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

by

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

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(ABSTRACT)

The effects of an aerobic walking program, from 22 to 30 wk gestation, on aerobic fitness, riboflavin and thiamin status, and birth outcome were studied in 28 healthy, pregnant women, 24-36 yr of age, receiving vitamin-mineral supplements. Aerobic capacity was evaluated by measurements of heart rate (HR) and relative oxygen consumption ($VO_2$, ml/kg/min) during two submaximal treadmill walking tests (22 and 30 wk). HR responses were significantly lower for walking subjects (W; n=18) compared to nonwalking (NW; n=10) at two (~2.9 METS) and four (~3.8 METS) min, and near significant at six (~4.6 METS) min of the 30 wk tests. HR at two min of exercise for NW subjects increased significantly from 22 to 30 wk. Oxygen consumption for W decreased significantly from 22 to 30 wk at two, four, and six min of exercise. Comparison of $VO_2$ changes (22 to 30 wk) between W and NW indicated a significant difference at six min and near significance at four min. Riboflavin and thiamin status, as determined by erythrocyte glutathione and transketolase activity coefficients, was adequate for 86% of the subjects with respect to riboflavin and 96% for

thiamin. There was a significant correlation ($r = 0.58$) between aerobic activity scores and ETKAC values (W group). Participation in a walking program slightly improved aerobic capacity without affecting birth outcome or riboflavin and thiamin status in pregnant women taking vitamin-mineral supplements.

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# CHAPTER I

## INTRODUCTION

During the last few years there has been increased participation by women in recreational and competitive athletics. Many women, in order to improve appearance and physical and mental health, are engaged in jogging programs, aerobic exercise classes, and other forms of physical exercise. The physiologically and psychological benefits of regular exercise are well accepted in the nonpregnant human population (NICHD, 1982). However, one area unique to the female and receiving increased attention, is the area of physical activity during pregnancy. Traditionally, physicians have not prescribed exercise as a component of prenatal health care. With the increased emphasis on physical fitness, physicians are requesting clinical guidance for recommendations to pregnant women on physical activity.

In September, 1982, the National Institute of Child Health and Human Development (NICHD) conducted a research planning workshop on physical activity in pregnancy. This workshop, which was attended by individuals in a variety of disciplines (i.e., obstetrics, nutrition, exercise physiology), was organized to assess the current state of knowledge and the research priorities of studies concerning the relationship of physical activity during pregnancy and the health of the mother and child (NICHD, 1982). One area of future research which was regarded as a priority was the area of

exercise during pregnancy and the effects on maternal stores of various nutrients.

In general, physical activity and training increases nutrient requirements for total kilocalories (kcal), amount of carbohydrate in the diet, thiamin, riboflavin, niacin, iron, water, sodium chloride, and possibly chromium (Manjarrez and Birrer, 1983; Nelson, 1982). Under normal health circumstances, increased nutrient needs with physical activity can be met by eating more of a varied diet (Nelson, 1982). Similarly, during pregnancy, nutrient requirements including energy are increased in order to provide for optimal fetal growth and development, while preserving maternal homeostasis. Gestational requirements are based on the Recommended Dietary Allowances (RDA) of the National Research Council (NRC, 1980). Most of the increased nutrient needs of the pregnant woman can be met by eating more of a varied diet, with the exception of folate and iron (Dwyer, 1982). There is a recommended increased energy intake (300 kcal) for pregnant women; however, little consideration was given to the increased energy cost of exercise (Nagy and King, 1983). Therefore the stress of exercise during pregnancy may increase demands beyond the present recommendations for energy and other nutrients.

Animal studies have suggested the possibility that acute exercise may jeopardize fetal oxygenation (Clapp, 1980) and fetal

growth (Dhindsa et al., 1978). However, several investigators have indicated that healthy pregnant women can engage in moderate aerobic exercise throughout pregnancy and improve or maintain physiological fitness without compromising maternal or fetal health (Collings et al., 1983; Erkkola, 1979; Dressendorfer and Goodlin, 1978).

Limited research is available regarding the effects of exercise and improved physical fitness on maternal or infant nutrient status. Intakes of the water soluble vitamins, riboflavin and thiamin, are directly related to energy intake, as a result of their roles in oxidative energy metabolism. Hence, the possibility exists that the added stress of physical activity to the already demanding state of pregnancy, may compromise riboflavin and thiamin nutriture. Therefore, the intent of this study is to examine the effects of a walking program during pregnancy (22 to 30 wk of gestation) on: 1) aerobic fitness, as measured by heart rate (HR) and oxygen consumption ($VO_2$) during two submaximal exercise tests; 2) maternal riboflavin and thiamin status, as measured by erythrocyte glututhione reductase activity coefficients (EGRAC) and erythrocyte transketolase activity coefficients (ETKAC), respectively; and, 3) pregnancy outcome as determined by birth weight, birth length, Apgar scores and duration of labor.

# CHAPTER II

# REVIEW OF LITERATURE

## Physiological Adjustments of Pregnancy

In order to discuss the effects of exercise during pregnancy on physiological or nutritional parameters, some of the physiological adaptations that occur during a normal healthy pregnancy were investigated and are described.

Blood volume and composition. Plasma volume and erythrocyte volume both increased during pregnancy, with plasma volume increasing greater than red cell volume (Hytten and Leitch, 1971a). The result is a decreased erythrocyte concentration which is sometimes referred to as the physiological anemia of pregnancy. Total plasma protein concentration decreased early in pregnancy and was attributed to the significant decline in albumin. In contrast, most plasma lipid fractions, including cholesterol, phospholipid, and free fatty acids, increased during pregnancy (Hytten and Leitch, 1971a).

Cardiovascular responses. Similar to the response which occurred during exercise, cardiac output (Q) was increased significantly during pregnancy from 4.5-5.0 to 6.0-7.0 liters/min (l/min) by the first half of pregnancy (Bader et al., 1955). Hytten and Leitch (1971b) have summarized the data of several investigators regarding Q during several stages of pregnancy. The

authors concluded that there was a significant rise in Q throughout pregnancy and the measurement was dependent on the position in which the subject was lying. A few researchers, reviewed by Hytten and Leitch (1971b), reported a fall in Q in late pregnancy. Hytten explained this decrease as the result of measurements being made in a lying down position in which the uterus seriously impedes venous return through the vena cava. Therefore, suggestions have been made to determine Q with the patient lying on her side. The increased Q during pregnancy was the result of an increased heart rate (HR) [Hytten and Leitch, 1971b; Knuttgen and Emerson, 1974] and stroke volume (SV) [Hytten and Leitch, 1971b]. Hytten stated that HR increased during pregnancy an average of 15 beats per minute (bpm), which is similar to the postpartum (66 bpm) and pregnancy (81 bpm) values reported by Knuttgen and Emerson (1974). A few investigators (Erkkola, 1976; Bader et al., 1954; Blackburn and Calloway, 1976b) indicated that HR increased approximately three to seven bpm as pregnancy progressed.

In one recent study (Clapp, 1985), the author measured the resting HR of 10 women runners prior to and during pregnancy, including a measurement each mo during gestation. The HR increased 16 bpm, from one mo prior to conception to 36 wk gestation, with only a five bpm increase from 16 to 32 wk gestation. Since Q and HR increased approximately, 1.5 l/min and 15 bpm, respectively, over nonpregnancy values, SV must also increase (64 to 71 ml) [Hytten and Leitch, 1971b]. This increase in SV was proportionally a smaller increase than HR.

Arterial blood pressure measurements made by Schwarz on 83 normal pregnant women indicated a decrease in systolic blood pressure (SBP) by about 3-4 mm Hg below the nonpregnant level (Hytten and Leitch, 1971b). However, there was a slight rise towards nonpregnant levels near term. Similarly, diastolic blood pressure (DBP) was observed at 4-5 mm Hg below the nonpregnant level in early pregnancy and increased near term.

Electrocardiographic (ECG) changes during pregnancy are attributed to changes in the position of the heart; these changes were also summarized by Hytten and Leitch (1971b). Some ECG readings showed a flattened T-wave in lead III, occasional depression of the S-T segment in both chest and limb leads, low voltage QRS complexes, and deep Q waves, and the occasional occurrence of U waves.

Respiratory responses. Knuttgen and Emerson (1974) and Hytten and Leitch (1971c) reported changes in selected pulmonary parameters which occur with pregnancy. Hytten and Leitch listed several studies which indicated that oxygen consumption ($VO_2$) during late pregnancy was greater than postpartum values by approximately 30-60 ml/min. Knuttgen and Emerson demonstrated that $VO_2$ (ml/min) increased from 191 for postpartum women to 249 for pregnant women at rest.

Similarly, Pernoll et al. (1975) and Bader et al. (1954) demonstrated that as pregnancy progresses from approximately 20 to 38 wk resting $VO_2$ increased significantly. Pernoll indicated an increase in resting $VO_2$ from 278 ml/min to 331 ml/min from the

19th to the 40th wk of pregnancy, while Bader showed an increase of 138 ml/min to 150 ml/min during the entire gestational period. In a recent review article (Gorski, 1985), the author stated that the approximate 15-20% increase in resting $VO_2$ above nonpregnant values, was initially due to increased cardiac and renal energy cost, and that later in pregnancy the increase was due primarily to the rapidly growing fetus, the enlarging placenta and the uterus.

Also occurring during pregnancy was an increased minute ventilation ($V_E$) [5.54 to 8.51 l/min], in excess of an appropriate $VO_2$ response, which resulted in an elevated ventilatory equivalent (28.3 to 33.2 l/min) [Knuttgen and Emerson, 1974]. Pernoll et al. (1975) also demonstrated that resting $V_E$ increases during pregnancy by approximately 50%. The increases in $V_E$ in pregnancy as compared to those of postpartum women was the result of an increased tidal volume (0.37 l to 0.56 l), with little change observed in frequency of breathing (15.6 to 15.2 breaths/min).

Weight gain in pregnancy. In the United States, the average woman gains approximately 11 kg during pregnancy (Gibbs and Scitchik, 1980) and the weight gain is composed of the fetus, placenta, amniotic fluid, uterus, breast, maternal blood and maternal interstitial fluid. Collings et al. (1983) and Nagy and King (1983) reported mean ($\bar{X}$) weight gains of 14 kg and 13 kg, respectively, in healthy pregnant women. Hytten and Leitch (1971d) suggested from a review of several studies that the range of weight gain was between 3.6 kg and 23 kg or more.

## Riboflavin and Thiamin Requirements During Pregnancy

The increased metabolism during pregnancy increases the dietary requirements for most nutrients including riboflavin and thiamin (NRC, 1980). During gestation an additional 300 kcal/day is recommended to cover the increased energy needs related to added maternal tissues, increased maternal metabolism, and work associated with activity and growth of the fetus and placenta (NRC, 1980). Riboflavin in the coenzyme forms of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), functions as prosthetic groups of flavoenzymes in the oxidative degradation of pyruvate, fatty acids, and amino acids, and also in electron transport (Lehninger, 1975). Thiamin, as thiamin pyrophosphate (TPP), serves as a coenzyme in carbohydrate metabolism, involving the decarboxylation of $\alpha$-ketols (Lehninger, 1975).

Therefore, the requirements of these vitamins have usually been related to energy intake. The RDA for riboflavin during pregnancy (22-50 yr) is an additional 0.3 mg/day or 1.5 mg/day and for thiamin, an extra 0.4 mg/day or 1.4 mg/day. Dwyer (1982), as well as a joint FAO/WHO expert group (1965), suggested that an ordinary, well balanced diet should adequately cover the modest increases for these vitamins during pregnancy without supplementation. However, Heller and coworkers (1974) recommended prenatal supplementation of both riboflavin and thiamin. Heller found that 25% of 651 pregnant women in the 1st trimester, increasing up to 40% at term were

significantly riboflavin depleted as determined by erythrocyte glutathione reductase (EGR) [NAD(P)H: oxidized-glutathione oxidoreductase; EC 1.6.4.2] activation tests. He also suggested that 25-30% of 599 pregnant women were thiamin depleted in respect to erythrocyte transketolase (ETK) [sedoheptulose-7-phosphate: D-glyceraldehyde-3-phosphate glycoaldehyde-transferase; EC 2.2.1.1] saturation measurements as compared to controls. Baker et al. (1975) measured the thiamin status in the blood of 174 pregnant women by a protozoological method and found that regardless of vitamin supplementation, 35% of the women had marginal thiamin status. Baker's data supports the observations reported by Heller et al. (1974).

Ramsey et al. (1983) also investigated the riboflavin and thiamin concentrations in maternal placenta, erythrocytes, and cord erythrocytes of 54 Kenyan women. Using the EGR activation test, Ramsey reported that 72% of the women were deficient with respect to maternal erythrocytes and 35% with cord erythrocytes. Thiamin status was assessed by the ETK activation test and 59% of the women were reported deficient as indicated by maternal erythrocytes, 15% by placenta values, and 41% by cord erythrocytes.

## Riboflavin and Thiamin Requirements with Exercise

Since riboflavin and thiamin requirements increase during pregnancy as a result of increased metabolism and caloric intake,

requirements for these vitamins may similarly be higher for individuals and athletes with increased energy expenditures and caloric intakes. Requirements for riboflavin and thiamin are elevated in active individuals with increased caloric intakes due to the vitamins' metabolic roles in energy metabolism (Nelson, 1982).

The data from several recent studies have suggested that exercise training increases the requirements for riboflavin in active normal weight young women (Belko et al., 1983), as well as in moderately obese subjects (Belko et al., 1984; 1985). Belko and coworkers (1983) demonstrated that 12 active normal weight women, jogging from 20 to 50 min/day for six wk increased their riboflavin requirements from a range of 0.62-1.21 mg/1000 kcal before exercise to 0.63-1.43 mg/1000 kcal after exercise. The increased riboflavin requirement was based on the amount of riboflavin needed in the diet to achieve an EGR activity coefficient (EGRAC) within the normal range of less than 1.20.

Belko et al. (1984) further determined that 12 moderately obese women who participated in a dance exercise program five days/wk, 50 min/day for five wk, also had increased riboflavin requirements, as indicated by increased EGRAC. The EGRAC increased from 1.28 $\pm$ 0.11 ($\bar{X}$ $\pm$ SD) to 1.49 $\pm$ 0.16 during the exercise period, which was indicative of biochemical riboflavin deficiency. In addition to the exercise related increase in riboflavin requirement, the requirement was elevated during the nonexercise period, as indicated by the increase in EGRAC from 1.28 $\pm$ 0.11 ($\bar{X}$ $\pm$ SD) at the

baseline period to 1.40 $\pm$ 0.12 in the nonexercise period. These data supported the earlier suggestion that healthy, active women required more riboflavin than the 1980 RDA to achieve EGRAC normality.

Belko et al. (1985), using two different levels of riboflavin intake, 0.94 mg/1000 kcal and 1.16 mg/1000 kcal, showed that moderately obese women, during exercise (bicycling) or nonexercise periods required the higher level of riboflavin intake to maintain a normal EGRAC. Increased riboflavin requirements were not shown to be related to weight loss in the subjects. Succinate dehydrogenase and palmityl CoA dehydrogenase are two enzymes that require FAD as a cofactor; both have been shown to be increased with endurance training (Belko et al., 1985). Therefore, increased utilization of FAD may accompany endurance training and possibly result in elevated EGRAC values. As a result of these studies, the suggestion of increased intakes of riboflavin with exercise is gaining attention even in the more popular magazines. For example, Daphne Roe, a colleague of Belko, discussed the idea of increased riboflavin requirements for women, in Health Magazine, entitled "Ribo-Loading" (Roe, 1984).

A higher thiamin requirement has also been associated with increased energy expenditure. Sauberlich et al. (1979) reported that young adult males, who consumed a high caloric diet (~ 3600 kcal), and at the same time increased physical activity to achieve weight maintenance, required more (0.15 mg/1000 kcal) thiamin in the diet to achieve ETKAC normality. Daum et al. (1949) also

showed that subjects consuming an intermediate (0.6 mg/day) or a low thiamin diet (0.2 mg/day) for 3-19 wk had a decreased maximum work output.

