INTERRELATIONSHIPS BETWEEN STRESS, DIETARY INTAKE, AND PLASMA ASCORBIC ACID DURING PREGNANCY

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INTRODUCTION

Research findings have established the importance of optimum nutrition during pregnancy for the proper development of the fetus and for the health of the mother (National Research Council, 1970). However, nutrition alone does not determine pregnancy outcomes. There is growing evidence that pregnancy outcomes may be affected by mental health. Researchers such as Davids and Devault (1962), Montagu (1962), Ferreira, A. J. (1960) and Blau et al. (1963) believe psychological reactions affect the developing fetus.

Anxiety has been implicated as a predicator of poor outcome. After reviewing the role of psychological stress in obstetrical complications, McDonald (1968) commented that "self-report of anxiety is to date the most discriminating behavioral measure for preassessing complications." Many research studies give support to the conclusion McDonald has made. Gunter (1963) linked anxiety to prematurity. Scott and Thomson (1956) found women experiencing more anxiety while pregnant had more delivery complications.

After reviewing the literature, one concludes that nutrition and psychological stress plays a determining role in pregnancy outcome. However, the current literature is lacking on how these two factors interrelate with each other. Some research indicates that stress can increase the requirements for certain nutrients such as ascorbic acid (Hodges, 1970). Briggs (1962) and Maas et al. (1961) suggest ascorbic acid metabolism is altered by psychological stress. Horwitt (1942), Leitner et al. (1956) and Ramsey et al. (1957), observed that mental patients have low plasma ascorbic acid levels. Also, previous research indicates that ascorbic acid plays some part in the release and/or formation of steroid hormones, such as cortisol, which are released during stress (Pirani, 1952).

Various researchers have observed that plasma ascorbic acid levels fall during pregnancy. It remains unknown as to whether this effect is due to physiological responses of pregnancy or due to increased demands. The effect psychological stress on ascorbic acid status during pregnancy on remains unknown.

Current information on the relationship of psychological stress and nutritional status is inadequate. An investigation of the role psychological stress has upon the nutritional intake and the requirements of pregnant women would lead to the awareness of particular problems among the pregnant. With a better understanding, of these problems, the nutritionist and other health care professionals can plan appropriate intervention strategies. The objectives of this study were:

1. To determine the amount of psychological stress and identify factors influencing this stress in 32 pregnant women during their third trimester.

2. To determine the adequacy of nutrient intake of these pregnant women by obtaining diet histories and 24 hour recalls.

3. To determine plasma ascorbic acid levels and ascorbic acid intake of these pregnant women.

4. To determine total plasma cortisol levels in these 32 pregnant women.

5. To examine what associations exist between stress measurements, nutrient intake, ascorbic acid intakes, and plasma ascorbic acid levels in pregnancy.

REVIEW OF LITERATURE

Physical Stress and Ascorbic Acid Status

A daily intake of 10 mg of ascorbic acid has been observed to be adequate to alleviate and cure the clinical signs of scurvy in human subjects (Hodges et al., 1969). However, 60 mg of ascorbic acid per day is recommended for adults of both sexes to maintain acceptable plasma ascorbic acid levels (above 0.30 mg/100 ml plasma) and an adequate body pool of ascorbate (Sauberlich et al., 1974 and Food and Nutrition Board, 1980).

A review of previous research indicates that various physical stresses may affect the requirements and metabolism of ascorbic acid. It is known ascorbic acid is necessary for the normal healing process in man (Food and Nutrition Board, 1980). A decrease in plasma ascorbic acid following trauma and surgical conditions have been demonstrated in many studies. Researchers such as Shulka (1969) suggest that there is a rapid utilization of ascorbic acid for the synthesis of collagen at the site of the wound. Abt et al. (1961) demonstrated in guinea pigs that ascorbic acid accumulates in scar tissue and persists long periods afterwards.

Crandon and associates (1961) found a reduction in leukocyte and plasma ascorbic acid concentrations in 561 surgical patients. Patients with deficient plasma ascorbic acid concentrations often had wound dehiscence. It has been a common and controversial practice to treat patients requiring surgery with large doses of ascorbic acid pre- and postoperatively (Goldsmith, 1961).

Some studies have shown that dose of ascorbic acid above that to maintain health, has little effect on the healing process. Hodges et al. (1969) reported no differences in the rate of healing in patients taking 4, 8, 16 or 32 mg of ascorbic acid daily. Crandon et al. (1958) found experimentally inflicted wounds healed normally in subjects deprived of ascorbic acid for 3 months. Andreas and Brown (1946) could find no indication that high ascorbic acid intakes influenced clinical progress of 7 burn patients.

Ascorbic acid requirements during infection also remain controversial and undetermined. Falkner (1935) suggested there was a greater increased metabolic need and use of ascorbic acid during periods with infection. Daum et al. (1939) observed a reduction in both blood and urinary ascorbic acid levels after patients were subjected to electrically induced artificial fever. Falkner and Taylor (1937) observed a similar trend. Banks (1965), and Macon (1956) have reported that ascorbic acid reduces the incidence and severity and duration of colds. Cowan et al. (1950), Frantz et al. (1956), Glickman et al. (1946), and Tebrock et al. (1956) observed no effect of massive doses of ascorbic acid on the incidence of the common cold. However, they did observe a significant decrease in the number of days missed from work due to illness in the treated as compared to the controls. This result was due to the reduction of nonspecific symptoms such as chills, fever and severe malaise.

Environmental stresses such as extreme heat and cold have been studied in regard to their effects on ascorbic acid requirements. The effect of elevated temperature upon ascorbic acid status remains unclear.

Hindson (1970) reported that Europeans who resided in the tropics for 4 to 36 months exhibited a significant drop in leukocyte ascorbic acid. Sheild et al. (1945) reported that men stressed by high environmental temperatures and humidity lost more ascorbic acid than men who worked in a temperate environment. The research findings of Stydrom et al. (1976) indicated the effectiveness of ascorbic acid may be due to reducing heat strain in unacclimatized individuals. In a study of controlled intakes of ascorbic acid, Henschel et al. (1944) produced results which failed to support that increased amounts of ascorbic acid were needed for heat stress.

In regard to cold acclimatization, experiments with mostly human subjects have not confirmed animals experiments in which ascorbic acid supplementation improved resistance to cold. Glickman et al. (1946) found cold acclimatization was not enhanced with the addition of 200 mg of ascorbic acid to the diet of 12 young men. Ryer et al. (1954) noted no change in ascorbic acid excretion or plasma ascorbic acid in soldiers engaged in activity in a cold climate for 9 weeks. Kuhl et al. (1952) found no significant changes in plasma ascorbic acid levels before and after exposing men for 8 minutes in cold water. However, urinary ascorbic acid rose significantly during the first and second hour after the stress.

The interrelationship between physical performance and ascorbic acid metabolism remains controversial. Fox et al. (1940) gave 40 mg of ascorbic acid daily to an experimental group of miners and no supplements to a control group. No differences were seen in strength, skill or endurance (putting the shot, sprinting 100 yards, and running 1 mile) between the groups. Bender and Nash (1975) studied the effects of 250-1,000 mg of ascorbic acid per day on normal athletic subjects. Work efficiency was measured by the Harvard Step test. Tests of competitive athletic performance in short and long events were conducted. Placebo and control groups were included. No significant differences were found between any groups and no significant change took place within any group.

In contrast, Howald et al. (1975), found in 13 athletically trained subjects, that ascorbic acid supplementation increased the physical working capacity. The research findings of Spioch et al. (1966) also gave support that ascorbic acid supplements increase physical efficiency. Margolis (1964) found ascorbic acid supplements were helpful in reducing fatigue and increasing muscular in young men.

Subramanian et al. (1973) has suggested that the beneficial effect of ascorbic acid in various stress conditions is due to its detoxification of excessive histamine produced during stress. Subramanian et al. (1973) have observed large doses of ascorbic acid, when given in a variety of stress situations, resulted in a marked decrease in urinary and blood histamine. Clemetson (1980) reported that ascorbic acid supplementation causes a decrease in blood histamine levels in humans also.

The Interrelationships Between Ascorbic Acid, Stress and Adreno-Cortical Functions

It is not yet possible to assign a definite role to ascorbic acid in adreno-cortical function, even though the adrenal cortex under normal condition is the tissue with the highest concentration of ascorbic acid in the human body (Kark, 1954). There are conflicting reports in the literature resulting in considerable confusion regarding the physiological role of ascorbic acid in steroidogenesis or steriod release (Pirani, 1952 and Liakakos et al., 1975).

In rats and guinea pigs, it is known that acute exposure to stress stimulates the pituitary-adrenal system which results in increased secretion of adrenocorticotropin hormone (ACTH), a fall of adrenal ascorbic acid and a subsequent release of adrenal corticoids. Sayers and coworkers (1946) administered ACTH and a variety of stressors to intact and hypophysectomized guinea pigs which resulted in decrease of adrenal ascorbic acid and cholesterol. Prolonged stress of ACTH administration resulted in adrenal hypertrophy. Many subsequent studies presented similar evidence (Pirani, 1952).

Results from these earlier studies lead many investigators to believe that ascorbic acid plays a role in steroidogenesis. Levenson et al. (1947) postulated that the depletion of ascorbic acid occurring upon stress was related to increased consumption of this vitamin by the adrenal gland. If the postulation of Levenson et al. holds true, then it would seem likely that a state of ascorbic acid deficiency should lead to an adrenocortical insufficiency. Kitabchi and Duckworth (1970) conducted a pituitary-adrenal evaluation in two scorbutic patients. Both patients had normal plasma cortisol levels (8 to 25 ug cortisol/100 ml plasma) and normal responses to ACTH stimulation. Clayton and Prunty (1951), Nadel and Schneider (1951) found an increased excretion of urinary-17-ketosteroids by scorbutic guinea pigs. Long (1947) observed the adrenals of scorbutic guinea pigs responded normally to stimulation to ACTH. May et al. (1954) found 17-ketosteriods levels remained normal in 18 scorbutic monkeys. These experiments demonstrated that adreno-cortical insufficiency is not necessarily associated with ascorbic acid deficiency.

Kark (1954) explained the disappearance of ascorbic acid from the adrenal gland upon ACTH and stress stimulation from results of Lauber and Rosenfield. These researchers studied the ascorbic acid content of healing wounds and scars of healthy and scorbutic guinea pigs. Ascorbic acid was mobilized from tissues and selectively concentrated in traumatized areas. Crandon et al. (1961) showed similar results in surgical patients. A study by Kitabchi (1967) indicated that ascorbic acid released from the adrenal could be recovered quantitively in the adrenal vein effulent.

Some researchers have postulated also, that if ascorbic acid is needed for steriodogenesis prolonged ACTH administration would produce scurvy in animal species requiring ascorbic acid (Pirani, 1952). No manifestations of scurvy have been reported for a large series of patients treated with ACTH over a long period of time (Pirani, 1952). ACTH treatment may produce scurvy in patients whose dietary intake is inadequate or tissue stores are markedly depleted. Dugal and Therien (1949) have reported the development of scurvy in some patients treated with ACTH for 9 to 110 days. Forsham et al. (1951) have observed that ACTH more specifically effects the ascorbic acid content of the adrenal gland than that of other organs.

When Dugal (1961) administered ascorbic acid to guinea pigs exposed to cold stress adrenal hypertrophy was prevented. Pirani (1952) hypothesized ascorbic acid requirements are likely to be greater under stress than ACTH administrations because in stress many tissues may present increased ascorbic acid utilization while ACTH effects the adrenal specifically. Also in conditions of adrenal hypertrophy such as Cushing's Syndrome, plasma ascorbic acid levels are normal and there is no evidence of scurvy (Goldsmtih, 1961). It is generally agreed that tissue requirements for ascorbic acid are increased under conditions of increased body metabolism (Hodges, 1970).

Kitabchi (1967) has suggested that a high concentration of adrenal ascorbic acid in the "resting" or nonstress conditions exert a "braking influence" on steroidogenesis. There is indirect evidence to support his theory. Lipscomb and Nelson (1960) demonstrated a temporal relationship between the release of ascorbic acid and steroids in vitro in rats. Ascorbic acid release was found to procede corticoid output. In the adrenal vein effulent, ascorbic acid concentration decreased as steroid levels rose. In the absence of steroid release, no significant ascorbic acid release was observed.

Cortisol and Ascorbic Acid

Only within recent years has the effect of ascorbic acid on cortisol secretion in humans been explored. Liakakos et al. (1975) assayed plasma cortisol levels in healthy children (4-14 years) maintained on high ascorbic acid levels (1 gram twice daily) and a control group receiving no supplementation for a period of 5 days. Both groups, consisting of 12 subjects, were treated with ACTH. Comparison of the mean values for plasma cortisol in experimental and control groups on the first day of the study revealed no significant difference before or after ACTH treatment.

On the fifth day of the experiment, after treatment with ACTH, the experimental and control group had mean plasma cortisol levels of 37.9 and 64.2 ug/100 ml plasma respectively. A Student t-test revealed the mean plasma cortisol values for the experimental group to be significantly lower than the mean plasma cortisol values for the control group (t-value = 2.62, $p < 0.02$). In the experimental group, the supplementation had no significant effects on "resting" state (before ACTH stimulation) cortisol levels. Liakakos et al. concluded that ascorbic acid excess exerted an inhibitory effect on cortisol secretion upon treatment with ACTH. These researchers further concluded, based on their results, that ascorbic acid excess may be of no value in conditions of stress. These results are consistent with the theory that ascorbic acid exerts a braking effect on steridogenesis.

Loh and Wilson (1973) conducted a study with healthy college students on the relationship between plasma cortisol, ascorbic acid taste threshold and plasma cortisol. Plasma ascorbic had maximum values at 1200 hours and a minimum at 2400 hours. Loh and Wilson found the circadian rhythm of plasma cortisol to be almost identical to that of plasma ascorbic acid. It was concluded by these researchers that the relationship between ascorbic acid thresholds and plasma concentrations, may be physiologically linked with changes in plasma cortisol.

Wilbur and Walker (1977) tested the hypothesis that ascorbic acid may affect the synthesis and release of cortisol by the adrenal. Using male guinea pigs, maintained on a fixed photoperiod, these researchers compared an experimental group fed 100 fold excessive ascorbic acid with a control group fed the recommended level of ascorbic acid and an ascorbic acid deficient group in regard to circadian rhythms of plasma cortisol. Plasma cortisol concentrations in the control and the excessively supplemented did not differ significantly at any time. The plasma cortisol concentrations were significantly greater in the scorbutic group than in the two supplemented groups. It was concluded that ascorbic acid deficiency imposed a stress on these animals and induced cortisol release. This finding is consistent with the theory of Kitabchi that lowered levels of adrenal ascorbic acid is necessary before steroidogenesis will commence.

Psychological Stress and Ascorbic Acid

Studies investigating the effects of psychological stress upon ascorbic acid status are few. Psychological stress is associated with physiological changes such as an increased plasma cortisol and epinephrine secretion which might change ascorbic acid status (Everly and Rosenfield, 1977).

