Groups A & B) | Complete Compliers (OK1) | Complete Compliers (OK2) |
|---|---|---|---|
| Placebo 1 | 1997 ± 265 (23)*<br>8.36 ± 1.11 | 1968 ± 260<br>8.23 ± 1.09 | NA |
| Placebo 2 | 2053 ± 390 (21)*+<br>8.59 ± 1.63 | NA | 1942 ± 277 (16)+++ |
| Supplement 1 | 1910 ± 386 (23)++<br>8.00 ± 1.62 | 1876 ± 363<br>7.85 ± 1.52 | NA |
| Supplement 2 | 2206 ± 351 (22)<br>9.24 ± 1.47 | NA | 2131 ± 303 (16)<br>8.59 ± 1.63 |

* Mean ± Standard Deviation (number of girls)
\+ Placebo 2 is lower than Supplement 2, $p < 0.01$
\++ Supplement 1 is lower than Supplement 2, $p < 0.01$
\+++ Placebo 2 is lower than Supplement 2, $p < 0.05$

**Table 14:** **Time allocation to activities with different levels of physical effort, minutes (% of 24 hours): Combining results of both groups.**

| | BOTH GROUPS | | | | | |
|---|---|---|---|---|---|---|
| | n | sleep | sedentary | light | moderate | heavy |
| Trimester 1 | 23 | 468 ± 52[•]<br>(32 ± 4) | 435 ± 190[a]<br>(30 ± 13) | 191 ± 114[b]<br>(13 ± 8) | 320 ± 158<br>(22 ± 11) | 26 ± 23<br>(2 ± 2) |
| Trimester 2 | 23 | 491 ± 49<br>(34 ± 3) | 291 ± 161<br>(20 ± 11) | 266 ± 131<br>(18 ± 9) | 363 ± 146<br>(25 ± 10) | 28 ± 20<br>(2 ± 1) |
| Trimester 3 | 20 | 492 ± 56<br>(34 ± 4) | 354 ± 173<br>(25 ± 12) | 233 ± 98[c]<br>(16 ± 7) | 333 ± 180<br>(23 ± 12) | 28 ± 34<br>(2 ± 2) |
| Trimester 4 | 21 | 486 ± 30<br>(34 ± 2) | 299 ± 132<br>(21 ± 9) | 291 ± 107<br>(20 ± 7) | 336 ± 128<br>(23 ± 9) | 29 ± 24<br>(2 ± 2) |
| Placebo 1 | 23 | 463 ± 52[d]<br>(32 ± 4) | 374 ± 151<br>(26 ± 10) | 193 ± 119[e]<br>(13 ± 8) | 377 ± 125<br>(26 ± 9) | 34 ± 24[f]<br>(2 ± 2) |
| Placebo 2 | 21 | 487 ± 42<br>(34 ± 3) | 341 ± 161<br>(24 ± 11) | 289 ± 102[g]<br>(20 ± 7) | 299 ± 144[h]<br>(21 ± 10) | 24 ± 26<br>(2 ± 2) |
| Mean of placebo 1 and 2 | 21 | 475 ± 39[i]<br>(32 ± 3) | 359 ± 144<br>(29 ± 9) | 237 ± 98<br>(14 ± 6) | 339 ± 118<br>(23 ± 6) | 30 ± 19<br>(2 ± 1) |
| Supplement 1 | 23 | 497 ± 47<br>(34 ± 3) | 352 ± 224<br>(24 ± 16) | 264 ± 127<br>(18 ± 9) | 307 ± 170<br>(21 ± 11) | 20 ± 17[j]<br>(1 ± 1) |
| Supplement 2 | 20 | 491 ± 42<br>(34 ± 3) | 310 ± 149<br>(22 ± 10) | 234 ± 105<br>(16 ± 7) | 372 ± 158<br>(26 ± 11) | 33 ± 32<br>(2 ± 2) |
| Mean of supplement 1 and 2 | 20 | 494 ± 38<br>(34 ± 3) | 329 ± 161<br>(20 ± 9) | 248 ± 97<br>(19 ± 7) | 344 ± 143<br>(25 ± 7) | 26 ± 20<br>(2 ± 1) |

[•] Mean ± standard deviation.
[a] Trimester 1 is higher than trimesters 2 and 4, $p < 0.01$.
[b] Trimester 1 is lower than trimesters 2 and 4, $p < 0.01$.
[c] Trimester 3 is lower than trimester 4, $p < 0.01$.
[d] Placebo 1 is lower than supplement 1, $p < 0.05$.
[e] Placebo 1 is lower than supplement 1, $p < 0.01$.
[f] Placebo 1 is higher than placebo 2 and supplement 1, $p < 0.05$.
[g] Placebo 2 is higher than placebo 1 and supplement 2, $p < 0.01$.
[h] Placebo 2 is lower than placebo 1 and supplement 2, $p < 0.05$.
[i] Mean of placebo 1 and 2 is lower than mean of supplement 1 and 2, $p < 0.05$.
[j] Supplement 1 is lower than supplement 2, $p < 0.05$.

**Table 15:** **$VO^2max$ (ml) as an indicator of physical fitness, for all Girls and Complete Compliers**

| Intervention Period | All Girls (Groups A & B) | Complete Compliers (OK1) | Complete Compliers (OK2) |
|---|---|---|---|
| Placebo 1 | 1312 ± 184 (22)* + | 1328 ± 207 (17) | NA |
| Placebo 2 | 1408 ± 156 (21) | NA | 1402 ± 159 (16) |
| Supplement 1 | 1317 ± 171 (24) + + | 1350 ± 188 (17) | NA |
| Supplement 2 | 1436 ± 185 (21) | NA | 1425 ± 185 (16) |

* Mean ± Standard Deviation (number of girls).
\+ Placebo 1 is lower than Placebo 2, $p < 0.01$
\+ + Supplement 1 is lower than Supplement 2, $p < 0.01$.

**Table 16:** **Intake of diet supplements of each trimester**

| | GROUP A | | GROUP B | |
|---|---|---|---|---|
| | S1* | S2 | S1 | S2 |
| Days with supplement | 91 | 102 | 91 | 89 |
| Mean Intake of** | | | | |
| - Cookies | 85 ± 11 | 90 ± 7 | 93 ± 10 | 90 ± 8 |
| - Tablets | 94 ± 6 | 93 ± 6 | 96 ± 4 | 96 ± 5 |
| - Beverage | 94 ± 7 | 97 ± 8 | 91 ± 14 | 91 ± 7 |

* S1, S2: Trimesters with dietary supplements
** % of supplements supplied to the girls on each trimester

**Table 17: Daily nutrient intake from home diet - Both Groups**

| | BASAL | DIETARY PERIOD | | | DIETARY PERIOD | | |
|---|---|---|---|---|---|---|---|
| | | PLACEBO 1 | PLACEBO 2 | MEAN P1,P2 | SUPPLEMENT 1 | SUPPLEMENT 2 | MEAN P1, P2 |
| Energy, kcal | 1453 ± 310 | 1541 ± 420 | 1611 ± 379 | 1575 ± 398 | 1471 ± 346 | 1446 ± 301 | 1458 ± 321 |
| kcal/kg | 54 ± 13 | 52 ± 13 | 51 ± 11 | 51 ± 12 | 49 ± 13 | 44 ± 11 | 47 ± 12 |
| Protein, g | 48 ± 11 | 52 ± 17 | 56 ± 13 | 54 ± 15 | 53 ± 19 | 52 ± 13 | 52 ± 17 |
| g/kg | 1.8 ± 0.4 | 1.7 ± 0.5 | 1.8 ± 0.4 | 1.8 ± 0.5 | 1.8 ± 0.7 | 1.6 ± 0.5 | 1.7 ± 0.6 |
| Fat, g | 35 ± 14 | 38 ± 17 | 43 ± 17 | 40 ± 16 | 39 ± 16 | 43 ± 16 | 41 ± 16 |
| Carbohydrate, g | 252 ± 58 | 268 ± 61 | 259 ± 66 | 264 ± 63 | 244 ± 54 | 221 ± 55 | 232 ± 55 |
| Calcium, mg | 495 ± 143 | 535 ± 185 | 544 ± 194 | 540 ± 187 | 522 ± 194 | 514 ± 163 | 518 ± 177 |
| Phosphorus, mg | 877 ± 198 | 923 ± 271 | 968 ± 214 | 944 ± 244 | 929 ± 283 | 880 ± 207 | 905 ± 246 |
| Iron, mg | 10.9 ± 2.6 | 10.8 ± 3.5 | 13.8 ± 4.4 | 12.3 ± 4.2 | 11.6 ± 3.8 | 11.8 ± 4.6 | 11.7 ± 4.2 |
| Thiamin, mg | 0.8 ± 0.2 | 0.9 ± 0.3 | 0.9 ± 0.2 | 0.9 ± 0.2 | 0.9 ± 0.3 | 0.8 ± 0.2 | 0.8 ± 0.3 |
| Riboflavin, mg | 0.7 ± 0.2 | 0.8 ± 0.3 | 0.8 ± 0.3 | 0.8 ± 0.3 | 0.8 ± 0.3 | 0.8 ± 0.2 | 0.8 ± 0.3 |
| Niacin, mg (NE) | 22 ± 6 | 24 ± 9 | 25 ± 6 | 24 ± 7 | 26 ± 5 | 22 ± 4 | 24 ± 5 |
| Vitamin C, mg | 41 ± 28 | 64 ± 80 | 51 ± 50 | 58 ± 67 | 65 ± 86 | 56 ± 28 | 60 ± 63 |
| Retinol equivalents, mcg* | 844 ± 542 (341 ± 496) | 737 ± 523 (272 ± 433) | 634 ± 199 (228 ± 127) | 684 ± 397 (251 ± 323) | 668 ± 291 (214 ± 148) | 629 ± 327 (267 ± 163) | 649 ± 306 (241 ± 156) |

