THE EFFECTS OF A WALKING PROGRAM ON AEROBIC FITNESS, VITAMIN B-6 STATUS, AND BIRTH OUTCOME IN PREGNANT WOMEN TAKING VITAMIN-MINERAL SUPPLEMENTS

by

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(ABSTRACT)

The effects of an aerobic walking program, from 22 to 30 wk gestation , on aerobic fitness, vitamin B-6 status, and birth outcome was studied in 28 healthy, pregnant women aged 21-36 yr receiving vitamin-mineral supplements. Aerobic fitness was assessed by heart rate (HR) and relative oxygen consumption (VO_2, ml/kg/min) during 2 submaximal treadmill walking tests (22 and 30 wk). HR responses were significantly lower for walking subjects (W; n = 18) compared to non-walking (NW; n = 10) at 2 and 4 min and near significance at 6 min of the 30 wk treadmill test. HR increased significantly at 2 min for NW from 22 to 30 wk. Oxygen consumption significantly decreased for W from 22 to 30 wk at 2, 4, and 6 min of exercise but remained unchanged for NW. Mean vitamin B-6 intake, minus the 10 mg supplement, was at least two-thirds the 1980 Recommended Dietary Allowance. Values for plasma total vitamin B-6 assessed microbiologically were in the low-normal range for only 3 subjects (1 W at 22 wk and 2 W at 30 wk). Values for plasma pyridoxal phosphate levels assessed radioenzymatically were in the low-normal range for only 2 subjects (1 W at 22 wk and 1 W at 30 wk). Birth weight, Apgar scores, and labor duration were similar for both groups. Participation in a walking program by pregnant women taking vitamin-mineral supplements slightly improved aerobic fitness without affecting vitamin B-6 status (probably due to the supplement usage) or birth outcome.

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INTRODUCTION

The physiological and psychological benefits of being physically fit have been well documented and highly publicized (1). In recent years there has been a growing number of Americans who are interested in physical fitness and who engage in some type of regular exercise program. As a result of this trend, more women are participating in recreational and competitive athletics than in the past. These activities include jogging, swimming, cycling, weight lifting, walking, aerobic dancing and a variety of other sports. Women are also interested in remaining physically active throughout pregnancy (2).

Traditionally, physicians have not recommended anything more strenuous than walking or gardening during pregnancy, and while much study has been done in the area of the effects of physical training with a nonpregnant population, comparatively little has been done with a pregnant population (2). With this increased emphasis on fitness, physicians are requesting information and guidance on what constitutes a safe exercise program for pregnant women. Some questions that need to be addressed are: (1) what type and how much exercise is appropriate? (2) what are the effects of exercise on the health of the mother and the fetus? (3) what are the effects of exercise on pregnancy outcome? (4) can fitness be maintained or achieved during pregnancy? (5) is there an effect on the nutritional status of the mother?

Several investigators have indicated that healthy pregnant women can participate in moderate aerobic exercise throughout pregnancy and maintain or improve their level of fitness without compromising maternal or fetal health (3-8) but there is very little data available regarding the effects of exercise on maternal nutritional status. The effect of exercise on vitamin B-6 status would be of particular interest since low plasma levels of this nutrient in pregnant populations has been well documented (9). There are few reports in the literature regarding vitamin B-6 and exercise. Leklem and Shultz (10) reported an increase in plasma total vitamin B-6 and pyridoxal phosphate (PLP) in males after an exercise bout and Critz et al. (11) reported an increase in aspartate aminotransferase activity, EC 2.6.1.1 (an enzyme that requires vitamin B-6 as a cofactor) in skeletal muscle of rats after an exercise bout. Research in this area is meager and there are still many questions that need to be addressed. However, more is known about changes in the metabolism of several other nutrients as a result of exercise and generally speaking, exercise and physical training increase requirements for calories, carbohydrates, thiamin, riboflavin, niacin, iron, water, sodium, and possibly chromium (12). Under normal health circumstances, these increased nutrient needs can be met by eating more of a varied diet.

Likewise in pregnancy there is an increased need for nutrients including vitamin B-6, to allow for optimal growth and development of the fetus (13), but the recommendations for nutrient intakes do not take the added stress of an exercise program into account. Therefore, exercise during pregnancy may increase nutrient requirements beyond present recommendations.

Since vitamin B-6 is a nutrient that is frequently marginal in pregnant women, the purpose of this study was to determine the effects of a walking program and vitamin-mineral supplementation during pregnancy on aerobic fitness, maternal vitamin B-6 status as ascertained by both a microbiological and a radioenzymatic method, and pregnancy outcome. In addition, dietary intakes of vitamin B-6, protein, and energy were determined.

REVIEW OF LITERATURE

Maternal Responses to Exercise During Pregnancy

This area has been receiving an increasing amount of attention recently and a variety of questions have been investigated. Some investigators have looked at specific cardiac, respiratory, and metabolic responses to exercise. Morton et al. (14) measured heart rate (HR) and stroke volume (SV) in late gestation and postpartum in 23 subjects at rest, at the onset, and at the end of a bicycle ergometer exercise session. The fit and nonfit subjects had similar HR and SV responses in late gestation (higher HR at rest, lower SV during exercise) but at postpartum, only the fit subjects had responses typical of trained individuals (lower HR and higher SV). The data suggested that cardiac output in late gestation was influenced by factors that affect venous return and was not related to physical fitness. Knuttgen and Emerson (15) observed 13 subjects throughout pregnancy for their responses to treadmill and bicycle ergometer exercises. There were significant respiratory changes with increases in resting vital capacity and inspiratory capacity and decreases in expiratory reserve volume and functional residual capacity, but no change in total lung capacity. No dypsnea or ventilatory impairment was observed. The O_2 cost of treadmill walking increased (as a result

of increased body weight) but remained constant for cycling exercise. The authors concluded that nonweight bearing exercise should pose no additional physiological stress to the pregnant woman.

Other investigators have looked at changes in fitness level throughout the course of pregnancy either as a result of an exercise program or independent of any regular exercise, and also the relationship of fitness level to pregnancy outcome. Artal et al. (16) observed the cardiovascular and metabolic responses to 15 min of light exercise on a treadmill of 23 untrained subjects all in their 3rd trimester. Increases in systolic blood pressure (SBP), HR, and plasma levels of glucagon, norepinphrine, and epinephrine were noted. These all promptly returned to baseline levels following exercise. Since these changes are the appropriate responses to exercise, the authors concluded that light exercise of a short duration during a normal pregnancy resulted in no abnormal or detrimental cardiovascular and metabolic changes. Erkkola (7) studied the effects of training that lasted from the 10th or 14th week of pregnancy until term on 62 subjects. One-half of the subjects were instructed to perform strenuous exercise that raised HR to 140 bpm for 1 h 3 times per wk. The various forms of exercise included walking, running, climbing stairs, bicycling, and swimming. Every 4 wk the women in the training group were examined and questioned about their exercise regimen and a bicycle ergometer test was performed. The physical work capacity (PWC, a quotient of the work load and HR) spontaneously increased 10% in the control group. When this spontaneous increase was taken into account, the PWC rose 17.6% (a significant difference) in the exercise group. There were no significant differences in HR and SBP between the 2 groups and no detrimental effects from the training were observed. The authors concluded that PWC can be improved during pregnancy with regular training without endangering the course of the pregnancy. Pernoll et al. (17) measured oxygen consumption (VO_2), which is a way to assess aerobic fitness, at rest and during exercise in 12 subjects. The measurements were taken on a bicycle ergometer every month during pregnancy and at 2, 6, 12 wk and 6 mo postpartum. There was an increase in exercise and resting VO_2 during pregnancy when compared with postpartum VO_2. It was concluded that the efficiency (defined as work performed/VO_2 steady state) of light activity decreased in pregnancy thought the authors were unable to explain this finding.

Hutchison et al. (18) did a case study on a woman who had been running 3 to 5 miles, 3 to 5 times a wk for 8 yr prior to pregnancy. The subject continued to run up to her 8th mo at which time it became too strenuous and she changed to walking 3 miles daily until term. She was given monthly, submaximal treadmill tests throughout her pregnancy. There was an increase in VO_2 (l/min) that was accounted for by the weight gain since VO_2 (in ml/kg/min) remained constant. Ventilation (V_E), ventilatory equivalent for oxygen (V_E/VO_2), and HR rose but not in proportion to weight gain, indicating increased physiological stress. The subject experienced no complications or discomfort. The authors concluded that while there were no apparent contraindications for running during pregnancy, running does become more stressful as pregnancy progresses. They suggested that the speed be gradually decreased in order to maintain a constant physiological stress. Dressendorfer (8) observed one subject over a 4 yr period that included 2 pregnancies. The subject began a running program at the beginning of her 1st pregnancy that increased from 10 miles per wk to 35 miles per wk at 11 mo postpartum. Mileage during the 2nd pregnancy averaged 15 miles per wk up to 20 miles per wk at 4 mo postpartum when the study ended. No complications were reported and the subject experienced 2 normal pregnancies and deliveries. There was no disturbance in milk production and VO_2 max increased during the 1st and 2nd pregnancies. Dressendorfer concluded that fitness level can be increased during pregnancy as indicated by the increase in VO_2 max and that lactation is not affected by an endurance training program. Regular exercise during a normal pregnancy appears to have no detrimental effects. Likewise, Collings et al. (5) noted a significant increase in VO_2 max in 20 untrained pregnant subjects in their 2nd and 3rd trimesters who were exercised on a bicycle ergometer 3 times a wk throughout pregnancy. They concluded that women enrolled in an aerobic exercise program are able to improve their aerobic capacity without compromising their health.

Concerning the question of fitness level and pregnancy outcome, Jarret and Spellacy (19) analyzed data obtained from a retrospective questionnaire of 67 women who ran an average of 3.8 yr prior to pregnancy and continued to run during pregnancy. The authors reported a low incidence of maternal and fetal complications and the data showed no relationships between number of miles run in either the 3rd trimester or in the entire pregnancy, and infant birth weight or gestational age.

The authors acknowledged the limitations of such a questionnaire but concluded that healthy women with a history of running can apparently continue to run without any adverse effects to the fetus.

Berkowitz et al. (20) also used a survey with 175 mothers of singleton preterm infants and 313 mothers of singleton term infants. Analysis of survey responses showed no relationship between housework, child care, employment, and physical activity and an increased risk of premature delivery. Women who engaged in physical exercise had a significantly lower risk of premature delivery than their more sedentary counterparts. The authors suggested that exercise may have a protective effect against premature delivery.

Pomerance et al. (6) observed 41 pregnant women in their 3rd trimester to determine if fitness level was related to pregnancy outcome. The subjects were tested on a treadmill to measure their aerobic capacity. The authors found no significant correlation between level of fitness and length of gestation, complications of pregnancy, length of labor, or birth weight, length, and head circumference, and concluded that the level of fitness seemed to have no effect on the fetus. Similarly, Diblee and Graham (21) found no relationships between level of fitness and length or type of labor, delivery, birth weight, or Apgar scores (a method for assessing infant health which is based on appearance, pulse, grimace, activity, and respiration).

However, not all investigators have found physical activity to have either a beneficial or nil effect on pregnancy outcome. Tafari et al. (22) compared infant birth weights of a group of Ethiopian women who performed heavy physical labor during pregnancy to that of a group of women who performed only light work. While both groups received less than 70% of the caloric intake recommended by WHO/FAO, the more active group had infants whose mean birth weight was significantly lower than that of the less active group. Naeye and Peters (23) reported, from the analysis of 7,722 pregnancy outcomes, that women who performed stand-up type work in the 3rd trimester had infants who weighed 150 to 400 g less than infants born to mothers who stayed at home. Inadequate blood flow from the uterus to the placenta was given as a possible cause for this trend.

Similarly, from a survey of 336 women, Clapp and Dickstein (24) reported an increased incidence of lower birth weight infants (~500 g), shorter gestational periods (~8 days), and lower maternal weight gains (~4.6 kg) for women who continued a vigorous exercise program during the 3rd trimester, and a reversal of this trend when vigorous exercise was stopped prior to 28 wk gestation. The authors stated that this data should be interpreted with caution since studies need to be done on women who begin a rigorous exercise program in pregnancy.

Lastly, certain types of exercise may be harmful to the fetus because of the violent jostling, and/or compression that occurs in activities such as wrestling, martial arts, skiing, and rodeo riding. A slightly increased incidence of birth defects was reported in a group of scuba divers who continued to dive during pregnancy, but contributing factors might have been hypothermia and hypoxia (25).

Fetal Responses to Exercise

A number of studies have focused more on the fetal responses to the stress of exercise in an attempt to answer part of the overall question of the effects of exercise during pregnancy. Dale et al. (26) electronically monitored fetal HR (FHR) patterns before, during, and after exercise. Four pregnant subjects who were in their 3rd trimester performed a treadmill running test at 80% of their predicted maximum HR. The subjects had participated in distance running throughout their pregnancy. Of the 3 electronic tracings that were acceptable, a transient fetal bradycardia was observed during the treadmill tests, but FHR returned to normal again within the same exercise period. The authors concluded that the bradycardia appeared to be short-lived and reversible but were unable to explain the etiology. Platt et al. (27) observed by ultrasound fetal breathing movements (FBM) and fetal movements (FM) before and after 17 untrained pregnant subjects in their 3rd trimester performed a mild treadmill walking test. There were no direct correlations between FBM

or FM and exercise, but positive correlations were found between maternal epinephrine levels and FBM and FM.

Dressendorfer and Goodlin (3) also looked at FHR response by ultrasound to aerobic exercise performed at up to 80% of the maximum HR of pregnant subjects. Five trained women in their 3rd trimester exercised on a bicycle ergometer and FHR responses were monitored throughout the exercise session. The FHR gradually increased to an average of 149 bpm (from a resting average of 142 bpm); the authors concluded that since the FHR fell within the normal range of 120 to 160 bpm and there were no persistent changes in FHR as a result of the exercise, that aerobic exercise of a submaximal level did not result in abnormalities in the FHR. Hauth et al. (4) determined that the FHR in 7 pregnant women in their 3rd trimester who jogged 1.5 miles 3 or more times a wk before and during pregnancy, increased after the jogging sessions (ranging from 155-204 bpm) but returned to baseline after exercise. Interestingly, the authors concluded that moderate exercise did not result in fetal distress as determined by FHR even though the FHR were well above the normal range as defined by Dressendorfer and Goodlin (3). Collings et al. (5) studied 20 untrained, pregnant women in their 2nd and 3rd trimester. The women were exercised on a bicycle ergometer at 65-70% VO_2 max 3 times a wk for from 7 to 19 wk. FHR increased in response to the exercise to an average of 148 bpm (from a resting average of 144 bpm).There were no significant differences in labor, delivery, and birth outcome between the exercise and control groups. The authors concluded that maternal aerobic exercise seemed to have no beneficial or detrimental effect on fetal growth, but they felt that larger samples were needed. They also concluded that the increase in FHR was not problematic. They stressed the importance of looking at the intensity of the exercise when prescribing programs for pregnant women.

Vitamin B-6 Requirement in Pregnancy

Vitamin B-6 is not one compound but the name given to a group of 3 pyridines--pyridoxine, pyridoxal, and pyridoxamine, which are metabolically and functionally interrelated. All 3 forms are nutritionally active and can be converted to the active, phosphorylated coenzyme form, PLP (13).

There is an increased need for vitamin B-6 during pregnancy because of several factors which include the demands of the growing fetus, increased protein in the diet (dietary protein and vitamin B-6 requirement being positively related) and increased circulating estrogens (which increase tryptophan oxygenase activity, EC 1.13.11.11, an enzyme which requires vitamin B-6) (13). The 1980 Recommended Dietary Allowance (RDA) for vitamin B-6 has been set by the National Academy of Sciences-National Research Council (NAS-NRC) at 2.6 mg/day for pregnant women, an increase of 0.6 mg over that for nonpregnant women.

A number of investigators have reported altered laboratory indices that imply a deficient vitamin B-6 status in pregnant women. Various groups of researchers have found that these biochemical changes can be prevented by supplementation of 6-10 mg/day of pyridoxine (13). However, the NRC states that a requirement of more than 2.6 mg/day could not generally be obtained through diet alone and would require a supplement; they do not feel that current evidence is strong enough to warrant such a recommendation.

Vitamin B-6 Status Parameters

There are a variety of methods for assessing vitamin B-6 status, each having advantages and disadvantages. In earlier years the tryptophan load test was a popular method for assessing status. This involves the measurement of various tryptophan metabolites (xanthurenic acid being the most easy to measure) in the urine after a load of 2 or 5 g of L-tryptophan (28). This method is good

for studying the status of individuals. Results must be carefully analyzed as this test is susceptible to influence by hormonal factors (pregnant women and oral contraceptive users may excrete abnormally high levels of xanthurenic acid).