Available evidence suggests that there is no need or advantage to riboflavin or thiamin supplementation for athletes or active individuals (Buskirk, 1981). Several researchers (Smith, 1976; Buskirk, 1981; Nelson, 1982) have recommended selecting a diet from a variety of foods to adequately cover the slight increases in riboflavin and thiamin.

## Physiological Responses to Exercise in Pregnant Animals

Many questions have been raised concerning the validity of using experimental animals to study the effects of maternal exercise on fetal well being in humans. In a summary report on physical activity in pregnancy (NICHD, 1982) the authors suggested that studies using sheep and goats (animals which are traditionally used) are of limited value because: 1) the magnitude of sympathetic response appears to be less in quadrupeds than in humans; 2) sheep and goats do not have the same susceptibility to venous pooling; 3) their mechanisms of heat loss differ from those of humans; and, 4) cooperation can be enlisted only to a limited extent in experimental animals and the various motivations determining human exercise behavior cannot be studied.

However, animal studies, investigating the effects of maternal exercise, are beneficial because more information can be obtained on fetal response and outcome, an area limited in human research. Researchers have identified several important physiological changes that occur during maternal exercise. One area of investigation is that of uterine blood flow during strenuous maternal exercise and the possibility of fetal hypoxia (Clapp, 1980). Clapp demonstrated in the untrained near term pregnant ewe, that exercise until exhaustion resulted in a 25% increase in maternal HR, which was directly related to a 28% decrease in uterine blood flow, without evidence of fetal compromise. Similarly, Curet and coworkers (1976) observed an increased HR in exercised pregnant ewes, but observed no changes in total uterine blood flow in either trained or untrained ewes. However, distribution of blood flow was altered in favor of the placenta with an 8% increase in blood flow to the cotylodons after exercise. This may represent a physiological adaptation mechanism for the fetus. Gorski (1985) in a review article points out that at the present time there is no correlation between increased intensity of exercise and decreased uterine blood flow.

Another study conducted by Clapp (1983) demonstrated that regardless of variations in uterine or umbilical blood flow during strenuous maternal exercise, there were no significant differences in $O_2$ extraction by the fetus. This again may represent compensatory mechanisms which protect the fetus during bouts of strenuous exercise.

Mottola and colleagues (1983) reported no significant differences compared to controls in birth weight or histological assessment of diaphragmatic muscles in pups born to rats mildly exercised for one h/day for 18 days during pregnancy. Similarly, Parizkova (1975) found no differences in body weights of the offspring from exercised pregnant rats. However, Parizkova observed that the number of capillaries and muscle fibers were higher in the heart muscles of offspring born to exercised mothers compared to sedentary controls.

Parizkova et al. (1978) also demonstrated that maternal exercise brought about significant changes in selected indicators of lipid metabolism in the liver of the pups. Male and female pups of mildly (14-16 m/min) exercised rat mothers had significantly higher cholesterol concentrations (2.41 g/dl vs 2.17 g/dl, $\bar{X}$) in the liver and lower lipid synthesis, while only male pups showed a lower total lipid and fatty acid concentration in the liver when compared to controls. These results suggested the possibility of altered lipid metabolism in later life of the offspring born to exercised mothers.

Nelson et al. (1983) and Smith et al. (1983) demonstrated that progressive (0 to 60 min) maternal exercise (9.7 m/min) in guinea pigs may be deleterious to the fetus. Nelson showed that maternal exercise of at least 30 min duration correlates with decreased fetal weight, while 15 min resulted in decreased placental weight and placental diffusing capacity. Smith further demonstrated that placental diffusing capacity and maternal surface area in the

peripheral labyrinth are highly correlated and that both decreased with increasing exercise.

The data of Dhindsa and associates (1978) suggested that exercise in the pregnant pygmy goat may compromise fetal growth and well being. The average birth weights of two sets of twins, born to mothers exercised during pregnancy, were significantly lower (1272 g vs 1592 g) than matched controls. The data suggested the possibility that maternal exercise may jeopardize fetal oxygenation and fetal growth.

Bell and coworkers (1982) have shown that acute exercise (2.5 km/h) in the pregnant ewe caused rapid and significant increases in maternal glucose, lactate, pancreatic glucagon, corticosteroids concentrations; no changes in insulin; and decreases in growth hormone and enteroglucagon. Similarly, fetal blood glucose, lactate and corticosteroids increased proportionally, accompanied by rapidly increased insulin levels. The exercise induced changes in maternal energy metabolism may result in increased fetal glucose utilization.

While many animal studies have reported the effects of maternal exercise on the offspring only, Wilson and Gisolfi (1980) investigated the effects of exercise training on maternal aerobic capacity, as well as on offspring aerobic capacity and mortality. Rats that were trained by treadmill walking had a higher $VO_{2max}$ (83.6 vs 67.0 ml/kg/min) and a greater offspring mortality rate than nonexercised controls, while no influence was observed on aerobic capacity of rat pups. Even though Wilson and Gisolfi

(1980) demonstrated an improved aerobic capacity of the pregnant rats, the data similar to that reported by other investigators, suggested the possibility that strenuous maternal exercise may be harmful to the fetus.

## Physiological Responses to Exercise in Pregnant Women

Traditionally, physical activity has been restricted during pregnancy in order to protect the mother and fetus from potential physiological complications. More women are now engaged in aerobic exercise programs during their pregnancy as a result of proposed labor and delivery benefits, improved postpartum fitness, and for continuation of already existing aerobic exercise routines. As mentioned earlier, animal studies indicated that there may be risk associated with maternal exercise, including reduced uterine blood flow (Clapp, 1980), lower birth weights (Dhindsa et al., 1978) and increased offspring mortality (Wilson and Gisolfi, 1980). Jopke (1983) suggested that there are discrepancies between animal and human data with regard to maternal exercise. Animal studies utilize extreme techniques such as shock treatment for exercise motivation (Wilson and Gisolfi, 1980) and rigorous nonrealistic exercise regimes. Therefore, data from pregnant animal studies should be used with caution when interpreting the effects of exercise in pregnant humans.

Responses to acute exercise. The physiological and metabolic responses to acute exercise during pregnancy have been reported by a few investigators. Artal et al. (1981) measured SBP and HR in 23 nontrained pregnant women (34 wk gestation) before, during and after 30 min of light [2.33 METS (Metabolic Equivalents)] treadmill walking. There were significant increases in HR (88 to 104 bpm), and SBP (111 to 121 mm Hg) immediately following exercise. HR recovered promptly after exercise, while SBP returned to normal within 30 min, suggesting an appropriate cardiovascular response of normal pregnancy. Artal and coworkers (1981) also reported significant increases in plasma glucagon, norepinephrine and epinephrine concentrations following exercise. The elevated levels of these hormones returned to normal within 30 min of recovery, reflecting normal metabolic responses to fuel utilization of light exercise. Similarly, Rauramo et al. (1983) found increases in maternal HR (93 to 157 bpm), SBP (120 to 148 mm Hg), epinephrine and norepinephrine in 10 healthy pregnant women after 10 min of submaximal bicycle exercise.

Guzman and Caplan (1970) measured $VO_2$, $V_E$, HR, Q, SV, and arteriovenous-oxygen ($a\text{-}vO_2$) difference in eight healthy pregnant women after a bicycle exercise test conducted each mo from the 1st trimester until delivery. The testing, which consisted of three exercise levels, produced increases in all cardiorespiratory parameters except in $VO_2$ where there was only a small nonsignificant increase and in $a\text{-}vO_2$ difference where a decrease occurred. HR, Q, and $V_E$ remained higher than controls throughout

gestation, while $VO_2$ did not significantly change throughout pregnancy. The lack of $VO_2$ increase may be the result of using nonweight bearing exercise. The authors suggested that the physiological responses to exercise in pregnant women were similar to women in the nonpregnant state, except that the responses were cumulative to an already established hyperkinetic state. They further stated that the pregnant woman most likely reached her maximum work capacity at a lower level of work than in the nonpregnant state. Pernoll et al. (1975), in contrast, found an increase in $VO_2$ throughout gestation in 12 healthy pregnant women at rest, during, and after submaximal bicycle exercise. The data suggested that $VO_2$ did increase with nonweight bearing exercise. The author concluded that the increased $VO_2$ was probably the result of decreased efficiency of performing light muscular work during pregnancy.

In a case study of a 32 year old trained pregnant woman, Hutchinson and coworkers (1981) reported an increase in $VO_2$ after 10 min of submaximal treadmill running tested at monthly intervals throughout pregnancy. When $VO_2$ was expressed as relative $VO_2$ (ml/kg/min), there was no significant increase, suggesting that weight gain was responsible for the increased $VO_2$. HR, respiratory exchange (R), $V_E$, and ventilatory equivalent ($V_E/VO_2$) all significantly increased throughout pregnancy, greater proportionally than the body weight gain. The authors concluded that as pregnancy progresses and body weight increases, running becomes more stressful as indicated by the increased $VO_2$,

$V_E$, and HR. Hutchinson et al. (1981) recommended that as a result of the increased stress, running speed or intensity should be reduced.

Knuttgen and Emerson (1974) studied the physiological response to pregnancy at rest and during treadmill walking and bicycling. The results, similar to Hutchinson's, indicated that $VO_2$ increased throughout gestation at rest and for treadmill walking, but not for cycling. The increases in $O_2$ cost of treadmill grade walking was due to the increases in body weight of the subjects. The $V_E/VO_2$ also increased during pregnancy at rest and during both modes of exercise, suggesting overbreathing or hyperventilation. At rest there were also increases in vital capacity (VC) and inspiratory reserve (IR) with significant decreases in expiratory reserve volume (ERV) and functional residual volume (FRV). The authors suggested that when weight lifting exercise was minimized, exercise does not impose a severe physiological stress during pregnancy.

Collings and coworkers (1983) predicted $VO_{2max}$ from HR and $VO_2$ values, using a submaximal bicycle exercise test, in eight nontrained pregnant women. The authors showed that from 22 to 30 wk of pregnancy the women had nonsignificant decreases in absolute $VO_{2max}$ (l/min) and relative $VO_{2max}$ (ml/kg/min) of 4% ($\bar{X}$) and 10%, respectively. Dibblee and Graham (1983), however, showed that unfit pregnant women had slight increases in predicted absolute $VO_{2max}$ (2.17 l/min, $\bar{X}$, to 2.42 l/min) throughout pregnancy, possibly the result of extra weight gain taxing the cardiovascular system enough to improve fitness.

Energy expenditure at rest and during selected aerobic activities has been reported by Blackburn and Calloway (1976b) and Nagy and King (1983). Blackburn and Calloway found that basal metabolic rate (BMR) during pregnancy was significantly higher than postpartum BMR and increased as gestation progressed. They also demonstrated that energy expenditure increased 4-5 fold with bicycling and 6-7 fold with walking over resting metabolism and the increased energy expenditure paralleled weight gain during pregnancy. HR measurements during exercise also indicated that pregnant women were less fit for a defined work load as pregnancy continued, and that the women may compensate for the increased energy expenditure with a slower work pace.

Nagy and King (1983) measured energy expenditure at rest and self-paced walking, either longitudinally (early to late pregnancy) or in a cross-sectional study. The cross-sectional study consisted of three groups including nonpregnant, early pregnant, and late pregnant. Similar to Blackburn and Calloway's data, the BMR increased approximately 28% during gestation. However, when expressed as kcal/kg bw/h, there were no significant differences due to stage of gestation. Total energy expenditure for self-paced walking was 17% greater in the LP group compared to the EP group suggesting that body weight, rather than pace, was the major determinant of total energy expenditure. The authors also noted that when the EP group was studied at 35 to 40 wk, their pace was only 4.5% slower than at 15 to 25 wk gestation. Therefore, it is possible that individual behavioral differences may influence pace more than stage of gestation (Nagy and King, 1980).

Responses to exercise training. The studies reviewed up to this point basically investigated the response of pregnant women to acute exercise at various stages of gestation. Recently there have been suggestions that pregnant women can maintain an exercise program and improve aerobic capacity without compromising fetal well-being. Collings et al. (1983), Erkkola (1976), and Dressendorfer and Goodlin (1978), have demonstrated that nontrained pregnant women, who engage in regular aerobic activity, can improve $VO_{2max}$ without deleterious effects on the fetus, while other investigators (Pomerance et al., 1974a; Dale et al., 1982; Jarrett and Spellacy, 1983) have shown that maternal exercise was not necessarily beneficial for the mother or detrimental on birth outcome. Ruhling et al. (1981) and Dibblee and Graham (1983) demonstrated that women, aerobically trained prior to pregnancy, can maintain, but not significantly improve, their aerobic capacity during gestation.

Collings and coworkers (1983) reported that $VO_{2max}$ (ml/kg/min) increased 8% ($\bar{X}$) in pregnant women cycling three days/wk for 13 wk at an intensity of 65 to 75% $VO_{2max}$. Pregnancy outcome was not significantly different between the trained and sedentary groups in regard to labor duration, Apgar scores, or fetal growth. Erkkola (1976) found that physical work capacity (PWC) was increased 18%, while HR and SBP decreased slightly in nontrained pregnant women performing various aerobic activities for approximately 25 wk. The exercises were performed 3 days/wk at an intensity which produced a HR of 140 bpm.

Dressendorfer and Goodlin (1978) in a case study of a 27 year old nontrained pregnant woman, demonstrated that functional aerobic capacity was improved 10% with an average of 24.2 km of jogging/wk. The weight bearing exercise, which was conducted throughout pregnancy, produced no harmful effects on either the mother or child.

Jarrett and Spellacy (1983) and Dale et al. (1982), using retrospective questionnaire methodologies, investigated the effects of running during pregnancy on birth outcome. Jarrett and Spellacy concluded that healthy pregnant women who were accustomed to running could continue their activity during pregnancy without harm to the infant. The data also indicated that the number of km run during pregnancy decreased as pregnancy progressed. Dale and coworkers (1982) found no significant differences between runners with respect to maternal weight gain and neonatal delivery weight. However, the authors did state that there was a suggestive trend in the runners of failure to progress (pitocin augmentation) during labor, resulting in increased Cesarean section rate.

Other investigators have shown that endurance exercise training during pregnancy may have a detrimental effect on the fetus. Clapp and Dickstein (1984) prospectively studied the effects of endurance exercise during pregnancy on fetal outcome. In contrast to the results of investigators reporting no influence of endurance exercise training on fetal outcome (Dressendorfer and Goodlin, 1978; Collings et al., 1983; Dale et al., 1982; Jarrett and Spellacy, 1983), Clapp and Dickstein (1984) suggested that pregnant

women who maintained regular vigorous exercise through the third trimester had an increased incidence of lower birth weight infants (~500 g), shorter gestational periods (~8 days), and less pregnancy weight gains (~4.6 kg). The authors further showed that when women reduced strenuous exercise prior to 28 wk, the incidence of these problems was less. Therefore, important factors to consider when evaluating the consequences of a prenatal training program are: 1) how long into the gestational period are the women training (i.e. 28 wk or 35 wk), and 2) what is the performance level during that gestational period.

Recent studies of Tafari et al. (1980) and Naeye and Peters (1982) demonstrated that women who performed physical labor during pregnancy had infants with lower birth weights than infants of women who were less active. Tafari et al. measured birth weights, maternal weight gains, and energy intakes of women who performed hard physical labor and those who performed light work only. The mothers who engaged in heavy physical labor and had energy intakes below 70% of the WHO/FAO recommended standards, had infants with $\bar{X}$ birth weights of 3.06 kg. In comparison, women on similar caloric diets, but less physically active, had $\bar{X}$ infant birth weights of 3.72 kg. Naeye and Peters (1982) analyzed the data from 7,722 pregnancy outcomes and concluded that women who worked (standup type) in the third trimester had newborns who weighed 150 to 400 g less than newborns of women who stayed at home. Inadequate blood flow from the uterus to the placenta was suggested as one possible cause for the fetal undergrowth.