* Including retinol in fortified sugar (in parenthesis: retinol equivalents provided by diet without considering fortified sugar)

Table 18: Daily nutrient intake from supplements - Both Groups

| | DIETARY PERIOD | | |
|---|---|---|---|
| | SUPPLEMENT 1 | SUPPLEMENT 2 | S1 AND S2 |
| Energy, kcal | 574 ± 98 | 611 ± 63 | 592 ± 84 |
| kcal/kg | 19 ± 4 | 18 ± 2 | 19 ± 3 |
| Protein, g | 7.6 ± 1.0 | 8.3 ± 0.9 | 8.0 ± 1.0 |
| Fat, g | 19 ± 3 | 19 ± 2 | 19 ± 2 |
| Carbohydrate, g | 93 ± 18 | 100 ± 10 | 97 ± 15 |
| Calcium, mg | 30 ± 4 | 33 ± 2 | 31 ± 4 |
| Phosphorus, mg | 27 ± 7 | 32 ± 3 | 30 ± 6 |
| Iron, mg | 17.1 ± 0.9 | 17.1 ± 1.1 | 17.1 ± 1.0 |
| Thiamin, mg | 9.5 ± 0.5 | 9.5 ± 0.6 | 9.5 ± 0.5 |
| Riboflavin, mg | 9.9 ± 0.6 | 10.0 ± 0.6 | 9.9 ± 0.6 |
| Niacin, mg | 19.4 ± 1.0 | 19.4 ± 1.0 | 19.4 ± 1.0 |
| Vitamin C, mg | 143 ± 20 | 156 ± 11 | 149 ± 17 |
| Retinol equivalents, mcg* | 1702 ± 114 | 1928 ± 150 | 1815 ± 174 |

* Orange-flavored supplement (152 kcal/cup) was introduced on day 30 of the study.

**Table 19: Total daily nutrient intake from home diet and supplement in the supplements in the two placebo and the two supplementation periods - Both Groups**

| | BASAL | DIETARY PERIOD | | | DIETARY PERIOD | | |
|---|---|---|---|---|---|---|---|
| | | PLACEBO 1 | PLACEBO 2 | MEAN P1, P2 | SUPPLEMENT 1 | SUPPLEMENT 2 | MEAN S1, S2 |
| Energy, kcal | 1453 ± 310 | 1541 ± 420 | 1611 ± 379 | 1575 ± 398 | 2045 ± 386 | 2056 ± 267 | 2050 ± 328 |
| kcal/kg | 54 ± 13 | 52 ± 13 | 51 ± 11 | 51 ± 12 | 69 ± 15 | 62 ± 12 | 65 ± 14 |
| Protein, g | 48 ± 11 | 52 ± 17 | 56 ± 13 | 54 ± 15 | 60 ± 19 | 60 ± 13 | 60 ± 16 |
| g/kg | 1.8 ± 0.4 | 1.7 ± 0.5 | 1.8 ± 0.4 | 1.8 ± 0.5 | 2.0 ± 0.7 | 1.8 ± 0.5 | 1.9 ± 0.6 |
| Fat, g | 35 ± 14 | 38 ± 17 | 43 ± 17 | 40 ± 16 | 57 ± 16 | 62 ± 15 | 60 ± 16 |
| Carbohydrate, g | 252 ± 58 | 268 ± 61 | 259 ± 66 | 264 ± 63 | 337 ± 61 | 321 ± 51 | 329 ± 56 |
| Calcium, mg | 495 ± 143 | 535 ± 185 | 544 ± 194 | 540 ± 187 | 551 ± 196 | 547 ± 162 | 549 ± 178 |
| Phosphorus, mg | 877 ± 198 | 923 ± 271 | 968 ± 214 | 944 ± 244 | 956 ± 285 | 912 ± 205 | 934 ± 246 |
| Iron, mg | 10.9 ± 2.6 | 10.8 ± 3.5 | 13.8 ± 4.4 | 12.3 ± 4.2 | 28.7 ± 3.7 | 29.0 ± 4.7 | 28.8 ± 4.2 |
| Thiamin, mg | 0.8 ± 0.2 | 0.9 ± 0.3 | 0.9 ± 0.2 | 0.9 ± 0.2 | 10.3 ± 0.6 | 10.3 ± 0.6 | 10.3 ± 0.6 |
| Riboflavin, mg | 0.7 ± 0.2 | 0.8 ± 0.3 | 0.8 ± 0.3 | 0.8 ± 0.3 | 10.7 ± 0.7 | 10.7 ± 0.6 | 10.7 ± 0.6 |
| Niacin, mg | 22 ± 6 | 24 ± 9 | 25 ± 6 | 24 ± 7 | 45 ± 6 | 41 ± 4 | 43 ± 5 |
| Vitamin C, mg | 41 ± 28 | 64 ± 80 | 51 ± 50 | 58 ± 67 | 208 ± 84 | 212 ± 34 | 210 ± 63 |
| Retinol equivalents, mcg* | 844 ± 542 (341 ± 496) | 737 ± 523 (272 ± 433) | 634 ± 199 (228 ± 127) | 684 ± 397 (251 ± 322) | 2370 ± 271 (1734 ± 170) | 2557 ± 383 (1883 ± 216) | 2464 ± 341 (1809 ± 206) |

* Including retinol in fortified sugar (in parenthesis: retinol equivalents provided by diet without considering fortified sugar)

**Table 20: Hematological status of all girls**

| Indicator | Placebo 1 | Placebo 2 | | Supplement 1 | Supplement 2 |
|---|---|---|---|---|---|
| Hemoglobin (g/dl) | 13.0 ± 1.0 | 13.2 ± 0.8 | | 13.1 ± 1.0 | 13.6 ± 1.0 |
| Hematocrit (%) | 40.1 ± 2.4 | 40.6 ± 1.9 | | 40.7 ± 2.1 | 40.7 ± 2.4 |

**Table 21: Parents or caretakers with whom the girls lived**

| | n | % |
|---|---|---|
| Father and mother | 10 | 42 |
| Only mother | 4 | 17 |
| Only father | 3 | 13 |
| Father and stepmother | 1* | 4 |
| Maternal or paternal grandparents | 5 | 20 |
| Other ** | 1 | 4 |
| Total | 24 | 100 |

* Abandoned study to live with paternal grandmother in another city, due to problems with stepmother.

** Elderly woman with 11 non-legally adopted children.

**Table 22: Living environment**

| | n=22 * | % |
|---|---|---|
| Own the house where they live | 11 | 50 |
| Number of families living in the house | | |
| 1 | 7 | 32 |
| >1, sharing common facilities | 12 | 54 |
| >1, independent facilities | 3 | 14 |

* Information on the 22 girls who completed the study

One girl had a parent or caretaker who owned a truck, another a car, and another a motorcycle.

**Table 23: Building materials for the houses**

| MATERIALS | n* | % |
|---|---|---|
| Tin walls | 3 | 14 |
| *Cement block walls* | 19 | 86 |
| Tin roof | 18 | 82 |
| Concrete roof | 4 | 18 |
| Earth floor | 3 | 14 |
| Cement floor | 3 | 14 |
| Brick floor | 16 | 72 |

* Information on the 22 girls who completed the study.

**Table 24: Characteristics of the living environment**

| | n=22* | % |
|---|---|---|
| Electricity | 21 | 95 |
| Running water | 18 | 82 |
| Shower inside the house | 14 | 64 |
| Hot water | 2 | 9 |
| Latrine | 4 | 18 |
| Toilet | 18 | 82 |
| Kitchen: | | |
| - separate from other rooms | 16 | 73 |
| - propane gas stove | 18 | 82 |
| - wood stove | 4 | 18 |
| - refrigerator | 12 | 54 |
| Number of bedrooms separate from livingroom/kitchen | | |
| - none | 1 | 4 |
| - 1 | 6 | 28 |
| - 2 | 7 | 32 |
| - 3 | 7 | 32 |
| - 4 | 1 | 4 |
| TV | 21 | 95 |
| Radio/audio cassette player | 20 | 91 |

* Information on the 22 girls who completed the study.