There is some evidence of a relationship between ascorbic acid and mental health. Horwitt (1942) obtained over 1,000 blood samples from subjects in a mental hospital. The average plasma ascorbic acid level of the patients, in this hospital, was less than 0.40 mg %. Ramsey et al. (1957) measured ascorbic acid in 28 older patients with chronic mental disorders, mean age 64 $\pm$ 9 years. Fasting levels of blood ascorbic acid were low (> 0.40 mg %) in all subjects and capillary strength as measured by a method of Hines and Parker were below normal. It was calculated that the hospital diet provided 80 mg of ascorbic acid daily. Supplements of 100 mg of ascorbic acid or 200 ml of orange juice were required by all subjects to obtain normal blood ascorbic acid levels. No information was provided of the type of medication subjects for mental patients.

Briggs (1962), after reviewing schizophrenia research literature, concluded that schizophrenics have higher plasma concentrations of copper and ceruloplasmin which suggests the existence of a defect in copper metabolism in these subjects. Briggs found schizophrenics excreted much more dehydroascorbic acid and diketogulonic acid in their urine than other patients or normal subjects receiving the same diet. It was suggested that ceruloplasmin catalyzes the oxidation of ascorbic acid. This could explain the earlier results of Ramsey et al. Briggs suggested that abnormal copper metabolism may form the basis for a biochemical hypothesis in the etiology of schizophrenia. He suggested early stages of schizophrenia might be controlled with high intakes of ascorbic acid (500 mg per day).

Maas et al. (1961) conducted a study comparing the plasma ascorbic acid levels of 20 anxious patients (10 schizophrenics and 10 patients with neuroses or personality trait disorders) and 20 non-anxious patients (10 schizophrenics and 10 patients with neuroses or personality trait disorders). Plasma ascorbic acid was lower for anxious patients, whether schizophrenic or nonschizophrenic. The mean plasma ascorbic acid values for anxious females and males was 0.49 mg/

100 ml and 0.28 mg/100 ml plasma respectively. For nonanxious females and males, the plasma ascorbic acid values were 0.69 mg/100 ml and 0.47 mg/100 ml plasma respectively. However, the difference between the plasma values of the nonanxious and anxious patients was not significant ($p < 0.08$). Maas et al. concluded from the uniformity of this finding that there was a real difference. No dietary information was included in this study. These researchers suggested that low ascorbic acid of anxious patients may be due to changes in dietary habits accompanying anxiety, to the increased utilization of ascorbic acid in anxiety or to other factors not clear.

Milner (1963) conducted a controlled blind saturation study involving 40 chronic psychiatric patients. He found after initial testing that psychiatric patients fell within the lowest limits of the normal range in execretion rates. A clinical state of subscurvy was found in these subjects. Milner reported follicular hyperkeratosis, minute haemorrhages around hair follicles, haemorrhagic gingivitis, and dyspepsia in patients and attributed these symptoms to vitamin C deficiency. The researcher found statistically significant improvement in depressive, manic and paranoid symptom complexes, together with an improvement in overall personality functioning following saturation with ascorbic acid. The M.M.P.I. (Minnesota Multiphasic Personality Inventory), the Wittenborn Psychiatric Rating Scale, and subjective assessments were used to make these assessments. Milner suggested that chronic psychiatric patients would benefit from the administration of ascorbic acid.

Pitt and Pollitt (1971) compared 32 schizophrenics wizh 24 demented control subjects. Buffy layer ascorbic acid of the white blood cells was determined over a 10 week period. Results of mean ascorbic acid levels showed the schizophrenic group had significant higher levels than the control group. No dietary information was recorded. In the second part of this study, schizophrenic and control subjects were matched for eating habits. Both groups received 500 mg of ascorbic acid twice daily for 14 days. During the period of study, the levels of the two groups were not significantly different. Signs of scurvy did not appear in either group during the experiment.

In a study of experimental scurvy produced in 4 prison inmates, Hodges (1970) found that one subject excreted more ascorbic acid in his urine, than he ingested during a period of intense anxiety. This subject began to retain ascorbic acid when the source of anxiety was removed.

Ascorbic acid depletion may produce a series of psychological disturbances. Kinsman and Hood (1971) depleted and repleted 5 prison inmates of ascorbic acid. Psychological tests were conducted at the beginning, at midnight, and at the end of the depletion period and after 15 days of load dosing. The most significant changes were observed in personality as measured by the Minnesota Multiphasic Personality Inventory (MMPI). The occurrence of depression, hypochondria, hysteria and social introversion significantly increased during the depletion stage. In 2 subjects, intakes of 6.5 mg ascorbic acid per day were sufficient to effect a reversal in personality changes.

Psychological Stress During Pregnancy

McDonald (1968), in a review on the role of psychological stress in obstetrical complications, commented that "self report of anxiety is to date the most discriminating behavioral measure for preassessing complications." Scott and Thompson (1956) found a low incidence of prolonged labor in subjects judged as "well-adjusted" by the Maudsley Medical Questionnaire. Davids (1962) administered the Taylor Manifest Anxiety Scale, the Thermatic Aperception Test (TAT), and a sentence completion test to pregnant women. He found that women who experienced complications in delivery or who gave birth to infants with abnormalities experienced a relatively higher amount of disturbing anxiety while pregnant.

Kapp et al. (1963) observed that 18 primigravidas experiencing labor difficulties, presented more anxiety and fear than matched controls. Weils and Tupper (1960) interviewed and administered 2 psychological tests to 18 habitual aborters prior to delivery. The habitual aborters were characterized by overt anxiety. Grimm (1962) compared 71 habitual aborters with 35 heterogenous controls. Rorschach and TAT tests showed that habitual aborters demonstrated poorer emotional control and greater anxiety than controls.

Prematurity may be linked to increased anxiety. Using the TAT, the Cornell Medical Index (CMI) and personal interviews, Gunter compared 20 patients with matched controls. The premature group obtained higher CMI scores (measuring anxiety and feelings of inadequacies) and more life stresses. Blau et al. (1962) also matched 30 mothers of premature infants with 30 mothers of normal infants. Women giving birth to premature infants showed an unconscious feeling of hostility and repulsion toward pregnancy.

Hyperemesis gravidum or disproportinate vomiting during pregnancy may be related to anxiety. Harvey and Sheffey (1954), on the basis of interviews and with the use of Rorscharch tests, related increased vomiting to high anxiety levels.

The incidence of obstetrical problems in women with stressful psychiatric disorders is another finding supporting a link between psychological stress and unfavorable outcomes in pregnancy. Rosen and Down (1968) found that women with a history of psychological disorders produced a disproportionate number of low-birth weight infants. Sameroff et al. (1975) reported that schizophrenic and neurotic depressive women had significantly more delivery complications than women diagnosed as normal or disordered. Further analysis indicated that the schizophrenics and neurotics as a group were more anxious.

Lederman et al. (1978) took the investigation of maternal anxiety and obstetrical complications further by measuring stress-related biochemical factors, anxiety and progress in 3 defined phases of labor in 32 normal pregnant women. It is known that anxiety stimulates adrenal secretion of cortisol and catecholamines (Mason, 1975). Measuring epinephrine and cortisol levels during labor, Burns (1976) found lower epinephrine and cortisol levels were associated with lower contractility and longer duration of labor. The results of Lederman et al. (1978) were similar to those of Burns. Plasma cortisol levels had a positive correlation with labor duration in the first stage. As expected, plasma cortisol levels had a significantly positive correlation with self-reported anxiety as measured by the A-State Anxiety Inventory scale of the Spielberger State-Trait Anxiety Inventory.

Ascorbic Acid Status During Pregnancy

Plasma ascorbic acid levels have been observed to decrease during pregnancy (Sauberlich, 1978). Mason and Rivers (1971) found in a sample of 37 unsupplemented pregnant women that the mean total plasma ascorbic acid was significantly lower in the third trimester ($\overline{X}$ = 0.55 mg %) than in the second ($\overline{X}$ = 0.71 mg %). Nearly half the samples obtained during the third trimester contained less than 0.40 mg ascorbic acid per 100 ml plasma. Martin et al. (1957) determined the ascorbic acid intake and serum levels in 2129 pregnant women. Except in a group of women taking 80 to 100 mg of ascorbic acid daily, plasma ascorbic acid levels decreased during pregnancy.

Macy and associates (1954) also found a decrease in plasma ascorbic acid in women between the first and third trimesters. Others to observe a decreased ascorbic acid level during pregnancy were Vobecky et al. (1974), Morse et al. (1975), Darby et al. (1953), Garcia et al. (1974) and Javert and Stander (1943). A few investigators have reported no decrease in ascorbic acid in pregnancy (Dawson et al., 1969; and Young et al., 1946).

Some researchers have contributed this downward trend in ascorbic acid levels during pregnancy to an increased need for the vitamin (Vobecky et al., 1974; and Martin et al., 1957). Other reasons given which may cause this downward trend are blood volume changes, hormonal changes, or decreased dietary intakes of ascorbic acid (River and Mason, 1971). During the progress of pregnancy, the production of estrogen is increased. Ingestion of oral contraceptives composed of synthetic estrogens have been observed to lower plasma levels of ascorbic acid (River and Devine, 1975 and McLeroy and Schendel, 1973). Thus increased estrogen levels produced in pregnancy may lower plasma ascorbic acid levels. Plasma volume increases to a maximum of about 50% over non-pregnant values by the thirty-fourth week of pregnancy (Hytten and Thomson, 1970). Hemodilution may be responsible in part for the decreased plasma ascorbic acid levels observed in pregnancies.

Pregnancy studies suggest ascorbic acid stores are smaller in pregnant than nonpregnant women. Toverud (1939) studied nonpregnant, pregnant, and lactating women whose diets were supplemented with 30 mg of ascorbic acid. These subjects also received orange juice which supplied 250 mg of ascorbic acid. Using the criteria that 50% urinary excretion of the test dose indicated saturation, Toverud concluded that the ascorbic acid intake of pregnant women should not fall below 75 mg per day.

Javert and Stander (1943) collected 376 blood samples from 246 pregnant women. They noted plasma ascorbic levels fell progressively during pregnancy. In 100 deficient pregnant women, they were able to affect a rise in plasma levels to normal nonpregnant levels. This was accomplished by administering 100 mg of ascorbic acid and 100 ml of orange juice daily to each patient. They concluded that pregnant women need about 200 mg of ascorbic acid daily. However, Martin et al. (1957) observed that 80-100 mg of ascorbic acid per day maintained high plasma levels throughout pregnancy.

The relationship between adequate ascorbic acid status and pregnancy outcome is still questionable. Toverud (1939) reported an association between decreased ascorbic acid levels during pregnancy and increased neonatal mortality. Wideman et al. (1964) found a positive association between decreased ascorbic acid intake of pregnant women and decreased birth weights. Martin et al. (1957) observed a weak relationship between low ascorbic acid intake, low serum levels during pregnancy, gingvitis, and premature birth and puerpual fever. Martin et al. concluded that ascorbic acid was a contributing factor in these conditions.

Other researchers have been unable to show any association between ascorbic acid and obstetrical complications. River and Devine (1975), in a study of more than 300 pregnant women, were unable to show a definite relationship between low plasma ascorbic acid and pregnancy outcome. Vobecky et al. (1974) were unable to make a definite conclusion between pregnancy outcome of 1500 pregnant women and ascorbic acid levels, however, the number of pregnancies which ended in abortions, stillbirths and other complications for whom plasma ascorbic acid levels were known was small.

It is known that fetal needs for ascorbic acid are met solely from the maternal diet and stores (Cochrane, 1965). Many researchers have shown that ascorbic acid content of the umbilical cord blood and fetal blood to be as much as 2 to 3 times higher than maternal blood (Barnes, 1947; Ingalls et al., 1937; Khattab et al., 1970; Ram, 1965; Slobody et al., 1947). Ascorbic acid crosses the placental membrane in the reduced form as dehydroascorbic acid (Khattab et al., 1970). It is then converted to L-ascorbic acid once inside the fetus. Ascorbic acid is impermeable to the placenta.

To meet fetal needs, the Committee on Dietary Allowances of the Food and Nutrition Board (Food and Nutrition Board, 1980) has recommended that the Recommended Dietary Allowances (RDA) of ascorbic acid be increased for pregnant women by 20 mg, in addition to 60 mg recommended for nonpregnant women. During lactation, a daily loss of 25-45 mg of ascorbic acid is excreted in 850 ml of human milk (FNB, 1980). Thus, the FNB added an additional allowance of 40 mg per day of ascorbic acid to assure a satisfactory level of the vitamin in lactating women.

River and Devine (1975) attempted to simulate the RDA amounts for pregnant and lactating women in pregnant guinea pigs and studied reproduction performance. Guinea pigs were divided into groups with low, intermediate and high intake levels of ascorbic acid for a total of three generations. The low intake was not adequate beyond weaning. The intermediate group was superior to the low intake group but inferior to the high intake group in conceiving, producing litters and carrying litters to term. Even high intake levels were insufficient to maintain some tissues at saturation levels. The researchers concluded that ascorbic acid requirements for pregnancy and lactation have been underestimated.

Pye et al. (1961) fed 2, 4, 6, or 8 mg of ascorbic acid to pregnant guinea pigs. Some females failed to produce at each level of ascorbic acid intake, but the largest number of nonproductive females fell within the 2 mg group. The rate of reproduction was highest and the number of young born was greatest in the 8 mg group.

The effect of large doses of ascorbic acid remain questionable. Some researchers report increased fertility and decreased complications during pregnancy when guinea pigs are given large doses (River and Devine, 1975). However, in humans it may be hazardous to take megadoses of ascorbic acid during pregnancy. It is theorized high levels during gestation may condition a higher than normal need for ascorbic acid in the infant. After birth, the infant receives less ascorbic acid than in the uterus. The infant exhibits scurvy until his body can adjust to lower intakes (Cochrane, 1965).

While excessive ascorbic acid intake may be a problem, inadequate intake may pose a greater problem. Ascorbic acid has been one of those nutrients cited as being inadequately met in the diets of many pregnant women (Mason and Rivers, 1970). Using the 1958 RDA, Payton et al. (1960) found that the mean intake of ascorbic acid for 571 southern pregnant women was slightly above 50 percent of the RDA. Darby and coworkers (1954) reported respectively a mean intake of 62 and 55 mg of ascorbic acid for 1222 second trimester and 1665 third trimester pregnant women. Steven and Olsen (1967) measured the estimated ascorbic acid intakes of 129 medically indigent pregnant women. The youngest (under 20) and older group (over 30) had mean ascorbic acid intakes less than the 1958 RDA.

The Ten State Survey (1970) of over 600 pregnant women revealed white and black women in low income ratios states had mean ascorbic acid intakes of 74.71 and 67.49 mg respectively. From this data, it seems likely a number of pregnant women in the U.S. are consuming less than 80 mg of ascorbic acid, the 1980 RDA for pregnant women.

EXPERIMENTAL PROCEDURES

Subject Criteria and Recruiting

The experimental protocol and subject criteria for this study were accepted by the University Review Board for Research involving Human Subjects on October 2, 1980. Subjects were 32 healthy pregnant women in their third trimester of pregnancy (7.00-8.75 months) from southwestern Virginia. The term "healthy" refers to disease-free or free of identified major health disorders. The age of these women ranged from 15-32 years.

Before entry into the study, subjects were required to read and sign a consent form (Appendix A). This form informed subjects of the purpose of the study, a brief description of procedures to be used, information of confidentiality and personnel to whom questions could be directed.