Urinary excretion of vitamin B-6 and 4-pyridoxic acid are useful methods for population studies (28,29). In the former, the 3 forms of vitamin B-6, pyridoxal, pyridoxine, and pyridoxamine, and vitamin B-6 are measured in a 24 h urine sample; the results of this method reflect the recent dietary intake of the individual so it is not useful in determining long-term status. In the latter method, 4-pyridoxic acid is determined in a 24 h urine sample. This is the major urinary metabolite of vitamin B-6. Again, the results of this method are indicative of recent intakes and the assay for 4-pyridoxic acid is tedious and subject to interference by 4-pyridoxic acid 5′-phoshate.

Levels of vitamin B-6 in erythrocytes, plasma, and whole blood can be determined, but there are many difficulties with the analytical procedures and normal ranges need to be established (28). The use of erythrocyte aminotransferases (alanine aminotransferase, EC 2.6.1.2 and aspartate aminotransferase) is considered a good method of assessing vitamin B-6 status (28,29). It involves comparison of the activity of the enzymes before (unstimulated) and after (stimulated) the addition of PLP. The ratio of stimulated to unstimulated activity is expressed as an index and there are established values for adequate and deficient status.

The determination of plasma PLP by radioenzymatic methods is a newer method for status assessment. This assay is based on the coenzyme dependent decarboxylation of L-tyrosine by tyrosine apodecarboxylase (EC 4.1.1.25) (30). This method is quick, sensitive, and reproducible. Norms for plasma PLP levels still need to be established, but this is becoming an increasingly popular method for status assessment.

Microbiological methods for determining total vitamin B-6 are well established and have been widely used (31). The yeast Saccharomyces uvarum, ATCC 9080 (formerly S. carlsbergensis) which requires vitamin B-6 for growth is employed in this method. The organism, which responds to all 3 forms of vitamin B-6, is incubated with the biological sample in question. A standard curve is prepared by incubating the yeast with known amounts of vitamin B-6. Growth is then measured

by turbidity. This method is sensitive and reproducible. Other advantages include being simple and convenient in that most any laboratory could easily be set up for this procedure and also is inexpensive with regard to the type of equipment and chemicals that are utilized. Normal values for plasma total vitamin B-6 still need to be established, but this is still a valuable method that is used by a number of investigators.

Vitamin B-6 Status and Intake in Pregnancy

There have been numerous studies conducted in this area with many investigators reporting an apparent deficient or marginal status in the pregnant population and intakes that are often below the RDA for vitamin B-6. Heller et al. (32) determined the vitamin B-6 status of 493 pregnant women by the erythrocyte aspartate aminotransferase activation test. Three hundred male and female blood donors were used as controls. Blood was taken from the pregnant subjects on one occasion at various stages of gestation. Forty to sixty percent of the subjects with uncomplicated pregnancies were deficient in vitamin B-6 in comparison to controls. Also, mothers that had fewer previous pregnancies had slightly better vitamin B-6 status than mothers that had more, and there was no correlation between vitamin B-6 status and pregnancy outcome. The authors concluded that while no deleterious effects on pregnancy outcome were observed as a result of poor maternal vitamin B-6 status, supplementation may still be necessary since it has been shown in animals that adequate vitamin B-6 is required for proper development of the fetus.

Vir et al. (33) assessed the vitamin B-6 status of 60 pregnant subjects and 20 nonpregnant controls by the erythrocyte alanine aminotransferase method (EALAT). Blood was taken from the pregnant subjects in the 2nd and 3rd trimester and 3 days postpartum and food records were obtained from some of the subjects. Fifty percent of the women in the 2nd trimester, 53% of those in the 3rd trimester, and 44% of postpartum subjects were deficient in vitamin B-6. The controls had a lower occurrence of deficiency which was significant in comparison to the 2nd and 3rd

trimester values. Intake of vitamin B-6 as calculated from food composition tables was ≥ 2.5 mg/day (1974 RDA for pregnant women) in only 3 of the 51 subjects. There was no correlation between status, intake, pregnancy outcome, or anthropometric measurements of infants. The authors were not certain whether vitamin B-6 should be supplemented during pregnancy based on their results. They felt that since there was such a big variation in status between pregnant subjects, that something else may have affected the outcome of the EALAT test when performed during pregnancy.

The purpose of a study by Baker et al. (34) was to prepare a vitamin profile of 174 women and infants at parturition. Circulating blood levels of several vitamins, including B-6, were determined by chemical and protozoologic methods. Vitamin B-6 was one of the vitamins that was low in plasma from mothers not taking supplements and while daily supplements of from 3-10 mg of vitamin B-6 decreased the incidence of hypovitaminemia, it did not totally abolish it. Also, infants whose mothers had vitamin levels below the mean ($\overline{X}$) also had the same. The authors concluded that a profile of this type (when compared to established norms) can help in the study of the effects of subclinical vitamin deficiencies.

Schuster et al. (35) determined the vitamin B-6 status of low-income pregnant adolescents and adults. The 127 subjects were participants in the Special Supplemental Food Program for Women, Infants, and Children (WIC) and were in their 2nd and 3rd trimesters. Blood samples were analyzed for vitamin B-6 by the EALAT method and diet records and birth outcome data were obtained from some of the subjects. The $\overline{X}$ vitamin B-6 intake was 53% of the 1980 RDA of 2.6 mg. Seventy-seven percent of the subjects consumed less than two-thirds of the RDA and 88% consumed less than the RDA. None of the subjects reported taking vitamin supplements before the time of the study. Sixty-eight percent of the subjects had inadequate vitamin B-6 status as defined by the EALAT test and there was a trend for black women to have higher EALAT percentage stimulation than white women, though there was no difference between age groups and stages of pregnancy. There was no correlation between intake and status. Finally, Apgar scores for infants whose mothers had adequate vitamin B-6 status were higher than the scores for infants of mothers who had inadequate status, but there were no significant differences in birth weights. It was con-

cluded that, in light of the findings, perhaps vitamin B-6 nutriture should be determined early in pregnancy.

Similarly, Bailey and co-workers (36) determined the plasma PLP levels and vitamin B-6 intakes of 43 participants in the WIC program and compared them to that of 58 controls. Eighty percent of the WIC participants and 86% of the controls had plasma PLP levels lower than 4.7 ng/ml which has been suggested to be the lower limit of the normal range. There was a significant difference between the 2 groups for vitamin B-6 intake. The WIC group consumed a $\overline{X}$ of 52% of the 1980 RDA while the controls consumed 41%. Finally, there were no differences in birth weights and Apgar scores between the 2 groups.

Reopke et al. (37) confirmed the reports of lower vitamin B-6 status and intakes in pregnant populations. These investigators observed 106 women during pregnancy and lactation. Serum levels of vitamin B-6 were determined microbiologically for 86 of the subjects at 5 and 7 mo gestation and at the time of delivery. Vitamin B-6 levels in the milk of 66 of the subjects were also determined at 3 and 14 days postpartum. Diet records were obtained from each subject and daily vitamin B-6 intakes were estimated by using food composition tables. The researchers found that the $\overline{X}$ intake was approximately 50% of the 1974 RDA without supplements and 250% of the RDA with supplements . Serum and cord levels of vitamin B-6 of women who consumed $\leq$ the RDA were lower than those of women who consume > RDA. The vitamin B-6 content of the milk of women who consumed > RDA was higher than those who consumed $\leq$ RDA at 3 but not 14 days postpartum. Serum vitamin B-6 concentrations at 5 mo gestation were positively correlated with cord vitamin B-6 levels. Lastly, mothers with lower intakes and serum vitamin B-6 levels had infants with lower Apgar scores. The authors concluded that vitamin B-6 inadequacy during pregnancy and lactation may be of real concern. Also, since 5 mo gestation precedes the period of rapid growth of the fetal central nervous system, this may be the best time to assess vitamin B-6 status and supplement if appropriate.

A number of investigators have attempted to determine the requirement for vitamin B-6 during pregnancy by finding the amount of this vitamin that is necessary to maintain normal biochemical indices. Cleary et al. (38) determined the vitamin B-6 status of 24 pregnant women at the time

of delivery. The subjects had been taking daily vitamin supplements that contained either 2 to 2.5 or 10 mg of vitamin B-6. Blood samples were also taken from the cord. Fifty-eight nonpregnant females who were not taking supplements served as controls. Mean plasma PLP levels of the controls were well above the lower limit of the normal range (4.7 ng/ml) and none of these subjects had levels below the lower limit. The $\overline{X}$ plasma PLP levels of the pregnant subjects was lower than that of the control group and 4 of the 11 women receiving 10 mg of vitamin B-6 had levels lower than 4.7 ng/ml while 10 of the 13 women receiving 2 to 2.5 mg of vitamin B-6 had levels lower than 4.7 ng/ml. Lastly, there was a positive correlation between maternal plasma PLP and cord PLP levels. The authors concluded that perhaps supplementation of more than 2.5 mg is necessary to maintain adequate vitamin B-6 status in pregnancy.

Lumeng and co-workers (39) assessed the effect of 2.5, 4, and 10 mg supplements of pyridoxine hydrochloride during pregnancy on maternal and fetal plasma PLP levels for 26 subjects. The authors reported a correlation between maternal plasma PLP levels with that of cord blood, and that more than 4 mg was necessary for the majority of the subjects to maintain plasma PLP levels in the same range as 1st trimester and nonpregnant ranges. In addition, most subjects had vitamin B-6 intakes of less than 2 mg/day. It was suggested that the status of the fetus is dependent on the circulating maternal levels of PLP, and that supplementation of more than 4 mg/day is needed.

Schuster and co-workers (40) also attempted to better ascertain the vitamin B-6 requirement during pregnancy. These investigators supplemented the diets of a group of pregnant subjects with either 2.6, 5, 7.5, 10, 15, or 20 mg of pyridoxine hydrochloride daily. A supplement of 7.5 mg or more prevented a decrease in maternal plasma PLP levels at delivery and cord levels of PLP peaked at this supplementation range. Apgar scores were higher at 1 min for infants whose mothers took 7.5 mg or more of pyridoxine hydrochloride than for infants whose mothers took 5 mg or less. Eighty-three percent of the subjects consumed less than the 1980 RDA for vitamin B-6. The authors concluded that maternal intakes of vitamin B-6 that range from 5.5 to 7.6 mg/day (diet plus supplement as pyridoxine equivalents) are necessary to maintain maternal plasma PLP at early and prepregnancy levels.

Several investigators have focused on maternal and fetal metabolism of vitamin B-6 as compared to nonpregnant populations in an attempt to find explanations for the apparent inadequacy that is often found in pregnant women. Contractor and Shane (41) studied a group of men and nonpregnant and pregnant women to compare vitamin B-6 metabolism in these groups. Each subject was given 50 mg of pyridoxine hydrochloride either orally or by injection. A specific chemical method developed by the authors was used to determine plasma PLP and urinary metabolites of vitamin B-6. It was determined that plasma PLP levels were significantly lower in pregnant subjects and fetal cord levels of PLP were high. There were no significant differences between the groups with respect to urinary 4-pyridoxic acid, the major metabolite of vitamin B-6, suggesting that absorption of the vitamin is unimpaired during pregnancy. The phosphorylation of pyridoxine hydrochloride was rapid in all subjects. Fetal plasma PLP levels peaked several hours after maternal plasma peaked indicating that this form of the vitamin crosses the placental barrier. The authors concluded that the relative deficiency state of pregnant women may be due to the high uptake of the vitamin by the fetus.

Reinken and Dapunt (42) offer the same explanation. They determined serum PLP concentrations in 16 pregnant women from mo 2 until term and also in the cord blood of their newborn infants. The authors reported the development of a gradual biochemical deficiency in vitamin B-6 as pregnancy progressed with the most significant decrease in plasma PLP levels occurring between mo 4 and 8, the time of the most intensive growth of the fetus. They also reported that maternal serum PLP levels at the beginning of pregnancy, decrease of serum PLP during pregnancy, and PLP concentrations of infant serum were all positively correlated to birth weight. Reinken and Dapunt (42) suggested that active transport of PLP across the placenta is the most important mechanism leading to the relative vitamin B-6 deficiency in pregnant women. The authors considered vitamin B-6 supplementation during pregnancy to be essential in light of their findings.

Similarly, Brin (43) analyzed paired samples of maternal and cord blood for the 3 forms of vitamin B-6 (vitamers), total vitamin B-6, and coenzyme stimulation of erthyrocyte alanine and aspartate aminotransferase activities. The cord blood had from 1.5 to 4 times the amount of the individual vitamers found in the mothers' blood, while the cord blood had at least twice the amount

of total vitamin B-6 found in the mothers' blood. The coenzyme stimulation for both enzymes was 50% higher in the cord erythrocytes than in the mothers' erythrocytes. The author suggested 2 possible mechanisms to explain the results: 1) the active transport of vitamin B-6 across the placenta and/or 2) an increase in the number of binding sites in fetal tissue due to increased levels of enzymes that require vitamin B-6. Either of these mechanisms could deplete maternal stores.

Finally, Reopke and Kirskey (44) approached vitamin B-6 nutriture in pregnancy from a slightly different angle. These investigators assessed vitamin B-6 status of pregnant subjects who were users of oral contraceptive agents (OCA). They reported that women who used OCA for more than 30 mo had significantly lower levels of serum vitamin B-6 at 5 mo gestation and delivery than women who used OCA for 1 to 30 mo or women who were nonusers of OCA prior to conception. The authors concluded that long-term use of OCA may compound the effects of the hormonal changes during pregnancy that alter vitamin B-6 status.

Vitamin B-6 and Exercise

It is generally accepted that regular exercise and training increases the need for calories, carbohydrates, thiamin, riboflavin, niacin, iron, water, sodium, and maybe chromium (12), and that these increased needs can be met by consuming more of a balanced diet. Very little has been done in the area of vitamin B-6 and exercise.

Leklem and Shultz (10) studied vitamin B-6 metabolism in 7 trained male adolescent athletes after a 4500 m run. Blood was collected before and after the run and a 24 h urine sample was collected on the day of the run. Plasma PLP and total vitamin B-6 concentrations and urinary excretion of 4-pyridoxic acid were determined. There were significant increases in plasma PLP and total vitamin B-6 levels, but urinary 4-pyridoxic acid excretion remained unchanged. It was concluded that while this study did not prove that there was an increased need for vitamin B-6 with exercise, there were definite changes in plasma vitamin B-6 levels following an exercise bout.

Leklem et al. (45) had similar results in another study done on trained male adolescent athletes. Plasma PLP concentrations increased after a 4500m run while urinary 4-pyridoxic acid excretion was not significantly changed.

A few animal studies have investigated vitamin B-6 metabolism and exercise. Critz et al. (11) reported an increase in aspartate aminotransferase activity in skeletal muscle, cardiac muscle, and liver in rats after exercise, but a decrease in serum activity of the enzyme. The authors stated that since aspartate aminotransferase converts aspartic acid to oxaloacetate, a TCA cycle intermediate and potential energy source, it is to the animal's advantage to accumulate the enzyme in active tissues. Chen and Marlatt (46) reported no change in EALAT activity in the tissue or serum of rats that were exercised 12 wk when compared to those of control rats.

Much more research needs to be done in this area before any conclusive statements can be made.

Summarization of Literature

There are still a number of questions that need to be addressed with regard to the effects of exercise during pregnancy, such as: 1) a closer look at the intensity, frequency, and duration of the exercise program; 2) whether weight bearing or nonweight bearing exercise is performed; 3) whether the program is started before or at the beginning of the pregnancy; 4) the quality of the diet; and 5) the effects that a training program may have on the nutritional status of the mother. However, it appears that at least a moderate exercise program is well tolerated by both mother and fetus and that there are generally no ill effects on birth outcome. The physiological responses to exercise by pregnant women are not tremendously different from those of nonpregnant women and research suggests that fitness level can even be improved during pregnancy.

With regard to vitamin B-6 nutriture in pregnancy, numerous reports in the literature have confirmed that low or deficient intake and status, as determined by biochemical parameters, is

usually seen in pregnant populations. Not all investigators agree on whether this phenomenum is detrimental to birth outcome. Explanations for suboptimal maternal vitamin B-6 status include active transport of the vitamin across the placenta and hormonal influences on its metabolism. While several investigators agree that pregnant women need a vitamin B-6 supplement over and above the current RDA, much more work needs to be done in the area of requirement and whether this apparent vitamin B-6 deficiency is actually harmful to the fetus.