**Table 25: Subjective appraisal by teachers of changes in the girls who participated in the study.**

| | Improved | No change | Decreased | No comment |
|---|---|---|---|---|
| General appearance | 90%* | | | 10% |
| Height | 95% | 5% | | |
| Weight | 76% | 5% | | 19% |
| General behavior | 67% | 19% | 14% | |
| Socializing (interactions with classmates) | 71% | 24% | 5% | |
| Participation in class | 76% | 14% | 10% | |
| Attitude towards school | 85% | 5% | 10% | |
| Performance of assignments | 62% | 14% | 19% | 5% |
| School grades | 62% | 28% | 10% | |
| Attendance | 48% | 38% | 14% | |

* Proportion of girls with or without change.

**Table 26: Age distribution of girls participating in the adolescent growth project**

| Age (yr) | Immigrants (n = 123) | U.S. Born (n = 135) |
|---|---|---|
| 8 | 12% | 15% |
| 9 | 27% | 20% |
| 10 | 25% | 29% |
| 11 | 19% | 22% |
| 12 | 17% | 14% |

**Table 27: Length of time in the U.S.A. of immigrant Latino girls (n = 123)**

| | |
|---|---|
| ≤ 3 years | 50% |
| 4-6 years | 38% |
| ≥ 7 years | 11% |
| Unknown | 1% |