Subjects were recruited by a variety of ways. A printed notice was published in the Spectrum, a weekly publication produced by Virginia Tech (Appendix B). Letters were sent to local physicians, obstetricians, and gynecologists informing them of the study, its purpose, personnel to be contacted and procedures to be used (Appendix C). They were requested to inform pregnant women who met the criteria of the study. Childbirth education classes (LaMaze) were also visited to recruit subjects. Prospective volunteers were given consent forms and a verbal description of the objectives and procedures to be used. Interested women were requested to give names and phone numbers where they could be reached. Appointments were set to meet the volunteers at Wallace Hall to collect blood samples and to conduct an intensive interview. See Appendices for forms used.

A letter regarding the study was sent to the New River Health District Director (Appendix D). Permission was obtained to interview and collect blood from selected volunteers from Maternity Clinics conducted at Montgomery County Health Department and Radford Health Department.

Interviews and Questionnaires

After signing the consent form, 20 ml of blood was obtained from subjects. For a further discussion on blood collection, see Collection of Biochemical Data section. Subjects were informed to avoid eating breakfast prior to reporting to the laboratory. It was also requested that they not take their vitamin supplements 24 hours before blood collection. The subjects were requested to avoid foods high in ascorbic acid content after 6:00 PM the day before the blood collection. See Appendix E for list of foods high in ascorbic acid the subjects were to avoid after 6:00 PM. These precautions were taken in an attempt to control sample variation. As a result of the above conditions, appointments with volunteers were set before 10:00 AM.

After the blood was collected, a 24-hour recall and diet history were obtained (Appendix F). The diet history was a modified method of Burke (1947). Both were conducted by interview with aid from food models and containers of graduated sizes to help establish accurate portion sizes. Cross checking occurred to make sure the volunteer had remembered all she had eaten.

From the diet history and 24-hour recalls form ascorbic acid, calories, protein, calcium, iron, vitamin A, thiamin, riboflavin and niacin intake were determined by use of a computer program designed by Wentworth and Choquette (1981). This program utilizes the nutrient content tables from the U.S. Department of Agriculture Home and Garden Bulletin No. 72 (U.S.D.A., 1978), Agriculture Handbook No. 456 (Adams, 1975) and Food Values of Portions Commonly Used (Church and Church, 1979). The history and recall forms were coded later for appropriate food items and quantity and retained on data cards.

The nutrient intake of each subject was compared to the 1980 Recommended Dietary Allowances (Food and Nutrition Board, 1980). The histories and recalls were rated using a modified procedure used in the Spring, 1965, Nationwide Household Food Consumption Survey (Clark, 1969). Nutrients meeting 100% or above the RDA were rated excellent and given four points; accordingly, at least 66% but less than 100% - rated good and given 3 points, above 50% but not greater than 65% were rated fair and given 2 points, below 50% were rated poor and given 1 point. The total score for the eight nutrients were combined. Diets which scored the highest possible total of 32 points were considered excellent. Diets which scored between 22 and 31 were rated good, while those diets which scored between 15 and 21 were rated fair. Any diet which scored a total less than 15 points was considered poor.

Personal data was also obtained from each subject prior to taking the diet history (Appendix F). Present height and weight, pre-pregnancy weight, eating habits and preferences during pregnancy, month of pregnancy as determined by physician and vitamin supplement used, and diagnosed health problems were covered by this form. Questions dealing with smoking habits were included since some research findings indicate cigarette smokers have lower serum ascorbic acid levels (Pelletier, 1970). The smoking habit was measured by a method developed by Keith and Driskell (1978). Pack years were determined for each smoker (number of packs per day times the number of years subject had been smoking).

A General Background Information Questionnaire was used to collect data on income, educational background, psychosocial assets and factors which may influence psychological stress during pregnancy (Appendix G). Also, this form provided a more detailed description of subjects who volunteered. This questionnaire was pretested on 15 pregnant women to determine if questions were appropriate and easily comprehended. Changes were made to shorten and eliminate some questions included in the first questionnaire draft.

Two self-evaluation questionnaires (Appendix H), the Spielberger State-Trait Anxiety Inventories - Forms X-1 and X-2 were used primarily to measure anxiety in subjects. These inventories were used due to their ease in scoring, brevity, in administration and high internal consistency. The Spielberger State-Trait Anxiety Inventory A-State scale (Form X-1) consists of 20 statements which determine how an individual feels at that particular moment of time. The A-Trait scale (Form X-2) also consists of 20 statements which describes how an individual generally feels. Each A-Trait item has been determined to be imprevious to situational stress and relatively stable over time (Spielburger, 1972). Spielburger, found that low scores on A-State scores indicated the presence of calmness and serenity, intermediate scores were designed to reflect moderate levels of tension and apprehensiveness, and high scores corresponded with intense states of fright and apprehension, near panic in the population used (Spielburger, 1972).

A Symptom Checklist (SCL), was used included to provide data about if physical symptoms which might be related to increased anxiety during pregnancy (Appendix I). Each symptom could be rated from 1-10. The higher the number indicated by the subject the more intense the symptom was experienced.

A Fitness Index Questionnaire (Appendix H) was included to provide information about various factors which may affect the plasma cortisol measurement. This schedule included questions about use of caffeine-containing beverages, sleep and relaxation patterns, smoking and exercising habits for the 24-hour period prior to blood collection.

Collection of Biochemical Data

Plasma ascorbic acid values of all subjects were determined by a modified method of Roe and Kuether (1943). Refer to Appendix J for a detailed description of this method. Plasma was acquired from 20 ml of whole blood collected via venipuncture in a 165 x 16 mm Vacutainer evacuated glass tube (Becton Dickinson Co.). Each tube contained approximately 357 U.S.P. units of sodium heparin to prevent clotting. Once collected, blood was placed immediately in ice for no longer than 2 hours and 30 minutes. Blood was centrifuged for 20 minutes at 5,000 x g at 0° Centigrade for 20 minutes to obtain plasma. Plasma was frozen in 5 ml plastic tubes and held until the analyses were done. All samples were analyzed within a 2 month period after collection. Total plasma cortisol levels were determined using the protocol suggested (with minor modifications) by Endocrine Sciences, Tarzana, California (1972). The antisera used for the cortisol assay was also obtained from Endocrine Sciences.

Methods of Statistical Analysis

The PROC MEANS program of the Statistical Analysis System (SAS, 1979) was used to calculate the mean standard deviation, minimum and maximum values for the following variables: plasma ascorbic acid levels, plasma cortisol levels, the Spielburger State-Trait scores for A-State and A-Trait, age (years), month of pregnancy, gravida number, ascorbic acid intakes as calculated from 24 hour recalls, ascorbic acid intakes as calculated from diet histories, ascorbic acid intakes from prenatal supplements alone, the amount of weight gain during pregnancy, total ascorbic acid intake values, symptom number, symptom severity, symptom index, income, education, packyears, nutrition scores as determined from diet histories, and nutrition scores as determined from 24 hours recalls.

The PROC CORR program of SAS was used to obtain Pearson product moment correlation coefficients between all the previously listed variables. The PROC CORR program also calculated the significance of probability of each correlation coefficient and the number of observation used to calculate each coefficient.

Using the PROC MEANS program of SAS, two tailed Students' t-test were performed to determine if there was any significant difference between the following listed values: 1) plasma ascorbic acid levels of smokers and nonsmokers, 2) ascorbic acid intakes values (mg) as calculated from 24 hour recalls and diet histories, 3) nutrition scores as determined from 24 hour recalls and diet histories.

The responses from the General Background Information Questionnaire were coded numerically. The PROC FREQ program of SAS was used to obtain frequencies, cumulative frequencies, percentages and cumulative frequencies of responses from each question. Cross tabulations were performed on appropriate responses to determine income and/or education groupings.

RESULTS AND DISCUSSION

Subject Description

Pregnant women in their third trimester were chosen. Previous research findings indicate that best time to record behaviors and attitudes is during the third trimester (McDonald, 1958). In an effort to control variations in biochemical parameters, women in their third trimester or very close to their eighth month of pregnancy were selected. For this group of subjects, the mean, minimum and maximum values for month of pregnancy were 8.03, 7.00 and 8.75 months, respectively. See Table 1 for a summary of variables.

The age of subjects ranged from 15 to 32 years with a mean age of 24.72. Forty-four percent of the women were 24 years of age or less. It has been observed that age is a determinant or reproductive efficiency. Mortality rates of infants increase with pregnant women who are younger than 17 and older than 35 years of age (Siegel and Morris, 1970). Only 2 subjects were 17 years of age or younger. None of the subjects were over 32 years of age.

An attempt was made to obtain subjects who were in their first or second pregnancy. Twenty-four subjects (75%) and 5 subjects (15.6%) were in their first and second pregnancy respectively. Only 2 subjects were experiencing their third and fourth pregnancy. There is sufficient evidence that birth order exerts an influence on reproductive performance. First pregnancies are often more difficult, regardless of maternal age (Siegel and Morris, 1970).

Table 1

Summary of Pregnancy Length, Age, Gravida Number, and Weight Gain in Subjects

Variable	Unit	Mean ± S.D.	Range Min.-Max.
Month of Pregnancy	Month	8.03 ± .39	7.00 - 8.75
Age	Years	24.71 ± 4.3	15 - 32
Gravida Number	Number	1.38 ± .75	1 - 4
Weight Gain	Pounds	25.25 ± 6.31	15.00 - 44.00
Weight Gain	Kilograms	11.47 ± 2.87	6.82 ± 19.82

The mean weight gain during pregnancy was 25.25 $\pm$ 6.31 lbs. (11.48 $\pm$ 2.87 kg), with a range of 15-44 lbs. (6.82 - 20.00 kg). There is evidence a weight gain from 26 to 30 pounds produces the most favorable outcome in healthy women (Naeye, 1979).

The General Background Information Questionnaire

Results from the General Background Information Questionnaire will provide further description of the subjects. This questionnaire provided information on psychosocial assets and factors which influence the perception of stress. See Appendix G for complete listing of frequencies and percentages of responses to this questionnaire. All subjects except one were married. The majority of subjects had attended college (56.3%) and/or graduate school (31.2%). Nine (28.1%) and 5 (15.6%) of the subjects had completed high school or grade school, respectively. The majority of spouses of the subjects had attended college or graduate school (56.3%). The spouses were distributed according to occupation as follows: 10 professionals (31.2%), 17 skilled workers (34.4%), 2 unskilled workers (6.3%) and 6 other types (18.8%). When other types were specified, the spouses were listed as students.

The combined income for the subject and her spouse were categorized into 6 groups. Eight subjects (25%) had a combined income of less than \$5,000, 8 subjects (25%) \$5,000-\$9,999, 7 subjects (22%) \$10,000-\$19,999, 5 subjects (16%) \$20,000-\$29,999, 3 subjects (9%) \$30,000-\$39,999, and 1 subject (3%) \$40,000 and above. Twenty-three of the subjects (71.88%) were not securely employed. The subjects rated their financial security as follows: 15.6% - no financial problems, 18.8% - very few financial problems, 40.6% - can make out, 18.8% - will have some financial problems and 6.3% - will have many financial problems. Previous research indicates the less socioeconomic stress upon a woman, the more likely she is to enjoy her pregnancy (Gordon, 1967; Sear et al., 1956; Poffenbarger, 1952).

The majority of the women (78.1%) had a recent job. More than half (68.8%) planned to return to work after the birth of the baby. The majority of the women (78.5%) who worked liked their jobs. Little or no job related stress was reflected through responses to the questionnaire.

Approximately 97% indicated they were very well satisfied with their present living situation. These subjects reported to be either very well satisfied, fairly satisfied or satisfied with their present living situation. Only 12.5% perceived their living situation as being crowded. Twenty-one subjects (65.6%) had lived in their present community for over one year. The environment or the perceived living condition appeared not to be stressful on these subjects. Gordon and Gordon (1959) found their subjects who had moved recently had more emotional upsets during and immediately following their pregnancies. In this study, the women rated their mobility (the ability to move from place to place in the community). In this study, the majority (78.13%) rated their mobility as either excellent or better than most.

Previous research has shown social support to have moderating effects or a preventive effect on strainful life events (Hefferin, 1980; Nuckolls et al., 1972). The results from the General Background Information Questionnaire indicated the majority of the subjects had a good social support system. When the subjects were questioned "Could you count on your family for help if you need it?", the subjects all answered positively: 68.75% of the subjects answered "yes in all matters" and 31.25% "yes, in some matters."

At least 90% had someone besides their relatives or spouse on whom they could count for help. Only 3 subjects had no close friends on whom they could count on. All 3 of these subjects were from the lowest income grouping. Some subjects (34%) felt deprived of social contacts. The social contacts missed most frequently were immediate family and close friends. Those subjects who felt deprived were usually missing only one type of contact.

Unacceptance or conflict of wanting the pregnancy has been associated with increased length of the labor process and increased levels of anxiety (McDonald, 1968). Increased risk of low birth weight, prematurity, higher infant mortality, increased physical symptoms during pregnancy and spontaneous abortions have been associated with unaccepted pregnancies (Morris et al., 1963; Gordon et al., 1959; Heinstein, 1967; Blau, 1963; and Coppen, 1959). In this study, all subjects felt either very happy (87.5%) or some happiness (12.5%) about their pregnancy. Increased acceptance of their pregnancy occurred in some subjects since first learning of their pregnancy: upon first learning, 47% were very pleased, 35% were pleased, 9% had accepted their pregnancy, 6% were a bit displeased and 1 subject (3.1%) was very displeased. Leifer (1977) and Werhoff (1956) reported a gradual acceptance of pregnancy in a majority of women who were first ambivalent about their pregnancy.

Most subjects rated the timing of their pregnancy favorably: thirty-four percent (34%) rated the timing very convenient, 28% at little convenient, and 20% convenient. Four subjects rated the timing of their pregnancy as inconvenient or very inconvenient. Two of those 4 subjects were from the lowest income category.

Women who feel anxious or frightened toward childbirth have been reported to have more delivery room difficulties and prolonged labor (Davids and Devault, 1962; McDonald et al., 1963; Lederman et al., 1978). Subjects were asked to rate their feeling toward childbirth. A majority of the subjects in this study felt either very confident (34.4%), quite confident (31.2%) or confident (12.5%) about childbirth. Seven subjects reported to be either a little frightened or very frightened (21.9%) of childbirth. Three of the subjects reported to be frightened or very frightened, were women in the low income gorup (less than $5,000). A majority of subjects appeared pleased with the physicians or obstetricians. Only 2 subjects rated their physician as "indifferent" (6.3%). The inability of taking care of the future baby caused little or no distress in the subjects. Only 2 subjects had even a "few doubts" about caring for their baby. Both subjects were low income.

Few studies have been conducted on the relationship of marital adjustment and pregnancy outcomes. It would seem logical that marital unhappiness would have an association with unfavorable pregnancy outcomes. Hetzel et al. (1961) found a positive association between marital unhappiness and toxemia in pregnant women. Heinstein (1967) found no relationship between marital unhappiness and complications in deliveries. The majority of subjects reported a positive marital situation. Sixty-nine percent (68.8%) of the subjects were "very happy" about their married life. About 19 percent were quite happy about their marital situation. One subject, in the lowest income group, was unhappy about her married life. The majority (93.8%) of subjects answered that their husbands were either "very pleased," "quite pleased" or "pleased" about their pregnancy. Only 2 subjects reported that their spouses were "a little displeased" or "very displeased." These 2 subjects were from the lowest income group (less than $5,000).