## Exercise Testing and Fetal Heart Rate

Measurement of fetal heart rate (FHR) in response to maternal exercise testing has been utilized as a possible indicator of uteroplacental insufficiency (Pomerance et al., 1974b; Collings et al., 1983; Hauth et al., 1981; Dressendorfer and Goodlin, 1980; Dale et al., 1982). Pomerance and coworkers (1974b) monitored the FHR in 54 pregnant women before and after bicycling to screen for uteroplacental insufficiency. The exercise testing was administered between 35 to 37 wk of gestation, and lasted six to eight min until the women reached a HR between 130 and 180 bpm. Four of 11 infants that were found to be stressed during labor and delivery, had altered FHR of $\pm$16 bpm after exercise. Dressendorfer and Goodlin (1980) examined FHR using the ultrasound method in five trained pregnant women. Graded cycling exercise, at an intensity which raised maternal HR to 80% maximum, resulted in an increase in FHR of 7 bpm. The authors stated that the increase in FHR was within the normal range (120 to 160 bpm) and, therefore, strenuous aerobic exercise did not tend to produce fetal bradycardia or tachycardia. Collings et al. (1983) also studied the effects of cycling exercise on FHR in nontrained women and observed small increases in FHR (144 to 148 bpm). Similar to Dressendorfer and Goodlin's observations, the FHR values were within the normal range (120 to 150 bpm) and there were no detrimental effects of the slight FHR increased on pregnancy outcomes.

In contrast to Dressendorfer and Goodlin (1980) and Collings et al. (1983), and similar to Pomerance et al. (1974), Clapp (1985) reported FHR elevations of approximately 16 bpm during treadmill running in six trained pregnant women. However, the highest reported FHR of 166 bpm, dropped 12 bpm by 10 min post exercise, and was still considered relatively close to the normal range. Dale and coworkers (1982) reported a transient fetal bradycardia in three women during treadmill running, with the fluctuating patterns returning to normal within the exercise session.

## Weight Changes With Exercise During Pregnancy

Recommendations regarding exercise during pregnancy are sometimes related to the possible benefit of weight gain control. Hutchinson et al. (1981) reported a weight gain of 8.5 kg for a 32 year old pregnant runner, a value significantly below the recommended weight gain (12.7 kg). Collings et al. (1983) however, described an average weight gain of 15.8 kg for 12 pregnant women who cycled for 13.4 wk, a value significantly higher than the sedentary controls (14 kg). Clapp and Dickstein (1984) reported $\bar{X}$ weight gains of 14.6, 16.8, and 12.2 kg in sedentary, active, and extremely active pregnant women, respectively. Erkkola (1976) found that 31 pregnant women, trained through various aerobic exercises throughout pregnancy, had $\bar{X}$ weight gains of 12 kg. Apparently the effects of exercise on weight gain during pregnancy

are dependent on the intensity and duration of the exercise, as well as the type of exercise (weight vs nonweight bearing).

## Summarization of Literature

There are still many questions that need to be answered regarding the effects of exercise during pregnancy. Future research should investigate the effects of exercise during pregnancy on nutritional status of both the mother and infant. Studies should examine various exercise protocols and include intensity, frequency, and duration of exercise, whether weight bearing was involved, fitness of the subject prior to and during pregnancy, progression of exercise through the gestational period, and FHR response. There are limited data regarding aerobic capacity with exercise during pregnancy, as well as limited information on labor and delivery parameters. In order to provide adequate guidelines for physicians regarding exercise during pregnancy and to protect the mother and fetus from potentially hazardous complications, more studies are needed. Therefore, this study was conducted to provide additional information on the effects of an aerobic training program (walking) during pregnancy on aerobic capacity, riboflavin and thiamin status, and pregnancy outcome. Not only will aerobic fitness be assessed using a weight bearing exercise test during two different trimesters (2nd and 3rd), but activity levels of the women will also be assessed using activity questionnaires. Unfortunately, due to time and equipment

limitations, the present study cannot evaluate, on a long term basis, the effects of aerobic exercise during pregnancy, or monitor FHR response to maternal exercise.

# CHAPTER III

# MATERIALS AND METHODS

## Subject Recruitment Procedures

Twenty eight healthy Caucasian pregnant women in their 2nd trimester (~22 weeks) were recruited from the Blacksburg, Christiansburg area following approval of the study by the Institutional Review Board (IRB) for Research Involving Human Subjects. Subjects were recruited from prenatal exercise classes through the Free University, Childbirth Education (Lamaze), and private physicians referral from September, 1984 to August, 1985. In addition, recruitment notices were announced in the Spectrum Newspaper, the University Newspaper (The Collegiate Times), and the Roanoke Times and World News. Flyers (Appendix A) describing the study were placed in strategic locations in Blacksburg resulting in many word of mouth referrals.

The women ranged in age from 24 to 36 yr and were apparently free from any illness, disease, or physical limitation, a prerequisite necessary to participate 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 inclusion of the physicians' signatures were necessary to ensure their knowledge of the experimental protocol, potential risk to the subjects, and their patient's participation. The women were told prior to participation that they could withdraw

from the study at any time. Also, all potential subjects were asked to complete a pre-experimental questionnaire (Appendix C) which provided information regarding prenatal history, vitamin-mineral supplementation, activity level, and general information (adapted from Christakis, 1973).

In order to provide some control on vitamin intake and to provide compensation for participating in the study, all subjects were given a prenatal vitamin-mineral supplement (Natalins Rx), approximately one month before the beginning of the experimental period (22 wk). The vitamins were provided by Meade-Johnson Pharmaceutical Division, Meade Johnson & Company, Evansville, IN; the composition of the supplement is listed in Appendix E.

## Experimental Design

After approval by the IRB, subjects were recruited and assigned to one of the two groups by their willingness to participate in either group. The two groups included the exercise or walking group (W) and the nonwalking group (NW). Once approval was obtained from each subject and their private physician for participation, the subjects were scheduled for exercise testing, blood collection, and completion of dietary and activity questionnaires. Exercise testing, blood collection and administration of questionnaires were conducted on both W and NW groups at approximately 22 and 30 wk of pregnancy. Those who selected the W group participated in a walking program from 22 to

30 wk of pregnancy. Those subjects assigned to the NW group tried to maintain their same activity level without participating in the walking program.

## Physical Activity Level Assessment

During the 22nd and 30th wk of pregnancy, the subjects were asked to respond to a questionnaire which listed the activities they had regularly participated in over the last 12 months (Appendix D). The questionnaire used is a modification of a method used by Reiff et al. (1967). The activity assessment helped determine the activity level of the subjects prior to and during the eight 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 activity scores for each individual were determined using the following formula:

Score = activity factor x h/wk (Reiff et al., 1967).

Activity factor is a value of the approximate MET level for various activities; values used were reprinted by the American College of Sports Medicine from Fox et al. (1972). Hours/wk of activity was determined by the following formulas: [(#mo x min)/60]/52 = h/wk if frequency of activity performed was monthly, not weekly; however if activity was performed on a weekly basis then the formula

[#mo x (#days/wk x min/day x 4)/60]/52 = h/wk

was used. After determination of the activity score for each activity, all scores were added to give the total activity score

for each subject. The total aerobic activity score was calculated as the sum of activity scores for those activities which were considered to be aerobic (ACSM, 1980).

## Dietary Assessment

Each subject completed a two-day dietary intake questionnaire and a 24 h recall (Appendix F and G) at 22 and 30 wk of pregnancy, to provide estimates of riboflavin, thiamin, and kcal intake (Sanjur, 1982; Christakis, 1973). The data base utilized to estimate vitamins and kcal intakes was derived from the old and updated version of the Agriculture Handbook No. Eight, Agriculture Research Division, United States Department of Agriculture (1963; 1976-84). The two-day dietary records included instructions and an example to help assure more accurate records (Sanjur, 1982). Food models were used when conducting the 24 h recalls to help subjects better estimate serving sizes.

## Exercise Testing

Each subject performed two submaximal exercise tests; one initial baseline test during the 2nd trimester (22 wk) and a 2nd test after the exercise program during the 3rd trimester (30 wk). A pilot study was conducted on one subject to determine the appropriate submaximal exercise testing protocol. The exercise test selected consisted of treadmill walking using a modification

of the Balke-Ware maximal exercise protocol (Froelicher, 1983) [Appendix H]. All subjects reported to the Health, Physical Education, and Recreation (HPER) Human Performance Laboratory one wk prior to the test in order to familiarize themselves with the equipment. Familiarization included walking on the treadmill, use of mouthpiece and noseclip, explanation of rate of perceived exertion (RPE), and general explanation of the test. Familiarization with the exercise equipment and the Borg RPE scale was necessary in order to try and reduce stress in the subjects and elicit more accurate data. Each subject was then instructed to meet at the Human Performance Lab on the day of their test and to allow sufficient time so that they would be as relaxed as possible.

After arriving, electrodes were placed on each subject with the standard 12-lead for the HR determination using electrocardiogram recordings (Froelicher, 1983). The subjects were then instructed to lay down and while resting, supine HR and BP were determined. Standing resting HR and BP were also recorded. Subjects walked on the treadmill at 4.02 km/h, beginning with a 0% grade and increasing 2.5% every two min (Appendix H). MET levels for minutes and % grade of exercise are also listed in Appendix H (ACSM, 1980).

HR, $V_E$, fraction of expired $O_2$ ($F_{EO_2}$), fraction of expired $CO_2$ ($F_{EO_2}$) and RPE were recorded every min, while BP was taken every two min. $F_{EO_2}$ and $F_{ECO_2}$ were determined by a S-3A Oxygen Analyzer and CD-3A Carbon Dioxide Analyzer, respectively, from Ametek, Thermox Instruments Division, Pittsburgh, PA. $V_E$ was measured using a Hewlett Packard model

46303-A Digital pneumotachometer from High Point, NC. Subjects exercised to either a HR that approximated 80% of their age predicted maximum HR (~150 bpm) [Fox, 1981], an RPE reading of 15, or until they requested to stop the test. After termination of the treadmill walking, subjects laid down and HR and BP values were recorded for four consecutive min post exercise.

In addition, immediately after the exercise, a small amount of blood (0.1 ml) was obtained via fingerprick for determination of lactic acid. A Model 23-L Lactate Analyzer from Scientific Division, Yellow Springs, OH was used for analyses. At least two values were obtained and an average recorded. The exercise tests were completed when each subject was weighed.

## Exercise Program

Exercise was individually prescribed for each woman participating in the exercise group based on their age predicted maximum heart rate. The target HR were calculated from a selected percentage (70%) of the maximum HR (Fox and Mathews, 1981). The subjects walked a minimum of three times/wk for approximately 30 min duration, at an intensity which approximated 70% of their maximum HR. The walking sessions took place on campus and at other convenient locations for the subjects. Several women in the study arranged their walking schedules with each other to assure adequate motivation. The walking sessions were initially supervised and then it was the responsibility of the subjects to continue the

walking program at the prescribed pace. Before the exercise program began, each woman was instructed on how to quickly approximate their HR by the palpitation method (Pollock et al., 1972). At the end of 30 min of walking the subjects would check their pulse rate for 10 sec and then multiply that value by six to approximate bpm. Subjects were also asked to maintain records of the frequency and duration of their walking program and the HR response. The length of the walking program was eight wk.

All of the subjects participating in the walking program discussed with the author, during the activity questionnaire interview, their success or difficulty in maintaining the requirements of the walking program. Those subjects that did not maintain the minimum requirements for the walking program of three days/wk, for 30 min a session, at 70% of their maximum HR, were not included in the W group. Participation in the walking program was documented and recorded in the 30 wk activity questionnaire.

## Blood Collection Procedure

Blood collection was conducted on both W and NW groups at the beginning (22 wk) and end (30 wk) of the eight wk walking session. A qualified technician obtained 20 ml of blood by venipuncture. Vacutainers with Ethylenedinitrilotetraacetic acid (EDTA) added as an anticoagulant were utilized to collect the blood and samples were kept in 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. After centrifugation, the plasma was removed and frozen for further analyses. The erythrocytes were washed with five times the volume of cold 0.9% sodium chloride solution and centrifuged at 2000xg for 15 min at 0-5°C (Glatzle et al., 1970). After centrifugation, the cold saline solution (supernatant) was discarded. This washing procedure was repeated twice more to ensure adequate removal of the buffy coat. After the erythrocytes had been washed three times, 10 ml of cold deionized water were added to 0.3 ml of erythrocytes. The erythrocyte solution was vortexed and centrifuged at 0-5°C for 15 min at 2000xg. The supernatant was then transferred to four dark colored glass vials and frozen for riboflavin and thiamin analyses.

## Biochemical Analysis

Riboflavin measurements. Riboflavin status was determined by a modification of Glatzle and coworkers' (1970) method utilizing EGRAC measurements (Appendix I) [Fordyce, 1985]. The EGRAC data represent the degree of stimulation from the in vitro addition of FAD.

Frozen erythrocytes were thawed the day of analysis and 0.1 ml was used for analysis. Once a sample was thawed, it was used that day or discarded. Each day of analyses, 2.0 mM B-nicotinamide Adenine Dinucleotide Phosphate, Reduced Form (B-NADPH), 0.25 mM FAD, and 7.5 mM Glutathione Oxidized Form (GSSG) were prepared

fresh. Potassium phosphate buffer, 0.1 M, was also prepared, refrigerated, and heated to the optimal reaction temperature of 37°C before use. EDTA, 80 mM, was prepared fresh each wk. All chemicals were ordered form Sigma Chemical Co., St. Louis, MO.

Triplicates were run on both samples with and without added cofactor (FAD). The FAD stimulated tubes had each solution added in the following order: 0.1 ml FAD, 2.2 ml phosphate buffer, 0.05 ml EDTA, 0.1 ml oxidized GSSG, and 0.1 ml hemolysate. The tubes without FAD contained the same except that the 0.1 ml of FAD was replaced by 0.1 ml of deionized water. After 0.1 ml of hemolysate was added to the first tube, the sample was covered with parafilm, vortexed, and placed into the 37°C water bath for 20 min. The hemolysate was then added to the other samples staggered by one min intervals between each and the same procedure followed.

Variability in absorbance readings was reduced with careful pipeting of the hemolysate. Two tubes containing 0.1 ml hemolysate and 2.5 ml of phosphate buffer were used as blanks to zero the spectrophotometer. After the 20 min incubation period, 0.1 ml NADPH was added to each tube, vortexed, and absorbance immediately measured at 340 nm on a Bausch and Lomb Spectronic, 2000 Double-Beam Spectophotometer from Bausch-Lomb Analytical Systems Division, Rochester, NY. Each sample was timed after the initial reading and then measured again after 10 min. Between each sample reading the Peltier Thermoelectric Flowcell, which was utilized to maintain samples at 37°C, was cleaned with 37°C phosphate buffer to prevent sample carry-over.

In order to determine the EGRAC, the final absorbance was subtracted from the initial absorbance to determine the change in absorbance ($\Delta$Abs) over a 10 min period. The $\bar{X}$ of the $\Delta$Abs was taken for the three tubes with added FAD and this value divided by the $\bar{X}$ $\Delta$Abs of the three samples without added FAD. EGRAC values of 1.00 to 1.19, 1.20 to 1.39, and greater than 1.40 represented adequate, marginal, and deficient riboflavin status, respectively (Glatzle, 1970). A sample was re-run if there was a greater than 5% error among triplicates.

Thiamin measurements. Thiamin status was determined by a modification of Smeets and coworkers' (1971) method utilizing ETKAC measurements (Appendix J) [Fordyce, 1985]. The ETKAC values represented the degree of stimulation from the in vitro addition of TPP.

Frozen erythrocytes were thawed the day of analysis and 0.075 ml was used for analysis (0.1 ml for blank). In order to keep absorbance readings within a reasonable range, the lesser amount of hemolysate was used and brought up to volume with Tris buffer. Once a sample was thawed it was used that day or discarded.

TPP 10 mM, 10 mM B-Nicotinamide Adenine Dinucleotide Reduced Form (B-NADH), and $\alpha$-glycerophosphate-dehydrogenase triosphosphate isomerase, type III ($\alpha$GDH-TPI), were prepared fresh each day, while ribose-5-phosphate (2.5% solution) and 0.1 M Tris buffer were prepared and stored until needed. The ribose-5-phosphate which was stored frozen, was heated to 37°C before use. All chemicals were ordered from Sigma Chemical Co., St. Louis, MO.