Figure A

# Pre/Post-Exercise Plasma hGH (pg/ml) for 20 Guatemalan Girls (Mean + s.e.m.)

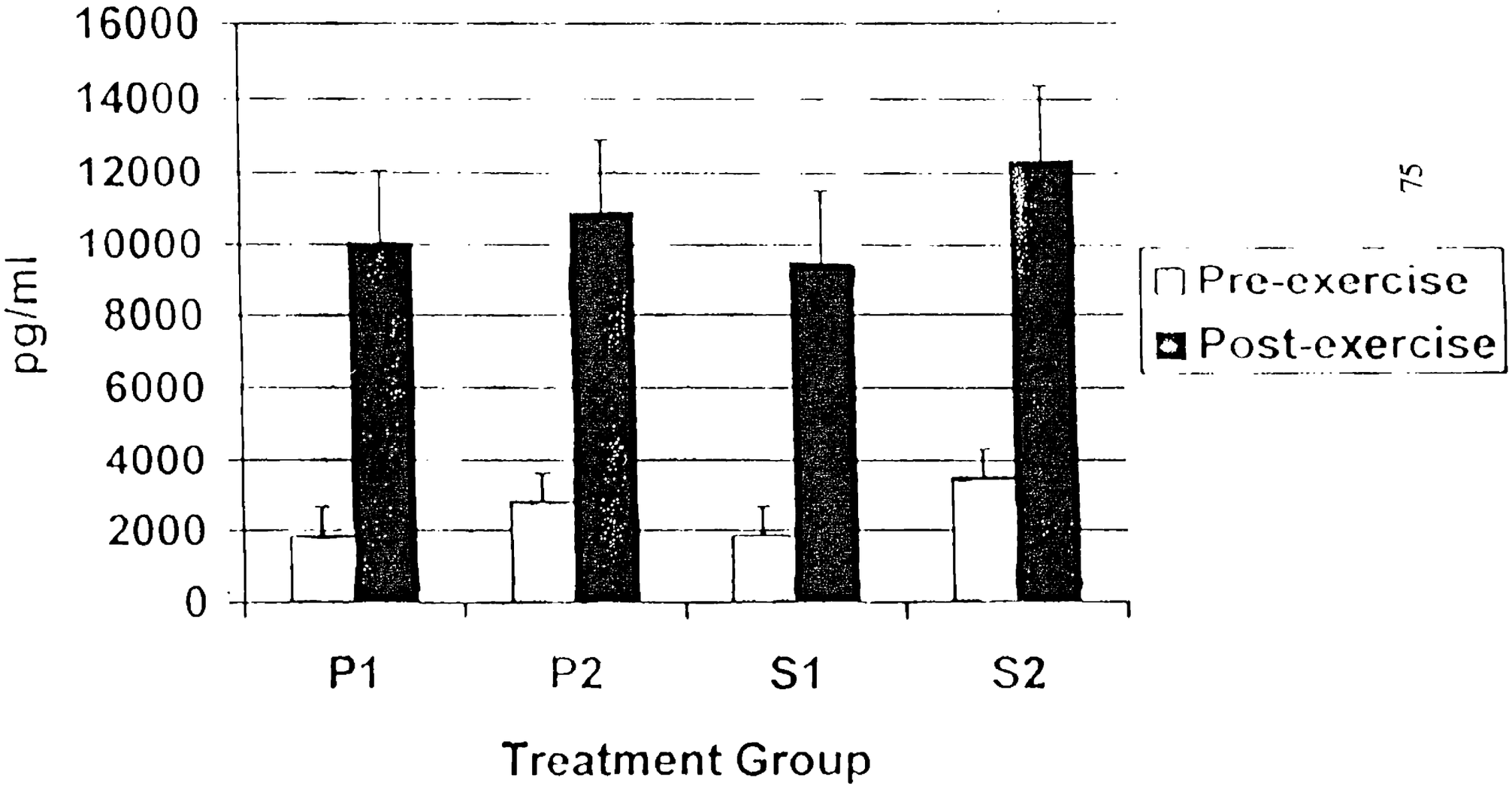


**Figure B**

# 24-hour Urinary hGH (pg/min) Before, During, and After Exercise (Mean + s.e.m.)

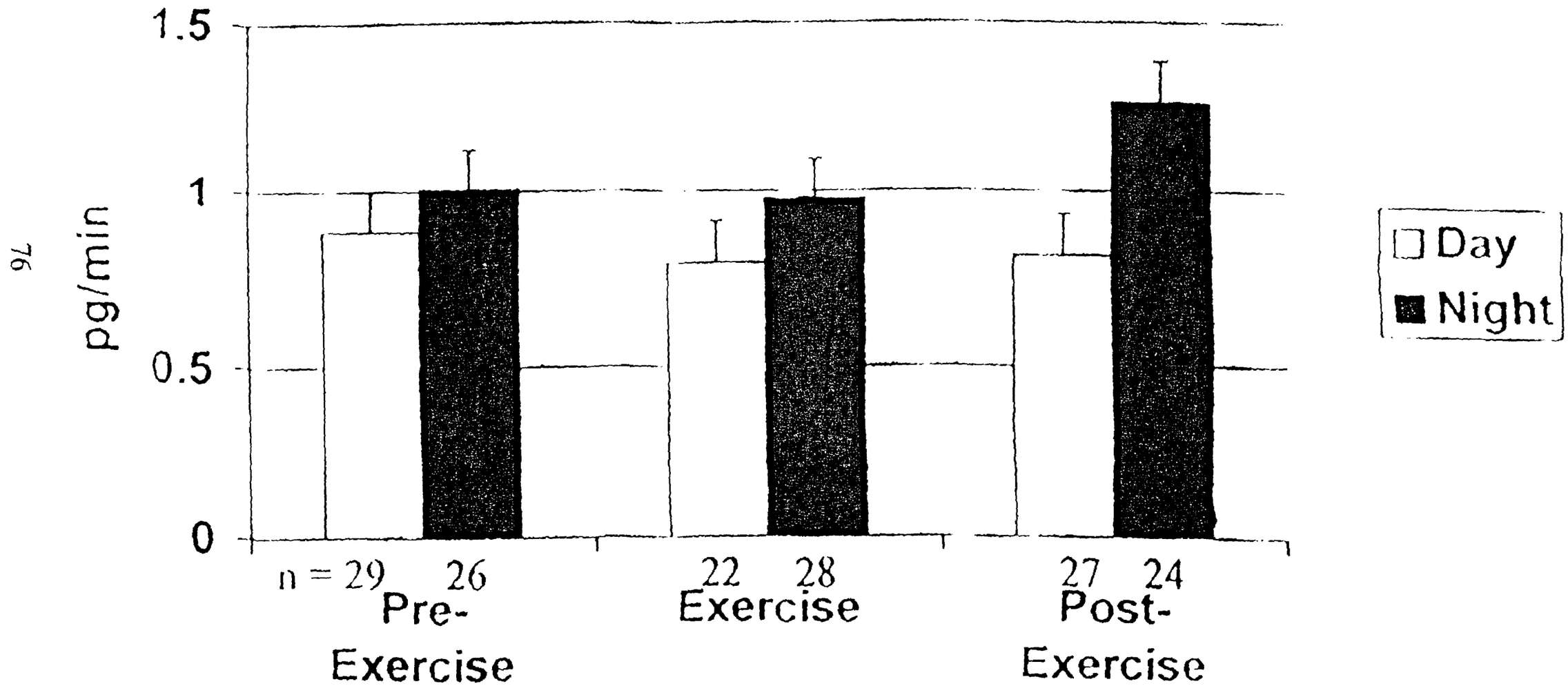


**Figure C**

# Basal Plasma IGF-1 (ng/ml) for 22 Guatemalan Girls (Mean + s.e.m.)

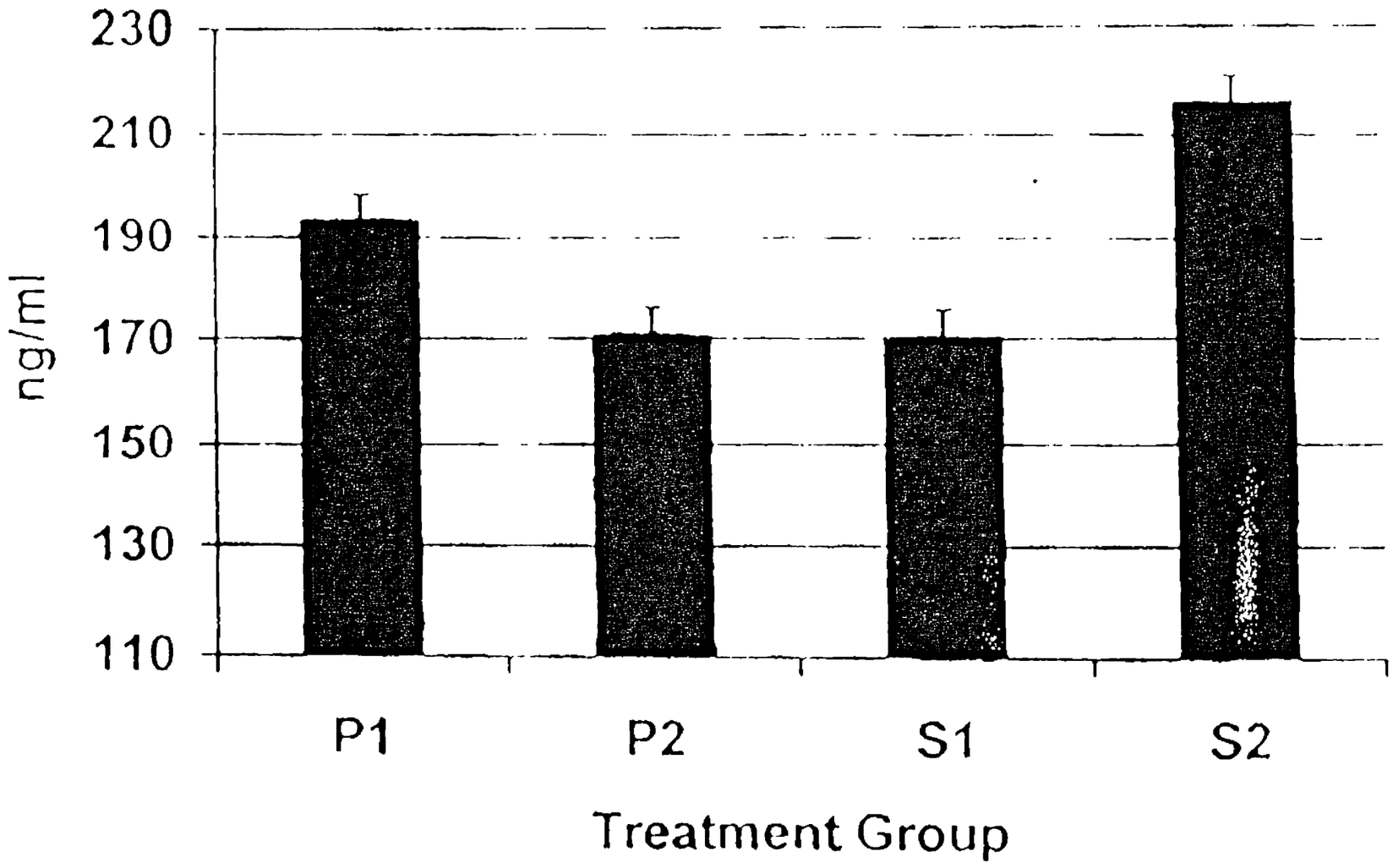