Healthy pregnant women were sought for this study. Nearly 41% perceived their health as excellent, and slightly more than 34% rated themselves as better than most. Twenty-five percent thought they were average in health.

Nutrient Intake Analysis of Subjects

The frequency and percentage distribution for nutrient intake ratings are summarized in Table 2. See Procedures section on how ratings were calculated. Nutrient analysis of 24-hour recalls revealed

Table 2

Distribution of Subjects by Rating for Nutrient Intakes for 24-Hour Recalls and Diet Histories

Nutrient Intake	Diet Rating							
	Excellent		Good		Fair		Poor	
	N	%	N	%	N	%	N	%
24 hour recalls	1	3.1	23	71.8	6	18.8	2	6.3
Diet histories	0	0.0	27	84.4	5	15.6	0	0.0

Excellent = 32 points

Good = 31-22 points

Fair = 21-16 points

Poor = less than 16 points

only one subject had a nutrient intake considered to be excellent (32 points). Approximately 72% of the women had nutrient intakes considered good as calculated for 24-hour recalls. Twenty-four hour recall analysis further showed 18.8% (6 subjects) had diets considered to be fair. Two subjects were assessed to have a poor nutrient intake as calculated from the 24-hour recall. There was an insignificant positive correlation between income and scores on 24-hour recalls (r = 0.12).

Diet histories, more representative of dietary patterns (Burke, 1947), indicated that the diets of a larger number of women were judged as being good diets, 84.4%, as compared to 71.8% rated good from 24-hour recalls. Nutrient analysis of diet histories revealed none of the subjects had diets rated as either poor or excellent. Approximately 15.6% of subjects had diet histories rated as fair. Increases in food intake in the diet histories over the 24-hour recalls were apparent. An insignificant negative correlation was found between income and scores on diet histories (r = -0.012). Positive correlations between socioeconomic status and dietary practice have been reported in the literature (Payton et al., 1960; Thomson, 1959; Murphy and Wertz, 1954).

Examination of Table 3 reveals individual nutrient intakes for diet histories were greater than that of 24-hour recalls. The intakes of 8 nutrients and calories at 5 levels of dietary adequacy as compared to the 1980 RDA are summarized in the forementioned table. For both methods, riboflavin was reported as one of the nutrients most

Table 3

Percentages of Pregnant Women Who Met Five Levels of the 1980 RDA for Eight Nutrients and Calories as Determined from 24-Hour Recalls and Diet Histories

Level of Intake	Calories g	Protein g	Calcium mg	Iron mg	Vitamin A I.U.	Thiamine mg	Riboflavin mg	Niacin mgNE	Ascorbic Acid mg
100% and over:									
24 hour recalls	31.2	53.1	43.8	9.4	56.2	46.9	68.7	65.6	46.9
Diet histories	56.3	75.0	75.0	9.4	78.1	9.4	65.63	68.8	87.5
66-100%:									
24 hour recalls	56.3	40.6	37.5	53.1	15.6	40.6	25.0	25.0	15.6
Diet histories	40.6	25.0	15.6	50.0	18.8	31.3	21.8	25.0	6.25
50-66%:									
24 hour recalls	9.4	6.3	9.4	21.9	12.5	6.3	6.3	3.1	9.4
Diet histories	3.1	0.0	3.1	25.0	3.1	21.8	9.4	6.3	3.1
33-50%:									
24 hour recalls	3.1	0.0	3.1	12.5	9.4	3.1	0.0	6.3	18.7
Diet histories	0.0	0.0	3.1	12.5	0.0	25.0	0.0	0.0	0.0

Table 3 (Continued)

Level of Intake	Calories g	Protein g	Calcium mg	Iron mg	Vitamin A I.U.	Thiamine mg	Riboflavin mg	Niacin mgNE	Ascorbic Acid mg
Less than 33%:									
24 hour recalls	0.0	0.0	6.2	3.1	6.3	3.1	0.0	0.0	9.4
Diet histories	0.0	0.0	3.1	3.1	0.0	12.5	3.12	0.0	0.0

frequently at levels of 100% or more of the RDA. This finding is similar to that found by Shavink (1974). Niacin and vitamin A were the next 2 nutrients to be frequently consumed 100% or more. Murphy and Wertz (1954) stated in their sample of pregnant women, the only nutrient "well" provided in the diets of pregnant women was vitamin A.

Iron intakes were least frequently at levels of 100% or more. The Food and Nutrition Board (1980) has suggested that increased requirements during pregnancy could not be met by the iron content of habitual American diets nor by existing iron stores of many women. The Board suggested the use of 30-60 mg of supplemental iron for pregnant women. The Ten State Nutrition Survey (1970) indicated that low iron intakes by pregnant women were also a nutrition problem in the United States.

Contrary to results from 24-hour recalls, ascorbic acid was most frequently at levels of 100% or more of the RDA. The discrepancy of levels reported for ascorbic acid in the 24-hour recalls and diet histories may be explained. The subjects were instructed not to consume foods high in ascorbic acid content after 6:00 PM the day before the schedule interview. Some subjects may have avoided these foods to excess by avoiding them all day. A Student's t-test was used to determine if a significant difference existed between the ascorbic acid intake as calculated from 24-hour recalls and diet histories (t value = 2.62, $p < 0.05$).

According to diet histories, seventy-five percent of the subjects consumed calcium and protein at levels of 100% or more of the RDA.

This finding is in agreement with the findings of a study by Shavink (1974). It has been observed that during pregnancy, women at all socioeconomic levels increase protein intakes due to increases in animal protein (Beal, 1971). The Ten State Nutrition Survey (1970) indicated that dietary protein of pregnant women were mostly at levels considered well above adequate.

All women in this study took prenatal vitamin and mineral supplements daily or as prescribed by the doctor. Only one subject who had been prescribed a prenatal supplement did not take it regularly. This subject complained of gastrointestinal discomfort upon taking the supplement. The most common brands prescribed were Materna-100, Stuart Natal 1+1, Natafort, Natalin and Pramilet. Many of the subjects were prescribed an additional iron supplement. Norman and Adams (1970), Shavink (1974) reported 83% and 86% of the women in their study had taken prenatal supplements.

Ascorbic Acid Status of Subjects

The Food and Nutrition Board of the National Research Council (1980) has recommended 80 mg of ascorbic acid daily for pregnant women. This provides an additional 20 mg over nonpregnant recommended levels to provide for fetal needs. For pregnant women, the dietary standard for the Hanes I study was 60 mg of ascorbic acid daily (USDHEW, 1979). Only 12.5% of the women in this study did not receive 80 mg or more of ascorbic acid according to diet histories. Analysis of the 24-hour recalls revealed that 59.4% (19 subjects) did not consume 80 mg or more of ascorbic acid from diet alone. The great discrepancy between these two percentages can be explained since subjects were asked to avoid consuming foods high in ascorbic acid after 6:00 PM the day before the scheduled interview.

In Table 4, summary data is presented for ascorbic acid intake and plasma ascorbic acid levels. A new variable was created called Total Ascorbic Acid. Total ascorbic acid intake is the combined intakes of ascorbic acid as calculated from diet histories and from prenatal supplements. All subjects received more than adequate levels of ascorbic acid when vitamin supplementation was considered.

All plasma levels of ascorbic acid were within the normal range. The guidelines for the Interdepartmental Committee on Nutrition for National Defense (ICNND) considered any level at 0.30 mg/100 ml plasma and above as an acceptable level (Sauberlich et al., 1974). The Ten State Nutrition Survey (1970) considered any plasma ascorbic acid levels less than 0.10 mg/100 ml as deficient and levels between 0.10-0.19 mg/100 ml as low. In this study, the plasma ascorbic acid levels ranged from 0.48-1.64 mg/100 ml plasma with a mean of 0.93 mg/100 ml plasma and standard deviation of 0.26 mg/100 ml plasma.

Correlation coefficients between plasma ascorbic acid and selected variables are summarized in Table 4 also. No significant correlations were found between plasma ascorbic acid and intake from diet or with supplements. Macy (1954) reported a positive correlation between calculated ascorbic acid content of maternal diet and corresponding plasma ascorbic acid levels in pregnant women. In this study,

Table 4

Correlations Coefficients Between Plasma Ascorbic Acid and Selected Variables

Variables	Correlation Coefficients
*Total Ascorbic Acid Intake	0.20
Ascorbic Acid Intake as calculated from diet history	0.12
Ascorbic Acid Intake as calculated from 24 hour recalls	-0.07
Ascorbic Acid Intake as calculated from vitamin supplementation only	0.27
Education	-0.35[1]
Income	-0.48[2]
Age	-0.30
Month of Pregnancy	-0.39[1]

*Total Ascorbic Acid Intake = ascorbic as calculated from diet histories plus ascorbic acid from vitamin supplementation

[1]Statistically significant at $p < 0.05$

[2]Statistically significant at $p < 0.01$

a positive correlation occurred between total ascorbic acid intakes and plasma ascorbic acid levels ($r = 0.20$).

There was a significant negative correlation, $r = -0.39$, ($p < 0.05$) between month of pregnancy and plasma ascorbic acid levels. This correlation may indicate that plasma ascorbic acid levels decreased as the month of pregnancy of subjects increased. Mason and River (1971), Vobecky et al. (1974), Darby et al. (1953) and Morse et al. (1975) found a decrease in plasma ascorbic acid levels as pregnancy advanced. The reasons for this decrease could be hemodilution since plasma volume can increase as much as 50% (Hytten et al., 1970). Increases in estrogen, cortisol and progesterone may play some role in decreased levels (Food and Nutrition Board, 1980).

A negative but nonsignificant correlation between age and plasma ascorbic acid level was found in this study. Garcia et al. (1974), Mason and Rivers (1970) found a positive association between increasing age and plasma ascorbic acid levels. Jalso et al. (1965) and Dodd (1969) found a negative correlation between age and plasma ascorbic acid levels.

There was an unexpected significant negative correlation between plasma ascorbic acid levels and level of education ($r = -0.32$, $p < 0.05$). Mason and Rivers (1970), Martin et al. (1957) and Garcia et al. (1974) found plasma ascorbic acid levels increased as level of education achieved and income increased. A significant negative correlation was found between plasma ascorbic acid levels and income (-0.48, $p \leq 0.01$). Again, this finding is in contradiction to most research findings. The individuals with low income had been instructed by Public Health doctors and nurses to take Pramilet, a prenatal supplement, at least once or twice a day. Some of these subjects had also received nutrition education during their pregnancy.

Since there is research indicating smoking cigarettes depresses plasma ascorbic acid levels (Pelletier, 1970; Irwin and Hutchins, 1976), a Student's t-test was performed to determine whether smokers had significant differences in plasma ascorbic acid levels. The "t" test revealed no significant differences in plasma ascorbic acid levels for nonsmokers and smokers ($p \leq 0.05$). Given the small number of smokers ($N = 8$), it is doubtful that much confidence can be placed in this finding. Ascorbic acid intakes of smokers and nonsmokers were similar. There was an insignificant negative correlation between packyears and plasma ascorbic acid levels. The correlation coefficient between plasma ascorbic acid and plasma cortisol will be presented and discussed under the plasma cortisol section.

Assessment of Anxiety by the Spielberger State-Trait Anxiety Scale (STAI) and Interrelationships Between Selected Variables

Anxiety has been consistently found to be related to obstetrical complications (McDonald, 1968). Anxiety may also affect the plasma ascorbic acid levels (Maas et al., 1961). In this study, an attempt was made to assess anxiety as measured by the STAI as a source of stress.

Scores on each part of the STAI (A-Trait and A-State) have a possible range of 1-4 points, with the higher score reflecting more stress. The range of scores for the A-Trait measurement was 1.05-2.85 with the mean of 1.63. (For a listing of scores of subjects, see Appendix K). According to Spielberger (1979), the low scores exhibited by these subjects indicated that the subjects are less likely to perceive a wide range of situations as threatening as would be reflected by high A-Trait scores. Low scores are indicative of more stable personalities.

Spielberger (1979) also indicated the A-State score may vary in intensity and duration and fluctuate over time and is a function of the amount of stress that impinges upon an individual at the time. These scores also depend upon how the subject interprets a situation as being threatening or dangerous. In this study, the range of A-State scores for subjects was 1.00-2.45 ($\bar{x}$ = 1.55). A mean of 1.55 indicates states of relative serenity and calmness, as reported by Spielberger (1972) from earlier studies. In this study, 6 subjects had scores of 2.00 and above. It is interesting to note that all of these six subjects were low income subjects interviewed at either Radford Health Department or Montgomery County Health Department.

Edwards and Jones (1970) administered the STAI A-State measurement tool at the beginning of the third trimester and each following week until delivery in 53 unwed pregnant women. Women having normal deliveries showed a reduction in STAI State scores until one week prior to delivery when a significant increase in A-State was observed.

Women having complications during delivery had consistently higher A-States until one week before delivery at which time their A-State scores dramatically decreased.

Gorsuch and Key (1974) found no statistically significant differences between normal and abnormal pregnant outcome groups in anxiety as measured by A-State during the ninth and tenth lunar months. However, women with obstetric complications were reported to have higher scores on A-State of the STAI during the first trimester.

Using the A-State of STAI, Srabstein et al. (1967) observed that women with abnormal perinatal outcomes were higher in anxiety during the second and third trimesters. Lubin et al. (1975), using the A-State measurement, found that anxiety was higher in the first and third trimesters of pregnancy.

It is apparent that current research does not provide adequate documentation of the normal psychological changes that occur during pregnancy (Leifer, 1980). There are some studies which suggest that pregnancy is experienced as a period of unusual well-being (Hooke and Marks, 1962; Lubin et al., 1975). The Kane, Lipton, and Ewing (1969) study suggested that psychopathology decreases throughout pregnancy. Rosenberg and Silver (1965) presented results indicating that pregnant women commit suicide much less frequently than nonpregnant women. A study by Poffenbarger (1964) revealed that psychotic reactions are rare during pregnancy. Dalton (1971) and Herzog and Detre (1976) have noted an elation of moods during pregnancy when levels of progesterone, estrogen and corticosteroids are highest.

Table 5 provides a summary of correlation coefficients concerning STAI. A significant negative correlation was found between age and A-State ($r = -0.42$, $p \leq 0.01$). This finding indicates that younger pregnant women exhibited more tension, apprehension, nervousness and worry than older women. It appears a more mature personality can more readily cope with the strain of pregnancy. Pregnant teenagers are at greater biological and psychological risk as reported in a review by the National Research Council (1970) after reviewing research projects involving this age group.

There was a negative but insignificant correlation coefficient between month of pregnancy and STAI (A-Trait and A-State) measures. There is some research to indicate that anxiety decreases near the end of pregnancy. Leifer (1977) found that pregnant women, by the end of their pregnancies, had moderately positive attitudes. Winokur and Werhoff (1959) noted an increase in the acceptance of the pregnancy as the pregnancy progressed.

Correlations between income and A-State and A-Trait were significant. The correlation between income and A-State ($r = -0.47$, $p \leq 0.01$) indicated that the low income women exhibited more signs of apprehension and tension according to Spielberger (1970). The correlation between A-Trait and income was also significant ($r = -0.34$, $p \leq 0.05$). According to Spielberger, this indicates that lower income women perceive more life situations as threatening.