Triplicates were run on both sets of samples with and without added TPP. The TPP stimulated tubes had each solution added in the following order: 0.1 ml TPP, 2.42 ml ribose-5-phosphate, 0.075 ml hemolysate, 0.02 ml GDH-TPI, and 0.10 ml B-NADH. The tubes without TPP were the same except that the 0.1 ml TPP was replaced with 0.1 ml extra ribose-5-phosphate. After 0.1 ml of NADH was added to the first tube, the sample was covered with parafilm, vortexed, and placed into a 37°C water bath for 15 min. The NADH was then added to the other samples staggered by 30 sec intervals between each and the same procedure followed. Two tubes containing 0.1 ml of hemolysate and 2.52 ml of Tris buffer were used as blanks to zero the spectrophotometer. After the 15 min incubation period each tube was removed individually from the water bath, absorbance measured on the Bausch and Lomb Spectronic 2000 at 340 nm, timed, and placed back in the water bath for 25 min. Between each reading Tris buffer was used to clean the flowcell to prevent sample carry-over. Similar to the EGR methodology, the thermoelectric flow cell was utilized to maintain the 37°C temperature of the samples while measuring absorbances. After the 25 min incubation period samples were removed and absorbances again measured.

In order to determine the ETKAC, the final absorbance was subtracted from the initial absorbance to determine the change in absorbance ($\Delta$Abs) over the 25 min incubation period. The $\bar{X}$ of the $\Delta$Abs was taken for the three tubes stimulated with TPP and this value divided by the $\bar{X}$ $\Delta$Abs of the three samples without added TPP. Similar to EGRAC, values of 1.00-1.19, 1.20-1.39, and

greater than 1.40 represented adequate, marginal, and deficient thiamin status, respectively (Fordyce, 1985). A sample was re-run if there was a greater than 5% error among triplicates.

## Pregnancy Outcome

Each subject was given a postpartum questionnaire after their 30 wk exercise test and asked to complete and return the form after delivery (Appendix K). The questionnaire included parameters similar to those reported by Dale et al. (1982) and Collings et al. (1983). The parameters were weight gain (kg) during pregnancy, infant birth weight (kg), infant length (cm), one and five min Apgar scores, and duration of 1st and 2nd stages of labor (min).

## Statistical Methods

All data were analyzed by the Statistical Analysis System (SAS) [SAS Institute Inc., 1985]. Mean values and standard deviations (SD) were calculated for all parameters. Analysis of variance (ANOVA) was used for statistical comparison of all variables between W and NW at 22 and at 30 wk.

Paired "t" tests (Hinkle et al., 1979) were used for within group comparisons of data at 22 wk and data recorded at 30 wk, after the 8 wk experimental period. The "t" test was used to determine the statistical significance between changes in dependent variables in the W group compared to the changes for the NW group.

In order to determine the significant differences between HR values at rest, two, four, and six min, as well as $VO_2$ at two, four and six min, repeated measures analyses were used (Morrison, 1976). Pearson product moment correlation coefficients were also used to determine if relationships existed between all variables. Correlations were considered significant at the 0.05 level or lower.

# CHAPTER IV

# RESULTS AND DISCUSSION

## Subject Description

Twenty eight Caucasian pregnant women volunteered for the study. Individual data for each variable measured in this study at 22 and 30 wk and at postpartum are listed in Appendices L, M, and N.

Table 1 lists the age, height, and weight values for the pregnant women participating in either the W or NW groups. The ages and heights of the subjects in both the W and NW groups were similar, with no significant differences between the two groups. The height/weight values, for all subjects, based on the publication by the Metropolitan Insurance Company (1983) on recommended height/weight, approached the recommended ranges for medium frame females 25 yr of age or older. There were no significant differences in prepregnancy weight values or weight values at 22 and 30 wk gestation. Subjects in both groups had a significant increase ($p<0.0001$) in body weight from 22 to 30 wk. The increase in weight for the W group was nonsignificantly greater than the weight gain for the NW group.

Collings and coworkers (1983) reported age and height values for 20 pregnant women and their data were similar to the values listed in Table 1 for this study. The authors, however, did not report

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 |
| Non-walking (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 ($p<0.0001$).

pregnancy weight gain values for the experimental period of 22 to 34 wk gestation.

Due to the readily available population within Blacksburg and the University, recruitment of the subjects was focused on the immediate area. Also, subjects were required to meet at the University to participate in the exercise tests and blood sampling. As a result, most subjects were from the immediate area and had similar economic, educational, and occupational backgrounds. All subjects participating in the present study except one had at least two years of college education, and most had graduated from four year universities. Individual occupations included secretaries, students, and homemakers. If subject recruitment had been conducted in a metropolitan area or areas surrounding Blacksburg, a more diverse pregnant population may have been obtained. Unfortunately, pregnant women who abstained from vitamin-mineral supplements were not available for this study.

The study was initiated at 22 wk of pregnancy to avoid a potential high risk period, since most miscarriages occur earlier in the 1st trimester of pregnancy. Also, many pregnant women experience nausea or vomiting early in pregnancy, and the 22 wk starting period avoided this problem. During the eight wk experimental period, none of the subjects reported any symptoms of nausea or other signs of distress during exercise testing or the walking program.

Three subjects reported previous miscarriages, while the other 25 subjects indicated no history of obstetrical complications. The

subjects reporting a previous miscarriage were allowed to participate in the study only through the consent of their physicians.

## Physical Activity Assessment

Total activity scores. Women were asked to respond to questions regarding their total activities including occupational and recreational related physical activities for the past year. The time span of one year was used to calculate the activity score in an attempt to control for any seasonal differences.

There were no significant differences in total activity between W and NW groups, perhaps the result of large SD, even though the W group had higher activity scores (Table 2). Both W and NW groups showed a nonsignificant decrease ($p<0.37$, $p<0.63$, respectively) in total activity from 22 to 30 wk gestation. One major component of the total activity score for most subjects was housework and this activity score appeared to remain constant for most subjects from 22 to 30 wk of pregnancy. Other activities contributing to the total activity scores included bicycling, swimming, dancing, running, calisthenics, stretching, and farming.

Blackburn and Calloway (1976b) reported activity patterns of mature pregnant women from 20 to 40 wk of gestation. Occupations of the pregnant women in his study were similar to those in the present study and included mostly students, homemakers, and secretaries. Similar to the present study, house cleaning did not significantly decrease throughout gestation; however, walking and

TABLE 2

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 are $\bar{X} \pm SD$.

cooking for subjects in Blackburn's study appeared to decline but not significantly. Review of NW subjects' activity records in the current study indicated that the small amount of walking was maintained throughout the eight wk experimental period. Blackburn and Calloway did not report recreational activity patterns of the subjects.

Approximately 20 of 28 subjects in the present study noted that after 30 wk gestation their involvement and pace in activities began to decrease. Jarrett and Spellacy (1983), and Dale et al. (1982), through the use of activity questionnaires, demonstrated that the number of miles run by pregnant women significantly decreased from the 2nd trimester until term. Therefore, even though there were no significant decreases in total activities from 22 to 30 wk gestation in the present study, the possibility exists that from 30 wk gestation to birth that there may have been a decrease in total activity.

Aerobic activity scores. Listed in Table 3 are the total aerobic activity scores for both W and NW groups. There were no significant differences between W and NW groups; however, the W group tended to have higher values at both 22 and 30 wk ($p<0.20$ and $p<0.09$, respectively). Similar to the total activity scores, large SD may be responsible for the lack of significant differences. Even with the slight increase in aerobic activity with W and the small decrease for NW from 22 to 30 wk, there were no significant differences ($p<0.18$), when comparing changes in aerobic activity scores between W and NW. The walking program used in this study

TABLE 3

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 are $\bar{X} \pm SD$

[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 ($p<0.18$).

was designed to provide minimal frequency, duration, and intensity, necessary to improve cardiovascular fitness and to avoid potential obstetrical complications. Therefore, the walking program contributed only slightly to the total aerobic activity score. A typical woman participating in the walking program three times/wk, for two mo, 30 min/session, at 5.6 km/h, could expect an activity score increase of 1.15 (Reiff et al., 1967). Therefore, other aerobic activities could possibly mask the walking activity scores of the subjects. A pregnant woman participating in the walking program in the current study, could possibly have had a decrease in aerobic activity score, even with the added score from the walking program, if other aerobic activities were decreased. The aerobic activity score in the present study may be more useful for interpretation of changes in aerobic activity levels when the training program is more rigorous and results in a larger individual score.

## Dietary Assessment

Two-day dietary records and 24 h recalls were compared using paired "t" tests to determine if riboflavin, thiamin, and energy intakes were significantly different. The paired "t" comparisons between 24 h recall and two-day dietary records were conducted at both 22 and 30 wk. There were no significant differences between the two-day records and the 24 h recalls, and, as a result, both were combined and three day averages for riboflavin, thiamin, and

energy intakes were used in statistical analyses. The intakes of riboflavin, thiamin, and energy for W and NW at 22 and 30 wk are reported in Table 4. There were no significant differences in riboflavin, thiamin, or energy intakes between W and NW at 22 wk. At 30 wk, vitamin intakes were similar; however, energy intakes were higher ($p<0.05$) for subjects in the W group vs subjects in the NW group. From 22 to 30 wk gestation, there were no significant changes regarding intakes of riboflavin, thiamin, or energy for W and NW subjects.

Riboflavin and thiamin requirements were also expressed on the basis of caloric intake (mg/1000 kcal), since these vitamins have roles in energy metabolism (NRC, 1980). Riboflavin intakes for women participating in the W group were approximately 1.2 mg/1000 kcal and 1.1 mg/1000 kcal at 22 and 30 wk, respectively, which were close to 160% of the RDA for this nutrient (~0.7/1000 kcal) [NRC, 1980]. Similarly, $\bar{X}$ intakes for riboflavin of 1.1 mg/1000 kcal and 1.0 mg/1000 kcal, for subjects in the NW group (22 and 30 wk, respectively), were also higher than the RDA (~150%). In contrast, thiamin intakes of women in W group were approximately 100% of the RDA or 0.6 mg/1000 kcal. For NW subjects, intakes of thiamin were approximately 0.65 mg/1000 kcal and 0.59 mg/1000 kcal at 22 and 30 wk, respectively. The recommended energy intake for pregnant women 23-50 yr, is approximately 2300 kcal (NRC, 1980). Mean energy intakes for women in the W group at 22 and 30 wk (Table 4) approached the recommended intake; however, women in NW group had adequate intakes at 22 wk and lower than the recommended energy intake at 30 wk.

TABLE 4

Dietary intakes of riboflavin, thiamin, and energy for walkers and nonwalkers at 22 and 30 wk gestation

| Group | 22 wk | | | 30 wk | | | Δ | | |
|---|---|---|---|---|---|---|---|---|---|
| | riboflavin (mg) | thiamin (mg) | energy (kcal) | riboflavin (mg) | thiamin (mg) | energy (kcal) | riboflavin (mg) | thiamin (mg) | energy (kcal) |
| Walking | 2.74 ± 0.88 | 1.38 ± 0.34 | 2273.8 ± 403.8 | 2.55 ± 0.83 | 1.37 ± 0.37 | 2328.2[a] ± 425.1 | -0.18 ± 0.59 | -0.01 ± 0.41 | 54.4 ± 476.3 |
| Nonwalking | 2.50 ± 1.48 | 1.47 ± 0.79 | 2251.9 ± 522.1 | 2.09 ± 1.26 | 1.17 ± 0.39 | 2025.1[a] ± 296.0 | -0.24 ± 0.67 | -0.28 ± 0.62 | -112.0 ± 402.7 |

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

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

Riboflavin and thiamin intakes for subjects in this study were comparable or slightly higher than the reported intakes of 2.2 mg (riboflavin) and 1.1 mg (thiamin) for a reference 70 kg pregnant women (Lu et al., 1981). Energy intakes for pregnant women in this study were higher than the reported $\bar{X}$ values of 2065 kcal (Blackburn and Calloway, 1976a) and 1500 kcal (Dibblee and Graham, 1983).

Dietary intakes of riboflavin, thiamin, and energy appeared to be adequate when compared to the RDA's for the nutrients. Even with the decreased caloric intake for NW subjects at 30 wk, riboflavin and thiamin intakes (mg/1000 kcal) were adequate and the $\bar{X}$'s approached 100% of the RDA. Vitamin supplements, which contained 3.00 mg of riboflavin and 2.55 mg of thiamin (Appendix E), also contributed to the total intake for these nutrients, but were not included in the estimations given in Table 4.

## Exercise Testing

Including both exercise tests at 22 and 30 wk, 91.1% of the subjects exercised to 80% of their maximum HR, 7.1% to an RPE of 15, and 1.8% until they requested to stop the test.

The subjects who exercised to an RPE of 15 were in the W group, while the one subject who requested to stop the test was in the NW group.

HR measurements. The HR responses at rest and during two, four, and six min of submaximal treadmill walking are listed in

Table 5 for both W and NW groups. The HR responses for all subjects during submaximal exercise appeared normal as both groups showed gradual HR increases ($p<0.05$) with elevated intensities. However, at 30 wk gestation, there were no significant differences between HR at two and four min of exercise for both groups.

There were significant correlations between resting HR, and HR at two min, four min, and six min of exercise for W at both 22 and 30 wk (Table 6). Similarly, there were significant correlations for NW between HR at two min, four min, and six min of exercise at 22 and 30 wk gestation; however, resting HR was not significantly correlated with the HR at two, four, or six min during 22 wk gestation, but was significantly correlated with HR at four min of exercise at 30 wk. Regardless, most HR values appeared to be positively correlated, and suggested that subjects apparently had a normal physiological HR response to increased intensity of exercise.

Resting HR values for the W and NW groups at 22 wk of gestation were similar to the $\bar{X}$ HR values of 85, 81, and 82 bpm, reported by Collings et al. (1983), Knuttgen and Emerson (1974), and Blackburn and Calloway (1985), respectively. The differences in resting HR between W and NW groups at 22 wk were not significant; however, at 30 wk gestation, the resting HR approached statistical significance ($p<0.07$) [Table 5]. The increases in $\bar{X}$ resting HR, of 4.12 bpm and 7.80 bpm, for W and NW respectively, were not statistically significant regardless of the lower HR increase in the W group. Similar to data reported by Erkkola (1976), Clapp (1985), and

TABLE 5

Comparison of heart rate measurements at rest and during two, four and six min of a submaximal treadmill walking test[1] for walkers and nonwalkers at 22 and 30 wk gestation

| 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[bcl] ± 12.2 | 139.1[fi] ± 9.2 | -2.83[o] ± 10.86 | 4.44[o] ± 11.98 |

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

[1]Conducted at 4.02 km/h with a 2.5% grade increase every two 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 $p<0.13$.

TABLE 6

Heart rate correlation coefficients[1] for walkers and nonwalkers at 22 and 30 wk gestation

| HR | Walkers | | | | Nonwalkers | | | |
|---|---|---|---|---|---|---|---|---|
| | 22 wk | | 30 wk | | 22 wk | | 30 wk | |
| | r | p | r | p | r | p | r | p |
| rest–2 min | 0.47 | 0.05 | 0.72 | 0.001 | 0.18 | NS | 0.26 | NS |
| rest–4 min | 0.56 | 0.02 | 0.66 | 0.004 | 0.16 | NS | 0.65 | 0.04 |
| rest–6 min | 0.46 | 0.06 | 0.56 | 0.02 | 0.55 | NS | 0.58 | 0.08 |
| 2–4 min | 0.76 | 0.0002 | 0.83 | 0.0001 | 0.95 | 0.0001 | 0.63 | 0.05 |
| 2–6 min | 0.67 | 0.003 | 0.86 | 0.0001 | 0.87 | 0.003 | 0.52 | NS |
| 4–6 min | 0.82 | 0.0001 | 0.88 | 0.0001 | 0.93 | 0.0003 | 0.76 | 0.01 |

[1]Correlation coefficients of heart rate responses during a submaximal treadmill walking test at 4.02 km/h with a 2.5% grade increase every two min.