**Figure D**

# 24-hour Urinary IGF-1 (ng/min) Before and After Exercise (Mean +- s.e.m.; n=13)

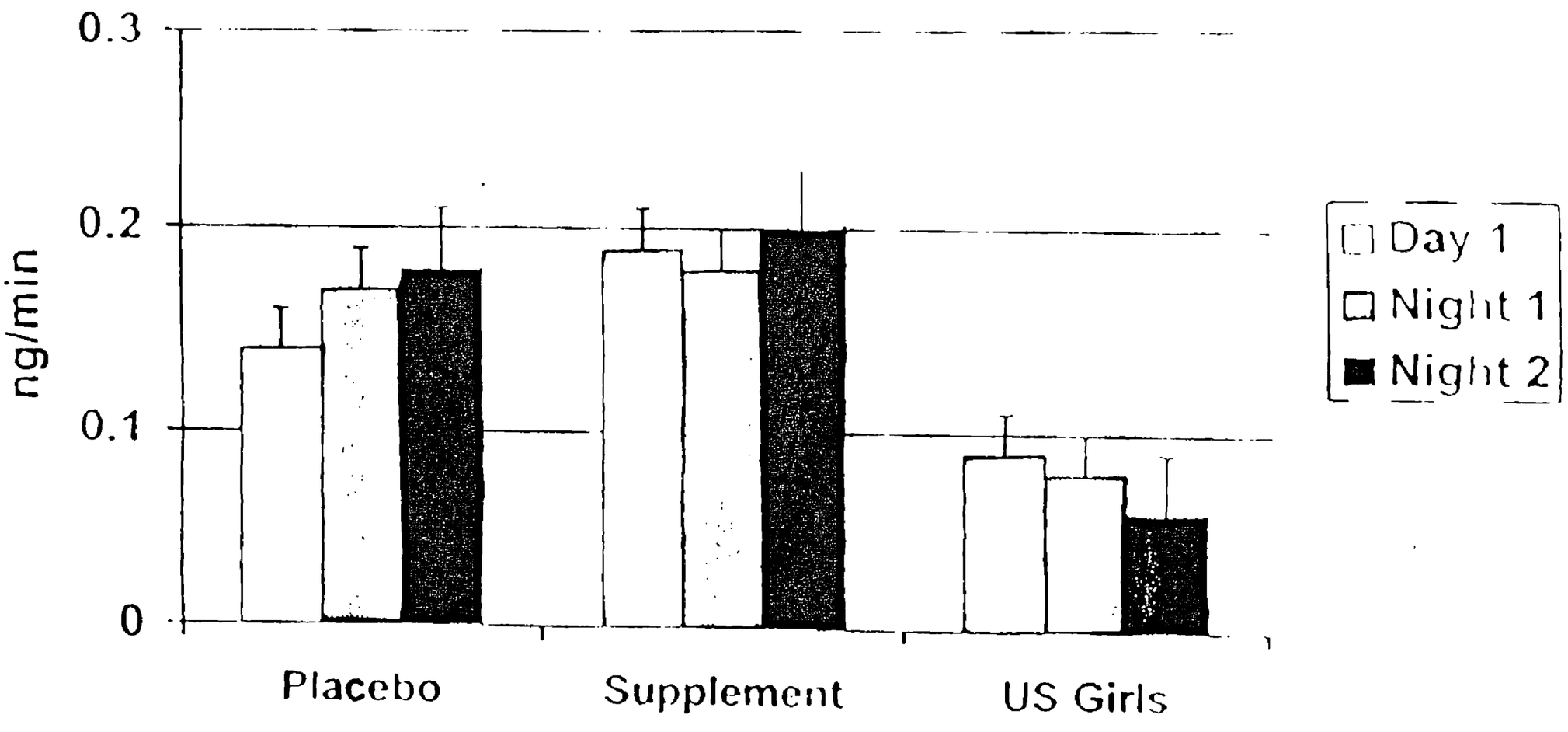


**Figure E**

# Height Growth Velocity
# (Mean + s.e.m.; n=17)

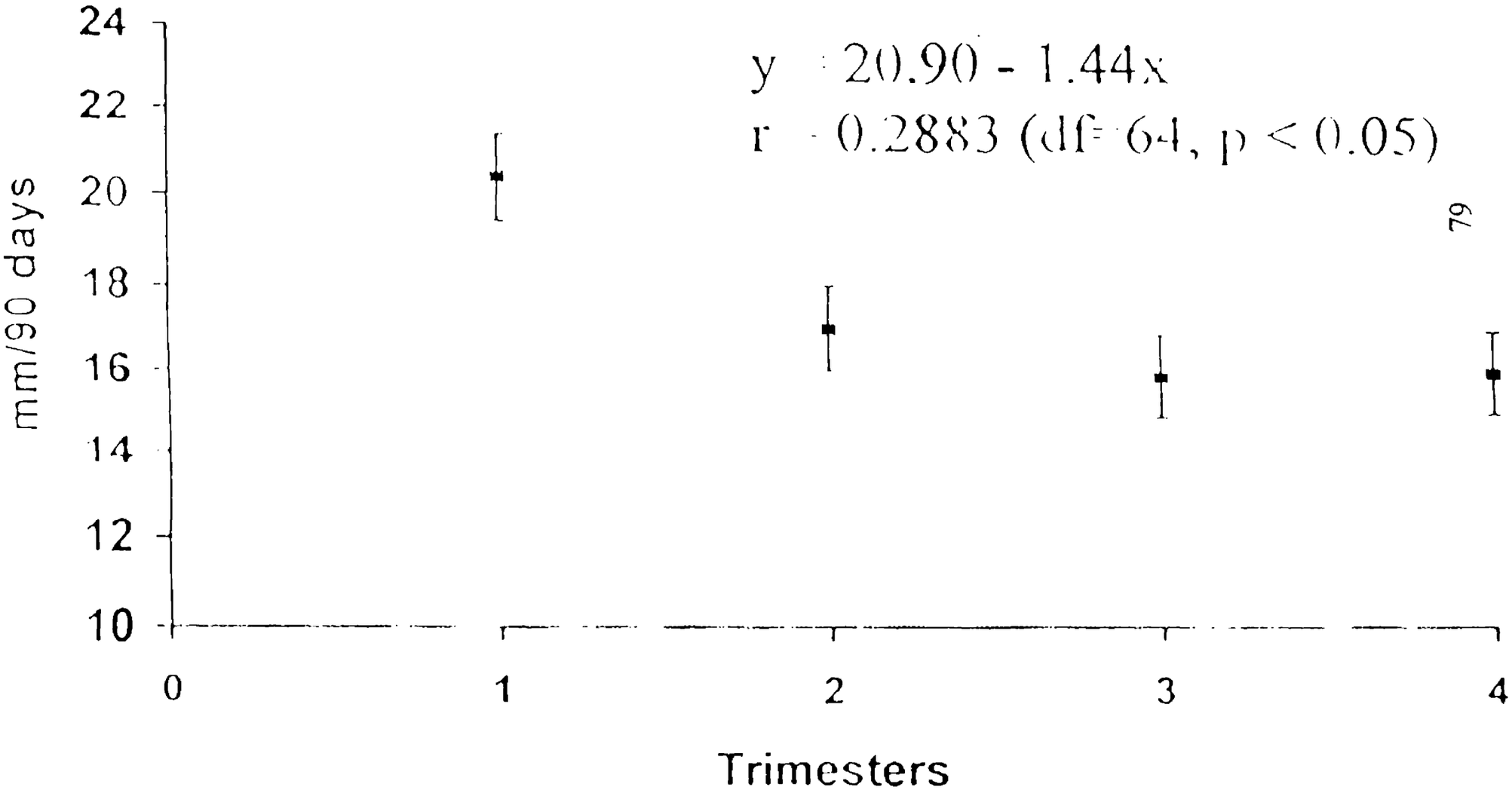


**Figure F**

# Total energy intake from home diet and