Also, there were negative correlations between education and A-State and A-Trait measures of STAI. The correlation coefficient

Table 5

Correlation Coefficients Involving
STAI with Various Variables

Variables	Correlation Coefficient
A - State and Month of Pregnancy	-0.26
A - State and Income	-0.47[2]
A - State and Education	-0.43[2]
A - State and Age	-0.42[2]
A - State and Nutrition Score as Calculated from Diet History	-0.11
A - State and Ascorbic Acid Intake as Calculated from Diet History	-0.34[1]
A - State and Total Ascorbic Acid Intake	-0.35[1]
A - State and Plasma Ascorbic Acid	0.24
A - State and A-Trait	0.76[2]
A - Trait and Month of Pregnancy	-0.25
A - Trait and Income	-0.34[1]
A - Trait and Education	-0.31
A - Trait and Age	-0.29
A - Trait and Nutrition Score as Calculated from Diet History	-0.03
A - Trait and Ascorbic Acid Intake as Calculated from Diet Histories	-0.11
A - Trait and Total Ascorbic Acid Intake	-0.12
A - Trait and Plasma Ascorbic Acid	0.20

[1] $p \leq 0.05$

[2] $p \leq 0.01$

between A-State and education was -0.43 ($p \leq .01$). This finding indicated that women with lower levels of educational attainment exhibited more anxiety. The correlation between age and education indicated that younger pregnant women had received less education ($r = 0.66$, $p < 0.01$). The results of this study confirm the hypothesis that maternal anxiety may play a major role in causing obstetrical problems in pregnant women of low socioeconomic status. Epidemiological studies have indicated a strong correlation between lower socioeconomic status and the occurance of a wide variety of abnormalities of reproduction including fetal death and growth retardation (Myers, 1979).

The correlation coefficient between A-State and A-Trait scores was positive and highly significant ($r = 0.76$, $p < 0.01$). This correlation indicates that pregnant women whose personalities were more disposed to perceive a wide range of circumstances as threatening exhibited more anxiety.

The effect of anxiety upon nutrient intake and nutritional status has not been well researched. In this study, there was a negative but insignificant correlation between nutrition scores obtained from diet histories and A-State of STAI ($r = -0.11$). This finding indicated a slight, but insignificant trend, that pregnant women scoring higher on the A-State measure received a lower nutrition score derived from diet histories. A significant negative correlation between A-State scores and ascorbic acid intake as calculated from diet histories was obtained ($r = -0.34$, $p \leq 0.05$). The correlation coefficient between total ascorbic acid intake and A-State was significant also ($r = -0.35$, $p < 0.05$). These two correlation coefficients indicated that ascorbic acid intakes decreased as A-State anxiety scores measured increased.

Some people under stress will resort to eating what is available - a meal from a fast food restaurant, vending machine items or convenience foods at home (King and Parham, 1981). This type of diet is usually limited in fruits and vegetables which provide ascorbic acid. The correlation found between ascorbic acid intake and A-State anxiety scores gives support to this explanation.

Positive correlation coefficients between plasma ascorbic acid and STAI measure (A-Trait and S-Trait) were found. Neither of these correlation coefficients were significant.

Plasma Cortisol

Higher plasma cortisol levels, attributed to psychological stress, have been associated with poor progress of labor (Burns, 1976). It is well established that emotional arousal such as anxiety stimulates the secretion of cortisol (Mason, 1975). Because these findings it was decided to measure total plasma cortisol as a biochemical parameter in this study.

Previous research indicates that a steady increase in total plasma cortisol occurs as pregnancy progresses (Burke and Roulet, 1970). In a normal adult with a normal sleep-wake cycle, total plasma cortisol levels range from 8 to 25 ug/100 ml plasma ($\bar{x}$ = 13 ug/100 ml) between 6:00 and 9:00 AM. Total plasma cortisol levels during the third trimester have been reported to be much greater. Burke and Roulet (1970) reported 16 third trimester pregnant women to have total plasma cortisol ranging from 25-46 ug/100 ml with a mean of 38.1 between the hours of 6:00 - 9:00 AM. Brown et al. (1972) reported a range of 19-50 ug/100 ml for total plasma cortisol in 43 third trimester pregnant women at 8:00 AM. Nolten et al. (1978) reported a mean and standard deviation of 31.6 $\pm$ 2.4 ug/100 ml for total plasma cortisol for 15 third trimester pregnant women at 8:00 AM.

Geigy Scientific Tables (1981) state levels of 32.00-72.0 ug/100 ml plasma for total plasma cortisol as normal for pregnant women between their 20 and 40th week of pregnancy. In the present study, the total plasma cortisol levels of the 32 women ranged from 16.12-77.18 ug/100 ml plasma. The mean and standard deviation for total plasma cortisol was 37 ug/100 ml plasma and 15 ug/100 ml plasma, respectively. All blood samples were taken from 8:00-10:00 AM since daily total plasma cortisol levels vary within a day due to circadian rhythm (Peterson and McGinley, 1976).

In this study, a review of the fitness index indicated that the 32 pregnant women had light physical activity or no strainful physical activity and normal sleep-wake cycles. Only 4 subjects had consumed caffeinated beverages within the 24-hour period prior to the interview. The forementioned factors were assessed because of their possible effect on plasma cortisol levels. However, research has shown that the circadian rhythm is dependent upon sleep-wake patterns and independent of food intake and exercise or light (Hellman et al., 1970; Krieger et al., 1971; Few, 1974; Orth et al., 1967 and Krieger et al., 1971).

Since anxiety increases cortisol secretion (Mason, 1975), one would expect to find a positive correlation between STAI measures and plasma cortisol levels. Lederman et al. (1978) found a correlation coefficient of 0.59 ($p < 0.01$) between the A-State Scale of STAI and plasma cortisol levels in 32 pregnant women in labor. However, Pancheri et al. (1979) found an insignificant negative correlation between the scores of the A-State Scale of STAI and plasma cortisol levels in pregnant women. In the present study, a negative but insignificant correlation was found between plasma cortisol levels and A-State scores of STAI ($r = -0.29$). See Table 6 for a summary of the correlation coefficients of cortisol with selected variables. A negative insignificant correlation coefficient was found between the A-Trait scores of STAI and plasma cortisol levels.

An insignificant correlation coefficient of -0.05 was found between plasma cortisol levels and the month of pregnancy of the subjects when in when blood samples were taken. There was a significant positive correlation between age of the pregnant women and plasma cortisol levels ($r = 0.39$, $p < 0.05$). An unexampled positive correlation of 0.42 between nutrition scores on the diet histories and plasma cortisol levels was found in these women ($p \leq 0.01$). Correlation coefficients between total plasma cortisol levels and education or income were not significant.

Table 6

Interrelationships of Total Plasma Cortisol and Selected Variables

Variables	Correlation Coefficient
A - State Scale of STAI	-0.29
A - Trait Scale of STAI	-0.20
Age	0.39[1]
Month of Pregnancy	-0.05
Nutrition Score from Diet Histories	0.42[2]
Income	-0.02
Education	0.26
Plasma Ascorbic Acid	0.03
Total Ascorbic Acid Intake	0.16

[1] $p < 0.05$

[2] $p < 0.01$

et al., 1980). Grimm (1962) indicated that biochemical changes occurring in pregnancy create a lower threshold for nausea and vomiting. Coppen (1959), using psychiatric interviews and personality questionnaires, studied 50 randomly selected primiparas. Twenty-nine had some vomiting and 21 were symptom free. There was no difference between the women who experienced vomiting and those who did not in extroversion, neurotic symptoms, or with stress or attitudes toward pregnancy. Using interview techniques to assess anxiety in first trimester pregnant women, Rosen (1955) concluded that severity of symptoms was caused by emotional stress.

The correlation coefficients between the three measures of SCL and income were negative but not significant. The negative correlations may indicate a trend that pregnant women from lower income may exhibit more symptoms and greater intensity in the symptoms experienced. There was a significant positive correlation between the month of pregnancy of the subject and the Symptom Number ($r = 0.34$, $p \leq 0.05$). The most frequent and severe symptom reported by women in this study was fatigue. Leifer (1980) reported similar results.

Although not significant, a positive correlation was found between Symptom Severity and plasma cortisol. The severity of symptoms may have contributed to physiological adjustments during pregnancy which results in increased cortisol secretion. It is known that physical or psychological stress stimulates cortisol secretion (Mason, 1975).

In this study, there was no significant correlation between plasma cortisol and ascorbic acid levels ($r = 0.03$). Loh and Wilson (1973) reported that plasma ascorbic acid had a similar daily rhythm to that of plasma cortisol. The present study revealed no indication of that relationship. Absence of any significant correlation between plasma cortisol and plasma ascorbic acid levels may be due to the great variation in plasma cortisol levels and the small number of subjects in this study.

The correlation coefficient between total ascorbic acid intake and plasma cortisol was insignificant ($r = 0.16$). The correlations coefficients between plasma cortisol and total ascorbic acid intake or plasma ascorbic acid failed to indicate any relationship between ascorbic acid and cortisol levels.

The Symptom Checklist (SCL)

Three scores were derived from the Symptom Checklist (SCL); 1) Symptom Number, 2) Symptom Severity, and 3) Symptom Index. Symptom number was obtained from the total number of symptoms a subject indicated experiencing. Symptom Severity was derived by totaling the symptoms scores and dividing the score by the number of symptoms identified. A 1 to 10 scale was used with the larger numbers indicating a greater severity of symptoms. The Symptom Index was calculated by totaling the numbers indicated for Symptom Severity. See Appendix I for the Symptom Checklist form.

The means ± standard deviations for the Symptom Number, Symptom Severity, and Symptom Index were 6.31 ± 4.23, 3.84 ± 1.34 and 24.87 ± 18.44 respectively. The ranges for the Symptom Number, Symptom Severity, and Symptom Index were 1-23.00, 1.67-6.11, and 4.00-77.00 respectively. A wide variation is readily noticeable in the Symptom Number and Symptom Index measurements. The variation for the Symptom Severity was markedly less. The mean of Symptom Severity, 3.84 ± 1.34, reflects less intense symptoms. Table 7 and 8 presents a summary of the relationships between SCL and selected variables. No statistically significant correlations were found between the three measures of SCL and A-State or SCL and A-Trait of STAI. Some research studies observed a positive correlation between somatic symptoms and self-reported anxiety measurements (Selby et al., 1980).

Lubin et al. (1975) found a significant positive relationship between somatic symptoms as measured by the Symptom Checklist (SCL) and Anxiety Adjective Checklist, and the STAI questionnaire. Their sample consisted of 93 white middle class pregnant women with a mean of 14 years of education. Zuckerman et al. (1963) also found a significant relationship between self-reported anxiety measures and somatic symptoms in pregnant women.

Deutsch (1945) suggested that symptoms such as nausea and vomiting were due to rejection of the pregnancy. It has been reported that vomiting during pregnancy occurs frequently in 50-70% of women and is generally unrelated to either emotional factors such as neurotism or to ambivalent feelings toward the unborn child (Selby

Table 7

Correlation Coefficients Involving Measures of Symptom Checklist (SCL) and Other Selected Variables

SCL Measure	Month of Pregnancy	A-Trait	A-State	Plasma Ascorbic Acid	Income	Education	Smoker's Pack Years	Nutrition Scores from Diet History	Nutrition Score from 24-Hour Recalls
Symptom Number	0.34[1]	0.02	0.03	-0.03	-0.02	-0.13	0.71[2]	0.21	0.05
Symptom Severity	-0.02	0.01	0.08	0.15	-0.18	0.12	0.53[2]	0.00	0.20
Symptom Index	0.32[1]	0.06	0.09	0.11	-0.21	-0.04	0.72[2]	0.14	0.16

[1] $p \leq 0.05$

[2] $p \leq 0.01$

Table 8

Correlation Coefficients Involving Measures of Symptom Checklist (SCL) and Other Selected Variables

SCL Measure	Plasma Cortisol	Age	Weight Gain	Pre-Pregnancy Weight	Pregnancy Weight	Ascorbic Acid as Calculated from Diet History	Ascorbic Acid as Calculated from 24-Hour Recalls	Total Ascorbic Acid Intake	Vitamin Supplement
Symptom Number	0.03	-0.15	0.11	0.26	0.29	0.08	0.09	0.07	-0.07
Symptom Severity	0.27	0.13	-0.07	-0.11	-0.13	-0.06	-0.23	-0.25	-0.08
Symptom Index	0.22	-0.11	0.03	0.18	0.18	0.09	-0.06	-0.07	-0.03

[1] $p \leq 0.05$

[2] $p \leq 0.01$

Only 8 smokers participated in this study, but a significant positive correlation was found between pack years (number of packs smoked per day times the number has smoking) and Symptom Number ($r = 0.710$, $p \leq .05$) and also with the Symptom Index measure ($r = 0.72$, $p \leq .05$). Smoking has been reported to contribute to obstetrical complications for pregnant women and their infants (Cole et al., 1972). It seems plausible that heavy smokers could have increased numbers of symptoms over light smokers.

Although not significant, a positive correlation between pregnancy weight and Symptom Number was found. It would seem likely that a large weight gain during pregnancy would contribute to an increase in the number of symptoms or their severity. In this study, there was no significant correlation between weight gain and Symptom Number or Symptom Severity.

Correlations between nutritional intake measures and SCL measures indicated no significant associations. There were no significant correlations between plasma ascorbic acid and the three measurements of SCL.

SUMMARY AND CONCLUSION

The objectives of this study were to assess psychological stress and factors which contribute to stress in 32 third trimester pregnant women, ranging in age from 15-32 years. Associations between psychological stress measurements, ascorbic acid intake, nutrient intake, plasma ascorbic acid and plasma cortisol were examined.

Spielberger State-Trait Anxiety Inventory (STAI) scores, used to assess anxiety, revealed little stress in the majority of subjects. Results from the General Background Information Questionnaire, used to assess psychosocial factors influencing stress, also indicated the presence of little stress in most of the subjects.

Nutrient analysis of diet histories revealed 84.4% of the women had diets rated as good and 15.6% were rated fair. Iron and thiamin were the nutrients least being met by the diets of these subjects. Because of this finding, it is recommended that nutritionist and other health care professionals instruct pregnant women to consume foods which are high in thiamin and iron. According to the diet histories, approximately 87% of these subjects met the RDA for ascorbic acid. All subjects received 100% of RDA for ascorbic acid when total intakes from prenatal supplements and diet histories were considered. The plasma ascorbic acid levels of all the subjects were within the acceptable range, 0.48-1.64 mg/100 ml plasma.

Unexpected negative correlations were found between plasma ascorbic acid levels and income, ($r = -0.48$, $p \leq 0.01$) and plasma ascorbic acid levels and education ($r = -0.32$, $p \leq 0.05$). Most low income subjects were from the Public Health Maternity Clinics. These subjects had been instructed to take a prenatal supplement, Pramilet, at least once a day. Some of the subjects had also received dietary counseling. All subjects within this study had taken prenatal vitamin and/or mineral supplements which were usually prescribed by their doctors. There was a heavy dependence on these supplements to insure proper nutrient intake in these subjects. This researcher suggests that more emphasis be placed on receiving nutrients from diet than from supplements. However, prenatal supplements, containing nutrients at levels recommended by Food and Nutrition Board (1980), appear to produce no harmful effects during pregnancy. However, vitamin-mineral supplements should not be regarded as substitutes for a balanced diet and continued nutritional counseling.