Finally, very little work has been done in the area of vitamin B-6 and exercise. Preliminary findings suggest an increased plasma level of the vitamin immediately following exercise. However, no conclusive statements can be made about the effects of physical training on vitamin B-6 metabolism.

MATERIALS AND METHODS

Subject Recruitment Procedures

Following approval of the study by the Institutional Review Board for Research Involving Human Subjects, recruitment notices were placed in the Roanoke Times and World News, the Spectrum Newspaper (the faculty, staff, and graduate student newspaper for Virginia Polytechnic Institute and State University (VPI & SU)), and the Collegiate Times (the university newspaper). Flyers (Appendix A) describing the study were posted in strategic locations in Blacksburg (churches, health spas, grocery stores, apartment complexes, and other area businesses) and on bulletin boards around the campus of VPI & SU. In addition, subjects were recruited by referral from private physicians, from prenatal exercise classes through the Free University, and from Childbirth Education (Lamaze) classes. Finally, word of mouth referrals resulted in recruitment of some subjects. The recruitment period lasted from September, 1984 to August, 1985.

Experimental Design and Procedures

The participants of the study consisted of 28 healthy Caucasian pregnant women in their 2nd trimester (~22 wk) from the communities of Blacksburg and Christiansburg. The women ranged in age from 21 to 36 yr old and were apparently free from any physical limitation, illness, or disease, a necessary prerequisite for taking part in the study. The subjects and their physicians were given a written explanation of the study and asked to sign a consent of participation and liability form (Appendix B). The physicians' signatures were required to ensure their understanding of the experimental design, potential risks to subjects and their patient's participation. All subjects completed a pre-experimental questionnaire (Appendix C) which provided some general background information regarding prenatal history, activity level, and the intake of vitamin-mineral supplements (adapted from Christakis (47)). The subjects were told during the recruitment that they would be free to withdraw from the study at any time.

All participants were given a vitamin-mineral supplement (Natalins Rx) approximately one mo before the beginning of the experimental period (22 wk gestation). This was done to ensure some measure of control on vitamin-mineral intake and to provide compensation for taking part in the study. The vitamins were provided by Meade-Johnson Pharmaceutical Division, Meade-Johnson and Company, Evansville, IN; the composition of the supplement is listed in Appendix D. The supplements contained 10 mg of vitamin B-6 per daily dosage.

After the consent forms and the pre-experimental questionnaires were completed, subjects were assigned to 1 of 2 groups based on their willingness to participate in either group. These groups were the walking group (W) and the nonwalking group (NW). Subjects from both groups were then scheduled for exercise testing, blood collection, and completion of activity and dietary questionnaires, which took place at approximately 22 and 30 wk of pregnancy. The women in the W group participated in a walking program for 8 wk from 22 to 30 wk gestation. The women in the NW group tried to maintain their same activity level during the 8-wk period.

Exercise Program

The exercise program was individually prescribed for each participant based on their age predicted maximum HR. The target HR was calculated by determining 70% of the maximum HR for each subject (1). The subjects walked a minimum of 3 times/wk, for approximately 30 min duration per session, at an intensity that approximated the target HR for a period of 8 wk. The walking sessions were initially supervised and then the subjects were instructed to continue with the prescribed exercise program on their own. The walking sessions took place at locations that were convenient for the subjects (on campus or in their neighborhoods) and some subjects walked together in order to provide mutual motivation.

The women were taught how to approximate their HR by the palpitation method (48). At the end of the 30 min walking session the subjects would check their pulse for 10 sec and then multiply that value by 6 to approximate bpm. The participants kept records of their walking programs (frequency, duration, and HR), and any subject that did not maintain the prescribed program of 3 times/wk, 30 min per session at target HR was not included in the W group.

Exercise Testing

All subjects from both the W and NW groups performed 2 submaximal exercise tests on a treadmill; 1 initial baseline test during their 2nd trimester (22 wk) and a 2nd test after the walking program during their 3rd trimester (30 wk). The exercise test was based on a modification of the Balke-Ware maximal exercise protocol (49).

The subjects were asked to report to the Health, Physical Education, and Recreation Human Performance Laboratory located on the campus of VPI & SU one wk prior to the test in order to become familiar with the test equipment and protocol. This was done to reduce the stress associ-

ated with an unfamiliar experience and to therefore elicit more accurate data. Subjects were asked to arrive early enough on the day of their test to allow sufficient time to relax before the start of the treadmill test.

A supine resting HR and BP and also a standing resting HR and BP were determined for each subject before the treadmill test began. HR, V_E, fraction of expired O_2 (F_{EO_2}), fraction of expired CO_2 (F_{ECO_2}), and rate of perceived exertion (RPE) were determined at regular intervals during the test.

Subjects walked on the treadmill at 4.02km/h, beginning with a 0% grade and increasing 2.5% every 2 min (Appendix E) until either a HR that approximated 80% of their age predicted maximum HR (~150/bpm), an RPE reading of 15, or until they requested to stop the test. Immediately following the test a blood sample (0.1 ml) was obtained via fingerprick for determination of lactic acid. Subjects then laid down and HR and BP were recorded for 4 consecutive min postexercise. Lastly, each subject was weighed. For a more detailed description of the exercise test protocol, see "Effects of a Walking Program on Aerobic Fitness, Riboflavin and Thiamin Status, and Birth Outcome in Pregnant Women Taking Vitamin-Mineral Supplements", Dissertation, R.D. Lewis, VPI & SU, 1986 (50).

Blood Collection

Blood was collected from all subjects, W and NW groups, at the beginning (22 wk) and end (30 wk) of the walking program. A qualified technician obtained 20 ml of blood by venipuncture. The blood was collected in vacutainers that contained ethylenedinitrilotetraacetic acid added as an anticoagulant. The vacutainers containing the blood samples were protected from the light and kept on ice for a maximum of 10 min until centrifugation. The blood samples were transferred to plastic centrifuge tubes and centrifuged at 2000xg for 30 min at 0-5°C. Following centrifugation, the plasma was removed and frozen and at a later time a microbiological assay of plasma total

vitamin B-6 was done. An assay for plasma PLP was also done at a later time by other investigators in this laboratory. Finally, erythrocytes were processed for riboflavin and thiamin analysis by another investigator in this laboratory (see Dissertation, R.D. Lewis(50)).

Physical Activity Level Assessment

During the 22nd and 30th wk of pregnancy, the subjects completed an activity questionnaire (Appendix F). The questionnaire contained a list of activities and subjects were asked to indicate which of the activities they had regularly participated in over the last 12 mo, along with the frequency, duration per session, and intensity of the activity. The activity questionnaire helped determine the activity level of the subjects prior to and during the 8 wk experimental period. A total activity score and a total aerobic activity score were calculated for each subject at 22 and 30 wk of pregnancy. The questionnaire used was a modification of a method used by Reiff et al. (51). For a detailed explanation of calculating activity scores see Dissertation, R.D. Lewis.

Dietary Assessment

Each subject completed a 2 day dietary intake record and a 24 h recall (Appendix G and H) at 22 and 30 wk of pregnancy to estimate intakes of vitamin B-6, protein and kilocalories (kcal) (47,52). The 2 day dietary record contained clear instructions and an example to facilitate as accurate of record keeping as possible. Food models were used during the 24 h recalls to help participants better estimate serving sizes. An old and updated version of the Agriculture Handbook No. 8 (53,54) and the Home Economics Research Report No. 36 (55), Agriculture Research Divi-

sion, United States Department of Agriculture (1963; 1976-84; 1969) provided the data base with which to estimate subject intakes of vitamin B-6, protein, and kcal.

Pregnancy Outcome

All subjects were given postpartum questionnaires (Appendix I) after their 30 wk exercise test which were completed and returned after delivery. The questionnaire covered several parameters including weight gain during pregnancy, infant birth weight and length, Apgar scores, and duration of 1st and 2nd stage labor.

Microbiological Analysis of Plasma Total Vitamin B-6

The instructions for preparation of the reagents for the analysis is given in Appendix J. The vendors and their addresses for certain of the chemicals used in the analysis are given in Appendix K. Plasma total vitamin B-6 was determined by a modification of a method described by Sutker et al. (56). Plasma PLP was determined by other investigators in this laboratory using a method described by Fries et al. (57).

Preparation of glassware

All glassware (test tubes, glass beads, pipettes, beakers, volumetric flasks, glass funnels, storage bottles, and innoculating pipettes) and plastic test tube caps were sterilized before use. Test tubes

were placed in a rack and 2 clean 6 mm glass beads were added to each one. The test tubes were then covered with aluminum foil and autoclaved for 15 min at 15 psi and 121°C. Likewise, other glassware and test tube caps were placed on metal trays, covered with foil, and autoclaved in the same manner. Glassware was then either allowed to dry overnight at room temperature or placed in a drying oven for 1 h.

Sample preparation

Frozen plasma samples were thawed overnight in the refrigerator. Then either 1, 2, or 3 ml, depending on what was available, was transferred to plastic centrifuge tubes. While keeping the centrifuge tubes on ice, sulfosalicyclic acid was added to each sample at the concentration of 0.05g sulfosalicyclic acid/ml plasma. If a sample was to be spiked, it was done at this point by adding 0.2 ml (2 ng pyridoxine, as pyridoxine hydrochloride) working stock standard. The tubes were covered with parafilm and vortexed at medium speed until the acid dissolved. The plasma samples were then centrifuged for 10 min at 5°C at 7000xg. The supernatant was decanted into a syringe that had been fitted with a 0.2 μm Acrodisc filter and the supernatant was filtered into a screw top glass test tube that was in ice. The precipitant was discarded. Three ml of 0.2 N hydrochloric acid were added to each tube through the syringe in order to rinse all sample through the filter. The samples were placed in a boiling water bath for 1 h and were then placed on ice to cool to room temperature. The pH of each sample was adjusted to 4.5 with 1 N potassium hydroxide and 10% glacial acetic acid. Each sample was then transferred to a 25 ml volumetric flask and brought up to volume with distilled, deionized water. Each sample was transferred to a screw top glass test tube and frozen until the microbiological analysis.

Maintenance of culture

Stock cultures of S. uvarum had been kept growing for 3 yr by another member of our research group prior to this author's microbiological assays, and were utilized to make fresh cultures. To make fresh cultures, agar slants were 1st prepared in bulk by dissolving 4.1g of Bacto YM agar in 100 ml distilled, deionized water over heat with constant stirring. Once dissolved, 5 ml agar was transferred to screw top glass test tubes, loosely capped, and autoclaved for 15 min at 15 psi and 121°C. The tubes were cooled at an angle so that they would form slants. Once cooled, the tube caps were tightened and the tubes were refrigerated until use.

Initially, a transfer of S. uvarum, using aseptic techniques, was made from the stock culture to 2 fresh agar slants. These agar slants were incubated overnight in a 28°C water bath. One slant was used to innoculate the broth for the current microbiological assay while the other was refrigerated and used to make new slants. Each week a transfer of S. uvarum was made from the refrigerated slant to 2 fresh agar slants where again, one slant was used to make innoculum for the current assay and the other was refrigerated for the next week's transfer.

Preparation of innoculum

The day prior to the microbiological assay the innoculum used in the standards and prepared samples was made. Into 20 ml distilled, deionized water, 0.54 g of Pyridoxine Y media was dissolved. Ten ml of this media were placed in each of 2 test tubes, covered with plastic caps, steamed for 10 min at 100°C, then cooled to room temperature in an ice water bath. Using aseptic techniques, a loop transfer from the fresh agar slant was made into both of the cooled media-containing tubes. The tubes were then placed in a water bath and incubated for 22 h at 28°C. Two tubes were innoculated to ensure that a culture suspension would be available for the test. After the incubation period, the cultures were washed with 0.85% sodium chloride solution in the following

manner. The contents of the innoculated media tubes were aseptically transferred into 2 screw top test tubes and spun at 2500xg at room temperature in a portable clinical centrifuge for 3-4 min. The supernatant was discarded, and 10 ml of sterile saline (room temperature) were added to the pellet, which was the culture, at the bottom of the tube. This mixture was gently shaken so that the pellet dispersed and then centrifuged at 2500xg for 3-4 min. Again the supernatant was discarded, and the saline wash was repeated twice more for a total of 3 saline washes. The final saline suspension was used to innoculate the standards and prepared samples.

Assay of vitamin B-6 standards

The individual working standards of 0.5, 1, 2, 3, 4, and 5 ng/10ml pyridoxine (as pyridoxine hydrochloride) were brought to room temperature by pouring a few ml of each from the storage bottles into small beakers and covering with plastic wrap. Each level of working standard was run in quadruplicate. Into glass test tubes containing 2 glass beads, 1 ml of each level of standard was added. Then 5 ml Pyridoxine Y media was added to the tubes followed by 4 ml of distilled, deionized water. The final setup was 6 groups of 4 tubes, each containing 1 ml of the appropriate working standard, 5 ml Pyridoxine Y media, and 4 ml distilled, deionized water for a total of 10 ml and standard curves of 0.5, 1, 2, 3, 4, and 5ng/tube of pyridoxine (as pyridoxine hydrochloride).

Plastic caps were placed on the tubes and then the tubes were steamed for 10 min at 100°C. After cooling the tubes to room temperature in an ice water bath, each tube was innoculated with 1 drop of the saline-washed innoculum using aseptic techniques. The tubes were incubated in a 28°C shaking water bath for 22 h. Following the incubation period, the tubes were steamed for 5 min at 100°C to stop growth of the organism, cooled to room temperature in an ice water bath, and read on the spectrophotometer.

Assay of plasma samples

The sample extracts were allowed to thaw overnight in the refrigerator. All samples were run in quadruplicate and at 2 levels. Into glass test tubes containing 2 glass beads, 1 or 2 ml cold sample extracts were added, followed by 5 ml Pyridoxine Y media and 4 or 3 ml of distilled, deionized water respectively. The final setup was 8 tubes for each sample; 4 tubes had 1 ml sample extract, 5 ml media, and 4 ml distilled, deionized water and 4 tubes had 2 ml sample extract, 5 ml media, and 3 ml distilled, deionized water for a total of 10 ml/tube. The tubes were then covered with plastic caps and steamed for 10 min at 100°C. After cooling the tubes to room temperature in an ice water bath, each tube was innoculated with 1 drop of the saline-washed innoculum using aseptic techniques. The tubes were then incubated in a 28°C shaking water bath for 22 h. Following incubation, the tubes were steamed for 5 min at 100°C to stop growth of the organism, cooled to room temperature in an ice water bath, and read on the spectrophotometer.

Blank samples were also run with the standards and samples in quadruplicate. The final setup was 8 tubes with 2 glass beads each, to which 1 ml distilled, deionized water was added followed by 9 ml of media for a total of 10 ml/tube. The tubes were then capped, steamed, and cooled in the same manner as the samples and standards and 4 of the tubes were innoculated with the saline-washed innoculum resulting in 4 innoculated blanks and 4 uninnoculated blanks. The blanks were incubated, steamed and read on the spectrophotometer in the same manner as the samples and standards. The uninnoculated blanks were used to zero the spectrophotometer while the value of the innoculated blanks was subtracted from the value of each standard and sample.

Calculation of vitamin concentration and % recoveries of spiked samples

The samples and standards were read at 550 nm on a Bausch and Lomb spectrophotometer 20 and the absorbances were recorded. The pyridoxine hydrochloride standards were plotted with

vitamin concentration in ng/tube on the X-axis and absorbance on the Y-axis. A typical standard curve is illustrated in Figure 1.

The samples were calculated from the standard curve, taking dilution factors into account. Percent recoveries of pyridoxine hydrochloride in spiked plasma samples were calculated as follows:

$$\frac{\text{(total [vitamin B-6] in plasma + pyridoxine hydrochloride spike)}}{\text{(total [vitamin B-6] in plasma)} + \text{(known [pyridoxine hydrochloride spike])}} \times 100 = \text{\% recovery of vitamer.}$$

Recoveries of 40-74% were obtained from plasma samples spiked with 20 ng pyridoxine (as pyridoxine hydrochloride). Normally, recoveries such as these would not be acceptable. However, there was an accident in the laboratory with the freezer and all of the plasma samples from the subjects were thawed. In order to salvage the plasma samples, the microbiological assay had to be performed immediately, even though more work on perfecting the method would have been preferable.

Note: This assay must be performed in the dark in order to prevent destruction of vitamin B-6 present in the plasma and in the standards. When transporting standards and samples to the autoclave, care should be taken to cover the tubes with a towel to avoid exposure to light. At the point when the tubes are to be read on the spectrophotometer, lights can be used again.