supplements

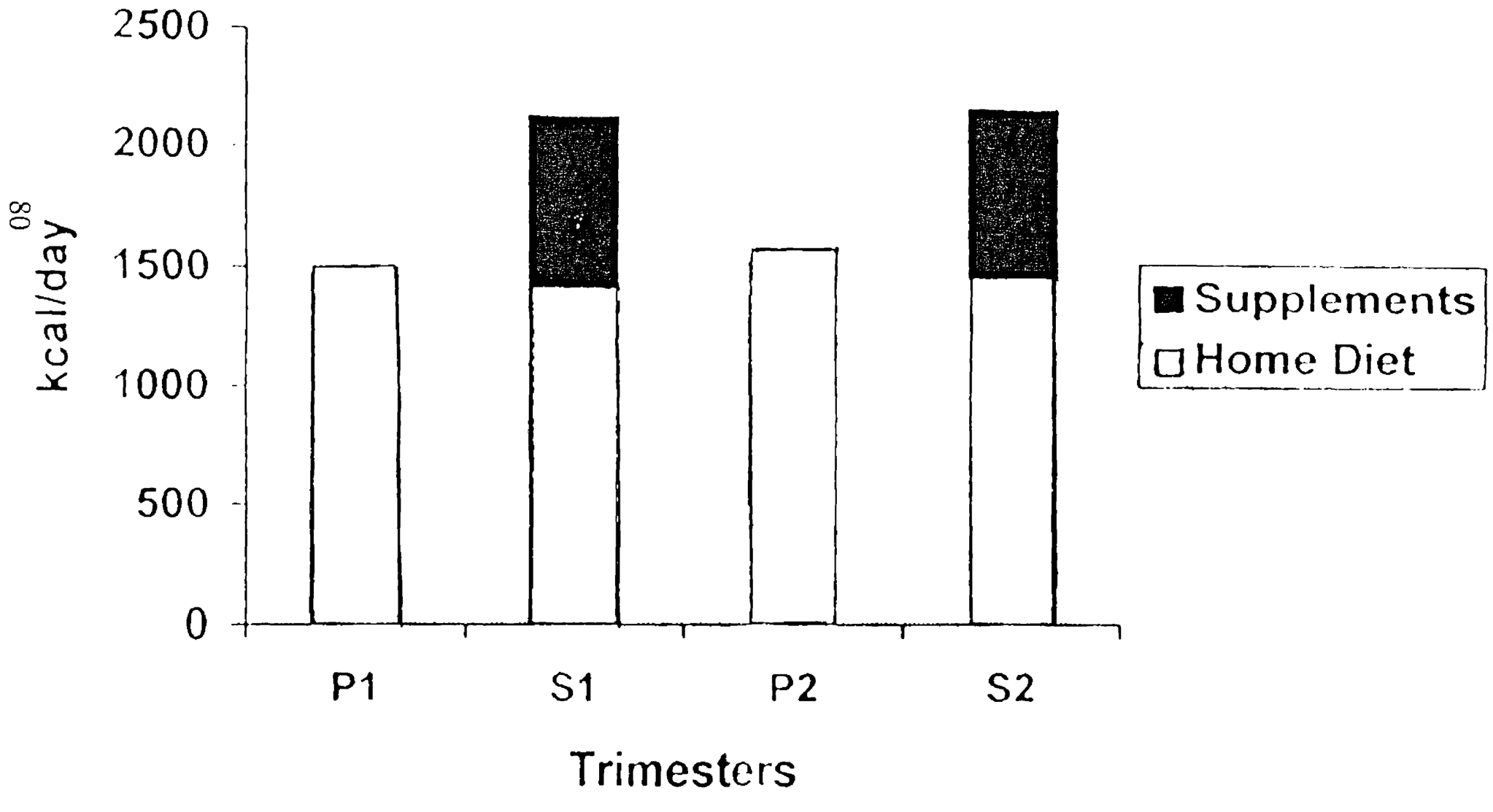


**Figure G**

# Weight (kg) of Immigrant and US-Born Latino Girls Compared to *NCHS*

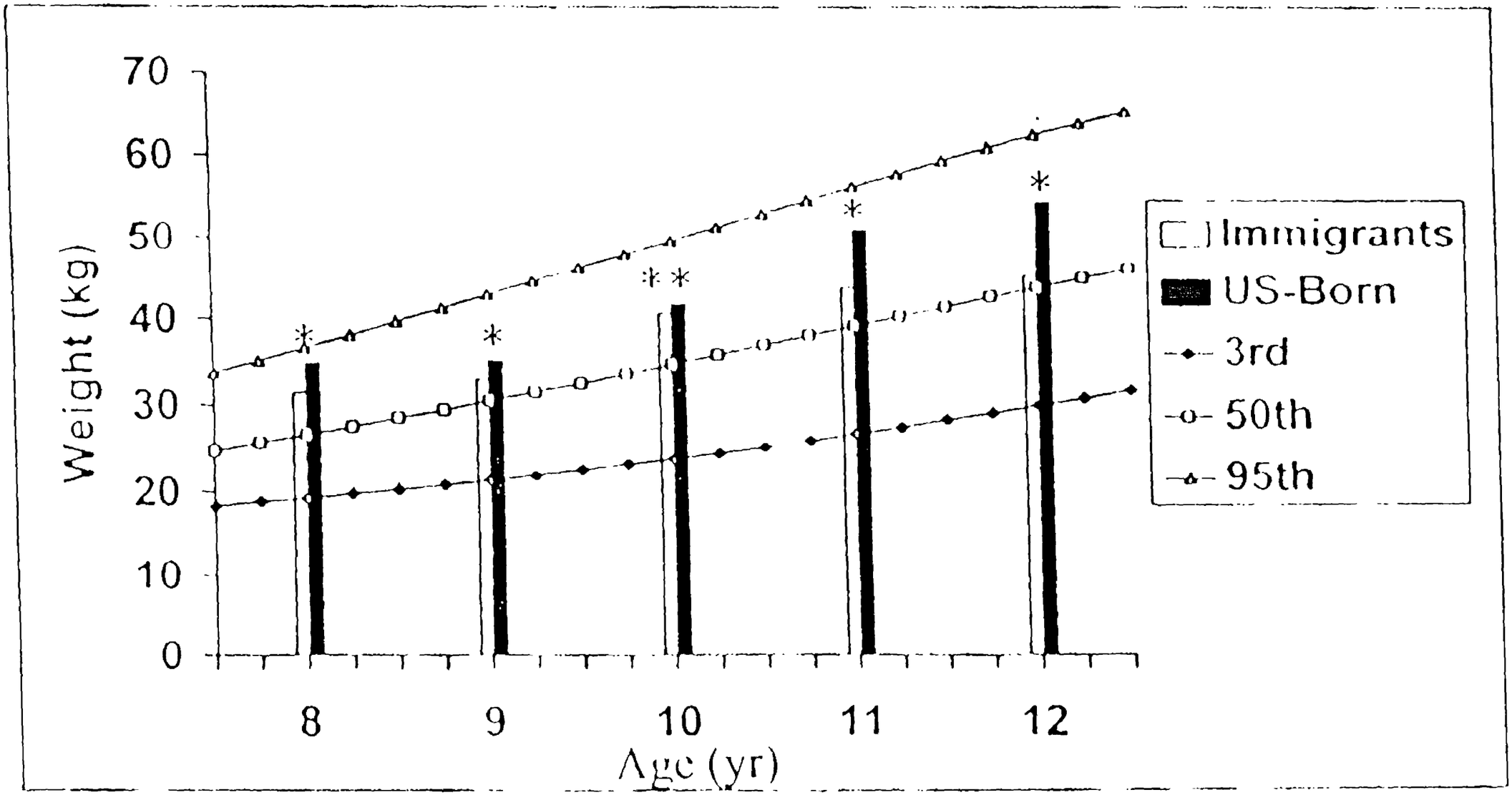


**Figure H**

# Height (cm) of Immigrant and US-Born Latino Girls Compared to *NCHS*

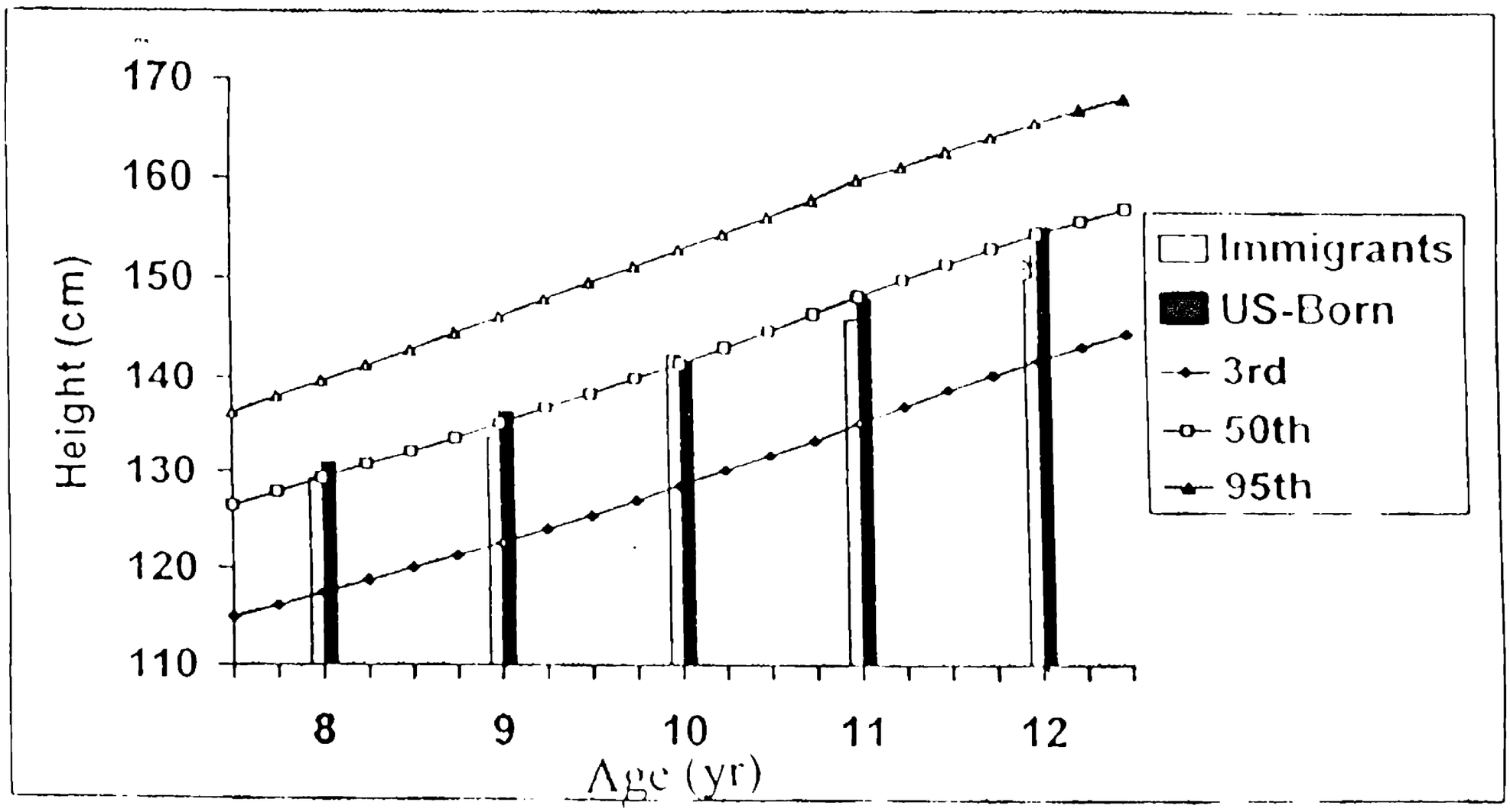
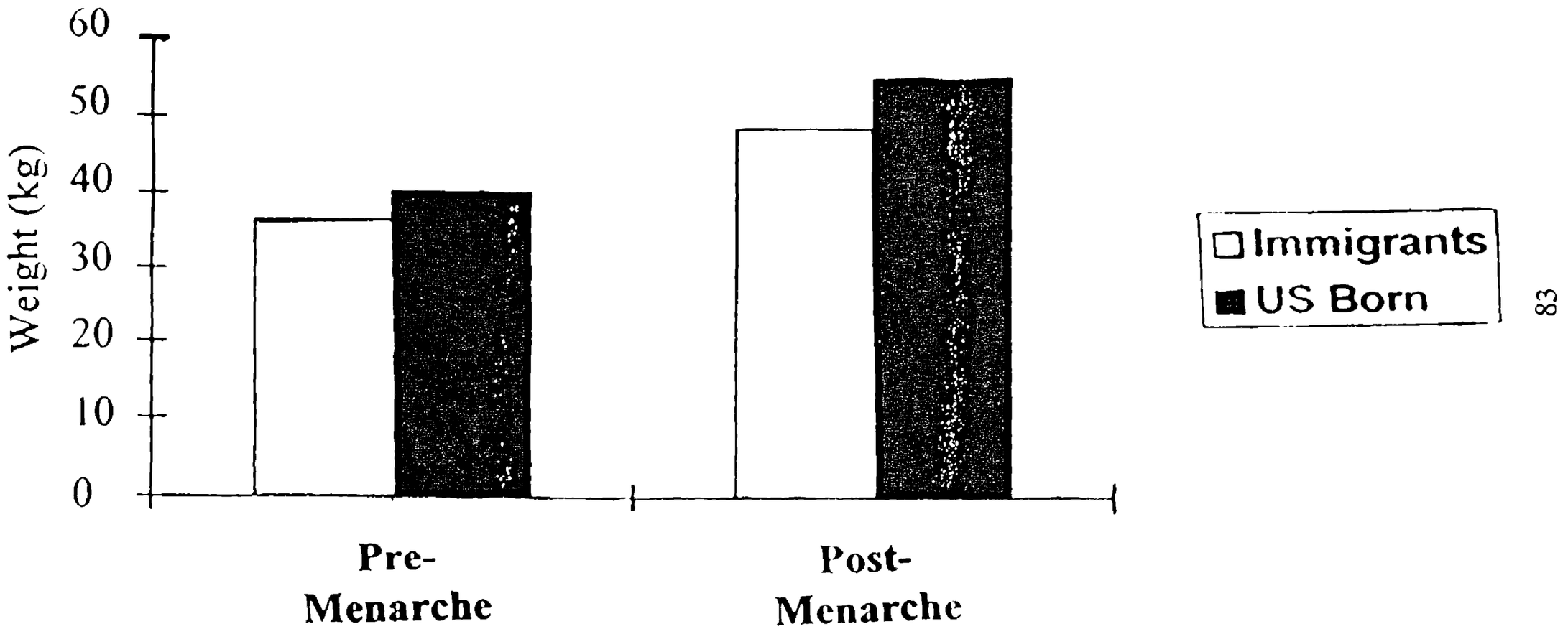