Blackburn and Calloway (1985), as pregnancy progressed resting HR increased and was within the reported range of three to seven bpm.

There were no significant differences between W and NW in HR response at two, four, or six min of walking during the 22 wk exercise test. However, at 30 wk gestation, the HR for W were significantly lower at two ($p<0.05$) and four min ($p<0.05$) of exercise and approached significance at six min ($p<0.07$). Hence, the author concluded that subjects in the W group tended to be working less than NW subjects for a given exercise intensity during the 30 wk walking tests. Subjects in the W group had nonsignificant decreases in HR for two, four, and six min of exercise after the 8 wk walking program, while NW subjects had nonsignificant increases at four and six min and a significant increase at two min ($p<0.05$) of exercise. Comparing HR changes between W and NW groups, values were significantly different at two min ($p<0.05$) and approached significance at four ($p<0.07$) and six min ($p<0.13$) of exercise.

Several investigators have suggested that pregnant women participating in a recommended training program can improve their aerobic capacity similar to their nonpregnant counterparts (Collings et al., 1983; Dressendorfer and Goodlin, 1978; and Erkkola, 1976). HR changes with training include a decreased resting HR, decreased submaximal exercise HR, and an increased maximal HR (McArdle et al., 1981). Resting HR in the W group in the current study did not decrease as a result of the walking program; however, the increased $\bar{X}$ HR (4.12 bpm) was not of the

magnitude of the increased $\bar{X}$ HR for the NW group (7.80 bpm). As mentioned previously, resting HR increases with advancing gestational age (Blackburn and Calloway, 1985). Therefore, if subjects in the W group did actually manifest training adaptations as a result of the walking program, the absence of a lowered resting HR at 30 wk may have been the result of the resting HR increases with pregnancy.

The lower HR response for W at two, four and six min of submaximal exercise during the 30 wk tests indicate the possibility of a HR training effect. In a recent study, Blackburn and Calloway (1985) demonstrated that similar to resting HR, HR increased 3-7 bpm from 20-28 wk gestation, to 29-36 wk gestation, in response to submaximal treadmill walking (4.83 km/h). Therefore, the possibility exists that subjects participating in the walking program in the present study had a slightly improved HR response (Table 5) after the 8 wk walking program, as indicated by the slight decrease to no change in the values. Subjects in the W group may have just maintained or slightly improved their previous HR response to submaximal exercise, while subjects in the NW group probably had a diminished HR response. Similarly, Ruhling et al. (1985) reported that a trained pregnant runner maintained her HR throughout pregnancy for a given exercise load. Guzman and Caplan (1970) also demonstrated that nontrained pregnant women had significant increases in HR of approximately 4 bpm during several stages of cycling exercise from 20 to 35 wk gestation. Collings et al. (1983) suggested that as a result of increased weight, pregnant

women not engaged in regular aerobic activity, show a decreased capacity for aerobic exercise.

Subjects in the W group were trained by walking, an exercise similar to the testing protocol (treadmill walking), thereby accommodating the training specificity recommendation. There is the possibility that if an alternative exercise test was used such as cycling or swimming, HR responses may have been different.

$VO_2$ measurements. Absolute $VO_2$ (l/min) was measured at two, four, and six min of submaximal exercise during the 22nd and 30th wk of pregnancy (Table 7). Similar to the report of Guzman and Caplan (1970), the $VO_2$ increased significantly ($p<0.05$) with elevated intensity of exercise. However, at the lower intensities of exercise (two and four min), there were no significant differences in $VO_2$ for the NW subjects. Significant correlations were determined between $VO_2$ at two, four, and six min of submaximal exercise for both W and NW at 22 and 30 wk gestation (Table 8). Similar to the HR responses, subjects apparently had a normal physiological response to exercise as $VO_2$ levels increased with elevated intensity of exercise.

There were no significant differences in $VO_2$ between W and NW subjects at both 22 and 30 wk gestation. Changes in $VO_2$ from 22 and 30 wk for both W and NW were also reported in Table 7. There were no significant changes in $VO_2$ for W, while the changes in $VO_2$ for NW subjects were also nonsignificant at two and four min but approached significance at six min ($p<0.10$). Comparison of the changes in $VO_2$ from 22 to 30 wk between W and NW indicated

TABLE 7

Comparison of relative $VO_2$ measurements during two, four and six min of a submaximal treadmill walking test[1] for walkers and nonwalkers at 22 and 30 wk gestation

| $VO_2$ | 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[h] ± 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 $\bar{X} \pm SD$.

[1]Conducted at 4.02 km/h with a 2.5% grade increase every two 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 $p<0.10$.

TABLE 8

$VO_2$ correlation coefficients[1] for walkers and nonwalkers at 22 and 30 wk gestation

| $VO_2$ | Walkers | | | | Nonwalkers | | | |
|---|---|---|---|---|---|---|---|---|
| | 22 wk | | 30 wk | | 22 wk | | 30 wk | |
| | r | p | r | p | r | p | r | p |
| 2-4 min | 0.80 | 0.0001 | 0.62 | 0.006 | 0.80 | 0.01 | 0.78 | 0.01 |
| 2-6 min | 0.73 | 0.0006 | 0.64 | 0.004 | 0.72 | 0.03 | 0.43 | NS |
| 4-6 min | 0.86 | 0.0001 | 0.84 | 0.0001 | 0.93 | 0.0003 | 0.78 | 0.01 |

[1]Correlation coefficients for $VO_2$ responses during a submaximal treadmill walking test at 4.02 km/h with a 2.5% grade increase every two min.

differences which approached significance ($p<0.10$) at four and six min of exercise.

Subjects participating in the W group had similar $VO_2$ values for both the 22 and 30 wk exercise tests, regardless of weight gain. Women in the NW group also tended to have similar $VO_2$ values at 22 and 30 wk; however, as indicated, their values approached an increase at six min ($p<0.10$). Guzman and Caplan (1970) reported small nonsignificant increases in $VO_2$ from 20 to 35 wk gestation for nontrained pregnant women during three bicycle ergometer workloads. The lack of significant change in $VO_2$ in Guzman and Caplan's study may have been the result of using a nonweight bearing exercise. Hutchinson et al. (1983), however, demonstrated that the $VO_2$ required to run at 9.6 km/h increased linearally from the 3rd through the 8th mo of pregnancy. The $VO_2$ values paralleled weight gain during pregnancy, which suggested that weight gain may have been responsible for the increased $VO_2$. In the present study, weight gain (Table 1) for W and NW were very similar; in fact, the weight gain for the W subjects were slightly higher ($\bar{X}$, 4.19 vs 3.83). Therefore, if weight gain were responsible for the increased $VO_2$ values during exercise with advanced gestational age, the changes in $VO_2$ from 22 to 30 wk for W and NW may have been more similar.

In order to assess changes in $VO_2$ values and control for weight gain, relative $VO_2$ (ml/kg/min) values at two, four, and six min of submaximal exercise were calculated (Table 9). Similar to the changes in absolute $VO_2$, relative $VO_2$ values increased

TABLE 9

Comparison of relative $VO_2$ measurements during two, four and six min of a submaximal treadmill walking test[1] for walkers and nonwalkers at 22 and 30 wk gestation

| $VO_2$ | METS ($\dot{}$) | 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 $\bar{X}$ ± SD.

[1]Conducted at 4.02 km/h with a 2.5% grade increase every two 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 $p<0.07$.

significantly ($p<0.05$) with elevated intensity of exercise for both groups except between two and four min of exercise in the NW group.

There was a significant difference ($p<0.05$) in relative $VO_2$ between W and NW during 22 wk gestation at four min of exercise, while values at 30 wk were similar for both groups. From 22 to 30 wk, relative $VO_2$ decreased significantly for W at two min ($p<0.001$), four min ($p<0.01$), and six min ($p<0.05$). In contrast, there were no significant changes from 22 to 30 wk for the NW group. Comparing the changes in relative $VO_2$ from 22 to 30 wk between W and NW, there was a significant difference at six min ($p<0.05$) and a near significant different at four min of exercise ($p<0.07$). The lack of significant difference at two min can possibly be explained by the large standard deviation ($\pm$ 2.5). Oxygen consumption during steady state, submaximal exercise is the same or slightly lower with training (Fox and Mathews, 1981). Whether considering the significantly decreased relative $VO_2$ values for W at two, four, and six min of exercise or the comparison of $VO_2$ changes between W and NW (four and six min), the data support the possibility that women participating in the W group had a slight training effect. Blackburn and Calloway (1985) recently demonstrated that $VO_2$ increased from 18.3 ($\bar{X}$) to 19.2 ml/kg/min from 20-28 wk to 29-36 wk gestation during treadmill walking at 4.83 km/h and a 10% grade. Subjects participating in the W group in the present study, and treadmill walking at 4.02 km/h on a 5% grade (6 min), had a significant decrease ($p<0.05$) in

$VO_2$ from 12.4 ($\overline{X}$) to 11.5ml/kg/min from 22 to 30 wk gestation. Therefore, the possibility exists that with endurance training during pregnancy, aerobic capacity can be improved.

Pernoll et al. (1975) stated that increased $VO_2$ during pregnancy may be attributed to the increased work associated with hyperventilation. In a review article Lotgering et al. (1985) suggested that the added weight of pregnancy causes a lower efficiency during treadmill exercise and may result in increased $VO_2$. The improved relative $VO_2$ response to submaximal exercise with subjects in the W group may be associated to training specificity (walking) and enhanced mechanical efficiency during treadmill walking.

As mentioned earlier, Collings et al. (1983) demonstrated that pregnant women not engaged in regular aerobic activity, had decreased functional aerobic capacities (nonsignificant) from 22 to 34 wk gestation. Subjects in the NW group had no change in relative $VO_2$ and an increase in the HR at two min of exercise only from 22 to 30 wk. Therefore, subjects in the NW group appeared to maintain their aerobic capacity over the eight wk training program.

Lactate, exercise test duration, and RPE measurements. Lactate and exercise test duration are reported in Table 10. There were no significant differences in lactate values between W and NW at 22 or 30 wk gestation. However from 22 to 30 wk gestation, lactate values for W increased significantly ($p<0.02$) while no change was observed for NW. Changes in lactate from 22 to 30 wk were compared

TABLE 10

Comparison of lactate, exercise test duration, and RPE for walkers and nonwalkers at 22 and 30 wk gestation

| | 22 wk | | 30 wk | | Δ | |
|---|---|---|---|---|---|---|
| | W | NW | W | NW | W | NW |
| Lactate (mmole/l) | 2.7[a] ± 0.9 | 3.0 ± 0.78 | 3.2[a] ± 1.2 | 2.8 ± 1.3 | 0.62[b] ± 0.86 | -0.33[b] ± 1.26 |
| Duration (min) | 10.3 ± 2.3 | 9.7 ± 1.7 | 10.8 ± 2.7 | 9.2 ± 2.5 | 0.53 ± 1.97 | -0.44 ± 1.94 |
| RPE | 12.8 ± 1.4 | 13.8 ± 1.9 | 13.3 ± 1.8 | 14.2 ± 1.3 | 0.56 ± 2.12 | 0.44 ± 1.01 |

All values $\bar{X} \pm$ SD.

[a] Values in the same line with like superscripts are significantly different at $p<0.02$.

[b] Values in the same line with like superscripts approaches significance at $p<0.06$.

between W and NW and the difference approached significance ($p<0.06$).

There were no significant differences in exercise test duration between W and NW at 22 or 30 wk gestation. Also, no significant changes in duration from 22 to 30 wk were apparent, regardless of the slight increase ($p<0.28$) for W ($\overline{X}$, 10.3 to 10.8).

RPE values, which represented the rate of perceived exertion prior to the termination of the exercise tests, are also reported in Table 10. Subjects in the W group had nonstatistically lower RPE values at 22 wk ($p<0.13$) and 30 wk ($p<0.19$).

Lactic acid production during submaximal exercise decreases with endurance training (Fox and Mathews, 1981). Hence, subjects participating in the W group would have been expected to have a decrease in lactate from 22 to 30 wk. Lactate production is related to intensity of exercise; therefore lactate to MET level (MET level prior to termination of exercise) ratios were calculated in an attempt to explain the increased lactate values for the W group. These data appeared similar to the lactate results listed in Table 10, and therefore, are not reported. Exercise test duration were not held constant at 22 and 30 wk; however, intensity of each test was controlled as subjects were exercised to 80% of their maximum HR. The slight increase in duration from 22 to 30 wk ($\overline{X}$, 10.3 vs 10.8) may possibly explain the significant increase in lactate observed for the W subjects. Subjects in the W group may have reached anaerobic threshold, since training adaptations may have been minimal and anaerobic threshold may not have been increased.

For both groups of subjects, there were significant negative correlations (22 wk , $r = -0.69$, $p<0.0001$; 30 wk, $r = -0.52$, $p<0.006$) between resting HR and duration of exercise. The possibility exists that the women with lower resting HR were better trained for treadmill walking. Similarly, for the W group only, there were significant relationships ($r = 0.57$, $p<0.002$) between duration of exercise and total aerobic activity scores at both 22 and 30 wk. The data indicated that subjects who performed more aerobic activity over the last year could walk for longer periods of time during the treadmill tests.

## Biochemical

Riboflavin measurements. EGRAC values for W and NW at 22 and 30 wk gestation are reported in Table 11. There were no significant differences in EGRAC values in regards to gestational age or participation in the walking program. Similarly, comparing the changes from 22 to 30 wk in EGRAC values between W and NW, there were no significant differences. Ramsey et al. (1983) and Heller et al. (1974) reported $\overline{X}$ EGRAC values in erythrocytes of pregnant women, of 1.31 and 1.17, respectively. The EGRAC values reported in Table 11 approached the values reported by Heller et al., but were lower than those reported by Ramsey et al. In both studies, there was no mention of vitamin-mineral supplementation. The European subjects in Heller and coworkers' study, as well as the Kenyan population in Ramsey and associates' study, probably did

TABLE 11

EGRAC measurements for walkers and nonwalkers at 22 and 30 wk gestation

| Group | 22 wk | 30 wk | Δ |
|---|---|---|---|
| Walking | 1.08 ± 0.09 | 1.07 ± 0.08 | -0.02 ± 0.08 |
| Nonwalking | 1.04 ± 0.08 | 1.06 ± 0.10 | 0.02 ± 0.09 |

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

not consume vitamin-mineral supplements. Lack of subject supplementation in these two studies could possibly explain the discrepancies in EGRAC values between the two studies and the present investigation. Heller et al. also demonstrated that $\overline{X}$ EGRAC values were similar at 19 to 36 wk gestation (1.17), but were marginally deficient (1.20) by term. The author's results support the suggestion that riboflavin requirements increase as pregnancy progresses. Similarly, the data in Table 11 indicated no significant changes in EGRAC from 22 to 30 wk gestation for subjects in the present study; however, no measurements were made after 30 wk and conclusions about riboflavin status for subjects at term cannot be made.

Ramsey and coworkers (1983) reported that 72% of the pregnant Kenyan subjects were biochemically deficient in riboflavin, as indicated by high EGRAC values ($\geq$ 1.20). Heller et al. (1974) also showed that 33% of the women participating in his study were riboflavin deficient at approximately 22 wk gestation and 42%, near term. In the present study, two subjects (W) at 22 wk and three subjects (2 W; 1 NW) at 30 wk, were marginally deficient ($\geq$ 1.20). Both subjects at 22 wk had intakes of riboflavin which met the RDA and total activity (21.5 and 9.9) and aerobic activity (3.1 and 4.3) levels which were not unusually high. One of the two subjects at 22 wk, after the 8 wk walking program, had an EGRAC level in the marginally deficient range. Two other subjects (1 W; 1 NW) at 30 wk had marginally deficient riboflavin status, as indicated by EGRAC values, and both had riboflavin intakes that exceeded the

RDA. One subject (NW), swam almost everyday during pregnancy and had a high total activity score (50.9) and aerobic activity score (25.1). The other subject (W) at 30 wk had a high total activity score (41.3) and a somewhat higher aerobic activity score (12.7), compared to other subjects in the study. She had been engaged in running (16 km/wk) prior to participation in the study, but then eliminated running and participated in the walking program. Only two of the four subjects with EGRAC values indicative of marginal deficient riboflavin status appeared to be extremely active as indicated by total activity and aerobic activity scores. However, the possibility that activity level was associated with EGRAC status exists.