Also, standards and samples do not have to read the same day they come out of the water bath. Following the steaming, the tubes can be refrigerated overnight and read the next day after they have been brought to room temperature in a warm water bath. Lastly, thawed plasma sample extracts can remain in the refrigerator for up to 3 days prior to performing the assay.

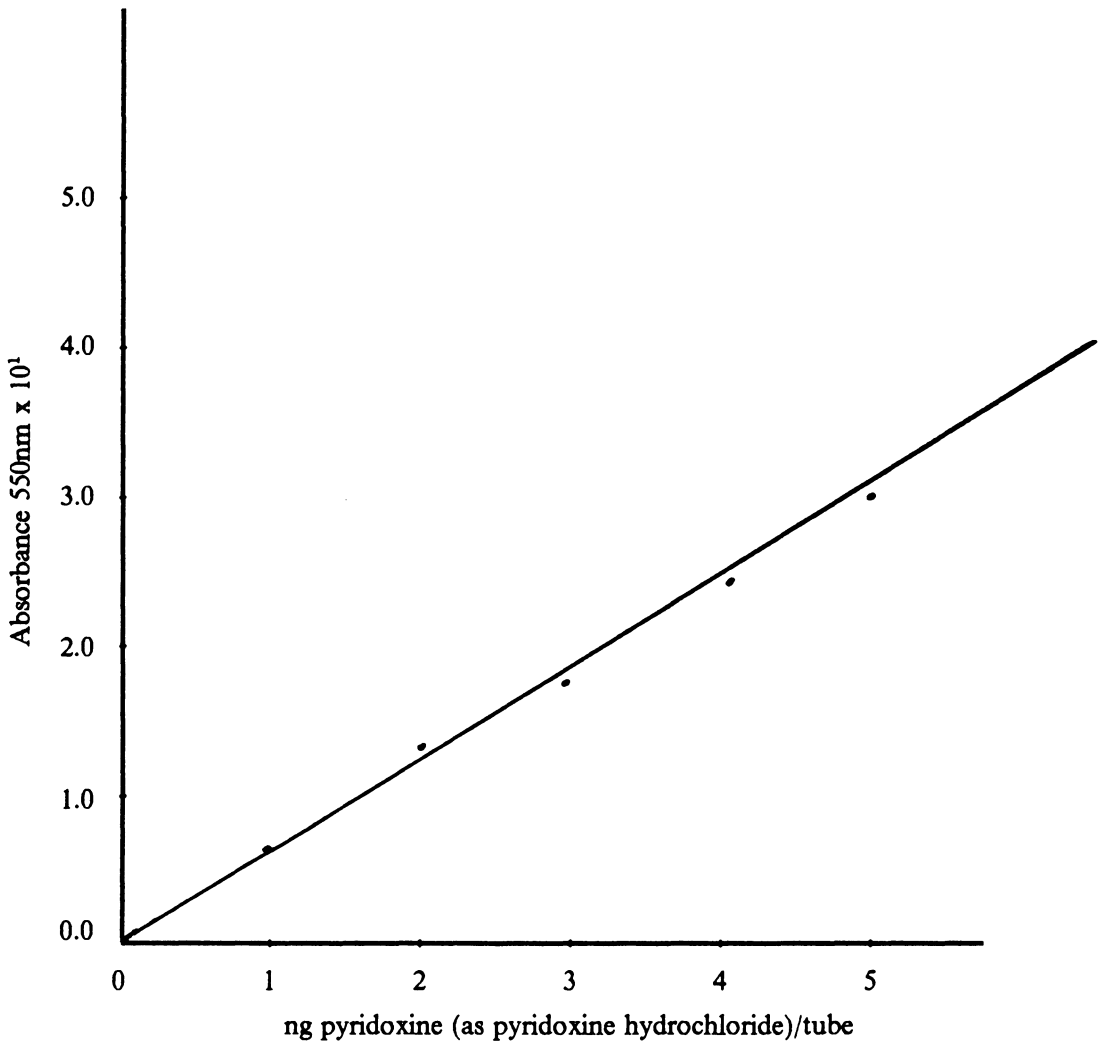

Figure 1. Typical microbiological standard curve for pyridoxine hydrochloride concentrations

Statistical Methods

Means ($\overline{X}$) and standard deviations (SD) were calculated for all parameters. Analysis of variance was used for statistical comparison of all variables between W and NW at 22 and 30 wk.

Paired t tests were used for comparisons within group of data at 22 and 30 wk. The t test was used to compare changes (Δ) in the dependent variables in the W group versus the changes in the NW group (58).

A repeated measures analysis was used to determine significant differences between HR values at rest, 2, 4, and 6 min, as well as VO_2 values at 2, 4, and 6 min. A Pearson r correlation coefficient was used to determine if any relationships existed between the various parameters (59).

Correlations and differences were considered significant at the 0.05 level or lower.

RESULTS AND DISCUSSION

Subject Description

Twenty-eight healthy, Caucasian, pregnant women ranging in age from 21 to 36 yr participated in the study. Individual data for selected variables are listed in Appendices L-N.

The majority of the women were from the Blacksburg area and had similar economic, educational, and occupational backgrounds. All participants except one had completed at least 2 yr of college and most had received 4 yr degrees. Occupations included secretaries, homemakers, and students. All of the subjects took vitamin-mineral supplements. Had the study been conducted in a large metropolitan area, a more diverse pregnant population may have been obtained. It may have also been possible to find women who abstained from taking vitamin-mineral supplements; unfortunately, such a group was not found for this study.

The values for age, height, and weight of the subjects are given in Table 1. The height/weight values for all subjects approached the recommended height/weight values for medium-frame females aged 25 yr or older that was published by the Metropolitan Insurance Company in 1983 (60). There were no significant differences between the W and NW groups for age, height, prepregnancy

Table 1. Age, height, and weight values for walkers and nonwalkers

Group	Age	Height	Weight			Δ
			Prepregnancy	22 wk	30 wk	
	yr	*cm*		*kg*		
Walking (n = 18)	28.4 ± 3.2	167.3 ± 6.7	59.4 ± 8.4	66.7[a] ± 7.7	70.9[a] ± 8.4	4.19 ± 1.48
Nonwalking (n = 10)	27.3 ± 3.4	166.5 ± 5.4	62.8 ± 10.6	68.4[b] ± 9.9	72.3[b] ± 9.9	3.83 ± 1.46

Values represent $\bar{X} \pm SD$.

[a,b] Values in the same line with like superscripts are significantly different ($p < 0.0001$).

weight, and weights at 22 and 30 wk gestation. There was a significant increase ($p < 0.0001$) in body weight from 22 to 30 wk for subjects in both groups as was expected.

Exercise Testing

HR measurements

The HR responses at rest and at 2, 4, and 6 min of the submaximal treadmill walking exercise tests are listed for both W and NW groups in Table 2. All subjects apparently had normal physiological HR responses as both groups showed gradual increases ($p < 0.05$) in HR with elevated intensities of exercise.

The values for resting HR at 22 wk gestation were similar to the $\overline{X}$ HR values of 85 and 81 bpm reported by Collings et al. (5) and Knuttgen and Emerson (15), respectively. While the differences in resting HR values between the W and NW group at 22 wk were not significant, the differences approached significance ($p < 0.07$) at 30 wk gestation. The increase in $\overline{X}$ resting HR for the W group was 4.12 bpm and 7.80 bpm for the NW group, neither of which was significant. Increases in resting HR with advancing gestational age is a common finding and Erkkola (7) and Clapp (61) also reported similar increases in resting HR as pregnancy progresses.

One of the physiological changes that occurs with increased fitness levels is a lower resting HR (1). Several investigators have suggested that pregnant women participating in a regular exercise program can improve their level of fitness (5,7,8). However, the resting HR of subjects in the W group in this study did not decrease as a result of the walking program. As was just mentioned, resting HR normally increases over the course of pregnancy, so it is possible that subjects in the W group could have improved their level of fitness even though the resting HR at 30 wk gestation

Table 2. Comparison of heart rate measurements at rest and during exercise between walkers and nonwalkers

HR	METS	22 wk		30 wk		Δ	
	(~)	W	NW	W	NW	W	NW
				bpm			
rest	1.0	80.6[a] ± 8.3	85.7[de] ± 7.5	85.3[bch] ± 11.0	93.5[fgh] ± 10.9	4.12 ± 11.9	7.80 ± 13.4
2 min	2.9	118.5[a] ± 9.2	120.7[del] ± 6.9	117.8[bj] ± 11.9	127.0[fjl] ± 7.5	-0.67[m] ± 9.28	7.11[m] ± 7.32
4 min	3.8	125.0[a] ± 10.5	126.8[d] ± 9.5	122.3[ck] ± 12.8	131.8[gk] ± 6.7	-2.67[n] ± 10.83	5.22[n] ± 10.08
6 min	4.6	133.4[a] ± 8.9	134.6[e] ± 10.8	130.6[bci] ± 12.2	139.1[fi] ± 9.2	-2.83[o] ± 10.86	4.44[o] ± 11.98

All values are $\overline{X} \pm SD$.

[1] Conducted at 4.02 km/h with a 2.5% grade increase every 2 min.

[abcdefg] Values in the same column with like superscripts are significantly different at $p < 0.05$.

[hin] Values in the same line with like superscripts approaches significance at $p < 0.07$.

[jklm] Values in the same line with like superscripts are significantly different at $p < 0.05$.

[o] Values in the same line with like superscripts approaches significance at $p < 0.13$.

were not lowered. Also, the increase in $\overline{X}$ resting HR for the W group (4.12 bpm) was smaller than that for the NW group (7.80 bpm).

With regard to HR responses during submaximal exercise, there were no significant differences between the W and NW groups at 22 wk gestation in HR response at 2, 4, or 6 min of the treadmill exercise test. In contrast, at 30 wk gestation the HR for W were significantly lower at 2 ($p < 0.05$) and 4 min ($p < 0.05$) of exercise and approached significance at 6 min ($p < 0.07$). Subjects in the W group had nonsignificant decreases in HR at 2, 4, and 6 min of treadmill exercise after the 8 wk walking program while subjects in the NW group had significant increases in HR at 2 min ($p < 0.05$) and nonsignificant increases at 4 and 6 min of treadmill exercise.

Another of the physiological changes that occur with increased fitness levels is a lower submaximal exercise HR. The lower HR response for the W group at 2, 4, and 6 min of submaximal treadmill exercise at 30 wk gestation suggests a HR training effect as a result of the 8 wk walking program. Blackburn and Calloway (62) reported increases in HR from the 2nd to 3rd trimester in response to submaximal treadmill walking while Ruhling et al. (63) reported that a trained runner maintained her HR throughout pregnancy for a given exercise load. Therefore, it is possible that the W group maintained or slightly improved their HR responses to submaximal exercise as indicated by the small decreases to no change in HR values. Subjects in the NW group probably had a diminished HR response as indicated by increases in HR values. Guzman and Caplan (64) reported that nontrained pregnant women had significant increases in HR during several stages of cycling exercise from 20 to 35 wk gestation. Collings et al. (5) suggested that as a result of increased weight, pregnant women that are not participating in a regular, aerobic exercise program demonstrate significantly decreased capacities for aerobic exercise.

VO_2 measurements

The values for absolute VO_2 (l/min) for both W and NW groups at 22 and 30 wk gestation during the submaximal treadmill exercise tests are in Table 3. As with the HR response, the sub-

Table 3. Comparison of absolute VO_2 measurements during exercise between walkers and nonwalkers

VO_2	METS	22 wk		30 wk		Δ	
	(~)	W	NW	W	NW	W	NW
			l/min				
2 min	2.9	0.70[a] ±0.12	0.64[b] ±0.17	0.67[d] ±0.10	0.72[e] ±0.14	-0.02 ±0.07	0.04 ±0.18
4 min	3.8	0.76[a] ±0.12	0.68[c] ±0.10	0.75[d] ±0.12	0.77[f] ±0.12	-0.01[h] ±0.09	0.06 ±0.13
6 min	4.6	0.82[a] ±0.12	0.76[bcg] ±0.13	0.81[d] ±0.12	0.87[efg] ±0.13	-0.01[i] ±0.12	0.08[i] ±0.12

All values are $\overline{X} \pm SD$.

[1] Conducted at 4.02 km/h with a 2.5% grade increase every 2 min.

[abcdef] Values in the same column with like superscripts are significantly different at $p < 0.05$.

[ghi] Values in the same line with like superscripts approaches significance at $p < 0.10$.

jects apparently had normal physiological responses to exercise as indicated by the increased VO_2 values with elevated intensities of exercise.

There were no significant differences in VO_2 between the W and NW groups at both 22 and 30 wk gestation. There were no significant changes in VO_2 for W subjects, while changes in VO_2 for NW subjects were nonsignificant at 2 and 4 min, but approached significance at 6 min ($p < 0.10$).

In order to control for weight gain, relative VO_2 values (ml/kg/min) were also calculated at 2, 4, and 6 min of submaximal exercise (Table 4). There was a significant difference ($p < 0.05$) in relative VO_2 between the W and NW groups at 22 wk gestation at 4 min exercise but values for both groups were similar at 30 wk. Relative VO_2 decreased significantly from 22 to 30 wk for W at 2 ($p < 0.001$), 4 ($p < 0.01$), and 6 min ($p < 0.05$) of exercise but there were no significant changes from 22 to 30 wk for NW.

With training, oxygen consumption during steady state, submaximal exercise stays the same or decreases slightly (1). The significantly decreased relative VO_2 values for W at 2, 4, and 6 min of exercise supports the possibility that subjects in the W group had a slight training effect as a result of the 8 wk walking program. Blackburn and Calloway (62) reported an increase from 18.3 ($\overline{X}$) to 19.2 ml/kg/min from the 2nd to 3rd trimester in pregnant subjects during treadmill walking at 4.83 km/h and 10% grade. In contrast, the subjects in this study in the W group had a significant decrease ($p < 0.05$) in relative VO_2 from 12.4 ($\overline{X}$) to 11.5 ml/kg/min from 22 to 30 wk gestation during treadmill walking at 4.02 km/h and 5% grade (6 min). Therefore, it seems possible that a regular, aerobic exercise program during pregnancy can result in an improved aerobic capacity. This finding is in agreement with Collings et al. (5) who also reported an improved aerobic capacity in a group of pregnant women, studied during their 2nd and 3rd trimester, who participated in an aerobic exercise program (cycling) for from 7 to 19 wk. The authors also reported, as previously mentioned, a decline in aerobic capacity in the controls who did not engage in any regular aerobic exercise. However, the subjects in the NW group in this study had no change in relative VO_2 and an increase in HR at only 2 min exercise from 22 to 30 wk gestation. It appears, therefore, that subjects in the NW group maintained their aerobic capacity over the 8 wk walking program.

Table 4. Comparison of relative VO_2 during exercise between walkers and nonwalkers

VO_2	METS	22 wk		30 wk		Δ	
	(~)	W	NW	W	NW	W	NW
			ml/kg/min				
2 min	2.9	10.4^{af} ± 1.2	9.4 ± 1.7	9.5^{bf} ± 1.2	9.8^{d} ± 1.6	-0.89 ± 0.95	-0.01 ± 2.5
4 min	3.8	11.4^{agk} ± 1.5	10.1^{ck} ± 1.3	10.5^{bg} ± 0.9	10.5^{e} ± 1.5	-0.87^{j} ± 1.35	0.36^{j} ± 1.92
6 min	4.6	12.4^{ah} ± 1.5	11.3^{c} ± 1.3	11.5^{bh} ± 1.2	11.8^{de} ± 1.2	-0.88^{i} ± 1.72	0.55^{i} ± 1.64

All values are $\overline{X} \pm SD$.

[1] Conducted at 4.02 km/h with a 2.5% grade increase every 2 min.

[abcde] Values in the same column with like superscripts are significantly different at $p < 0.05$.

[f] Values in the same line with like superscripts are significantly different at $p < 0.001$.

[g] Values in the same line with like superscripts are significantly different at $p < 0.01$.

[hik] Values in the same line with like superscripts are significantly different at $p < 0.05$.

[j] Values in the same line with like superscripts approaches significance at $p < 0.07$.

Physical Activity Assessment

Total activity scores

The subjects in both the W and NW groups were asked to respond to a questionnaire regarding their total amount of physical activity including both occupational and recreational activities. The questionnaire covered the time span of 1 yr in order to control for seasonal differences. The values for total activity scores are in Table 5. Activities that contributed to the total activity scores included running, swimming, dancing, bicycling, stretching, calisthenics, and farming. There were no significant differences between W and NW in total activity, possibly due to the large SD. The W group did, however, have higher activity scores. Both W and NW groups showed a nonsignificant decrease in total activity from 22 to 30 wk gestation. For the majority of the subjects, housework was a major component of the total activity score and this activity apparently remained constant from 22 to 30 wk.