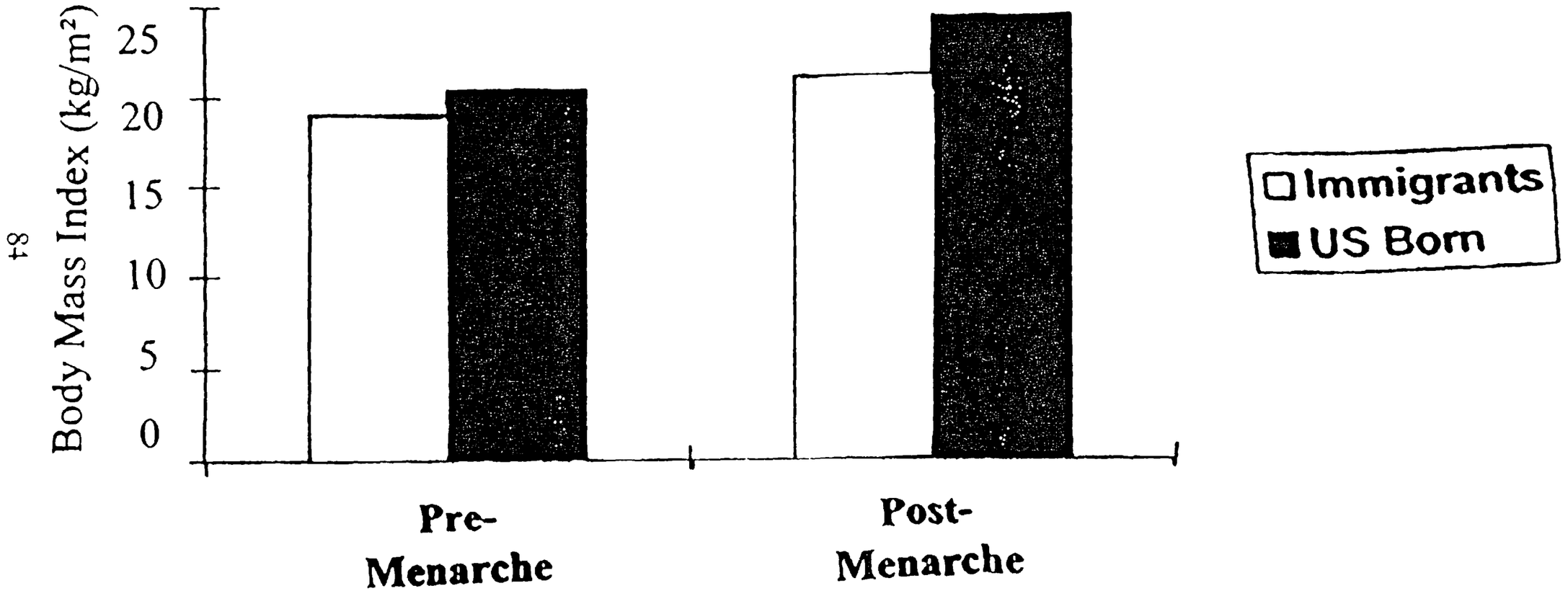
Figure I




| | | |
|---|---|---|
| POB | 0.002 | 0.058 |
| Age | 0.006 | 0.692 |
| MS | 0.000 | 0.490 |

**Figure J**

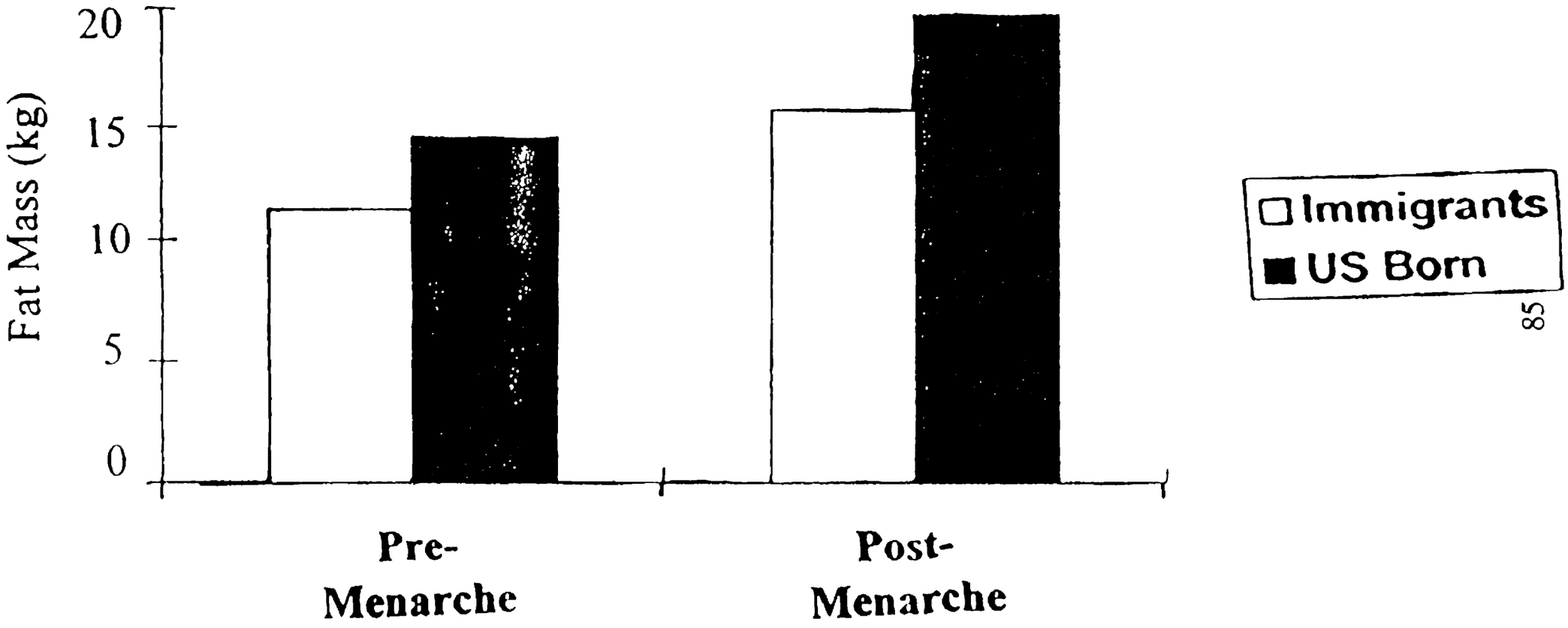
# Adjusted Body Mass Index (kg/m²) for Immigrant and US-Born Latino Girls




| | | |
|---|---|---|
| POB | 0.011 | 0.031 |
| Age | 0.656 | 0.728 |
| MS | 0.000 | 0.487 |