Negative correlations were found between A-State Anxiety scores and income ($r = -0.47$, $p \leq 0.01$) and education ($r = -0.43$, $p \leq 0.01$). These correlations indicated women of lower income or had received less education tended to be more anxious. The researcher concluded from these results that it would be advisable for Public Health physicians to instruct their clients in methods or strategies to cope with stress.

This study found some associations which suggest nutrient intake was poorer with increased anxiety. There was a negative but insignificant correlation of -0.11 between nutrition scores obtained from diet histories and A-State Anxiety scores. Also, a significant negative correlation of -0.34 ($p \leq 0.05$) was found between total ascorbic acid intake and A-State scores.

The correlation coefficients were insignificant between plasma ascorbic acid levels and A-State or A-Trait Anxiety scores. Even if a highly significant negative correlation between plasma ascorbic acid levels and A-State scores was found, the researcher could not speculate that anxiety had a lowering effect of plasma ascorbic acid levels. The researcher could not support such a claim due to consistently low scores obtained from the A-State or Trait Anxiety Scales.

There was no significant correlation between total plasma cortisol levels and A-State Anxiety scores. A high positive correlation was expected since cortisol secretion is increased in anxiety (Mason, 1975). There was a wide range in cortisol levels (16.12 - 77.18 ug/ 100 ml plasma) for a small number of subjects which may account for any lack of significant findings. This reason could also explain the weak positive correlation ($r = 0.03$) between plasma ascorbic acid levels and cortisol levels. A higher positive correlation was expected since Loh and Wilson (1973) reported plasma ascorbic acid and plasma cortisol to have similar circadian rhythms.

One limitation of this study were the low to moderate levels of anxiety exhibited by the majority of subjects. In conclusion, the effect of psychological stress upon ascorbic acid status could not be determined. Further research should be conducted, using high risk pregnant subjects, to determine the effect of psychological stress on ascorbic acid status. Also animal studies, such as with guinea pigs, could be used to control for exercise, sleep-wake cycles, ascorbic acid intake and other factors which affect either plasma ascorbic acid levels and/or plasma cortisol levels.

Further studies on the relationship between stress, nutrient intake and/or ascorbic acid status should use other statistical methods in addition to correlation coefficients. Statistical methods to help clarify cause and effect relationships are necessary. An interdisciplinary approach among nutritionists, biochemists and psychologists is needed to determine whether psychological variables such as stress influences intakes of nutrients, their requirements, and/or metabolism.

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Appendix A

CONSENT FORM

I do hereby voluntarily agree and consent to participate in a research project entitled "The Effect of Psychological Stress During Pregnancy on Ascorbic Acid Status" conducted by the personnel and a graduate student of the Human Nutrition and Foods Department of Virginia Polytechnic Institute and State University.

The purpose of the study is to determine if the pregnant women under stress have an increased requirement for Vitamin C during pregnancy. This study will involve the following procedures:

1. At the beginning of the study, a venipuncture blood sample of approximately 20 ml will be taken by a Registered Medical Technologist. Risks involved are slight or none since precautionary procedures are used. Infrequently slight bruising can occur. However, the University cannot be held responsible for any medical complications arising from the taking of the blood sample.

2. Levels of stress will be examined by self-rating pen and paper questionnaires. No sensitive questions will be asked.

3. A diet history will be taken during pregnancy.

4. A personnel data sheet to gather information about socio-economic status and general health of each subject will be included.

Benefits of participants in this project include assessment of Vitamin C and dietary status.

I understand that my participation is voluntary, and I may terminate it at any time. Any information obtained will be kept anonymous by using a numbering system. Any published data to be used will not be identified with me or other subjects.

I have read the above statements and I was encouraged to ask questions about the procedures. Participating project personnel can be contacted to be asked questions. The people to be contacted are listed below along with their phone numbers.

Mary Ann McFarland, Master's Candidate
Dr. Jane Wentworth, Project Director
Dr. Milton Stombler, Associate Dean of Research

______________________ ______________________________________

DATE SIGNATURE

Appendix B

SUBJECT RECRUITMENT NOTICE

Spectrum

PREGNANT WOMEN ARE NEEDED!

Pregnant women in their 7-9 months of pregnancy are needed as volunteers. A research study designed to determine the effect of stress during pregnancy upon Vitamin C requirements is being conducted by Tech's Human Nutrition and Food Department. Those interested in participating call Mary Ann McFarland at Or call Dr. Jane Wentworth at

Appendix C

COLLEGE OF HOME ECONOMICS

VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY

Blacksburg, Virginia 24061

DEPARTMENT OF HUMAN NUTRITION AND FOODS

February 9, 1981

Dear

Very little research has been carried out regarding the effect of stress on nutritional requirements in humans and virtually none on pregnant women. The intent of this letter is to inform you of a research study designed to determine if pregnant women are under any psychological stress and the effect on ascorbic acid requirements during pregnancy. We need your assistance in identifying subjects who would be interested in participating.

This research will be carried out by a graduate student, Mary Ann McFarland, under the direction of her faculty advisor Dr. Jane Wentworth. It has been approved by Dr. Milton Stombler, Associate Dean of Research, VPI & SU, and meets the Federal Regulations governing the use of human subjects. Pregnant women around their 8th month of pregnancy are needed for volunteers. The study will require a veni-puncture for one blood sample, a set of self-administered psychological questionnaires, a diet history, and a personal data form (to determine socioeconomic status) from each subject. All information will be kept anonymous and blood and dietary information can be sent to the subject's private physician upon her requrest. Dietary counseling can also be provided to patients who might be interested.

As a physician you can inform potential volunteers about this research and help us to begin long overdue research in this field. Thank you for your assistance, and please check the desired space below if you want a copy of the results. Dr. Barbara Turner, psychologist and Dr. Judy Driskell, nutritional biochemist are participating in this study also.

A few copies of the sheet on the Criteria for Participation in the Study are enclosed. Please hand these to potential participants or post in your office. We greatly appreciate your assistance and hope that this study will lead into one in the near future which identifies factors leading to stress and mechanisms of coping.

Sincerely,

Mary Ann McFarland, Master Candidate

Project Director
Assistant Professor

Attachments: as noted

____Yes, send a copy of the results

____
Name of Physician

COLLEGE OF HOME ECONOMICS

VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY

Blacksburg, Virginia 24061

DEPARTMENT OF HUMAN NUTRITION AND FOODS

Criteria for Participation in the Study
Stress and Vitamin C in Pregnant Women

I. Criteria for subjects for the project "The Effect of Stress During Pregnancy Upon Ascorbic Acid Status"

Subjects should be:

1. Free of identified diseases, such as diabetes, hypertension, and others

2. Experiencing a normal pregnancy without any complications

3. Women in their first, second, or third pregnancy

4. Women in their third trimester ($\pm$ 2 weeks) of pregnancy

5. Women agreeing to provide one blood sample and fill out several questionnaires

Data collected will be anonymous and kept confidential. Interpretation of the data will be provided to the physician and subject.

Contact one of the individuals below if interested.

Mary Ann McFarland, Master Candidate
Phone: 961-6387 or 552-5754

Dr. Jane Wentworth, Project Director
Phone 961-5866 or 961-6943

Appendix D

New River Health District

In cooperation with the Virginia State Department of Health

April 20, 1981

Administrative Office
210 Third Avenue
Radford, VA 24141
703-639-9656

Floyd County Health Dept
Highway 221 South
P.O. Box 157
Floyd, VA 24091
703-745-2141

Giles County Health Dept.
722 Wenonah Avenue
Pearisburg, VA 24134
703-921-3545

Montgomery Co. Health Dept.
Depot Street
P.O. Box 449
Christiansburg, VA 24073
703-382-6154

Pulaski Co. Health Dept.
4th Street and Randolph Ave.
P.O. Box 784
Pulaski, VA 24301
703-980-1490

Radford City Health Dept
210 Third Avenue
Radford, VA 24141
703-639-9656

Assistant Professor
Department of Human Nutrition and Foods
VPI and SU
Blacksburg, VA 24061

Dear

In reply to your letter of April 3, 1981, which asked for our cooperation in a study regarding the effect of stress on nutritional requirements of pregnant women, we would be glad to support your efforts.

We understand that a venipuncture will be done, a diet history taken and a questionnaire be filled out by the clients. We, in our busy maternity clinics, do not have the personnel to do this. However, if you were to supply us with the personnel to assist the clients to complete their portion of the paperwork, we would be glad to draw bloods, as we routinely do this in any case, but we will also draw a tube for your purposes. We think that this could be done with minimal effort in an on-going manner if you were to outline in particular a protocol for our cooperation with you.

We hope to hear from you in the near future. It behooves me to notify the Bureau of Maternal and Child Health in Richmond and our Regional Health Director in Roanoke of our willingness to cooperate in helping you in your studies in your new program.

Sincerely yours,

Health Director

WHH:MSG:jk

cc; Director, Bureau of Maternal and Child Health

COLLEGE OF HOME ECONOMICS

VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY

Blacksburg, Virginia 24061

DEPARTMENT OF HUMAN NUTRITION AND FOODS

May 7, 1981

Dear

Thank you for the opportunity to recruit participants in the study of the effect of stress on nutritional requirements of pregnant women.

Women should be in their last trimester of pregnancy, willing to sign a consent form to participate in the study and willing to fill out 3 short psychological questionnaires, and provide information on their previous twenty-four hour food intake. A dietary history will be carried out on each person at a later time to gain more information about their food patterns.

Mary Ann McFarland, Graduate Student in the Department of Human Nutrition and Foods, and I would like to attend Montgomery Maternal Clinic on May 14 and 28 and Radford Clinic on May 21 and June 4th to obtain participation by subjects. We would fill out necessary paper work while in the clinic (except for the diet history) and obtain the blood sample on the patient. A fasting blood sample from pregnant women who are in their last trimester is desirable. If patients have eaten just prior to coming to clinic, we would still like to have their blood. We will provide the sterile tubes for blood collection since plasma is required for the vitamin C, cortosol and protein tests. As an incentive to volunteer for the study we will provide the patient with a $5.00 bill upon completion of the paper work, a small amount for their effort.

We will call on Monday to confirm the first visit. Thanking you for your assistance I remain,

Sincerely yours,

Assistant Professor

cc:
Mary Ann McFarland
ds

Appendix E

If possible, please refrain from eating the foods listed below after 6:00 PM the day before and the day of your scheduled interview. Please do not take any vitamin supplements within the 24 hour period before your interview. Thank you for your help.

Fruits

Tomatoes, or juice, sauce
Apricots
Avocados
Blackberries
Canteloupe
Grapefruit
Lemons
Oranges or juice
Pineapple or juice
Strawberries
Watermelon
Tangerines or juice

Vegetables

Vegetable soup
Asparagus
Lima beans
Green beans
Broccoli
Brussel sprouts
Cabbage
Cauliflower
Greens
Green peppers
White potatoes
Squash
Sweet potatoes

Meats

Liver

Appendix F

PERSONAL DATA FORM, 24-HOUR RECALL AND DIET HISTORY FORMS

No.____________

ALL INFORMATION IS CONFIDENTIAL!

Date____________

(Circle the appropriate response)

Personal Data Form

1. Name ______________________________

   Address______________________________

   Phone______________________________

   Date Due ______________________________

2. Age ____________ 4. Current Weight ______________

3. Height ______________________

5. What is your pre-pregnancy weight? ________________

6. Are you under the care of a doctor during this pregnancy?

   1) Yes 2) No

7. How many months are you at this time (as determined by the doctor)?

   ____

8. Is this your first pregnancy? 1) Yes 2) No

9. If not, how many times have you been pregnant? ________________

10. Have the other pregnancies been successful? 1) Yes 2) No

11. If not, describe the problems? ______________________________

    ____

    ____

12. When you became pregnant, did you change the type or amount of food you ate or drank? 1) Yes 2) No

No.____________

Date____________

13. Have your food habits changed in any way? 1) Yes 2) No

3) If yes, how? ____

____

14. Has your appetite increased during pregnancy? 1) Yes 2) No

15. Do you have cravings for any particular foods? 1) Yes 2) No

3) If yes, what are these foods? ________________________________

____

16. Are there any foods you do not eat? 1) Yes 2) No

3) If yes, what are these foods? ________________________________

____

17. Are you on a special diet? 1) Yes 2) No

3) If yes, what kind of diet is it? _____________________________

____

18. Have you avoided any particular food due to symptoms such as heartburn or indigestion? 1) Yes 2) No 3) If yes, what food have you avoided? ____________________________________

____

19. Do you take any vitamin and/or mineral supplements? 1) Yes 2) No

3) If yes, what kind of supplements (brand names if known)?

____

20. Type of supplement? 1) multi-vitamin 2) ascorbic acid?

______50 mg ______100 mg _____250 _____500 _____more than 500

3) Vitamin A & D 4) iron 5) other ________________specify

6) mineral and vitamins? ____

No.____________

Date____________

21. How many do you take per day? _____________per week?____________
22. Who suggested you take this supplement? ________________________
23. Do you smoke cigarettes? 1) Yes 2) No
24. If yes, how many cigarettes per day do you typically smoke?

    1/2 pack _________ 1 pack _________ 1-1/2 packs___________

    2 packs __________ more than 2 packs ___________(specify)
25. How long have you been smoking? 1-5 years_________

    5-10 years___________ 11-15 years__________ over 15 years________

    (specify)
26. Has the doctor ever diagnosed you as having any medical problems or diseases? 1) Yes 2) No
27. If yes, specify____

Subject Number_________

24-Hour Recall

Name ____

Date and time of interview ______________________________

Length of interview ___________________________________

Date of Recall _______________________________________

Day of the week of Recall _______________________________

"I would like you to tell me everything you ate and drank from the time you got up on the morning until you went to bed and what you ate during the night. Be sure to mention everything you ate or drank at home, work and away from work."

What time did you get up yesterday? ______________________

What was the first time you ate or had anything to drink yesterday morning? ____

Where did you eat?_____________________________________

Now tell me -- What you had to eat and how much?

(Occasionally ask)

When did you eat again? Or, is there anything else?

Did you have anything to eat or drink during the night?