In a study that looked at the activity patterns of pregnant women from 20 to 40 wk gestation, Blackburn and Calloway (65) reported similar results. There was a nonsignificant decrease in housework, walking, and cooking by their subjects. Occupations of the subjects were similar to those of women in the present study and included mostly students, secretaries, and homemakers.

Approximately 20 of the 28 subjects in the present study reported that their involvement and pace in various activities began to decrease after 30 wk gestation. Dale et al. (26) and Jarrett and Spellacy (19) both reported significant decreases in the number of miles run by pregnant women from the 2nd trimester until term. Therefore, it is possible in the present study that from 30 wk gestation to birth there may have been a decrease in total activity even though there was no significant decrease from 22 to 30 wk gestation.

Table 5. Total activity scores for walkers and nonwalkers at 22 and 30 wk gestation

Group	22 wk	30 wk	Δ
Walking	38.0 ± 21.6	36.9 ± 20.4	-1.09 ± 5.05
Nonwalking	28.8 ± 15.3	28.1 ± 14.6	-0.71 ± 4.46

Values represent $\overline{X} \pm SD$.

Aerobic activity scores

The values for the total aerobic activity scores are in Table 6.While the W group had higher scores at both 22 and 30 wk, there were no significant differences between the 2 groups, again possibly due to the large SD. The walking program employed in this study (3 times/wk, for 8 wk, 30 min/session, 5.6 km/h) was designed to provide the minimal intensity, frequency, and duration required to illicit a training effect and at the same time avoid potential obstetrical risks and complications. Therefore, the walking program contributed very slightly to the total aerobic activity score. A typical woman participating in the walking program would have an activity score increase of 1.15 (51). With such a small increase, other aerobic activities could possibly mask the walking activity scores of the subjects. Therefore, a participant in the present study could actually have experienced a decrease in the aerobic activity score, even with the walking program, if other aerobic activities were decreased. The aerobic activity score would possibly have been more useful in this study if the training program had been more rigorous thereby resulting in larger individual scores.

Dietary Assessment

The 24 h recalls and 2 day dietary records at both 22 and 30 wk were compared using paired t tests to determine if vitamin B-6, protein, and energy intakes were significantly different. Since there were no significant differences between the values obtained using the 24 h recalls and the 2 day dietary records, both were combined and 3 day averages for vitamin B-6, protein, and energy intakes were used in further statistical analysis. The intakes of vitamin B-6 (minus the 10 mg supplement), protein, and energy for both groups at 22 and 30 wk are reported in Table 7. There were no significant differences between theW and NW groups for vitamin B-6, protein, and energy intakes at 22 wk. At 30 wk gestation, vitamin B-6 and protein intakes were similar; however, energy

Table 6. Total aerobic activity scores for walkers and nonwalkers at 22 and 30 wk gestation

Group	22 wk	30 wk	Δ
Walking	11.4[a] ± 8.4	11.6[b] ± 8.1	0.23[c] ± 1.49
Nonwalking	6.9[a] ± 9.1	6.2[b] ± 7.2	-0.76[c] ± 2.33

Values represent $\overline{X} \pm SD$.

[a] Values in the same column with like superscripts approaches significance ($p < 0.20$).

[b] Values in the same column with like superscripts approaches significance ($p < 0.09$).

[c] Values in the same column with like superscripts approaches significance ($p < 0.18$).

Table 7. Dietary intakes of vitamin B-6, protein, and energy for walkers and nonwalkers at 22 and 30 wk gestation

Group	22 wk			30 wk			Δ		
	vitamin B-6	protein	energy	vitamin B-6	protein	energy	vitamin B-6	protein	energy
	mg	*g*	*kcal*	*mg*	*g*	*kcal*	*mg*	*g*	*kcal*
Walking	1.81 ±0.52	103.0 ±23.2	2274 ±404	1.81 ±0.58	94.1 ±22.3	2328[a] ±425	-0.004 ±0.75	8.9 ±27.7	54 ±476
Nonwalking	2.19 ±0.61	100.9 ±17.3	2252 ±522	1.85 ±0.23	99.9 ±20.0	2025[a] ±296	0.35 ±0.62	-2.9 ±23.4	-112 ±403

All values are $\overline{X} \pm SD$.

[a] Values in the same column with like superscripts are significantly different ($p < 0.05$).

intakes for the W group were significantly higher ($p < 0.05$) than that of the NW group, possibly due to the increased activity. Lastly, there were no significant changes from 22 to 30 wk in vitamin B-6, protein, and energy intakes for W and NW.

The current RDA for pregnant women 23-50 yr for vitamin B-6 is 2.6 mg (13). The $\overline{X}$ dietary vitamin B-6 intake of the W group was 70% of the RDA at 22 and 30 wk, while that of the NW group was 84% of the RDA at 22 wk and 71% of the RDA at 30 wk. However, all W and NW did take a nutrient supplement. Vitamin B-6 intakes in pregnant populations that are below the RDA are reported to be common. Reynolds et al. (66) reported $\overline{X}$ daily dietary intakes of 1.14 mg for women at 37 wk gestation as calculated by analysis of 3 consecutive days of food composites. Reopke and Kirskey (37), using 3 day dietary records, reported $\overline{X}$ daily intakes of 1.24 mg for women 5 to 7 mo pregnant, while Schuster et al. (40) reported $\overline{X}$ intakes of 1.43 mg for women at 30 wk gestation as estimated by 24 h recalls. Finally, Schuster et al.(35) in another study, reported $\overline{X}$ dietary intakes of vitamin B-6 of 1.37 mg in a group of women at various stages of gestation. The range of reported intakes could be due to a variety of reasons such as the use of different data bases and the completeness of the data bases used for estimating dietary intakes, accuracy of record keeping by the subjects, and differences in time of sampling (66). Also, the studies done by Reopke and Kirskey (37) and Schuster et al. (35,40) had some or all of their subjects coming from the low socioeconomic sector of the population. This could possibly explain why the vitamin B-6 intakes of subjects in the current study were somewhat higher than the intakes reported by these investigators.

Because of numerous reports in the literature of suboptimal vitamin B-6 intakes by pregnant women, a number of investigators have expressed concern, especially since this vitamin is not routinely prescribed in prenatal supplements. The women in the present study received a 10 mg supplement of vitamin B-6 which boosted their intakes to over 3 times the RDA.

The current RDA for protein during pregnancy is 74 g (13). Subjects in the W group had $\overline{X}$ protein intakes of 103 and 94.1 g at 22 and 30 wk respectively, while subjects in the NW group had protein intakes of 100.9 and 99.9 g at 22 and 30 wk respectively. Individual protein intakes that were below the RDA included 2 W subjects at 22 wk (63.7 and 68.5 g) and 2 W subjects at 30 wk

gestation (66.0 and 60.0 g). The $\overline{X}$ ranged from 127-139% of the RDA. Other investigators have had similar findings. Schuster et al. (40) reported $\overline{X}$ daily protein intakes of 81.7 g in a group of pregnant women based on 24 h recalls while Bailey et al. (36) reported $\overline{X}$ protein intakes of 90 g for WIC participants and 105 g for nonparticipants in WIC, also based on 24 h recalls.

There was a significant correlation between protein and vitamin B-6 intakes for W at 22 wk ($r = 0.77$, $p < 0.01$) and at 30 wk ($r = 0.80$, $p < 0.01$) but this same relationship was not seen with the NW group, possibly due to the small n. However, when values for W and NW were grouped together at 22 wk, there was a significant relationship between protein and vitamin B-6 intakes ($r = 0.59$, $p < 0.01$). The same was true when the values for the groups were combined at 30 wk ($r = 0.70$, $p < 0.01$). This relationship is not surprising since vitamin B-6 is commonly found bound to proteins in food (67). Sutker et al. (56) also reported a significant correlation between the intakes of these 2 nutrients in a group of adolescent females.

Finally, the recommended energy intake for pregnant women 23-50 yr is 2300 kcal with an acceptable range of 1900 to 2700 kcal (13). The $\overline{X}$ energy intakes of both W and NW at 22 and 30 wk approached or slightly exceeded the recommended intake. Individual intakes that were below the acceptable range included 3 W subjects at 22 wk (1522, 1805, 1720 kcal); 2 NW subjects at 22 wk (1790, 1547 kcal), 5 W subjects at 30 wk (1858, 1869, 1712, 1878, 1881 kcal); and 4 NW subjects at 30 wk (1714, 1689, 1772, 1873 kcal). Finley et al. (68) reported $\overline{X}$ daily energy intakes of 2200 kcal in a group of pregnant women based on 24 h recalls and 2 day dietary records. Similarly, Schuster et al. (40) reported $\overline{X}$ energy intakes of 2152 kcal for pregnant subjects as determined from 24 h recalls.

Biochemical Assessment

Plasma total vitamin B-6 measurements

The values for plasma total vitamin B-6 for W and NW are in Table 8. There were no significant differences between the values for the 2groups for plasma total vitamin B-6 at 22 or 30 wk gestation. Also, there were no significant differences between W at 22 wk and at 30 wk and likewise for NW. There are no reports in the literature as to what constitutes normal vitamin B-6 status in a pregnant population when determined microbiologically using S. uvarum. Reopke and Kirskey (37) reported $\overline{X}$ total vitamin B-6 values of 16.3 ng/ml (97.0 nM) and 12.5 ng/ml (74.4 nM) for women 20-24 and 28-32 wk gestation, respectively. In another study by Reopke and Kirskey (44), the authors compared the values for plasma total vitamin B-6 for the women in the above mentioned study who previously used OCA to those of nonusers. For users of OCA, $\overline{X}$ values of 12.3 ng/ml (73.2 nM) and 10.7 ng/ml (63.7 nM) at 20-24 and 28-32 wk gestation respectively, were reported. For nonusers, the investigators reported $\overline{X}$ values of 16.8 ng/ml (100.0 nM) and 12.2 ng/ml (72.6 nM) at 20-24 and 28-32 wk gestation respectively. Some of the women took vitamin supplements ($\overline{X}$ intake from supplements was approximately 5.0 mg) while others did not. This could explain why their values are somewhat lower than the values in the present study. Brin (43) determined total plasma vitamin B-6 in a group of pregnant subjects and reported a $\overline{X}$ of 11.8 ng/ml (70.2nM). There was no mention of vitamin supplementation or stage of gestation in this study, making comparisons a little difficult.

Sauberlich et al. (69), in a review article on assessment of vitamin B-6 status in humans, tentatively suggested that plasma total B-6 vitamer concentrations that are $\geq$ 50 ng/ml ($\geq$ 297.5 nM) be classified as acceptable status and $<$ 25 ng/ml ($<$ 148.8 nM) as marginal or inadequate status. Willet (70) analyzed plasma from a group of nonpregnant, female adults between the ages of 33 and 75 yr for total vitamin B-6 content. He reported a median of 94.0 nM and a 10% -ile value of 64.8

Table 8. Plasma total vitamin B-6 measurements for walkers and nonwalkers at 22 and 30 wk gestation

Group	22 wk	30 wk	Δ
		nM	
Walking	127.9 ± 30.9	117.8 ± 39.9	11.3 ± 29.8
Nonwalking	135.1 ± 34.5	142.2 ± 46.4	-2.38 ± 25.6

All values represent $\overline{X} \pm SD$.

nM. Willet's findings are more in agreement with values in this and other studies done with pregnant populations. When comparing values from his subjects to those determined in the present study, only 1 W subject at 22 wk and 2 W subjects at 30 wk gestation fell below the 10% -ile value (49.4 and 53.6, 64.3 nM, respectively). Values of all other subjects were either very close to or well above the median. Since there are no definite guidelines for what constitutes adequate and deficient status, discussing data in terms of normal ranges may be more desirable. Although Willet's study was done with a nonpregnant population, the values he reported could possibly serve as a rough guideline as to what constitutes normal and low ranges for plasma total vitamin B-6 values. As just mentioned, almost all of the subjects from the present study would fall into the normal range for plasma total vitamin B-6 if Willet's values were used. Even if his values were not used, the $\overline{X}$ from this study were somewhat higher than the $\overline{X}$ reported by other investigators. This is not surprising in light of the fact that all subjects were taking a relatively large supplement of vitamin B-6 (10mg) that was more than 3 times the RDA. Although a number of investigators (34-37) have reported low vitamin B-6 status in pregnant populations, the subjects in some of these studies were not taking supplements or were taking supplements that were less than the 10 mg given in the present study.

Lumeng et al. (39) suggested that based on a study they had done, a supplement of at least 4, but not greater than 10 mg of pyridoxine hydrochloride was required to maintain plasma PLP levels in the nonpregnant and 1st trimester ranges. Schuster et al. (40) administered various levels of pyridoxine hydrochloride to a group of pregnant women in an attempt to better ascertain vitamin B-6 requirements in pregnancy. The authors reported that a supplement of 7.5 mg was required to maintain plasma PLP levels at term comparable to those found in early (6-21 wk gestation) pregnancy. These authors concluded from their results that a daily vitamin B-6 intake of 5.5 to 7.6 mg (diet plus supplement as pyridoxine equivalents) is necessary for maintaining status during pregnancy.

These 2 studies differ from the present study in that plasma PLP was determined rather than total vitamin B-6, and status was assessed at several stages of gestation and at term. However, one might reasonably conclude that the 10 mg supplement of vitamin B-6 given in this study was

enough to maintain status in the normal range at 22 and 30 wk gestation for a large majority of the subjects (even subjects whose values were just outside the normal range could be possibly classified as low-normal), and that the 8 wk walking program had no effect on vitamin B-6 status, probably due to supplement usage.

Plasma pyridoxal phosphate measurements

The values for plasma PLP for W and NW are given in Table 9. There were no significant differences between the 2 groups for plasma PLP at 22 or 30 wk gestation. There were also no significant differences between W at 22 wk and at 30 wk and likewise for NW. Bailey et al. (36) determined plasma PLP at 30 wk gestation for a group of WIC participants and for a group of non-WIC controls. The investigators reported $\overline{X}$ plasma PLP values of 4.6 ng/ml (18.6 nM) and 3.3 ng/ml (13.4 nM) for WIC participants and controls, respectively. These values are somewhat lower than the values reported here possibly due to the fact that subjects in the study by Bailey et al. (36) took supplements that contained only iron and folacin.

Lumeng et al. (39), taking a different approach, administered pyridoxine hydrochloride supplements of either 2.5, 4, or 10 mg to 3 different groups of pregnant women. For subjects that took the 2.5 mg supplement $\overline{X}$ plasma PLP values were approximately 17.0 ng/ml (68.8nM) and 8.0 ng/ml (32.4 nM) at 19-24 and 31-36 wk gestation, respectively. For subjects that took the 4.0 mg supplement $\overline{X}$ plasma PLP values were approximately 17.0 ng/ml (68.8 nM) and 6.0 ng/ml (24.3 nM) at 19-24 and 31-36 wk gestation, respectively. Finally, for subjects that took the 10 mg supplement $\overline{X}$ plasma PLP values of approximately 28 ng/ml (113.3 nM) and 20 ng/ml (80.9 nM) at the previously mentioned gestational periods were reported. This last set of values compares favorably with values in the present study, where a 10 mg supplement of pyridoxine hydrochloride was also used. Similarly, Schuster et al. (40) also administered several different levels of pyridoxine hydrochloride to groups of pregnant women attending a prenatal clinic for low-income women. For the group that took the 10 mg supplement $\overline{X}$ plasma PLP values were 25.8 nM (6.4 ng/ml)

Table 9. Plasma PLP measurements for walkers and nonwalkers at 22 and 30 wk gestation

Group	22 wk	30 wk	Δ
		nM	
Walking	77.7 ± 20.6	72.4 ± 19.4	8.1 ± 12.5
Nonwalking	78.9 ± 23.1	74.5 ± 19.4	0.81 ± 7.7

All values represent $\overline{X} \pm SD$.

at the initial clinic visit (gestational age ranged from 6-21 wk) and 66 nM (16.3 ng/ml) at 30 wk gestation. These values are somewhat lower than the values of the present study. This could be explained by the fact that the subjects in the present study had been taking the 10 mg supplement for at least a mo prior to the assessment of plasma PLP at 22 wk while women in the study by Schuster et al. (40) had not been taking any vitamin B-6 supplements. Also, the small n (5 at initial clinic visit, 4 at 30 wk gestation) reported by these investigators could possibly account for the lower values.