All of the subjects with marginally deficient EGRAC values were given the Natalin vitamin-mineral supplement one mo prior to participation in the study. Therefore, riboflavin intakes which met the RDA, in addition to that in the vitamin-mineral supplement, were not sufficient to prevent marginal riboflavin deficiency which was observed in these four subjects. Evaluation of EGRAC status at term in the present study would have been of interest in determining if the vitamin-mineral supplement would have prevented the onset of biochemical riboflavin deficiency during a time in which Heller and coworkers (1974) reported a greater incidence of deficiency.

Belko et al. (1983) have demonstrated that normal weight nonpregnant women, after beginning an aerobic exercise program, required approximately 0.20 mg/1000 kcal more riboflavin to achieve

normality in regards to EGRAC. The same investigators (Belko et al., 1984) reported that moderately obese women required more riboflavin during exercise (0.80 mg/1000 kcal to 1.16 mg/1000 kcal), independent of weight loss. The results of the present study show no significant effects of a walking program during pregnancy on EGRAC values, but all subjects took vitamin-mineral supplements. There were no significant correlations between EGRAC and other variables measured in the study. The lack of any significant changes in EGRAC may be the result of the vitamin-mineral supplement, but also a minimal aerobic training program. In contrast, the aerobic training programs in Belko et al. studies, were more rigorous, involving jogging or dance five to six days/wk, for approximately 20 min a session. Also, dietary intakes of riboflavin without including the amounts from the supplement, for most of the subjects in the present study, were above the RDA. Future studies investigating the effects of exercise during pregnancy on riboflavin status should include: 1) exercise programs which are slightly more rigorous (increased frequency and duration) than the walking program in the present study; 2) an additional status measurement such as urinary riboflavin excretion after a test dose; 3) inclusion of supplemented and nonsupplemented groups; and, 4) riboflavin status measurements at term. If riboflavin requirements should increase with advancing gestational age (Heller et al., 1974) and with exercise (Belko et al., 1983), there may be a need to examine the effects of vitamin-mineral supplementation in the pregnant population.

Thiamin measurements. ETKAC values are listed in Table 12 for W and NW. Twenty-two wk ETKAC values were similar for W and NW groups; however, at 30 wk gestation, values for subjects in the W group were slightly higher than the NW group and approached significance ($p<0.06$). ETKAC values for W from 22 to 30 wk gestation increased from a $\overline{X}$ value of 0.92 to 0.99, which was a near significant ($p<0.06$) change. No significant changes ($p<0.37$) occurred in ETKAC values for NW over the 8 wk experimental period. Comparison of the changes in ETKAC from 22 to 30 wk between W and NW indicated a difference that approached significance at the $p<0.10$ level.

Heller et al. (1974) reported $\overline{X}$ ETKAC values higher (~1.13) than the $\overline{X}$'s of 0.89 to 0.99 reported in the present study. The discrepancies between their data and ETKAC values from the present study may be explained by vitamin-mineral supplementation of subjects in the current study. The authors made no mention of supplementation, while subjects in the present study were all given Natalin vitamin-mineral supplements. Also, they found no change in $\overline{X}$ ETKAC values from the 7th to the 36th wk of pregnancy. However, as mentioned previously, subjects in the W group in the present study had a slight increase in ETKAC which did approach significance at the $p<0.06$ level. The possibility exists that the slightly elevated ETKAC values may have been the result of participation in the walking program.

Nagy and King (1983) demonstrated that energy expenditure for 400 m of self paced walking, conducted at five wk intervals,

TABLE 12

ETKAC measurements for walkers and nonwalkers at 22 and 30 wk gestation

| Group | 22 wk | 30 wk | Δ |
|---|---|---|---|
| Walking | 0.92[a] ± 0.15 | 0.99[a b] ± 0.15 | 0.06[c] ± 0.13 |
| Nonwalking | 0.93 ± 0.10 | 0.89[b] ± 0.14 | -0.04[c] ± 0.13 |

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

[a,c] Values in the same line or column with like superscripts approaches significance at $p<0.06$.

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

significantly increased ($p<0.05$) from 15 to 40 wk gestation in a longitudinal study of six pregnant women. Increased energy expenditure was explained by the increased body weight and not pace of walking. Blackburn and Calloway (1985) reported that energy expenditure slightly increased for pregnant women during treadmill walking from 20 to 36 wk gestation. Sauberlich et al. (1979) reported that there was a significant relationship between thiamin requirement and caloric intake and expenditure in adult males. Subjects who consumed 3600 kcal/day and at the same time increased their physical activity to maintain their present weight, had increased thiamin requirements as determined by ETKAC and urinary thiamin excretion. Daum and colleagues (1949) also showed that young adults consuming an intermediate to low thiamin diet (0.63 mg/day to 0.20 mg/day), from three to 19 wk, tended to have a decreased maximum work output.

As mentioned previously, TPP is directly involved in energy metabolism as a result of the vitamin's role in the oxidative decarboxylation of pyruvic acid to acetyl-SCoA and $\alpha$-ketoglutarate to succinyl-SCoA. The recommended allowance for energy in pregnant women is increased 300 kcal above nonpregnant requirements (NAS, 1980). Dietary thiamin and energy intakes met or approached the RDA for most of the pregnant women in the present study. Therefore, the increased caloric requirement, in addition to the potential increased energy expenditure of the walking program, may result in increased utilization of thiamin. Even though there may have been increased utilization of thiamin as indicated by the

increased ($p<0.06$) ETKAC value in the W group at 30 wk, most values were still within the range classified as being adequate.

The possibility exist that if subjects participating in the W group had abstained from vitamin-mineral supplementation, ETKAC values may have been higher and maybe even be classified as marginally deficient. Unfortunately, nonsupplemented pregnant women were not available for the present study.

Horwitt (1984) suggested the changes in ETKAC values should be interpreted with care. He stated that in using indirect analyses of vitamin concentration in the blood, one has to depend upon the constancy of the enzyme concentration in the erythrocyte. McLaren et al. (1981) showed that stressful situations, such as alcoholism, can cause differences in apoenzyme levels in the erythrocyte. Regardless, ETKAC measurements are one of the most reliable and sensitive indicators of thiamin status (Smeets et al., 1970).

Ramsey et al. (1983) reported that 59% of a pregnant Kenyan population were thiamin deficient, as measured by ETKAC, while Heller et al. (1974) indicated that approximately 30% were deficient at 25-30 wk gestation. Baker and coworkers (1975), using a protozoologic method for status assessment, found that 30% of a low to middle income pregnant population, taking a vitamin-mineral supplement (1.5 to 15 mg of thiamin) were thiamin deficient. The authors did not indicate how long supplements had been taken. Including both groups of W and NW in the present study at 22 and 30 wk, only one subject (W group) at 30 wk was classified as marginally deficient ($\leq 1.20 < 1.40$), with a value of 1.29. The

subject was a runner who ran approximately 6 km/day until 22 wk gestation and then stopped running and participated in the walking program. Regardless, she did have a total activity score (42.8) and an aerobic activity score (24.0) which were generally higher than those of most of the subjects at 30 wk in the study. The thiamin intake for the subject was only 75% of the RDA, not including her thiamin intake from the vitamin- mineral supplement. Conclusions cannot be drawn regarding one subject; however, similar to the subject (swimmer) that was marginally riboflavin deficient at 30 wk, in the present study, total activity and aerobic activity levels appeared to be higher for this woman. Even though one subject had marginally deficient levels, in contrast to the findings of Baker et al., the vitamin-mineral supplement in this study may have negated the possibility of a significant number of this pregnant population becoming marginally deficient or deficient in thiamin.

There was a significant correlation ($r = 0.58$, $p<0.01$) between ETKAC and aerobic activity scores in the W group at 22 wk gestation, but not in the NW group. There were no significant correlations of thiamin status and other variables for the NW group. There appears to be a trend in the data which supports the possibility of a relationship between participation in aerobic activities and ETKAC values. Similar to riboflavin, future studies should examine the effects of: 1) a more demanding or longer duration training program on the thiamin status; 2) comparison of supplemented and nonsupplemented pregnant women; and, 3) thiamin

status measurements at term. Finally, additional status measurements of thiamin (urinary thiamin excretion with test dose) could possibly assist in interpretation of ETKAC data, especially values not within the normal range.

## Pregnancy Outcome

Table 13 summarizes the postpartum parameters in the W and NW groups. There were no significant difference between the two groups regarding any of the parameters. Several researchers reported that there was no significant relationship between birth outcome and physical fitness (Pomerance et al., 1974a) or endurance training during pregnancy (Collings et al., 1983; Dibblee and Graham, 1983; Jarrett and Spellacy, 1983; Ruhling et al., 1985). In contrast, Clapp and Dickstein (1984) reported that women who maintained their preconceptual exercise habits above a minimum conditioning level, well into the 3rd trimester, had infants which weighted less by 500 g (X). Those women were compared to pregnant women who stopped exercising prior to 28 wk gestation. Naeye and Peters (1982) also showed that women who continued standup work during the 3rd trimester, had newborns that weighed 150 to 400 g less than newborns of mothers who stayed at home.

In the present study, activity level was determined up to 30 wk gestation, with no assessment of activity level from 32 to 40 wk gestation. The possibility exists that activity levels or other maternal factors during the time period of 30 wk gestation to

TABLE 13

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 are $\bar{X} \pm SD$.

delivery, could have influenced pregnancy outcome. Therefore future studies should include documentation of the intensity and level of activity during the 3rd trimester until birth. The information could further help elucidate the relationship between fitness and activity level during pregnancy and birth outcome.

There are still many questions regarding physical fitness and labor and delivery parameters. In a review of physical fitness and delivery, Gorski (1985) discussed the possibility that intensive sport activities stiffen the pelvis, therefore making labor more difficulty. She also stated that some obstetricians are of the opinion that the strengthened abdominal muscles in athletes may possibly be beneficial during the 2nd stage of labor. The walking program in the present study is probably not comparable in intensity to the intensive sports activities discussed in Gorski's review. Regardless, there were no significant effects of the walking program on labor duration or type of delivery. Labor duration data does include one woman with a 29 h labor, which is partially responsible for the high SD for the W group.

Four women, two in the NW group and two in the W group, had Cesarean deliveries. One of the subjects in the W group had a Cesarean, perhaps as a result of carrying twins, while the other subjects reported failure to progress as responsible for Cesarean delivery.

Pearson product moment correlation coefficient analyses were conducted on the postpartum parameters vs 22 and 30 wk data, and no significant relationships were observed. Lack of significant

correlation with labor duration may have been the result of large SD. Metcoff et al. (1981) suggested that nutrient intakes (excluding riboflavin and thiamin) and maternal characteristics such as weight gain and age, are related to fetal outcome. In the present study, no significant correlations regarding dietary intakes, riboflavin or thiamin status, weight gain, or age with birth outcome were apparent. The small sample size in this study, in combination with the lack of a diverse population, may have contributed to the lack of significant relationships.

The literature appears to recommend moderate aerobic exercise during pregnancy excluding exercise which involves weight lifting activities. The intensity of the exercise should probably be recommended on an individual basis and depends on the initial fitness level of the woman. Also, studies of active or trained pregnant women have demonstrated that there is little effect on birth outcome, whether detrimental or beneficial, as a result of increased exercise.

In the present study, participation in a walking program had no apparent influence on birth outcome, while mothers had a slight improvement in aerobic capacity. Riboflavin and thiamin status were not affected as a result of the participation in the walking program, even though there was a slight increase in ETKAC values for the W subjects. There are still many unanswered questions regarding the relationships between maternal aerobic exercise, vitamin status, and birth outcome. Future studies should consider the following factors: 1) longer duration and increased frequency

of aerobic exercise programs including assessment until term; 2) greater control of subject fitness levels prior to participation; 3) inclusion of both vitamin-mineral supplemented and nonsupplemented groups; and, 4) larger sample size.

# CHAPTER V

## SUMMARY AND CONCLUSIONS

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

Total activity and aerobic activity scores appeared to be higher for the W group but not significantly. Lack of significant differences may have been the result of large SD.

Dietary intakes of riboflavin and thiamin were similar for the two groups, with most subjects consuming at least 100% of the RDA. Prenatal vitamin-mineral supplements, which were taken by all subjects, were not considered with dietary intakes and provided 3.00 mg and 2.55 mg of riboflavin and thiamin, respectively. Energy intakes for the two groups were similar at 22 wk; however, at 30 wk the NW group had a significantly lower ($p<0.05$) energy intake.

HR responses to a submaximal treadmill walking test were significantly or near significantly lower for W compared to NW after the eight wk walking program (30 wk). The lower HR response was evident at two ($p<0.05$) and four ($p<0.05$) min, and near significant at six ($p<0.07$) min of exercise. While the values for

the W group decreased nonsignificantly from 22 to 30 wk, the HR response at two min of exercise for the NW group increased significantly ($p<0.05$). Also comparison of HR changes from 22 to 30 wk between W and NW indicated a significant difference at two min ($p<0.05$) and near significant differences at four min ($p<0.07$) and six min ($p<0.13$). For several exercise intensities (~2.9, ~3.8, ~4.6 METS), subjects in the W group apparently had improved HR responses when compared to the responses of the NW group.

Relative $VO_2$ measurements during submaximal treadmill walking decreased significantly for the W group after the eight wk walking program. Responses at 30 wk were significantly lower than 22 wk values at two min ($p<0.001$), four min ($p,0.01$), and six min ($p<0.05$) of exercise, while values for women in the NW group increased nonsignificantly. Comparison of changes in relative $VO_2$ as a result of the walking program, between W and NW, indicated a significant difference at six min ($p<0.05$) and a near significant difference at four min ($p<0.07$). These data, in conjunction with the HR responses, tend to support the hypothesis that pregnant women can improve their aerobic capacity with participation in a walking program. The improvement was slight and may have been more substantial had the frequency, duration, and intensity of the walking program been increased.

No significant differences were observed between W and NW in

regard to EGRAC or ETKAC; however, there was a near significant increase ($p<0.06$) in ETKAC for subjects in the W group after the eight wk experimental period. Mean values for EGRAC and ETKAC in both groups were well within the normal range. However, even the inclusion of vitamin-mineral supplements and riboflavin intakes that were 100% of the RDA, four subjects (3 W; 1 NW) had EGRAC values indicative of marginal riboflavin deficiency. One subject (W) with a dietary thiamin intake approximately 75% of the RDA, had an ETKAC value indicative of marginal thiamin deficiency. Two of the subjects who were marginally deficient in riboflavin and the one woman marginally thiamin deficient, had total activity and aerobic activity scores higher than most subjects in the present study. The possibility exists that there may be a relationship between high total activity or aerobic activity levels and increased utilization of riboflavin and thiamin.

Participation in the walking program had no significant influence on birth outcome parameters, whether beneficial or detrimental. Similarly, there appeared to be no relationship between birth outcome and vitamin status or other variables investigated in the study.

In conclusion, pregnant women participating in the walking program had a slight improvement in aerobic capacity, without any apparent benefits or risk to the developing fetus. The majority of women engaged in the maternal aerobic exercise program and taking vitamin-mineral supplements, were apparently not at risk for

clinical riboflavin or thiamin deficiency; however, even vitamin-mineral supplementation was not sufficient to prevent marginal riboflavin or thiamin deficiency in 17% of the present population.