Was this intake unusual in any way? Yes No

How? ____

____

____

Subject Number________

Record Form for 24-Hour Recall

Time	Where Food Was Eaten	Food Type	Preparation	Amount	Food Code	Amount Code

Code	Total Grams Taken	Food Item	Amount	Frequency DA	WK	MO	Preparation
		MILK					
		Whole					
		Skim					
		2%					
		Evap					
		Buttermilk					
		Powder wh.					
		Powder skim					
		CHEESE					
		Cheddar					
		Swiss					
		American (Porcessed)					
		Cottage					
		Cream					
		CREAMS					
		Whipping					
		Half & Half					
		Sour cream					
		(Imitation Whipped)					
		(Toppings)					
		MILK BEVERAGES					
		Chocolate milk					
		cocoa					
		Milk Shakes					
		MILK DESSERTS					
		custard					
		puddings					
		ice cream					
		ice milk or sherbert					
		yogurt					
		EGGS					
		eggs					
		MEATS					
		bacon					
		fat back					
		beef (hamburger)					
		Poultry					
		Organ meats					
		Lamb					
		pork (fresh)					
		cured					
		etc.					
		sausage					
		bologna					

Code	Total gm Taken	Food Item	Amount	Frequency DA	WK	MO	Preparation
		MEATS (cont)					
		hot dog					
		other luncheon meats					
		veal					
		fish					
		fish sticks					
		shell fish					
		tuna fish					
		LEGUMES					
		Can. dry beans (soy)					
		lima					
		pinto					
		etc.					
		Can. dry peas (chick)					
		green					
		etc.					
		pork and beans					
		chili					
		Nuts (peanuts)					
		cashews					
		etc.					
		Peanut butter					
		soybean flour (meal)					
		other					
		VEGETABLES					
		green beans (snap)					
		pole					
		wax					
		etc.					
		sprouts					
		artichokes					
		broccoli					
		turnip greens					
		kale					
		mustard					
		spinach					
		collards					
		sweet potato					
		corn					
		carrot					
		squash					
		parsnips					
		mushrooms					
		cabbage					
		celery					
		lettuce					
		onions					

Code	Total gm Taken	Food Item	Amount	Frequency DA	WK	MO	Preparation
		VEGETABLES (cont)					
		beets					
		potatoes					
		turnips					
		tomato					
		FRUITS & FR PRODUCTS					
		juices (orange)					
		grape					
		apple					
		etc.					
		avocado					
		orange					
		grapefruit					
		lemons					
		apple					
		banana					
		fruit cocktail					
		peach					
		pear					
		cherries					
		watermelon					
		cantaloupe					
		grapes					
		raisins					
		dates					
		prunes					
		GRAINS					
		breads (cracked wt)					
		white					
		whole wheat					
		bagals					
		barley					
		cereals (cold)					
		cereals hot					
		oatmeal					
		grits					
		biscuits					
		muffins					
		corn bread					
		cakes					
		cookies					

Code	Total Gram Taken	Food Item	Amount	Frequency DA.	WK	MO	Preparation
		GRAINS (cont)					
		brownies					
		donuts					
		pastries					
		macaroni					
		spaghetti noodles					
		pancakes					
		pies					
		pizza					
		popcorn					
		rice					
		yeast (brewers)					
		wheat germ					
		pretzels					
		crackers (rye)					
		wheat					
		etc.					
		FATS AND OILS					
		butter					
		margarine					
		peanut (oils)					
		safflower					
		thousand is. (dress)					
		Italian					
		SNACK					
		chips (potato)					
		sodas (coke)					
		honey					
		molasses					
		jam					
		jelly					
		preserve					
		sugar					
		Koolaid					
		tea					
		coffee					
		beer					
		wine					
		whiskey					
		soups (veg)					
		gravy					
		jello					
		vit supplements					
		gelatin					
		pickles					
		plain water					

Appendix G

GENERAL BACKGROUND INFORMATION QUESTIONNAIRE

________ No.

INSTRUCTIONS: Answer the following questions as honestly as possible. Put a check near the answer that applies

1. Is this your first pregnancy? 1) _____Yes 2) _____No

2. If no, in which pregnancy are you? 1) _____2nd 2) _____3rd 3) _____Other

3. Do you have a job now or had one in the recent past?

   1) _____Yes 2) _____No

4. Do you plan to go to work after the baby is born?

   1) _____Yes 2) _____No

5. If so, how soon do you plan to go to work after the baby's birth?

   ____________________________specify

6. Rate how you feel or felt toward your work.
   1) _____Like it very much
   2) _____Like it
   3) _____Liked it a little
   4) _____Disliked it
   5) _____Dislike very much

7. Rate the degree of satisfaction with your present living situation.
   1) _____Very well satisfied
   2) _____Fairly satisfied
   3) _____Satisfied
   4) _____Dissatisfied
   5) _____Very dissatisfied

8. Do you live under crowded conditions? 1) _____Yes 2) _____No

9. How long have you lived in your present community?
   1) _____Less than 3 months
   2) _____3 to 6 months
   3) _____6 months to one year
   4) _____Over one year _______________specify

_________ No.

10. Of your friends how many are close friends in whom you can really confide?
 1) _____None
 2) _____One
 3) _____2 to 4
 4) _____Over 4

11. Do you feel deprived of any social contacts? 1) _____Yes 2)_____No

12. If so, what kinds of social contacts do you miss?
 1) _____Immediate family
 2) _____Other relatives
 3) _____Close friends
 4) _____Neighbors
 5) _____School friends
 6) _____Others

13. Is there anyone besides your relatives or spouse on whom you can count on for help? 1) _____Yes 2) _____No

14. Could you count on your family for help if you need it?
 1) _____Yes, in all matters
 2) _____Yes, in some matters
 3) _____No

15. Rate your perception of your state of health.
 1) _____Excellent
 2) _____Better than most
 3) _____Average
 4) _____Below average
 5) _____Poor

16. Rate your mobility (Your ability to go from one place to another).
 1) _____Excellent
 2) _____Good
 3) _____Fair
 4) _____Have some difficulty
 5) _____Very poor

17. Rate your feelings toward married life or living with your spouse.
 1) _____Very happy
 2) _____Quite happy
 3) _____Average
 4) _____Unhappy
 5) _____Extremely unhappy

____
No.

18. Rate your spouse's feelings toward your pregnancy.
   1) _____Very pleased
   2) _____Quite pleased
   3) _____Pleased
   4) _____A little displeased
   5) _____Very displeased

19. Is the timing of your pregnancy --
   1) _____Very convenient
   2) _____A little convenient
   3) _____Convenient
   4) _____Inconvenient
   5) _____Very inconvenient

20. Rate how you feel toward childbirth.
   1) _____Very confident
   2) _____Quite confident
   3) _____Confident
   4) _____A little frightened
   5) _____Very frightened

21. Rate your feeling when you first learned of your pregnancy?
   1) _____Very pleased
   2) _____Pleased
   3) _____Accepted it
   4) _____A bit displeased
   5) _____Very displeased

22. Presently how do you feel about your pregnancy?
   1) _____Very happy
   2) _____Kind of happy
   3) _____Unsure
   4) _____Unhappy
   5) _____Very unhappy

23. Rate your feelings about caring for your baby.
   1) _____Confident that I will be able to care for him
   2) _____Kind of confident that I will be able to take care of him
   3) _____Have a few doubts
   4) _____A little worried
   5) _____Afraid I will be unable to care for him properly

24. Rate your physician.
   1) _____Very caring
   2) _____Quite caring
   3) _____A little caring
   4) _____Indifferent
   5) _____Very indifferent

_______ No.

25. What is the highest grade of regular school you have completed?
    1) _____Grade school
    2) _____High school
    3) _____College
    4) _____Graduate school

26. Have you had any other schooling? 1) _____Yes 2) _____No
    (for skills ie. secretary, hairdresser,
    computer operator, etc.)

27. Present Status:
    1) _____Married
    2) _____Single

28. What is the highest grade of education your spouse has completed
    1) _____Grade school
    2) _____High school
    3) _____College
    4) _____Graduate school

29. Is your spouse securely employed? 1) _____Yes 2) _____No

30. What is his occupation?
    1) _____Professional - requires college degree
    2) _____Skilled - requires some training
    3) _____Unskilled
    4) _____Other - list _______________

31. Rate your feelings about your financial security.
    1) _____No financial problems
    2) _____Very few financial problems
    3) _____Can make out
    4) _____Will have some financial problems
    5) _____Will have many financial problems

32. What is your and spouse's combined income (gross)?
    1) _____Less than $5,000
    2) _____5,000 to 9,999
    3) _____10,000 to 19,999
    4) _____20,000 to 29,999
    5) _____30,000 to 39,999
    6) _____40,000 and above

Appendix G (Continued)

Frequency, Percentage of Responses to General Background Information Questionnaire

Question Number and Coded Response	Frequency	Cumulative Frequency	Percent	Cumulative Percent
Q1				
1	24	24	75.00	75.00
2	8	32	25.00	100.00
Q2				
.	24	.		
2	5	5	62.50	62.50
3	2	7	25.00	87.50
4	1	8	12.50	100.00
Q3				
1	25	25	78.13	78.13
2	7	32	21.87	100.00
Q4				
1	22	22	68.75	68.75
2	10	32	31.25	100.00
Q5				
.	10	.	.	.
1	5	5	22.73	22.73
2	7	12	31.82	54.55
3	1	13	4.55	59.10
4	3	16	13.64	72.73
5	1	17	4.55	77.27
6	5	22	22.73	100.00
Q6				
.	4	.	.	
1	9	9	32.14	32.14
2	13	22	46.43	78.57
3	3	25	10.71	89.28
4	2	27	7.14	96.43
5	1	28	3.57	100.00
Q7				
1	14	14	43.75	
2	9	23	28.13	
3	8	31	25.00	
4	1	32	3.13	
Q8				
1	4	4	12.50	12.50
2	28	32	87.50	100.00
Q9				
1	3	3	9.37	9.37
2	3	6	9.37	18.75
3	5	11	15.63	34.37
4	21	32	65.63	100.00

Question Number and Coded Response	Frequency	Cumulative Frequency	Percent	Cumulative Percent
Q10				
1	3	3	9.38	9.38
2	14	17	43.75	53.13
3	10	27	31.25	84.38
4	5	32	15.62	100.00
Q11				
1	11	11	34.38	34.38
2	12	32	65.62	100.00
Q12				
.	20	.		
1	6	6	50.00	50.00
2	4	10	33.33	83.33
3	2	12	16.67	100.00
Q13				
1	29	29	90.63	90.63
2	3	32	9.37	100.00
Q14				
1	22	22	68.75	68.8
2	10	32	31.25	100.0
Q15				
1	13	13	40.63	40.63
2	11	24	34.40	75.00
3	8	32	25.00	100.00
Q16				
1	11	11	34.38	34.38
2	14	25	43.75	78.12
3	4	29	12.50	90.25
4	3	32	9.40	100.00
Q17				
1	22	22	68.75	68.75
2	6	28	18.75	87.50
3	3	31	9.38	96.88
4	1	32	3.12	100.00
Q18				
1	23	23	71.87	71.88
2	3	26	9.38	81.25
3	4	30	12.50	93.75
4	1	31	3.13	96.88
5	1	32	3.13	100.00
Q19				
1	12	12	37.50	37.50
2	7	19	21.87	59.38
3	9	28	28.13	87.50
4	3	31	9.39	96.88
5	1	32	3.12	100.00

Question Number and Coded Response	Frequency	Cumulative Frequency	Percent	Cumulative Percent
Q20				
1	11	11	34.38	34.38
2	10	21	31.25	65.63
3	4	25	12.50	78.12
4	5	30	15.63	93.75
5	2	32	6.30	100.00
Q21				
1	16	16	50.00	50.00
2	10	26	31.25	81.25
3	3	29	9.38	90.63
4	2	31	6.30	96.88
5	1	32	3.12	100.00
Q22				
1	28	28	87.50	87.50
2	4	32	12.50	100.00
Q23				
1	26	26	81.25	81.25
2	4	30	12.50	93.75
3	2	32	6.25	100.00
Q24				
1	13	13	40.63	40.62
2	9	22	28.12	68.75
3	7	29	21.87	90.62
4	2	31	6.25	96.87
5	1	32	3.12	100.00
Q25				
1	5	5	15.62	16.63
2	9	14	28.13	43.75
3	8	22	25.00	68.75
4	10	32	31.25	100.00
Q26				
1	8	8	25.00	25.00
2	24	32	75.00	100.00
Q27				
1	31	31	96.88	96.88
2	1	32	3.12	100.00
Q28				
1	4	4	12.50	12.50
2	10	14	31.25	43.75
3	12	26	37.50	81.25
4	6	32	18.75	100.00
Q29				
1	23	23	71.87	71.88
2	8	31	25.00	96.88
3	1	32	3.12	100.00

Question Number and Coded Response	Frequency	Cumulative Frequency	Percent	Cumulative Percent
Q30				
1	10	10	31.25	31.25
2	11	21	34.38	65.63
3	2	23	6.25	71.88
4	6	29	18.75	90.63
5	3	32	9.37	100.00
Q31				
1	5	5	15.63	15.63
2	6	11	18.75	34.38
3	13	24	40.62	75.00
4	6	30	18.75	93.75
5	2	32	6.25	100.00
Q32				
1	8	8	25.00	25.0
2	8	16	25.00	50.0
3	7	23	21.88	71.88
4	5	28	15.63	87.50
5	3	31	9.38	96.88
6	1	32	3.12	100.00

Appendix H

SELF-EVALUATION QUESTIONNAIRE

Developed by C.D. Spielberger, R.L. Gorsuch and R. Lushene
STAI Form X-1

Name____Date__________________

DIRECTIONS: A number of statements which people have used to describe themselves are given below. Read each statement and then circle the appropriate number to the right of the statement to indicate how you feel right now, that is, at this moment. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe your present feelings best.

	Not at all	Somewhat	Moderately so	Very much so
1. I feel calm	1	2	3	4
2. I feel secure	1	2	3	4
3. I am tense	1	2	3	4
4. I am regretful	1	2	3	4
5. I feel at ease	1	2	3	4
6. I feel upset	1	2	3	4
7. I am presently worrying over possible misfortunes	1	2	3	4
8. I feel rested	1	2	3	4
9. I feel anxious	1	2	3	4
10. I feel comfortable	1	2	3	4
11. I feel self-confident	1	2	3	4
12. I feel nervous	1	2	3	4
13. I am jittery	1	2	3	4
14. I feel "high strung"	1	2	3	4
15. I am relaxed	1	2	3	4
16. I feel content	1	2	3	4
17. I am worried	1	2	3	4
18. I feel over-excited and "rattled"	1	2	3	4
19. I feel joyful	1	2	3	4
20. I feel pleasant	1	2	3	4

Appendix H

SELF-EVALUATION QUESTIONNAIRE

STAI Form X-2

Name______________________________________Date____________________

DIRECTIONS: A number of statements which people have used to describe themselves are given below. Read each statement and then circle the appropriate number to the right of the statement to indicate how you generally feel. There are no righr or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe how you generally feel.

		Almost never	Sometimes	Often	Almost Always
21.	I feel pleasant	1	2	3	4
22.	I tire quickly	1	2	3	4
23.	I feel like crying	1	2	3	4
24.	I wish I could be as happy as others seem to be	1	2	3	4
25.	I am losing out on things because I can't make up my mind soon enough	1	2	3	4
26.	I feel rested	1	2	3	4
27.	I am "calm, cool, and collected"	1	2	3	4
28.	I feel that difficulties are piling up so that I cannot overcome them	1	2	3	4
29.	I worry too much over something that really doesn't matter	1	2	3	4
30.	I am happy	1	2	3	4
31.	I am inclined to take things hard	1	2	3	4
32.	I lack self-confidence	1	2	3	4
33.	I feel secure	1	2	3	4
34.	I try to avoid facing a crisis or difficulty.	1	2	3	4
35.	I feel blue	1	2	3	4
36.	I am content	1	2	3	4
37.	Some unimportant thought runs through my mind and bothers me	1	2	3	4
38.	I take disappointments so keenly that I can't put them out of my mind	1	2	3	4
39.	I am a steady person	1	2	3	4
40.	I get in a state of tension or turmoil as I think over my recent concerns and interests .	1	2	3	4

Appendix H

Number________

Date__________

FITNESS INDEX

CONFIDENTIAL - FOR PROFESSIONAL USE ONLY

1. Have you smoked within the last 24 hours? ___yes ___no

If so, what have you smoked? (Check appropriate box(es) below.