There are no guidelines for normal plasma PLP values in pregnant populations, but there have been a few suggestions for nonpregnant populations. Rose et al. (71) suggested 2 possible values for a lower limit of the normal range. The values were arrived at as follows: The investigators assessed vitamin B-6 status in 617 males, ages 18 to 90, by coenzyme stimulation of aspartate aminotransferase performed on both plasma and erythrocytes and by the plasma PLP method. Since the former method has tentative guidelines for status classification, and 30% of the subjects were classified as marginal or inadequate, the authors selected the PLP value that would yield an equal percentage of unsupplemented subjects being classified as marginal or inadequate. Thus, the value of 8.5 ng/ml (34.4 nM) for plasma PLP was selected as the lower limit of the normal range, resulting in 30% of the subjects being classified as marginal or inadequate by both methods. The authors also selected 7.5 ng/ml (30.4 nM) as a possible lower limit of the normal range, this value arrived at by arbitrarily assigning 25% of the subjects to the marginal or inadequate category.

If the value of 8.5 ng/ml (34.4 nM) were used as the lower limit of the normal range, only 1 W subject at 22 wk and 1 W subject at 30 wk (same 2 subjects that were classified as low-normal by the microbiological method; no plasma PLP value was available for the 3rd subject that was in the low-normal category by the microbiological method) would have values that fall just below the limit, with plasma PLP levels of 30.8 and 34.0 nM respectively. If the value of 7.5 ng/ml (30.4nM) were used as the lower limit, all of the subjects in the present study had values above this level and would be classified as having normal status.

Shultz and Leklem (72) have also suggested some tentative guidelines that are very close to those suggested by Rose et al. (71). These authors correlated dietary vitamin B-6 intakes or vitamin

B-6/protein ratios (taken from other studies) with selected vitamin B-6 status parameters and extrapolations from these were used to obtain marginal range limits using intakes of 1.25 to 1.50 mg of vitamin B-6/day. For adult females, plasma PLP levels in the range of < 7.8-8.8 ng/ml (< 31.6-35.6 nM) based on the aforementioned vitamin B-6 intakes, would be considered marginal. Plasma PLP levels in the range of < 5.5-6.2 mg/ml (22.3-25.1 nM) based on the vitamin B-6/protein ratios would be considered marginal. Similarly, Driskell and Moak (73) suggested that < 28.3 nM (< 7.0 ng/ml) might be indicative of vitamin B-6 deficiency in adolescent females, while levels between 28.3 and 40.4 nM (7.0-10.0 ng), might indicate marginal status. Like Rose et al. (71), these values are based on a comparison of the percentage of subjects found to have inadequate status by another method (EALAT).

Cleary et al. (30) suggested that 4.7 ng/ml (19.0 nM) (arrived at by taking the $\overline{X}$ minus 2 S.D. after logarithmic transformation of each plasma PLP value of the controls in their study) be assigned as the lower limit of the normal range for nonpregnant females between the ages of 20 and 34 yr. Again, all of the subjects in the present study had values that were above this level and would be classified as having normal status.

Lastly, another way to interpret data from the current study would be to compare it to the data from the previously mentioned study by Willet (70) that was done on nonpregnant females ages 33 to 75 yr. For plasma PLP, Willet reported a median of 49.0, and a 10% -ile value of 21.0 nM. No subjects had values below or even close to the 10% -ile value and values from most were at or above the median.

There was a significant correlation between plasma PLP values and total plasma vitamin B-6 values for W at 22 wk ($r = 0.85$, $p < 0.01$) and 30 wk ($r = 0.95$, $p < 0.0001$) and for NW at 22 wk ($r = 0.90$, $p < 0.01$) and 30 wk ($r = 0.83$, $p < 0.02$). Also, when the data from all the subjects were combined the correlation of the 2 status parameters was significant ($r = 0.87$, $p < 0.0001$). This correlation supports the fact that both methods provided similar information. Willet (70) also noted a significant correlation ($r = 0.57$, $p < 0.05$) between plasma PLP and plasma total vitamin B-6 for the women in his study.

A significant negative correlation was seen between plasma PLP and age ($r = -0.38$, $p < 0.05$) and plasma total vitamin B-6 and age ($r = -0.39$, $p < 0.05$) for all subjects at 22 wk. This same correlation was not seen for all subjects at 30 wk, possibly due to missing data. Baker et al. (74) reported similar findings in a study done with elderly and young populations. The authors noted that depressed plasma vitamin B-6 levels were associated with aging despite an apparently good diet. Lee et al. (75) also reported significantly lower plasma PLP concentrations in older women as compared to younger women when both groups were fed a constant diet providing what the authors referred to as normal amounts of vitamin B-6.

There were no significant correlations between vitamin B-6 status parameters and vitamin B-6 intake. Sutker et al. (56) also reported a nonsignificant correlation between vitamin B-6 intake and plasma PLP and plasma total vitamin B-6. This finding could possibly be due to variations in individual requirements, the incompleteness of food tables with regard to vitamin B-6 content of foods (56), or a low n.

In conclusion, there are no definite guidelines for acceptable and inadequate status for plasma PLP values. When taking the various suggestions in the literature for status classifications into account, and the fact that the 2 status parameters are in agreement, one might reasonably conclude that plasma PLP values for a large majority of the subjects were within the normal range at both 22 and 30 wk gestation. As mentioned in the previous section, subjects whose values were just outside the normal range could possibly be classified as low-normal. Lastly, the 8 wk walking program had no effect on vitamin B-6 status, probably due to the use of the 10 mg vitamin B-6 supplement.

Pregnancy Outcome

A summary of the postpartum parameters for W and NW is in Table 10. Two W subjects and 2 NW subjects had Cesarean deliveries. One of the W subjects had a Cesarean, possibly due

Table 10. Postpartum parameters for walkers and nonwalkers

Group	Birth weight	Birth length	Weight gain	Apgar 1 min	Apgar 5 min	Labor 1st stage	Labor 2nd stage
	(kg)	*(cm)*	*(kg)*			*(min)*	*(min)*
Walking	3.4 ± 0.7	52.2 ± 2.4	14.4 ± 3.1	8.5 ± 0.6	9.2 ± 0.6	496.6 ± 416.8	177.2 ± 425.0
Nonwalking	3.7 ± 0.5	52.5 ± 2.1	13.2 ± 1.7	8.7 ± 1.0	9.2 ± 0.7	269.3 ± 124.7	51.0 ± 36.8

All values represent $\overline{X} \pm SD$.

to twin births, and the other subjects reported failure to progress as the reason for Cesarean deliveries. There were no significant differences between the 2 groups for any of the postpartum parameters. Other investigators have reported similar findings. Pomerance et al. (6) reported no correlation between level of fitness and birth outcome and Dibblee and Graham (21), Jarret and Spellacy (19), and Ruhling et al. (63)also reported no relationship between endurance training during pregnancy and birth outcome. In contrast, Tafari et al. (22) reported significantly lower birth weights for infants born to mothers who performed heavy physical labor during pregnancy as compared to infants whose mothers performed only light work. Naeye and Peters (23) analyzed 7,722 pregnancy outcomes and reported that women who performed stand-up type work in the 3rd trimester had infants who weighed 150 to 400 g less than infants born to mothers who stayed at home. Similarly, Clapp and Dickstein (24) reported an increased incidence of lower birth weight infants ($\sim$500 g) born to mothers who continued a vigorous exercise program during the 3rd trimester. There was no assessment of activity level from 31 to 40 wk gestation in the current study. There is a possibility that the activity level during this period could effect pregnancy outcome. Also, the intensity of the activity reported in the studies just mentioned was greater than the intensity of activity in the present study.

There were no significant correlations between the postpartum parameters and any of the other parameters at 22 or 30 wk. Schuster et al. (35) reported significantly lower Apgar scores at 1 min for infants whose mothers were vitamin B-6 deficient when compared to the Apgar scores of infants whose mothers had adequate status. Similarly, Reopke and Kirskey (37) reported unsatisfactory Apgar scores (< 7) at 1 min for infants whose mothers had significantly lower intakes of vitamin B-6, and lower levels of the vitamin in serum at delivery and in the milk at 3 and 14 days postpartum when compared to mothers of infants with satisfactory scores (≥ 7). In contrast, Bailey et al. (36) found no differences in Apgar scores or birth weights between a group of WIC participants and their non-WIC controls. However, the 2 groups were not significantly different from each other with regard to vitamin B-6 status (plasma PLP). Although 80% of the WIC participants and 86% of the controls had plasma PLP values below the lower limit of the normal

range (defined here as 4.7 ng/ml or 28 nM), the $\overline{X}$ Apgar scores at 1 min were 7.5 and 7.4 for the WIC group and controls respectively, and at 5 min $\overline{X}$ scores were 8.8 and 7.9, respectively.

In the present study, none of the subjects had infants with unsatisfactory (< 7) Apgar scores. Only 1 W and 1 NW subject had infants with Apgar scores of 7 at 1 min, while all other subjects had infants with scores of 8 or better out of a possible total of 10. At 5 min, 1 W and 1 NW subject had infants with Apgar scores of 8 while all other Apgar scores were 9 or 10. Also, the vast majority of subjects in the current study had normal vitamin B-6 status and intakes well above the RDA as a result of the use of a 10 mg supplement.

There are still many unanswered questions regarding the relationship of physical fitness to labor and delivery parameters. In a review article on pregnancy and exercise, Gorski (76) stated that some obstetricians feel that strong abdominal muscles in athletes could possibly be beneficial during the 2nd stage of labor. She also discussed the possibility of increased difficulty during labor as the result of exercise-stiffened pelvic muscles.

In spite of these controversies, there does appear to be a consensus in the literature that participation in a moderate, aerobic exercise program during pregnancy (excluding weight-lifting or activities where violent jostling or compression may harm the fetus) is appropriate and will not compromise the health of the mother or have any effect, detrimental or beneficial, on birth outcome. As was previously mentioned, the 8 wk walking program in the current study was not comparable in intensity to the intensive athletics discussed in some of the other studies, nor was the exercise program continued into the 3rd trimester. However, it appears that participation in the walking program resulted in a slightly improved aerobic capacity for the mothers with no influence on birth outcome. Future studies should consider the following factors: 1) inclusion of both vitamin-mineral supplemented and nonsupplemented groups; 2) greater control of the fitness levels of subjects prior to participation; 3) an aerobic exercise program that is of a longer duration and greater frequency than that of the present study; 4) an assessment of participation in an aerobic exercise program until term; and 5) larger sample size.

SUMMARY AND CONCLUSIONS

The 2 groups of subjects, W and NW, participating in the current study were similar in age, height, prepregnancy weight, and socioeconomic background. Both W and NW subjects had significant weight gains ($p < 0.0001$) after the 8 wk experimental period of 4.19 and 3.83 kg, respectively.

Following the 8 wk walking program (30 wk) HR responses to a submaximal treadmill walking test were significantly or near significantly lower for W compared to NW. The lower HR response was seen at 2 ($p < 0.05$) and 4 min ($p < 0.05$), and was near significance at 6 min ($p < 0.07$) of the exercise test. The HR values for the W group decreased nonsignificantly from 22 to 30 wk. However, for the NW group, HR response increased significantly at 2 min ($p < 0.05$) and nonsignificantly at 4 and 6 min of exercise from 22 to 30 wk. The W group apparently had improved HR responses for several exercise intensities when compared to the HR responses of the NW group.

There was a significant difference ($p < 0.05$) in relative VO_2 between W and NW during 22 wk gestation at 4 min of exercise only while values at 30 wk were similar for both groups. Relative VO_2 values during submaximal treadmill walking decreased significantly for the W group and increased nonsignificantly for the NW group following the 8 wk walking program. The lower VO_2 values for W were seen at 2 ($p < 0.001$), 4 ($p < 0.01$), and 6 min ($p < 0.05$), of exercise. The

VO_2 data and the HR responses seem to support the hypothesis that pregnant women can improve aerobic fitness by participation in a walking program such as the one utilized in the present study. The improvement was small and may have been more substantial had the frequency, duration, and intensity of the walking program been greater.

Total activity and total aerobic activity scores were higher, but not significantly, for the W group. The large SD may have been the reason for the lack of significant differences between the 2 groups.

Dietary intakes of vitamin B-6 and protein at 22 and 30 wk were similar for both the W and NW groups, with most subjects consuming at least two-thirds the RDA for vitamin B-6 and 100% of the RDA for protein. However, all W and NW subjects did take a nutrient supplement that provided 10 mg of vitamin B-6 (more than 3 times the RDA of 2.6 mg) that was not considered with the dietary intakes. Energy intakes for the 2 groups were similar at 22 wk but at 30 wk the NW group had a significantly lower ($p < 0.05$) energy intake than the W group.

There were no significant differences between the values of W and NW for plasma total vitamin B-6 and plasma PLP at 22 or 30 wk gestation. Similarly, there were no significant differences for W or NW from 22 to 30 wk. The values for both parameters were within the normal range for most subjects. Only 1 W subject at 22 wk and 2 W subjects at 30 wk were found to have plasma total vitamin B-6 levels in the low-normal range, while 1 W subject at 22 wk and 1 W subject at 30 wk (same 2 subjects that were just mentioned; no plasma PLP value was available for the 3rd subject) had plasma PLP levels in the low-normal range.

There appeared to be no relationship between birth outcome and any of the other parameters investigated in the study. Birth outcome parameters were similar for both groups indicating that participation in the walking program had neither a beneficial nor detrimental effect on birth outcome.

In conclusion, pregnant women participating in the 8 wk walking program were able to slightly improve their aerobic fitness level without any apparent benefit or risk to themselves or the developing fetus. The majority of the women engaged in the aerobic exercise program and taking the vitamin-mineral supplement were apparently not at risk for clinical vitamin B-6 deficiency.

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Appendix A. Recruitment Notice

PREGNANT WOMEN NEEDED

PREGNANCY, NUTRITION, EXERCISE

A STUDY IS BEING CONDUCTED BY THE DEPARTMENT OF HUMAN NUTRITION AND FOODS AT VIRGINIA TECH.

VALUABLE INFORMATION ON PREGNANCY, NUTRITION AND EXERCISE WILL BE OBTAINED FROM THIS STUDY.

ANY PREGNANT WOMAN, 10-22 WEEKS PREGNANT, WHO IS INTERESTED, PLEASE CALL DURING THE DAY RICK LEWIS OR CHARLENE YATES AT

OR

EVENINGS
RICK
CHARLENE

Appendix B. Consent for Participation

Subject Code Number _____

CONSENT FOR PARTICIPATION
in
Nutrition, Pregnancy, Exercise Study
Virginia Polytechnic Institute and State University

I have received a verbal explanation of the study and have had an opportunity to ask questions regarding the procedures. I understand the following:

Purpose:

The purpose of this study is to provide information on the effects of exercise training and B-vitamin supplementation during pregnancy on cardiovascular fitness, riboflavin, thiamin, and vitamin B-6 status and on birth outcome.

I am a healthy, white, _____ pregnant woman, aged 19-29, in my second trimester (about 22 weeks). Prior to pregnancy, my weight was within 20% of my ideal weight. I do not have any illness, disease, physical limitation, or previous history of obstetrical complications. Descriptions of physical activity, 3 day dietary intake, and general information will be provided. I will participate in one of the following groups which include exercise, B-vitamin supplemented; exercise, non-supplemented; sedentary, B-vitamin supplemented; and sedentary, non-supplemented. Participation in the non-supplemented group will be allowed only if I voluntarily abstain from B-vitamin supplementation. If possible, the type (brand) of vitamin supplement will be controlled for those participating in the vitamin B-supplemented groups.

Submaximal exercise tests will be administered two times: one initial baseline test during the second trimester and a second test after the exercise program and during the third trimester. The tests will be conducted on a treadmill apparatus in the Human Performance Laboratory. The walking intensity will be determined via a pilot study conducted prior to the exercise testing. These are not maximal exercise tests and the intensity during the tests will not exceed 70% of maximal oxygen consumption ($\dot{V}O_2$ max). Heart rate, oxygen consumption ($\dot{V}O_2$), and blood pressure will be monitored during each exercise test. A physician will be on call and available during the entire exercise testing period. I will have the opportunity to report to the performance laboratory before the exercise test to familiarize myself with the equipment.