**Figure K**

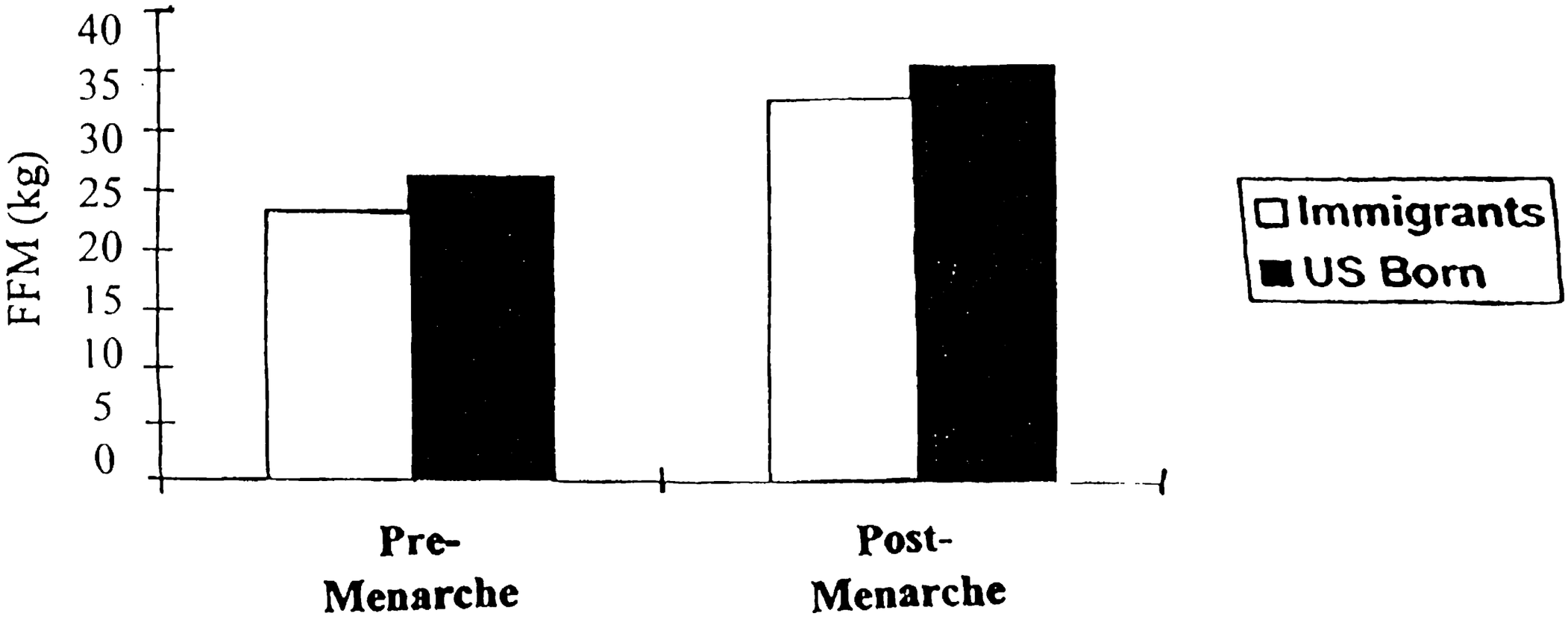
# Adjusted Fat Mass (kg) for Immigrant and US-Born Latino Girls




| | | |
|---|---|---|
| POB | 0.001 | 0.082 |
| Age | 0.313 | 0.850 |
| MS | 0.000 | 0.560 |

**Figure L**

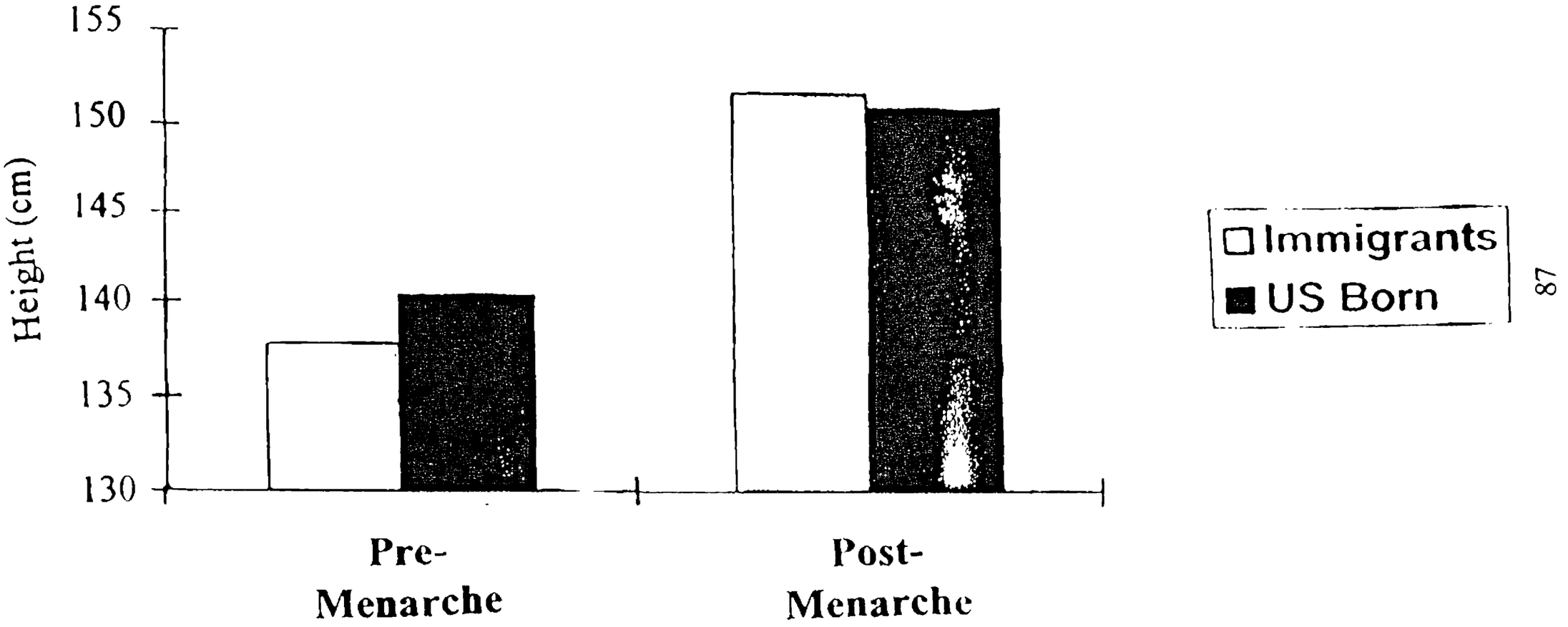
# Adjusted Fat Free Body Mass (KG) for Immigrant and US-Born Latino Girls




| | | |
|---|---|---|
| POB | 0.002 | 0.186 |
| Age | 0.000 | 0.289 |
| MS | 0.000 | 0.888 |

**Figure M**

# Adjusted Height (cm) for Immigrant and US-Born Latino Girls




87

| | | |
|---|---|---|
| POB | 0.020 | 0.758 |
| Age | 0.000 | 0.084 |
| MS | 0.000 | 0.988 |

## About the Nutrition of Adolescent Girls Research Program

The Nutrition of Adolescent Girls Research Program was established at ICRW in 1990 through a cooperative agreement with the Office of Nutrition, U.S. Agency for International Development. The premise for the Research Program was that the period of adolescence should be viewed as a window of opportunity for enhancing the lives of adolescents in developing countries, both for their present and future adult roles. Investments during adolescence can help girls and boys realize social and educational opportunities, manage their home and market responsibilities, and improve their nutritional and health status. However, little information is available to guide the formulation of policies and programs to address adolescent nutrition. The objective of the Research Program was to provide information on the many factors that are related to nutritional status, including physical growth, morbidity, food intake, energy expenditure, education level, family structure, intrahousehold food distribution, social and economic status, and self-perceptions. The program includes 11 research projects: five in Latin America and the Caribbean, four in Asia, and two in Africa.

**Publications from the Nutrition of Adolescent Girls Research Program**

The Nutrition and Lives of Adolescents in Developing Countries: Findings from the ICRW Nutrition of Adolescent Girls Research Program *by* Kathleen M. Kurz and Charlotte Johnson-Welch.

Investing in the Future: Six Principles for Promoting the Nutritional Status of Adolescent Girls in Developing Countries *by* Kathleen M. Kurz, Nancy L. Peplinsky, and Charlotte Johnson-Welch.

Addressing Needs and Opportunities: A Survey of Programs for Adolescents *by* Nancy L. Peplinsky.

Report Series:

No. 1. Nutritional and Health Determinants of School Failure and Dropout in Adolescent Girls in Kingston, Jamaica *by* S.P. Walker, S. Grantham-McGregor, J.H. Himes, S. Williams, and F. Bennett. (Available also in Spanish.)

No. 2. Nutrition of Adolescent Girls Research Program *by* B. Torún, F. Viteri, M. Ramírez-Zea, M.M. Rodríguez, and K. Guptill.

No. 3. Nutrition, Health and Growth in Guatemalan Adolescents *by* R. Martorell, J. Rivera, and P. Melgar.

No. 4. Early Nutrition and Physical and Mental Development in Mexican Rural Adolescent Women *by* A. Chávez, C. Martínez, B. Soberanes, L. Domínguez, and A. Avila. (Available also in Spanish.)

No. 5. A Multidimensional Study of Nutritional Status of Adolescent Filipinas *by* A.T. Roldan, V. Bautista, and R. Manalo.

No. 6. A Study on the Factors Influencing Nutritional Status of Adolescent Girls in Nepal *by* S. Regmi, and R. Adhikari.

No. 7. Understanding Gender-Differentiated Constraints to Philippine Farm Household Investments in Adolescents: Implications for their Nutritional Status *by* H.E. Bouis, M. Palabrica-Costello, O. Solon, and A.B. Limbo.

No. 8. Influence of Women's Social Status on the Nutritional Status of Adolescent Girls in Bénin *by* E. Alihonou, S. Inoussa, S. Vissoh, V. Capo-Chichi, C. Quenum, and A. Sagbohan. (Available also in French.)

No. 10. Study of the Factors that Influence the Nutritional Status of Adolescent Girls in Cameroon *by* K.M. Kurz, and Julienne Ngo Som.

No. 11. Improving Nutritional Practices of Ecuadorean Adolescents *by* Y. de Grijalva, and I. Grijalva.

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