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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

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

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

PREGNANCY, NUTRITION,
EXERCISE STUDY

CODE NO._____
DATE_____
ACTIVITY SCORE_____

## EXERCISE ACTIVITY LEVELS

| | | | |
|---|---|---|---|
| 1. | Dancing (includes aerobic dancing) | Frequency_____<br># of months_____<br># of days/week_____ | minutes_____<br>intensity_____<br>activity factor 6.4 |
| 2. | Bicycling | Frequency_____<br># of months_____<br># of days/week_____ | minutes_____<br>intensity_____<br>activity factor 5.0 |
| 3. | Swimming | Frequency_____<br># of months_____<br># of days/week_____ | minutes_____<br>intensity_____<br>activity factor 4.0 |
| 4. | Gymnastics | Frequency_____<br># of months_____<br># of days/week_____ | minutes_____<br>intensity_____<br>activity factor 10.5 |
| 5. | Stretches | Frequency_____<br># of months_____<br># of days/week_____ | minutes_____<br>intensity_____<br>activity factor 4.5 |
| 6. | Golfing | Frequency_____<br># of months_____<br># of days/week_____ | minutes_____<br>intensity_____<br>activity factor 5.0 |
| 7. | Baseball or Softball | Frequency_____<br># of months_____<br># of days/week_____ | minutes_____<br>intensity_____<br>activity factor 3.0 |
| 8. | Basketball | Frequency_____<br># of months_____<br># of days/week_____ | minutes_____<br>intensity_____<br>activity factor 8.0 |
| 9. | Waterskiing | Frequency_____<br># of months_____<br># of days/week_____ | minutes_____<br>intensity_____<br>activity factor 6.0 |
| 10. | Soccer | Frequency_____<br># of months_____<br># of days/week_____ | minutes_____<br>intensity_____<br>activity factor 12.0 |
| 11. | Frisbee | Frequency_____<br># of months_____<br># of days/week_____ | minutes_____<br>intensity_____<br>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_____<br># of months_____<br># of days/week_____ | minutes_____<br>intensity_____<br>activity factor_____ |
| 24. | Canoeing | Frequency_____<br># of months_____<br># of days/week_____ | minutes_____<br>intensity_____<br>activity factor_____ |
| 25. | Ping Pong | Frequency_____<br># of months_____<br># of days/week_____ | minutes_____<br>intensity_____<br>activity factor_____ |
| 26. | Pitching Horseshoes | Frequency_____<br># of months_____<br># of days/week_____ | minutes_____<br>intensity_____<br>activity factor_____ |
| 27. | Racquetball | Frequency_____<br># of months_____<br># of days/week_____ | minutes_____<br>intensity_____<br>activity factor_____ |
| 28. | Weight Lifting | Frequency_____<br># of months_____<br># of days/week_____ | minutes_____<br>intensity_____<br>activity factor_____ |
| 29. | Wood cutting | Frequency_____<br># of months_____<br># of days/week_____ | minutes_____<br>intensity_____<br>activity factor_____ |
| 30. | Volleyball | Frequency_____<br># of months_____<br># of days/week_____ | minutes_____<br>intensity_____<br>activity factor 4.0 |
| 31. | Other (Please specify) _____ | Frequency_____<br># of months_____<br># of days/week_____ | minutes_____<br>intensity_____<br>activity factor_____ |

APPENDIX E

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

| Each Natalins 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:** Natalins Rx tablets help ensure 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 F

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 G

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 | | | | | |
| | | | | | | |
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| | | | | | | |
| | | | | | | |
| | SNACK | | | | | |
| | | | | | | |
| | | | | | | |
| | DINNER | | | | | |
| | | | | | | |
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| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | SNACK | | | | | |
| | | | | | | |
| | | | | | | |
| | ANY OTHER TIME | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |

# APPENDIX H

## 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 I

## Glutathione Reductase For Riboflavin Determination

| With Cofactor 2 tubes | Without Cofactor 2 tubes | Blank 2 tubes |
|---|---|---|
| 0.1 ml FAD | ----- | ----- |
| ----- | 0.1 ml D-$H_2O$ | ----- |
| 2.2 ml Phosphate Buffer | 2.2 ml Phosphate Buffer | 2.5 ml Phosphate Buffer |
| 0.05 ml EDTA | 0.5 ml EDTA | ----- |
| 0.1 ml Oxidized Glutathione | 0.1 ml Oxidized Glutathione | ----- |
| **0.1 ml Hemolysate | 0.1 ml Hemolysate | 0.1 ml Hemolysate |

*Heated to 37°C before use

**0.4 ml RBC/4 ml D-$H_2O$ - centrifuge 10' @ 1000xg, take supernatant

(1) Incubate 20 min @ 37°C to bring the mixture to opital temp.

(2) Add 0.1 ml -NADPH to the first 4 cuvettes, stir with plastic stirrer, set time for 10' and measure ABS @ 340 nm. (initial)

(3) Add 0.1 ml -NADPH to the second set of cuvettes, stir, set timer for 10' and read (initial)

(4) After 10' measure ABS @ 340 nm. again (final)

NOTE: Only 2 or 3 sets of cuvettes can be handled between the reading of the first and the final reading of the first set.

Calculations: Each subject has 6 tubes:
2 w/cofactor (+a, +b)
2 w/out cofactor (-a, -b)
2 blanks - use to zero spectrophotometer - No NADPH

| ex | Sample | Initial ABS | Final ABS | ΔABS | $\bar{X}$ ABS | ACTIVITY COEFFICIENT |
|---|---|---|---|---|---|---|
| with cofactor | +a | .585 | .531 | .054 | .0535 | 1.09 |
| | +b | .522 | .469 | .053 | | |
| without cofactor | -a | .560 | .511 | .049 | .049 | |
| | -b | .538 | .489 | .049 | | |

| ACCEPTABLE | MARGINAL | DEFICIENT |
|---|---|---|
| up to 1.20 | 1.20 - 1.40 | 1.40 + |

## $B_2$ SOLUTIONS

(1) POTASSIUM PHOSPHATE BUFFER: 0.1 m, Ph 7.4. Dissolve 16.41 g $K_2HPO_4$ (diabasic) and 0.79 g $KH_2PO_4$ (monobasic) in approx. 800 ml D-$H_2O$, adjust pH with 2 N HCl, qs to 1 L with D-$H_2O$

(2) -NADPH - 2.0 mM — Dissolve 16.6 mg of -NADPH in 10.0 ml 1% Sodium Bicarbonate
- prepare fresh each day

(3) GLUTHATHIONE, OXIDIZED — 7.5 mM Dissolve 46 mg of Glutathione in 10.0 ml 1 % NaOH
- prepare fresh each day

(4) FAD - 0.25 Mm — Dissolve 2.4 mg of FAD in 10.0 ml D-$H_2O$, keep the tube covered with aluminum foil
- prepare fresh each day

(5) EDTA - 80 mM
- Dissolve 1.6 g EDTA in D-$H_2O$, qs to 50 ml
- stable for 1 week when refrigerated

(6) SODIUM BICARBONATE — 1% (w/v) Dissolve 5.0 g $NaHCO_3$/ 500 ml D-$H_2O$

(7) NaOH — 1% (w/v) Dissolve 5.0 g $NaHCO_3$/ 500 ml D-$H_2O$

(8) HCl - 2 N — 166 ml conc HCL/1000 ml D-$H_2O$

# APPENDIX J

## Transketolase Method For Determination of Thiamin

| With Cofactor 2 tubes | Without Cofactor 2 tubes | Blank 2 tubes |
|---|---|---|
| 0.1 ml TPP | ----- | ----- |
| *2.42 ml Substrate (R-5-P) | 2.52 ml Substrate (R-5-P) | ----- |
| **0.075 ml Hemolysate | 0.075 ml Hemolysate | 0.100 Hemoslyate |
| 0.02 ml -GDH-TPI | 0.02 ml -GDH-TPI | ----- |
| 0.10 ml -NADH | 0.10 ml -NADH | ----- |
| ----- | ----- | 2.52 ml TRIS BUFFER |

*Heated to 37°C before use

**0.4 ml RBC+ 4 ml D-$H_2O$ - centrifuge 10' @ 1000xg, take supernatant

(1) Incubate 25 min @ 37°C to bring the mixture to optical temp.

(2) Measure ABS. @ 340 nm (initial)

(3) Place samples back into 37°C incubator for 20 min.

(4) Measure ABS @ 340 nm. again (final)

Calculations: Each subject has 6 tubes:
2 w/cofactor (+a, +b)
2 w/out cofactor (-a, -b)
2 blanks - use to zero spectrophotometer

| ex | Sample | Initial ABS | Final ABS | ΔABS | $\bar{X}$ ABS | ACTIVITY COEFFICIENT |
|---|---|---|---|---|---|---|
| with cofactor | +a | .585 | .531 | .054 | .0535 | 1.09 |
| | +b | .522 | .469 | .053 | | |
| without cofactor | -a | .560 | .511 | .049 | .049 | |
| | -b | .538 | .489 | .049 | | |

| ACCEPTABLE | MARGINAL | DEFICIENT |
|---|---|---|
| up to 1.20 | 1.20 - 1.40 | 1.40 + |

## SOLUTIONS - TRANSKETOLASE - $B_1$

(1) TRIS BUFFER — 0.1 M, pH 7.6. Dissolve 12.12g TRIZMA HCl and 2.78g TRIZMA BASE in D-$H_2O$, adjust pH with HCl, qs to 1 L with D-$H_2O$

(2) SUBSTRATE — RIBOSE - 5 - PHOSPHATE, BARIUM SALT
- Dissolve 1.0 g R-5-P in 2.0 ml 2N HCl + vortex until dissolved.
- Add 1.0M SODIUM SULFATE until all Ba++ ions are precipitated (approx 2.7 ml)
- Centrifuge at 2000xg for 15 min.
- Transfer supernatant to a clean test tube
- Adjust pH of supernatant to 7.6 with 10 N NaOH, then 1 N NaOH
- qs with TRIS BUFFER to 190 ml

(3) 2N HCL - 166 ml conc HCl/1000 ml D-$H_2O$

(4) SODIUM SULFATE - 1.0 M
14.2 g $Na_2SO_4$ (anhydrous)/100ml D-$H_2O$

(5) 10 N NaOH — 100 g/250 ml D-$H_2O$
1 N NaOH — 10 ml 10 N NaOH 90 ml D-$H_2O$

(6) TPP - 10 mN
- Dissolve 30 mg in 6.5 ml TRIS BUFFER
- prepare fresh each day

(7) B-NADH - 10 mM
- Dissolve 46.2mg in 6.5 ml TRIS BUFFER
- prepare fresh each day

(8) α-GDH-TPI -
a) 7.4 mg protein/ml - pipette out 138 λ/1ml TRIS BUFFER
b) 8.2 mg protein/ml 124 λ/1ml TRIS BUFFER
c) 12.0 mg protein/ml 85 λ/1ml TRIS BUFFER
d) 19.8 mg protein/ml 50 λ/1ml TRIS BUFFER

APPENDIX K

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 L
22 Wk Data

| Observation | Age (yr) | Height (cm) | Pregnancy weight (kg) | Weight (kg) | Total activity score | Aerobic activity score | Riblfavin intake (mg) | Thiamin intake (kcal) | Energy intake (kcal) | HR-rest (bpm) | HR-2 (bpm) | HR-4 (bpm) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Walkers** | | | | | | | | | | | | |
| 1 | 26 | 174.0 | 56.8 | 63.9 | 53.8 | 27.7 | 1.46733 | 0.98390 | 2012.02 | 60 | 100 | 110 |
| 2 | 27 | 171.5 | 53.6 | 62.8 | 44.9 | 22.7 | 2.50500 | 1.13813 | 2158.42 | 70 | 120 | 120 |
| 3 | 27 | 158.8 | 61.4 | 69.2 | 21.5 | 3.1 | 1.47733 | 0.96160 | 2269.71 | 90 | 110 | 136 |
| 4 | 27 | 170.2 | 57.7 | 63.4 | 29.9 | 6.4 | 3.46833 | 1.52643 | 2312.92 | 88 | 125 | 130 |
| 5 | 31 | 162.6 | 65.5 | 72.8 | 10.5 | 4.1 | 2.29600 | 1.08187 | 2092.61 | 89 | 130 | 136 |
| 6 | 31 | 180.3 | 67.3 | 74.1 | 14.8 | 7.1 | 4.45267 | 1.56467 | 2688.19 | 88 | 120 | 136 |
| 7 | 30 | 172.7 | 56.8 | 66.4 | 45.1 | 25.4 | 3.86967 | 2.25563 | 2599.41 | 74 | 107 | 107 |
| 8 | 24 | 162.6 | 55.5 | 62.3 | 31.3 | 10.2 | 3.13400 | 1.43907 | 2834.87 | 79 | 130 | 136 |
| 9 | 30 | 157.5 | 50.9 | 62.8 | 9.9 | 4.3 | 2.17400 | 1.33503 | 2828.18 | 75 | 120 | 122 |
| 10 | 36 | 174.6 | 63.6 | 73.8 | 99.9 | 26.9 | 2.62867 | 1.19493 | 1521.81 | 75 | 115 | 125 |
| 11 | 32 | 172.7 | 60.9 | 70.5 | 51.9 | 6.9 | 2.29300 | 1.51670 | 2728.84 | 85 | 125 | 130 |
| 12 | 28 | 156.2 | 45.5 | 52.3 | 20.9 | 10.4 | 3.69833 | 1.59697 | 1977.91 | 83 | 115 | 115 |
| 13 | 31 | 167.6 | 69.1 | 75.5 | 62.2 | 2.0 | 2.72233 | 1.51613 | 2219.56 | 94 | 128 | 135 |
| 14 | 23 | 172.7 | 79.5 | 81.5 | 31.5 | 9.8 | 1.75233 | 0.78273 | 1805.07 | 77 | 110 | 118 |
| 15 | 26 | 166.4 | 65.5 | 69.2 | 45.9 | 9.5 | 3.81533 | 1.46590 | 2836.02 | 78 | 120 | 120 |
| 16 | 28 | 162.6 | 45.5 | 51.2 | 32.4 | 9.5 | 2.78433 | 1.76750 | 1720.15 | 82 | 131 | 131 |
| 17 | 25 | 162.6 | 55.9 | 63.0 | 45.9 | 12.3 | 1.70133 | 1.28310 | 2049.86 | 77 | 122 | 136 |
| 18 | 29 | 166.4 | 57.7 | 66.2 | 31.6 | 6.7 | 3.09767 | 1.44837 | 2273.22 | 86 | 105 | 107 |
| **Nonwalkers** | | | | | | | | | | | | |
| 19 | 25 | 170.2 | 61.4 | 68.7 | 34.0 | 8.5 | 2.00933 | 0.84310 | 1789.95 | 79 | 125 | 130 |
| 20 | 29 | 174.0 | 66.4 | 72.0 | 60.2 | 30.4 | 1.94067 | 1.15890 | 1547.20 | 80 | . | . |
| 21 | 30 | 172.7 | 75.0 | 76.2 | 28.2 | 0.5 | 2.79600 | 2.09607 | 2542.39 | 98 | 120 | 120 |
| 22 | 24 | 170.2 | 63.6 | 68.6 | 9.8 | 2.6 | 4.04567 | 1.60690 | 3285.02 | 88 | 122 | 130 |
| 23 | 31 | 162.6 | 61.4 | 72.2 | 43.5 | 7.8 | 1.73700 | 1.47290 | 1996.90 | 94 | 120 | 130 |
| 24 | 21 | 167.6 | 84.5 | 89.5 | 30.2 | 3.9 | 1.93167 | 0.95047 | 2048.34 | 83 | 125 | 135 |
| 25 | 26 | 165.1 | 58.4 | 63.4 | 17.4 | 11.3 | 0.89067 | 1.05800 | 2021.73 | 83 | 125 | 130 |
| 26 | 28 | 160.0 | 49.1 | 58.4 | 32.1 | 0.9 | 1.38233 | 0.97217 | 2871.67 | 94 | 118 | 125 |
| 27 | 32 | 165.1 | 55.8 | 58.8 | 21.0 | 3.1 | 2.32633 | 1.07667 | 2059.67 | 83 | 127 | 136 |
| 28 | 27 | 157.5 | 52.3 | 56.5 | 11.2 | 0.1 | 5.96267 | 3.43553 | 2355.66 | 75 | 104 | 105 |

APPENDIX L (cont.)