Participation in the exercise groups will include 8 weeks of physical training involving walking for 30 minutes a day, 3 days a week. The intensity of the walking will approximate 60-70% $\dot{V}O_2$ max or less. The walking will take place at a convenient location in the area, will be supervised, and blood pulse rates recorded in order to assure my safety and to assure a proper training intensity. Before the exercise program begins, I will be instructed on how to quickly approximate my heart rate. I will be encouraged to discuss any discomfort or feelings regarding the exercise with the investigators.

A qualified technician will obtain blood samples (20 ml) prior to the two exercise testing sessions. The investigators will use the blood to assess my thiamin, riboflavin, and vitamin B-6 status.

My physician or I will provide information regarding labor, delivery, and birth outcome. The parameters will include duration of pregnancy, weight gain during pregnancy, duration of first and second stage of labor, infant length, infant birthweight, and Apgar scores.

All information obtained in the study will be held strictly confidential and will be used for statistical purposes only.

No compensation will be offered if injury is incurred as a result of my participation in this project. The probability of injury is very low. I will be expected to advise the researchers of any medical problems that might arise in the course of the study and I am free at any time to withdraw consent and discontinue participation in the project. A physician will be on call and available if necessary during the entire testing period.

Any inquiries I may have concerning the procedures utilized in this study will be answered at any time.

I understand the above and agree to participate in this study.

_____ (Date) _____ (Name)

_____ (Date) _____ (Physician)

Principle Investigators: Dr. Judy Driskell
(961-5939)
Richard Lewis
(961-5375)

Chairman, Institutional Review Board for Research Involving Human Subjects: Dr. Charles Waring (961-5283).

Appendix C. Pre-Experimental Questionnaire

PRE-EXPERIMENTAL QUESTIONNAIRE

NAME_____ CODE NO._____

ADDRESS_____ DATE_____

PHONE NUMBER (HOME/OFFICE)_____

PHYSICIAN'S NAME (OR CLINIC)_____

HEIGHT_____ WEIGHT_____ PRE-PREGNANCY WEIGHT_____

WEEKS GESTATION_____ *EDC_____ AGE_____ RACE_____

NUMBER OF PREVIOUS PREGNANCIES_____

1. Have you had any history of complicated pregnancy or delivery? Yes_____ No_____
   Not applicable_____
   If yes, please describe_____
   _____

2. Do you or have you had any illness or disease? Yes_____ No_____
   If yes, please describe_____
   _____

3. Do you take vitamin or mineral supplements? Yes_____ No_____
   If yes, please specify what brands and for how long_____
   _____

4. Do you take any other nutritional supplements? Yes_____ No_____
   If yes, please specify what type of supplement, the brand name and for how long
   _____
   _____

5. Did you take vitamin, mineral or other nutritional supplements before you became pregnant? Yes_____ No_____
   If yes, please specify_____
   _____

6. Are you on a special diet? Yes_____ No_____
   If yes, please specify what kind_____
   _____

7. Did you take oral contraceptives? Yes_____ No_____
   If yes, please specify what kind and for how long. Also include the dates during which the contraceptives were used (i.e. for 1 year 8/82-8/83)
   _____
   _____

8. Do you take any kind of medication? Yes_____ No_____
   If yes, please specify the medication and for how long_____
   _____

*Expected date of confinement.

9. How physically active are you? (**See activity level guide)
sedentary_____light_____moderate_____very active_____exceptionally active_____

10. Do you attend any child birth or prenatal exercise classes? Yes_____No_____
If yes, please specify what kind of class and for how long_____
_____

11. Do you plan to be out of town soon? Yes_____No_____
If yes, please specify when and for how long_____
_____

12. What approximately is your family income per year?

_____under 5,000
_____5,000-8,000
_____8,000-10,000
_____10,000-15,000
_____15,000-20,000
_____over 20,000

13. How many individuals are in your family? _____

14. What is your occupation?
_____

15. What is your education level?

_____12 or less
_____4 years college
_____1-2 years college
_____graduate school

**Activity Level Guide

Sedentary - Virtually no activity during the day (sitting at a desk all day). Little or no activity upon returning home (reading, watching TV). No exercise program.

Light - Office workers with some movement around office, most professional persons (doctors, lawyers, teachers, secretaries). Some form of mild exercise 1-2 times per week (tennis, jogging 1/2 mile, golf).

Moderate - Light industry workers, active students, building workers (excluding heavy laborers), homemakers, light farm workers. Some form of mild exercise 3 times per week (jogging one mile, hard tennis, swimming, cycling 5-10 miles).

Very Active - Heavy farm worker, heavy manual laborers, mine workers, steel workers. Strenuous exercise program 4-5 times per week (jogging 5 miles, swimming a mile, hard cycling 15-20 miles).

Exceptionally Active - Lumberjacks, blacksmiths, very strenuous exercise program 6-7 times per week (marathon running, swimming long distances, hard long distance cycling 50+ miles).

Appendix D. Multivitamin and Multimineral Supplement

100 TABLETS *NDC* 0087-0702-01

MULTIVITAMIN AND MULTIMINERAL SUPPLEMENT

WITH 1 MG. FOLIC ACID AND 60 MG. IRON

CAUTION: FEDERAL LAW PROHIBITS DISPENSING WITHOUT PRESCRIPTION

DISPENSE IN A TIGHT CONTAINER AS DEFINED IN THE USP.

Johnson

Description: Nutralins Rx tablets provide twelve vitamins and six minerals to supplement the diet during pregnancy or lactation.

Each Nutralins Rx tablet supplies:		% U.S. RDA Pregnant or Lactating Women
Vitamins		
Vitamin A, IU	8,000	100
Vitamin D, IU	400	100
Vitamin E, IU	30	100
Vitamin C (Ascorbic acid), mg	90	150
Folic acid (Folacin), mg	1.0	125
Thiamine (Vitamin B_1), mg	2.55	150
Riboflavin (Vitamin B_2), mg	3.0	150
Niacin, mg	20	100
Vitamin B_6, mg	10.0	400
Vitamin B_{12}, mcg	8	100
Biotin, mg	0.05	16
Pantothenic acid, mg	15.0	150
Minerals		
Calcium, mg	200	15
Iodine, mcg	150	100
Iron, mg	60	333
Magnesium, mg	100	22
Copper, mg	2.0	100
Zinc, mg	15.0	100

Ingredients: Vitamin A acetate, ergocalciferol, dl alpha tocopheryl acetate, sodium ascorbate, folic acid, thiamine mononitrate, riboflavin, niacinamide, pyridoxine hydrochloride, cyanocobalamin, biotin, calcium pantothenate, calcium carbonate, calcium iodate, ferrous fumarate, magnesium hydroxide, cupric oxide and zinc oxide.

Indications and Usage: Nutralins Rx tablets help assure an adequate intake of the vitamins and minerals listed above. Folic acid helps prevent the development of megaloblastic anemia during pregnancy.

Contraindications: Supplemental vitamins and minerals should not be prescribed for patients with hemochromatosis or Wilson's disease.

Mead Johnson

PHARMACEUTICAL DIVISION
Mead Johnson & Company
Evansville, Indiana 47721 U.S.A.

P 7222 11

Appendix E. Modification of Balke-Ware Maximal Exercise Test

Min	Speed (km/h)	Grade (%)	~METS
0	4.02	0	2.9
1			
2		2.5	3.8
3			
4		5.0	4.6
5			
6		7.5	5.5
7			
8		10.0	6.3
9			
10		12.5	7.2
11			
12		15.0	8.1
13			
14		17.5	8.9
15			

Appendix F. Activity Questionnaire

PREGNANCY, NUTRITION,
EXERCISE STUDY

CODE NO._____
DATE_____
ACTIVITY SCORE_____

EXERCISE ACTIVITY LEVELS

1.	Dancing (includes aerobic dancing)	Frequency_____ # of months_____ # of days/week_____	minutes_____ intensity_____ activity factor 6.4
2.	Bicycling	Frequency_____ # of months_____ # of days/week_____	minutes_____ intensity_____ activity factor 5.0
3.	Swimming	Frequency_____ # of months_____ # of days/week_____	minutes_____ intensity_____ activity factor 4.0
4.	Gymnastics	Frequency_____ # of months_____ # of days/week_____	minutes_____ intensity_____ activity factor 10.5
5.	Stretches	Frequency_____ # of months_____ # of days/week_____	minutes_____ intensity_____ activity factor 4.5
6.	Golfing	Frequency_____ # of months_____ # of days/week_____	minutes_____ intensity_____ activity factor 5.0
7.	Baseball or Softball	Frequency_____ # of months_____ # of days/week_____	minutes_____ intensity_____ activity factor 3.0
8.	Basketball	Frequency_____ # of months_____ # of days/week_____	minutes_____ intensity_____ activity factor 8.0
9.	Waterskiing	Frequency_____ # of months_____ # of days/week_____	minutes_____ intensity_____ activity factor 6.0
10.	Soccer	Frequency_____ # of months_____ # of days/week_____	minutes_____ intensity_____ activity factor 12.0
11.	Frisbee	Frequency_____ # of months_____ # of days/week_____	minutes_____ intensity_____ activity factor_____

12.	Walking (circle) Outdoor: a) slowly b) moderately c) rapidly d) upstairs	Frequency_____ # of months_____ # of days/week_____	minutes_____ intensity_____ activity factor 5.0
13.	Tennis	Frequency_____ # of months_____ # of days/week_____	minutes_____ intensity_____ activity factor 7.0
14.	Running	Frequency_____ # of months_____ # of days/week_____	minutes_____ intensity_____ activity factor 14.0
15.	Skating, Ice	Frequency_____ # of months_____ # of days/week_____	minutes_____ intensity_____ activity factor 7.0
16.	Skating, Roller	Frequency_____ # of months_____ # of days/week_____	minutes_____ intensity_____ activity factor 7.0
17.	Hiking or Mt. Climbing	Frequency_____ # of months_____ # of days/week_____	minutes_____ intensity_____ activity factor 8.0
18.	Bowling	Frequency_____ # of months_____ # of days/week_____	minutes_____ intensity_____ activity factor 3.0
19.	Calisthenics (Prenatal Class)	Frequency_____ # of months_____ # of days/week_____	minutes_____ intensity_____ activity factor 4.5
20.	Farming or Gardening	Frequency_____ # of months_____ # of days/week_____	minutes_____ intensity_____ activity factor 3.7
21.	Snowskiing	Frequency_____ # of months_____ # of days/week_____	minutes_____ intensity_____ activity factor 8.0
22.	Horseback Riding	Frequency_____ # of months_____ # of days/week_____	minutes_____ intensity_____ activity factor_____

23.	Housework (Standup work)	Frequency_____ # of months_____ # of days/week_____	minutes_____ intensity_____ activity factor_____
24.	Canoeing	Frequency_____ # of months_____ # of days/week_____	minutes_____ intensity_____ activity factor_____
25.	Ping Pong	Frequency_____ # of months_____ # of days/week_____	minutes_____ intensity_____ activity factor_____
26.	Pitching Horseshoes	Frequency_____ # of months_____ # of days/week_____	minutes_____ intensity_____ activity factor_____
27.	Racquetball	Frequency_____ # of months_____ # of days/week_____	minutes_____ intensity_____ activity factor_____
28.	Weight Lifting	Frequency_____ # of months_____ # of days/week_____	minutes_____ intensity_____ activity factor_____
29.	Wood cutting	Frequency_____ # of months_____ # of days/week_____	minutes_____ intensity_____ activity factor_____
30.	Volleyball	Frequency_____ # of months_____ # of days/week_____	minutes_____ intensity_____ activity factor 4.0
31.	Other (Please specify) _____	Frequency_____ # of months_____ # of days/week_____	minutes_____ intensity_____ activity factor_____

Appendix G. Two Day Dietary Food Record Form

PREGNANCY, NUTRITION, EXERCISE STUDY
TWO DAY DIETARY FOOD RECORD FORM

DATE OF RECORD_____ SUBJECT CODE NO._____

DAY OF WEEK TAKEN: M T W TH F S SUN (CIRCLE)

Please list all the foods and drinks you have consumed for two consecutive days. Make sure that at least one of the two days occurs on a typical weekday. Please be sure to record the amount you eat (for example, 1 medium potatoe, 1-8 oz. glass of milk, 2 slices of bread, 1/2 cup of peas), and the cooking method (for example, hamburger-baked with no fat, or fried in 2 tbs. of margarine). Also list the time and activity (for example, 8:00 am, watching T.V.) while eating. If foods are eaten out, such as at McDonalds, just list the food, amount, time of day and the activity (for example, 1 Big Mac, 1 small fry and 1 large coke, 2:00 pm, no activity). To insure accuracy, try to record food eaten immediately after each meal.

FOOD AND BEVERAGE CONSUMED

DAY OF WEEK TAKEN: M T W TH F S SUN (CIRCLE)

CODE NO.		WHAT DID YOU EAT?	AMOUNT	COOKING METHOD	TIME OF DAY	ACTIVITY WHILE EATING
	Example:	Eggs	2 med.	fried	7:30 a.m.	talking with
		Oil	1 tbs.			family
	BREAKFAST					
	SNACK					
	LUNCH					
	SNACK					
	DINNER					
	SNACK					
	ANY OTHER TIME					

FOOD AND BEVERAGE CONSUMED

DAY OF WEEK TAKEN: M T W TH F S SUN (CIRCLE)

CODE NO.		WHAT DID YOU EAT?	AMOUNT	COOKING METHOD	TIME OF DAY	ACTIVITY WHILE EATING
	Example:	Eggs	2 med.	fried	7:30 a.m.	talking with
		Oil	1 tbs.			family
	BREAKFAST					
	SNACK					
	LUNCH					
	SNACK					
	DINNER					
	SNACK					
	ANY OTHER TIME					

Appendix H. 24-Hour Recall Questionnaire

PREGNANCY, NUTRITION, EXERCISE STUDY

24-HOUR RECALL QUESTIONNAIRE

DATE OF RECORD_____SUBJECT CODE NO._____

DAY OF WEEK TAKEN: M T W TH F S SUN (CIRCLE)

FOOD AND BEVERAGE CONSUMED

CODE NO.		WHAT DID YOU EAT?	AMOUNT	COOKING METHOD	TIME OF DAY	ACTIVITY WHILE EATING
	BREAKFAST					
	SNACK					
	LUNCH					
	SNACK					
	DINNER					
	SNACK					
	ANY OTHER TIME					

Appendix I. Postpartum-Delivery Questionnaire

Post Partum-Delivery

Questionnaire

NAME_____

Birth Weight (g) __ __ __ __ . __

Birth Length (cm) __ __ __ . __

Apgar Score

1 min __ __

5 min __ __

Duration of Labor

1st stage (min) __ __ __ __

2nd stage (min) __ __ __ __

Weight gain (kg) __ __ . __
(during pregnancy)

Delivery Date_____

Type of Delivery

_____

_____

_____

Send to: Richard Lewis
Dept. Human Nutrition & Foods
Wallace Hall
VPI&SU
Blacksburg, VA 24061

Appendix J. Reagent Preparation

YM Agar: 4.1 g Bacto YM agar dissolved in 100 ml distilled, deionized water over heat with constant stirring. Boil until dissolved. Place 5 ml in each screw top test tube, loosely cap, and autoclave 15 min at 121°C and 15 psi. Tighten caps and cool in slanted position at room temperature. Store refrigerated.

Pyridoxine Y media for innoculum: Dissolve 0.54 g Pyridoxine Y media in 20 ml distilled, deionized water. Make fresh for each assay.

Saline: Dissolve 8.5 g NaCl in a l distilled, deionized water. Place 10 ml in each test tube, cap, and autoclave 15 min at 121°C, 15 psi. Store refrigerated.

0.2 N HCl: 1.67 ml concentrated HCl brought up to 500 ml with distilled, deionized water.

1 N KOH: Dissolve 28 g KOH in 500 ml distilled, deionized water.

10% Glacial Acetic Acid: 10 ml glacial acetic acid brought up to 100 ml with distilled, deionized water.

Pyridoxine Y media for sample assay: To prepare for 100 tubes dissolve 26.5 g pyridoxine Y media in 500 ml distilled, deionized water, stirring mechanically. Make fresh for each assay.

Stock standard-50mg/ml pyridoxine as pyridoxine hydrochloride: Place 0.0304 g PN in a 500 ml volumetric flask. Bring to volume with 25% ETOH. Store refrigerated in amber bottle. Stock refrigerated in amber bottle. Stock standard is stable for 2-3 mo.

Working stock standard-100 mg/ml pyridoxine as pyridoxine hydrochloride: Volumetrically measure 2 ml stock standard into a 1 volumetric flask. Bring to volume with distilled, deionized water. Store refrigerated in amber bottle. Working stock standard is stable for 2-3 wk.