22 Wk Data

| Observation | 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) | EGRAC | ETKAC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Walkers | | | | | | | |
| 1 | 118 | 0.68 | 0.88 | 0.87 | 10.6416 | 13.7715 | 13.6150 | 12 | 14 | 1.6 | 1.15 | 1.15 |
| 2 | 140 | 0.58 | 0.66 | 0.74 | 9.2357 | 10.5096 | 11.7834 | 13 | 14 | 4.0 | 1.11 | 0.95 |
| 3 | 145 | 0.69 | 0.72 | 0.77 | 9.9711 | 10.4046 | 11.1272 | 10 | 8 | 1.8 | 1.20 | 0.62 |
| 4 | 135 | 0.70 | 0.65 | 0.71 | 11.0410 | 10.2524 | 11.1987 | 14 | 13 | 3.0 | 1.03 | 0.99 |
| 5 | 136 | 0.97 | 0.89 | 0.93 | 13.3242 | 12.2253 | 12.7747 | 15 | 8 | 1.3 | 1.08 | 0.67 |
| 6 | 143 | 0.79 | 0.87 | 0.86 | 10.6613 | 11.7409 | 11.6059 | 13 | 7 | 2.1 | 0.94 | 0.95 |
| 7 | 125 | 0.59 | 0.67 | 0.82 | 8.8855 | 10.0904 | 12.3494 | 12 | 12 | 3.8 | 1.10 | 1.10 |
| 8 | 150 | 0.66 | 0.65 | 0.65 | 10.5939 | 10.4334 | 10.4334 | 14 | 11 | 4.4 | 1.12 | 0.92 |
| 9 | 130 | 0.64 | 0.68 | 0.69 | 10.1911 | 10.8280 | 10.9873 | 12 | 11 | 2.5 | 1.25 | 0.84 |
| 10 | 133 | 0.74 | 0.72 | 0.94 | 10.0271 | 9.7561 | 12.7371 | 11 | 11 | 3.5 | 1.11 | 1.00 |
| 11 | 140 | 0.58 | 0.70 | 0.81 | 8.2270 | 9.9291 | 11.4894 | 13 | 8 | 1.6 | 1.09 | 0.96 |
| 12 | 125 | 0.54 | 0.70 | 0.83 | 10.3250 | 13.3843 | 15.8700 | 12 | 8 | 2.2 | 0.94 | 1.05 |
| 13 | 136 | 0.74 | 0.86 | 0.91 | 9.8013 | 11.3907 | 12.0530 | 13 | 9 | 3.3 | 0.91 | 0.71 |
| 14 | 120 | 0.88 | 0.91 | 1.01 | 10.7975 | 11.1656 | 12.3926 | 11 | 11 | 3.3 | 1.03 | 1.04 |
| 15 | 126 | 0.86 | 0.92 | 1.02 | 12.4277 | 13.2948 | 14.7399 | 13 | 10 | 2.4 | 1.07 | 0.88 |
| 16 | 139 | 0.50 | 0.49 | 0.57 | 9.7656 | 9.5703 | 11.1328 | 14 | 8 | 3.0 | 1.19 | 0.92 |
| 17 | 136 | 0.75 | 0.90 | 0.94 | 11.9048 | 14.2857 | 14.9206 | 13 | 13 | 2.8 | 1.05 | 0.75 |
| 18 | 125 | 0.66 | 0.81 | 0.77 | 9.9698 | 12.2356 | 11.6314 | 15 | 9 | 1.9 | 1.10 | 1.12 |
| | | | | | Nonwalkers | | | | | | | |
| 19 | 136 | 0.83 | 0.75 | 0.81 | 12.0815 | 10.9170 | 11.7904 | 15 | 11 | 3.0 | 1.01 | 0.84 |
| 20 | | | | | | | | | | | 1.07 | 0.82 |
| 21 | 140 | 0.57 | 0.57 | 0.68 | 7.4803 | 7.4803 | 8.9239 | 11 | 8 | 2.2 | 1.11 | 0.97 |
| 22 | 136 | 0.61 | 0.71 | 0.74 | 8.8921 | 10.3499 | 10.7872 | 14 | 10 | 3.2 | 0.92 | 1.10 |
| 23 | 140 | 0.63 | 0.68 | 0.76 | 8.7258 | 9.4183 | 10.5263 | 13 | 8 | 3.3 | 1.08 | 1.03 |
| 24 | 145 | 0.94 | 0.88 | 1.07 | 10.5028 | 9.8324 | 11.9553 | 13 | 8 | 4.1 | 1.19 | 1.01 |
| 25 | 136 | 0.73 | 0.73 | 0.75 | 11.5142 | 11.5142 | 11.8297 | 12 | 12 | 6.1 | 0.97 | 0.83 |
| 26 | 135 | 0.40 | 0.66 | 0.78 | 6.8493 | 11.3014 | 13.3562 | 17 | 10 | 4.0 | 1.07 | 0.94 |
| 27 | 136 | 0.57 | 0.55 | 0.60 | 9.6939 | 9.3537 | 10.2041 | 16 | 8 | 2.4 | 0.99 | 0.81 |
| 28 | 107 | 0.51 | 0.62 | 0.68 | 9.0265 | 10.9735 | 12.0354 | 13 | 12 | 2.1 | 0.94 | 0.90 |

## APPENDIX M

### 30 Wk Data

| Observations | Age (yr) | Height (cm) | Prepregnancy weight (kg) | Weight (kg) | Total activity score | Aerobic activity score | Riboflavin intake (mg) | Thiamin intake (mg) | Energy (kcal) | HR-rest (bpm) | HR-2 (bpm) | HR-4 (bpm) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Walkers | | | | | | |
| 1 | 26 | 174.0 | 56.8 | 66.4 | 42.8 | 24.0 | 1.77433 | 1.05760 | 2291.99 | 75 | 107 | 115 |
| 2 | 27 | 171.5 | 53.6 | 66.2 | 34.2 | 21.3 | 3.76367 | 2.24917 | 2607.53 | . | 115 | 125 |
| 3 | 27 | 158.8 | 61.4 | 75.5 | 22.4 | 3.8 | 1.38300 | 0.94877 | 2173.04 | 90 | 110 | 123 |
| 4 | 27 | 170.2 | 57.7 | 64.6 | 30.8 | 6.8 | 2.43533 | 1.45170 | 1858.12 | 90 | 125 | 120 |
| 5 | 31 | 162.6 | 65.5 | 76.8 | 14.0 | 3.5 | 2.37400 | 1.19277 | 2514.22 | 75 | 112 | 118 |
| 6 | 31 | 180.3 | 67.3 | 80.0 | 18.3 | 8.6 | 3.22533 | 1.44133 | 2142.21 | 84 | 118 | 125 |
| 7 | 30 | 172.7 | 56.8 | 70.0 | 46.3 | 26.6 | 3.83567 | 1.96720 | 2995.86 | 77 | 108 | 90 |
| 8 | 24 | 162.6 | 55.5 | 67.3 | 32.3 | 11.0 | 2.53800 | 1.19357 | 2526.72 | 96 | 135 | 136 |
| 9 | 30 | 157.5 | 50.9 | 69.5 | 11.2 | 5.0 | 1.54167 | 0.90963 | 2208.30 | 83 | 130 | 134 |
| 10 | 36 | 174.6 | 63.6 | 76.5 | 99.9 | 28.7 | 1.94433 | 1.18187 | 1869.10 | 78 | 110 | 123 |
| 11 | 32 | 172.7 | 60.9 | 75.6 | 53.7 | 8.7 | 2.66400 | 1.35790 | 1712.83 | 107 | 125 | 128 |
| 12 | 28 | 156.2 | 45.5 | 55.5 | 21.6 | 11.1 | 2.82700 | 1.31953 | 2506.53 | 72 | 94 | 100 |
| 13 | 31 | 167.6 | 69.1 | 80.0 | 60.2 | 1.4 | 2.80433 | 1.32553 | 2459.59 | 91 | 125 | 134 |
| 14 | 23 | 172.7 | 79.5 | 86.8 | 32.6 | 11.0 | 1.62700 | 1.17543 | 2464.11 | 77 | 100 | 110 |
| 15 | 26 | 166.4 | 65.5 | 73.7 | 34.5 | 7.2 | 4.11833 | 1.78273 | 3380.80 | 107 | 132 | 136 |
| 16 | 28 | 162.6 | 45.5 | 53.4 | 37.7 | 11.2 | 2.69000 | 0.90140 | 1878.23 | 94 | 136 | 140 |
| 17 | 25 | 162.6 | 55.9 | 68.0 | 41.3 | 12.7 | 1.54700 | 1.35560 | 1881.45 | 79 | 120 | 125 |
| 18 | 29 | 166.4 | 57.7 | 70.5 | 30.5 | 6.5 | 2.91767 | 1.85340 | 2436.60 | 75 | 119 | 120 |
| | | | | | | Nonwalkers | | | | | | |
| 19 | 25 | 170.2 | 61.4 | 73.6 | 31.8 | 6.3 | 2.82867 | 1.36447 | 2198.17 | 100 | 123 | 136 |
| 20 | 29 | 174.0 | 66.4 | 78.0 | 50.9 | 25.1 | 1.98233 | 1.11693 | 1714.42 | 90 | 120 | 130 |
| 21 | 30 | 172.7 | 75.0 | 80.0 | 27.9 | 0.5 | 1.40100 | 1.01567 | 2359.41 | 100 | 136 | 136 |
| 22 | 24 | 170.2 | 63.6 | 73.9 | 11.3 | 3.9 | . | . | . | 77 | 120 | 122 |
| 23 | 31 | 162.6 | 61.4 | 75.4 | 52.0 | 4.2 | 1.17000 | 0.74367 | 1689.76 | 94 | 135 | 128 |
| 24 | 21 | 167.6 | 84.5 | 91.3 | 28.7 | 4.0 | 1.37233 | 0.96820 | 1772.10 | 106 | 136 | 142 |
| 25 | 26 | 165.1 | 58.4 | 68.5 | 16.3 | 9.8 | 1.24567 | 1.16513 | 2528.59 | 75 | 125 | 126 |
| 26 | 28 | 160.0 | 49.1 | 60.2 | 29.7 | 2.3 | 1.58833 | 1.02920 | 2111.47 | 100 | 130 | 140 |
| 27 | 32 | 165.1 | 55.8 | 62.3 | 23.0 | 4.8 | 2.07900 | 0.98763 | 1873.33 | 88 | 130 | 133 |
| 28 | 27 | 157.5 | 52.3 | 59.4 | 8.9 | 0.6 | 5.14967 | 2.10540 | 1978.61 | 105 | 115 | 125 |

APPENDIX M (cont.)
30 WK Data

| Observation | 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) | EGRAC | ETKAC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Walkers | | | | | | | | | | | | |
| 1 | 125 | 0.76 | 0.75 | 0.92 | 11.4458 | 11.2952 | 13.8554 | 12 | 15 | 2.9 | 1.12 | 1.29 |
| 2 | 120 | 0.63 | 0.68 | 0.87 | 9.5166 | 10.2719 | 13.1420 | 13 | 16 | 2.3 | 1.01 | 0.88 |
| 3 | 126 | 0.60 | 0.84 | 0.99 | 7.9470 | 11.1258 | 13.1126 | 14 | 11 | 5.7 | 1.14 | 0.74 |
| 4 | 130 | 0.68 | 0.62 | 0.66 | 10.5263 | 9.5975 | 10.2167 | 15 | 11 | 2.1 | 0.95 | 1.08 |
| 5 | 125 | 0.84 | 0.86 | 0.95 | 10.9375 | 11.1979 | 12.3698 | 15 | 8 | 4.2 | 1.06 | 1.03 |
| 6 | 140 | 0.70 | 0.95 | 0.93 | 8.7500 | 11.8750 | 11.6250 | 13 | 8 | 3.5 | 0.96 | 1.05 |
| 7 | 110 | 0.61 | 0.79 | 0.80 | 8.7143 | 11.2857 | 11.4286 | 13 | 15 | 2.4 | 0.99 | 1.13 |
| 8 | 149 | 0.66 | 0.63 | 0.77 | 9.8068 | 9.3611 | 11.4413 | 13 | 9 | 5.5 | 1.04 | 0.89 |
| 9 | 140 | 0.70 | 0.71 | 0.82 | 10.0719 | 10.2158 | 11.7986 | 12 | 8 | 5.6 | 1.20 | 0.88 |
| 10 | 136 | 0.59 | 0.85 | 0.85 | 7.7124 | 11.1111 | 11.1111 | 12 | 11 | 2.4 | 1.08 | 0.85 |
| 11 | 136 | 0.53 | 0.65 | 0.67 | 7.0106 | 8.5979 | 8.8624 | 16 | 9 | 5.5 | 1.08 | 1.05 |
| 12 | 103 | 0.51 | 0.54 | 0.63 | 9.1892 | 9.7297 | 11.3514 | 15 | 13 | 2.0 | 0.95 | 1.05 |
| 13 | 136 | 0.79 | 0.87 | 0.91 | 9.8750 | 10.8750 | 11.3750 | 13 | 9 | 2.3 | 0.99 | 0.67 |
| 14 | 120 | 0.80 | 0.81 | 0.90 | 9.2166 | 9.3318 | 10.3687 | 15 | 9 | 2.7 | 1.06 | 1.05 |
| 15 | 136 | 0.79 | 0.85 | 0.82 | 10.7191 | 11.5332 | 11.1262 | 8 | 10 | 2.0 | 1.14 | 0.92 |
| 16 | 150 | 0.52 | 0.53 | 0.57 | 9.7378 | 9.9251 | 10.6742 | 13 | 8 | 3.2 | 1.12 | 0.92 |
| 17 | 133 | 0.76 | 0.79 | 0.85 | 11.1765 | 11.6176 | 12.5000 | 14 | 13 | 6.2 | 1.25 | 1.13 |
| 18 | 136 | 0.66 | 0.75 | 0.74 | 9.3617 | 10.6383 | 10.4965 | 14 | 9 | 4.5 | 1.06 | 1.13 |
| Nonwalkers | | | | | | | | | | | | |
| 19 | 136 | 0.62 | 0.61 | 0.77 | 8.4239 | 8.2880 | 10.4620 | 15 | 10 | 1.3 | 1.07 | 1.06 |
| 20 | 140 | 0.78 | 0.93 | 1.08 | 10.0000 | 11.9231 | 13.8462 | 14 | 9 | . | 1.28 | 0.62 |
| 21 | 150 | 0.65 | 0.70 | 0.92 | 8.1250 | 8.7500 | 11.5000 | 12 | 8 | 2.8 | 1.04 | 0.86 |
| 22 | 128 | 0.77 | 0.75 | 0.85 | 10.4195 | 10.1488 | 11.5020 | 15 | 14 | 3.5 | 0.98 | 0.91 |
| 23 | 130 | 1.00 | 0.89 | 0.83 | 13.2626 | 11.8037 | 11.0080 | 14 | 7 | 1.9 | 1.02 | 1.00 |
| 24 | 159 | 0.79 | 0.89 | 1.00 | 8.6528 | 9.7481 | 10.9529 | 14 | 6 | 2.2 | 1.15 | 0.99 |
| 25 | 136 | 0.66 | 0.79 | 0.92 | 9.6350 | 11.5328 | 13.4307 | 12 | 12 | 2.6 | 1.08 | 0.91 |
| 26 | 140 | . | . | . | . | . | . | 16 | 7 | 5.2 | 1.02 | 0.98 |
| 27 | 136 | 0.65 | 0.79 | 0.80 | 10.4334 | 12.6806 | 12.8411 | 15 | 8 | 1.9 | 1.01 | 0.66 |
| 28 | 136 | 0.53 | 0.58 | 0.64 | 8.9226 | 9.7643 | 10.7744 | 15 | 11 | . | 0.92 | 0.86 |

# APPENDIX N

## Post-Partum Data[1]

| | Age (yr) | Birth weight (kg) | Birth length (cm) | Weight gain (kg) | Apgar-1 min | Apgar-5 min | Labor duration 1st stage (min) | 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).

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