Working standards-0.5, 1, 2, 3, 4, 5 ng/10 ml pyridoxine, as pyridoxine hydrochloride: Volumetrically measure 0.5, 1, 2, 3, 4, and 5 ml respectively of working stock standard into 100 ml volumetric flasks. Bring to volume with distilled, deionized water. Store refrigerated in amber bottles. Working standards are stable for 2-3 wk, however this author made fresh working standards weekly.

Appendix K. Chemicals and Vendors

American Type Culture Collection (Rockville, MD)

Saccharomyces uvarum

stock culture-ATCC 9080

Sigma Chemical Company (St. Louis, MO)

Pyridoxine HCl - P 9755

Difco Laboratories (Detroit, MI)

Bacto YM agar - 07-2-01

Pyridoxine Y Media - 0951-15-2

Fischer Scientific Company (Raleight, NC)

NaCl ACS

HCl ACS

Acetic acid, glacial ACS

KOH ACS

0.2 um Gelman Arodisc filters - 4192

Appendix L. 22 Wk Data

22 wk Data

Observation	Age (yr)	Height (cm)	Prepregnancy weight (kg)	Weight (kg)	Total activity score	Aerobic activity score	Vitamin B-6 intake (mg)	Protein intake (g)	Energy intake (kcal)	HR-rest (bpm)	HR-2 (bpm)	HR-4 (bpm)	HR-6 (bpm)	VO_2-2 (l/min)	VO_2-4 (l/min)	VO_2-6 (l/min)	VO_2-2 (ml/kg/min)	VO_2-4 (ml/kg/min)	VO_2-6 (ml/kg/min)	RPE	Duration of exercise test (min)	Lactate (mmole/l)	Plasma total vitamin B-6 (nM)	Plasma PLP (nM)
Walkers																								
1	26	174.0	56.8	63.9	53.8	27.7	0.99	63.7	2012.02	60	100	110	118	0.68	0.88	0.87	10.6416	13.7715	13.6150	12	14	1.6	189.2	96.3
2	27	171.5	53.6	62.8	44.9	22.7	1.63	101.1	2158.42	70	120	120	140	0.58	0.66	0.74	9.2357	10.5096	11.7834	13	14	.	139.8	88.6
3	27	158.8	61.4	69.2	21.5	3.1	1.48	89.0	2269.71	90	110	136	145	0.69	0.72	0.77	9.9711	10.4046	11.1272	10	8	4.0	154.1	89.8
4	27	170.2	57.7	63.4	29.9	6.4	2.36	124.7	2312.92	88	125	130	135	0.70	0.65	0.71	11.0410	10.2524	11.1987	14	13	1.8	169.6	92.7
5	31	162.6	65.5	72.8	10.5	4.1	1.21	83.4	2092.61	89	130	136	136	0.97	0.89	0.93	13.3242	12.2253	12.7747	15	8	3.0	124.9	78.5
6	31	180.3	67.3	74.1	14.8	7.1	2.26	158.5	2688.19	88	120	136	143	0.79	0.87	0.86	10.6613	11.7409	11.6059	13	7	1.3	139.2	110.5
7	30	172.7	56.8	66.4	45.1	25.4	2.39	96.1	2599.41	74	107	107	125	0.59	0.67	0.82	8.8855	10.0904	12.3494	12	12	2.1	114.8	70.4
8	24	162.6	55.5	62.3	31.3	10.2	2.58	123.7	2834.87	79	130	136	150	0.66	0.65	0.65	10.5939	10.4334	10.4334	14	11	3.8	113.1	46.9
9	30	157.5	50.9	62.8	9.9	4.3	1.84	114.6	2828.18	75	120	122	130	0.64	0.68	0.69	10.1911	10.8280	10.9873	12	11	4.4	114.2	57.5
10	36	174.6	63.6	73.8	99.9	26.9	1.07	68.5	1521.81	75	115	125	133	0.74	0.72	0.94	10.0271	9.7561	12.7371	11	11	2.5	118.4	70.8
11	32	172.7	60.9	70.5	51.9	6.9	1.86	117.6	2728.84	85	125	130	140	0.58	0.70	0.81	8.2270	9.9291	11.4894	13	8	3.5	119.6	74.0
12	28	156.2	45.5	52.3	20.9	10.4	1.43	97.4	1977.91	83	115	115	125	0.54	0.70	0.83	10.3250	13.3843	15.8700	12	8	1.6	168.9	116.5
13	31	167.6	69.1	75.5	62.2	2.0	1.96	110.1	2219.56	94	128	135	136	0.74	0.86	0.91	9.8013	11.3907	12.0530	13	9	2.2	133.3	85.4
14	23	172.7	79.5	81.5	31.5	9.8	1.32	83.2	1805.07	77	110	118	120	0.88	0. 91	1.01	10.7975	11.1656	12.3926	11	11	3.3	111.9	72.0
15	26	166.4	65.5	69.2	45.9	9.5	1.79	119.3	2836.02	78	120	120	126	0.86	0.92	1.02	12.4277	13.2948	14.7399	13	10	2.4	116.6	73.2
16	28	162.6	45.5	51.2	32.4	9.5	1.55	84.7	1720.15	82	131	131	139	0.50	0.49	0.57	9.7656	9.5703	11.1328	14	8	3.0	49.4	30.8
17	25	162.6	55.9	63.0	45.9	12.3	2.05	96.3	2049.86	77	122	136	136	0.75	0.90	0.94	11.9048	14.2857	14.9206	13	13	2.8	108.3	68.8
18	29	166.4	57.7	66.2	31.6	6.7	2.75	121.6	2273.22	86	105	107	125	0.66	0.81	0.77	9.9698	12.2356	11.6314	15	9	1.9	111.9	75.3
Nonwalkers																								
19	25	170.2	61.4	68.7	34.0	8.5	1.67	96.1	1789.95	79	125	130	136	0.83	0.75	0.81	12.0815	10.9170	11.7904	15	11	3.0	162.4	107.6
20	29	174.0	66.4	72.0	60.2	30.4	2.30	83.4	1547.20	80	.	.	.	.	.	.	.	.	.	.	.	.	104.7	58.3
21	30	172.7	75.0	76.2	28.2	0.5	3.00	111.9	2542.39	98	120	120	140	0.57	0.57	0.68	7.4803	7.4803	8.9239	11	8	2.2	187.4	122.2
22	24	170.2	63.6	68.6	9.8	2.6	2.17	135.6	3285.02	88	122	130	136	0.61	0.71	0.74	8.8921	10.3499	10.7872	14	10	3.2	119.6	68.0
23	31	162.6	61.4	72.2	43.5	7.8	2.73	92.9	1996.90	94	120	130	140	0.63	0.68	0.76	8.7258	9.4183	10.5263	13	8	3.3	96.9	46.1
24	21	167.6	84.5	89.5	30.2	3.9	1.50	87.5	2048.34	83	125	135	145	0.94	0.88	1.07	10.5028	9.8324	11.9553	13	8	4.1	111.9	66.8
25	26	165.1	58.4	63.4	17.4	11.3	1.36	76.1	2021.73	83	125	130	136	0.73	0.73	0.75	11.5142	11.5142	11.8297	12	12	6.1	194.6	94.3
26	28	160.0	49.1	58.4	32.1	0.9	2.56	105.8	2871.67	94	118	125	135	0.40	0.66	0.78	6.8493	11.3014	13.3562	17	10	4.0	121.9	80.9
27	32	165.1	55.8	58.8	21.0	3.1	2.97	110.7	2059.67	83	127	136	136	0.57	0.55	0.60	9.6939	9.3537	10.2041	16	8	2.4	122.6	68.8
28	27	157.5	52.3	56.5	11.2	0.1	1.72	108.6	2355.66	75	104	105	107	0.51	0.62	0.68	9.0265	10.9735	12.0354	13	12	2.1	126.7	77.3

Appendix M. 30 Wk Data

30 wk Data

Observation	Age (yr)	Height (cm)	Prepregnancy weight (kg)	Weight (kg)	Total activity score	Aerobic activity score	Vitamin B-6 intake (mg)	Protein intake (g)	Energy intake (kcal)	HR-rest (bpm)	HR-2 (bpm)	HR-4 (bpm)	HR-6 (bpm)	VO_2-2 (l/min)	VO_2-4 (l/min)	VO_2-6 (l/min)	VO_2-2 (ml/kg/min)	VO_2-4 (ml/kg/min)	VO_2-6 (ml/kg/min)	RPE	Duration of exercise test (min)	Lactate (mmole/l)	Plasma total vitamin B-6 (nM)	Plasma PLP (nM)
Walkers																								
1	26	174.0	56.8	66.4	42.8	24.0	1.59	75.0	2291.99	75	107	115	125	0.76	0.75	0.92	11.4458	11.2952	13.8554	12	15	2.9	146.9	82.9
2	27	171.5	53.6	66.2	34.2	21.3	2.51	130.9	2607.53	.	115	125	120	0.63	0.68	0.87	9.5166	10.2719	13.1420	13	16	2.3	117.8	75.7
3	27	158.8	61.4	75.5	22.4	3.8	0.99	66.0	2173.04	90	110	123	126	0.60	0.84	0.99	7.9470	11.1258	13.1126	14	11	5.7	109.5	71.6
4	27	170.2	57.7	64.6	30.8	6.8	2.08	74.9	1858.12	90	125	120	130	0.68	0.62	0.66	10.5263	9.5975	10.2167	15	11	2.1	223.1	113.3
5	31	162.6	65.5	76.8	14.0	3.5	1.81	107.5	2514.22	75	112	118	125	0.84	0.86	0.95	10.9375	11.1979	12.3698	15	8	4.2	93.4	62.7
6	31	180.3	67.3	80.0	18.3	8.6	2.10	110.2	2142.21	84	118	125	140	0.70	0.95	0.93	8.7500	11.8750	11.6250	13	8	3.5	117.8	84.2
7	30	172.7	56.8	70.0	46.3	26.6	1.76	104.7	2995.86	77	108	90	110	0.61	0.79	0.80	8.7143	11.2857	11.4286	13	15	2.4	105.3	64.3
8	24	162.6	55.5	67.3	32.3	11.0	1.62	103.8	2526.72	96	135	136	149	0.66	0.63	0.77	9.8068	9.3611	11.4413	13	9	5.5	53.6	34.0
9	30	157.5	50.9	69.5	11.2	5.0	0.99	60.0	2208.30	83	130	134	140	0.70	0.71	0.82	10.0719	10.2158	11.7986	12	8	5.6	91.0	54.6
10	36	174.6	63.6	76.5	99.9	28.7	1.67	80.1	1869.10	78	110	123	136	0.59	0.85	0.85	7.7124	11.1111	11.1111	12	11	2.4	112.5	67.6
11	32	172.7	60.9	75.6	53.7	8.7	0.99	74.7	1712.83	107	125	128	136	0.53	0.65	0.67	7.0106	8.5979	8.8624	16	9	5.5	76.2	47.3
12	28	156.2	45.5	55.5	21.6	11.1	2.17	110.6	2506.53	72	94	100	103	0.51	0.54	0.63	9.1892	9.7297	11.3514	15	13	2.0	169.6	106.4
13	31	167.6	69.1	80.0	60.2	1.4	2.21	126.0	2459.59	91	125	134	136	0.79	0.87	0.91	9.8750	10.8750	11.3750	13	9	2.3	125.6	73.6
14	23	172.7	79.5	86.8	32.6	11.0	1.84	94.4	2464.11	77	100	110	120	0.80	0.81	0.90	9.2166	9.3318	10.3687	15	9	2.7	143.9	89.4
15	26	166.4	65.5	73.7	34.5	7.2	3.33	130.5	3380.80	107	132	136	136	0.79	0.85	0.82	10.7191	11.5332	11.1262	8	10	2.0	124.4	63.9
16	28	162.6	45.5	53.4	37.7	11.2	1.32	77.3	1878.23	94	136	140	150	0.52	0.53	0.57	9.7378	9.9251	10.6742	13	8	3.2	64.3	.
17	25	162.6	55.9	68.0	41.3	12.7	2.00	77.2	1881.45	79	120	125	133	0.76	0.79	0.85	11.1765	11.6176	12.5000	14	13	6.2	.	64.7
18	29	166.4	57.7	70.5	30.5	6.5	1.62	90.3	2436.60	75	119	120	136	0.66	0.75	0.74	9.3617	10.6383	10.4965	14	9	4.5	123.2	74.0
Nonwalkers																								
19	25	170.2	61.4	73.6	31.8	6.3	2.05	141.3	2198.17	100	123	136	136	0.62	0.61	0.77	8.4239	8.2880	10.4620	15	10	1.3	167.2	103.6
20	29	174.0	66.4	78.0	50.9	25.1	1.63	90.0	1714.42	90	120	130	140	0.78	0.93	1.08	10.0000	11.9231	13.8462	14	9	.	88.7	56.6
21	30	172.7	75.0	80.0	27.9	0.5	1.81	75.2	2359.41	100	136	136	150	0.65	0.70	0.92	8.1250	8.7500	11.5000	12	8	2.8	160.7	.
22	24	170.2	63.6	73.9	11.3	3.9	.	.	.	77	120	122	128	0.77	0.75	0.85	10.4195	10.1488	11.5020	15	14	3.5	.	.
23	31	162.6	61.4	75.4	52.0	4.2	1.93	103.6	1689.76	94	135	128	130	1.00	0.89	0.83	13.2626	11.8037	11.0080	14	7	1.9	110.1	52.2
24	21	167.6	84.5	91.3	28.7	4.0	1.60	82.0	1772.10	106	136	142	159	0.79	0.89	1.00	8.6528	9.7481	10.9529	14	6	2.2	.	62.7
25	26	165.1	58.4	68.5	16.3	9.8	2.01	97.0	2528.59	75	125	126	136	0.66	0.79	0.92	9.6350	11.5328	13.4307	12	12	2.6	225.5	93.1
26	28	160.0	49.1	60.2	29.7	2.3	1.79	90.0	2111.47	100	130	140	140	.	.	.	.	.	.	16	7	5.2	132.1	73.2
27	32	165.1	55.8	62.3	23.0	4.8	2.23	101.7	1873.33	88	130	133	136	0.65	0.79	0.80	10.4334	12.6806	12.8411	15	8	1.9	89.8	61.5
28	27	157.5	52.3	59.4	8.9	0.6	1.58	118.5	1978.61	105	115	125	136	0.53	0.58	0.64	8.9226	9.7643	10.7744	15	11	.	162.4	92.2

Appendix N. Postpartum Data

Postpartum Data[1]

	Age (yr)	Birth weight (yr)	Birth length (cm)	Weight gain (kg)	Apgar-1 min	Apgar-5 min	Labor duration 1st stage	Labor duration 2nd stage (min)
				Walkers[2]				
1	26	3.0	53.3	10.4	9	9	270	20
2	27	3.4	51.4	12.7	9	9	240	75
3	27	2.8	49.5	16.4	9	10	1750	1757
4	27	4.1	55.2	9.6	8	9	570	15
5	31	3.4	55.0	15.9	9	10	340	83
6	31	4.2	53.3	15.9	8	10	.	.
7	30	4.6	52.5	13.5	9	9	180	20
8	24	4.2	54.0	17.7	8	9	330	60
9	30	2.9	49.3	20.5	9	9	540	60
10	36	4.1	55.9	14.5	9	9	530	90
11	32	3.1	50.8	15.5	8	9	315	45
12	28	3.0	49.5	11.8	8	9	1230	30
13	31	3.7	52.1	15.9	8	9	360	150
14	23	3.8	52.7	11.4	9	10	360	90
15	26	3.1	53.3	10.5	9	9	480	40
16	28	3.5	53.3	11.4	9	10	210	60
17	25	3.3	54.0	17.3	7	8	240	240
18a	29	2.3	47.0	18.2	.	.		.
18b		2.2	49.5					
				Nonwalkers				
19	25	4.1	56.5	12.3	9	10	390	120
20	29	4.7	54.6	12.7	8	9	.	.
21	30	2.9	52.1	14.1	8	8	450	67
22	24	.	.	.	.	.	.	.
23	31	3.5	50.8	15.9	10	10	255	45
24	21	3.6	52.1	12.3	10	10	.	.
25	26	3.4	50.8	10.5	9	9	300	60
26	28	3.5	54.0	12.9	8	9	250	5
27	32	3.7	50.8	15.8	9	9	120	30
28	27	3.9	50.8	12.7	7	9	120	30

[1]Observations 6, 18, 20 and 24 had Cesarean delivery and therefore, labor durations were not reported.

[2]Observation 18 included twins (a,b).

The vita has been removed
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