	Amount	Brand
___cigarettes	______	______
___cigars	______	______
___pipes	______	______

2. Have you used alcohol within the last 24 hours? ___yes ___no

If so, what have you consumed in the last 24 hours?

	Amount
___wine	______glasses
___liquor	______drinks
___beer	______cans

3. Have you consumed any beverages containing caffeine in the last 24 hours? If so, please specify. ___yes ___no

	Amount
___coffee	______cups
___sanka or decaffinated	______cups
___tea	______cups
___Coke/Pepsi	______glasses
___cocoa	______cups

Number___________

4. Did you exercise within the last 24 hours? ___yes ___no

If so, in what manner?

	Hours	
___jogging	_____	_____miles
___calesthenics (including exercycle)	_____	
___indoor (e.g. bowling, ping pong)	_____	
___outdoor (e.g. softball, tennis)	_____	
___swimming or water sports	_____	
___dancing	_____	
___martial arts	_____	
___yoga	_____	
___weight lifting	_____	
___horseback riding	_____	
___bicycling	_____	
___walking for exercise	_____	
___other (please specify below)	_____	

5. Have you taken any medications within the last 24 hours?

If so, please specify. ___yes ___no

	Amount
___Aspirin	______
___Antacids	______

Number__________

___Prescription Meds (specify) ______

___Other (specify) ______

6. Have you taken time out to relax within the last 24 hours?

If so, please specify. ___yes ___no

	Number of Times
___Took a nap	____________
___Sat quietly	____________
___Did relaxation exercise/ meditation	____________

FITNESS INDEX (continued)

1. What time did you wake up this morning?______________________________

2. What time did you go to sleep last night?____________________________

_____hours _____minutes

3. What time do you normally go to sleep?_______________________________

4. What time do you normally wake up?___________________________________

_____hours _____minutes

5. Do you take a nap during the day? ____yes ____no

6. If so, how long is this nap? _________hours ______minutes

Appendix I

SYMPTOM CHECKLIST

Of the symptoms listed below, please put a check by the ones you experienced during the last month and indicate the severity of each checked symptom by circling one of the numbers on the 10-point scale. If you did NOT experience a particular symptom, leave the space BLANK and DO NOT CIRCLE a number.

	Very Mild									Very Severe
___fatigue	1	2	3	4	5	6	7	8	9	10
___insomnia	1	2	3	4	5	6	7	8	9	10
___headaches	1	2	3	4	5	6	7	8	9	10
___backaches	1	2	3	4	5	6	7	8	9	10
___muscular pain	1	2	3	4	5	6	7	8	9	10
___skin disorder	1	2	3	4	5	6	7	8	9	10
___Gastro-intestinal disorder	1	2	3	4	5	6	7	8	9	10
___flu and/or cold	1	2	3	4	5	6	7	8	9	10
___accident	1	2	3	4	5	6	7	8	9	10
___Ulcer pain	1	2	3	4	5	6	7	8	9	10
___asthma	1	2	3	4	5	6	7	8	9	10
___contractions	1	2	3	4	5	6	7	8	9	10
___intestinal problems	1	2	3	4	5	6	7	8	9	10
___fluid retention	1	2	3	4	5	6	7	8	9	10
___nausea	1	2	3	4	5	6	7	8	9	10
___vomiting	1	2	3	4	5	6	7	8	9	10
___soreness of breasts	1	2	3	4	5	6	7	8	9	10
___cramping, abdominal	1	2	3	4	5	6	7	8	9	10
___cramping, muscular	1	2	3	4	5	6	7	8	9	10

	Very Mild									Very Severe
___food allergies	1	2	3	4	5	6	7	8	9	10
___infection	1	2	3	4	5	6	7	8	9	10
___spotting or hemoharraging	1	2	3	4	5	6	7	8	9	10
___other (specify)	1	2	3	4	5	6	7	8	9	10

____

Appendix J

PLASMA ASCORBIC ACID ASSAY

A. Standard Curve - One gram of acid - washed Norite and 50 ml of ascorbic acid working standard (20 ug/ml) was added to 125 ml Erlenmeyer flask. This was shaken for 1.0 min. and filtered using a Whatman No. 42 filter paper. A standard curve was then established using 0.0, 0.5, 1.0, and 2.0 ml of working standard filtrate. Each of these volumes were delivered to three 20 ml glass test tubes and then diluted to 4.0 ml using 4% metaphosphoric acid.

B. Plasma Ascorbic Acid - Four milliliters of plasma were added dropwise, with continuous mixing, to a 50 ml centrifuge tube containing 16 ml of 6% meta-phosphoric acid. The mixture was then allowed to stand 5.0 min. which was enough time to cause complete precipitation of plasma proteins. At this time centrifugation of the tubes was carried out at room temperature and 2,500 x g for 10 min. The supernatant resulting from the centrifugation was decanted into a 25 ml glass test tube containing 250 mg of acid-washed Norite and mixed thoroughly for 1.0 min. The mixture was next filtered through Whatman 42 filter paper and collected in another 25 ml glass test tube. Exactly 4.0 ml of each of the unknown filtrates was delivered into a set of three 20 ml glass test tubes. At this point both standard and unknown tubes were placed in the same test tube rack for continuation as under "Development of Color."

C. Development of Color - One drop of 10% thiorurea was added to all tubes and the tubes were mixed on a Vortex mixer for 5.0 sec. Next, 1.0 ml of 2,4-dinitrophenylhydrazine reagent was added to two of the set of three test tubes for each sample, the third tube of each set being reserved as a blank.

Tubes receiving the 2,4-dinitrophenylhydrazine were then mixed on a Vortex mixer for 15 sec. All tubes were covered with a double layer of Parafilm and placed in a covered boiling water bath for exactly 10 min. Tubes were removed from the boiling water bath and immediately embedded in a crushed ice bath which had been sprinkled liberally with sodium chloride.

At this point, all tubes were removed individually from the crushed ice bath and placed in a large breaker containing a crushed ice-ice water mixture. The Parafilm cover was removed from the tube and the breaker containing the tube was positioned under a 50 ml buret containing 85% sulfuric acid. Sulfuric acid (5.0 ml) was added slowly, dropwise, over a period of 1.0 min. to the tube while the tube remained in the ice mixture. Vigorous mixing of the tube was carried out while the sulfuric acid was being added. After the addition of the sulfuric acid, each tube was mixed on the Vortex for 15 sec. and returned to a large container of crushed ice. When sulfuric acid had been added to all the tubes, the blank tubes for each standard and unknown were removed from the ice bath and 1.0 ml of 2,4-dinitrophenylhydrazine reagent was added. Each blank tube was then mixed for 15 sec. All tubes were removed from the ice bath and allowed to stand at room temperature for 15 min.

Color development was measured by reading each tube in a spectrophotometer (Spectronic 20, Bausch and Lomb) at 515 . Unknowns were plotted against the standard calibration curve (Appendix) and data were expressed as mg of ascorbic acid per 100 ml of plasma.

1. 2,4-Dinitrophenylhydrazine (Eastman Chem. Co.) - Dissolve 2.0 g in 100 ml of 9 N sulfuric acid. This solution was allowed to stand overnight, filtered, and stored in the dark and cold (5°C) for no longer than 10 days.

2. Acid Washed Norite (Sigma Chem. Co.) - Activated charcoal.

3. Metaphosphoric Acid, 4 and 6% (Fisher Sci. Co.) - Dissolve 40 or 60 g, respectively, of meta-phosphoric acid in distilled deionized water and dilute to 1.0 liter. This reagent was stored at 5°C for no longer than 14 days.

4. Sulfuric Acid, 85% (Baker Chem. Co.) - Add, very carefully, concentrated sulfuric acid to 100 ml of distilled, deionized water. Bring the total volume to 1.0 liter.

5. Thiourea, 10% (Fisher Sci. Co.) - Dissolve 10 g of thiourea in 100 ml of 50% (by volume) aqueous ethyl alcohol (U.S. Industrial Chem. Co.). This reagent was stored in the dark at room temperature for no longer than 2.0 months.

6. L-Ascorbic Stock Standard (Fisher Sci. Co.) - Dissolve 100 mg of L-ascorbic acid and dilute to 100 ml (1.0 mg/ml). This standard was made fresh for every trail and was stored in the dark and cold (5°C) until time for use.

7. L-Ascorbic Acid Working Standard - Dilute 2.0 ml of stock standard to 100 ml with 4% meta-phosphoric acid, adding 1.0 ml of 10% thiourea to the solution before diluting to volume. This solution was made fresh for each trail and was stored in the dark and cold (5°C) until time for use.

Appendix K

Individual Data

Subject Number	Age	Height	Weight	Month of Pregnancy	Plasma Ascorbic Acid (mg/100)	Cortisol (ug/100 ml)	A-State Score	A-Trait Score	Nutrient Rating 24-Hour Recall	Nutrient Rating Diet History	Ascorbic Acid Intake from Recall (mg)	Ascorbic Acid Intake from Diet History (mg)
01	20	5.7	155	8.75	0.97	34.87	1.60	1.65	28	27	159	082
02	21	5.5	180	7.50	0.72	47.12	1.45	1.35	20	27	115	094
03	25	5.5	143	8.00	1.00	55.62	1.45	1.55	12	23	075	091
04	30	5.3	128	8.00	0.81	53.32	1.60	2.40	28	31	267	215
05	29	5.1	132	8.25	0.62	45.52	1.15	1.05	27	29	082	142
06	22	5.4	142	7.5	1.32	56.69	1.50	1.55	25	31	064	317
07	22	5.5	172	8.00	1.24	48.19	1.45	1.80	19	25	278	167
08	32	5.7	189	8.50	0.99	77.18	1.30	1.15	24	28	024	083
09	27	5.3	166	8.50	0.90	52.65	1.45	1.40	27	29	134	150
10	26	5.1	132	7.50	0.74	65.68	1.05	1.20	27	31	150	284
11	25	5.4	132	7.75	0.88	45.18	1.55	1.45	26	23	034	064
12	30	5.3	144	7.50	0.94	46.88	1.40	1.35	28	30	079	180
13	28	5.4	177	8.25	1.16	32.08	1.15	1.20	17	26	017	192
14	28	5.7	167	8.50	0.86	42.76	1.80	1.75	23	21	045	129
15	27	5.6	151	8.00	0.85	30.07	1.80	2.05	20	27	017	126
16	22	5.8	168	8.50	0.58	24.54	1.40	1.55	30	26	134	228
17	24	5.4	173	8.00	0.57	22.03	1.15	1.20	29	26	047	148
18	29	5.1	140	8.00	0.64	30.08	1.25	1.40	23	26	491	430
19	27	5.3	158	8.50	0.48	37.68	1.00	1.50	31	18	052	080
20	30	5.11	156	8.00	0.98	16.12	1.70	2.00	27	16	084	111
21	29	5.6	138	8.25	0.71	23.30	1.20	1.15	22	28	025	044
22	27	5.1	132	8.00	1.09	21.10	1.15	1.25	30	29	061	198
23	28	5.5	142	8.50	0.92	49.79	1.15	1.35	29	27	269	242

Appendix K (Continued)

Individual Data

Subject Number	Age	Height	Weight	Month of Pregnancy	Plasma Ascorbic Acid (mg/100)	Cortisol (ug/100 ml)	A-State Score	A-Trait Score	Nutrient Rating 24-Hour Recall	Nutrient Rating Diet History	Ascorbic Acid Intake from Recall (mg)	Ascorbic Acid Intake from Diet History (mg)
24	17	5.2	138	8.50	0.78	16.89	1.65	1.65	14	21	027	036
25	22	5.6	165	8.00	1.24	31.95	1.30	1.90	24	29	034	355
26	15	5.8	158	8.00	0.98	22.95	2.05	1.85	24	25	058	169
27	24	5.5	174	8.00	0.68	40.36	2.45	2.85	29	30	36	81
28	18	5.4	142	8.00	1.18	19.56	2.20	1.80	26	19	72	73
29	26	5.2	178	8.00	1.18	29.80	2.35	1.70	32	24	404	115
30	21	5.7	195	7.00	1.36	33.42	2.35	2.45	31	28	92	113
31	21	5.4	118	7.75	0.78	21.23	2.25	1.95	27	30	127	143
32	19	5.2	129	7.50	1.64	33.14	1.40	1.75	16	26	14	187

Appendix K (Continued)

Individual Data

Subject Number	Ascorbic Acid from Vitamin Supplement	Total Ascorbic Acid Intake (mg)	Income	Education	Symptom Number	Symptom Severity	Smokers (S) or Non-Smokers (N)	Pack - Years
01	060	142	2	3	14	5.5	N	
02	060	154	1	2	03	4.0	N	
03	090	181	3	4	04	5.5	N	
04	090	305	5	3	02	2.0	N	
05	090	232	4	3	05	5.8	N	
06	060	377	2	2	07	4.8	N	
07	120	287	1	3	12	5.5	N	
08	090	173	2	2	07	3.8	N	
09	090	240	3	4	09	6.1	N	
10	120	404	3	4	03	3.0	N	
11	120	184	3	3	10	4.3	N	
12	060	240	4	4	04	4.8	N	
13	120	312	4	4	05	1.8	N	
14	120	249	3	3	11	3.8	N	
15	120	246	3	4	06	3.3	N	
16	060	288	5	2	23	2.2	N	
17	060	208	2	3	06	2.0	N	
18	060	490	4	4	05	2.0	N	
19	060	140	5	3	04	3.0	S	2.5
20	090	201	4	4	01	5.0	N	
21	090	134	6	4	02	4.0	S	5.0
22	090	288	3	2	06	4.5	N	

Appendix K (Continued)

Individual Data

Subject Number	Ascorbic Acid from Vitamin Supplement	Total Ascorbic Acid Intake (mg)	Income	Education	Symptom Number	Symptom Severity	Smokers (S) or Non-Smokers (N)	Pack - Years
23	060	302	2	4	05	3.6	N	
24	060	98	2	1	02	2.5	N	
25	060	415	1	1	08	3.5	N	
26	120	289	1	1	03	1.6	S	2.5
27	060	141	1	2	08	5.2	S	8.0
28	120	193	2	2	05	2.2	N	
29	060	175	2	1	07	4.1	S	8.0
30	060	173	1	2	05	3.0	S	5.0
31	060	203	1	2	05	5.8	S	5.0
32	120	307	1	1	05	4.4	S	2.5

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INTERRELATIONSHIPS BETWEEN STRESS,
DIETARY INTAKE AND PLASMA ASCORBIC
ACID DURING PREGNANCY

BY

Mary Ann McFarland

(ABSTRACT)

The relationships between stress, ascorbic acid status, and the adequacy of nutrient intake during the third trimester of pregnancy were studied. Adequacy of nutrient and ascorbic acid intake were measured by diet histories and 24 hour recalls. Plasma ascorbic acid and cortisol levels were determined. Stress was assessed by Spielberger State-Trait Anxiety Inventories (STAI) and Symptom Checklists (SCL). Factors which may affect stress were assessed by a General Background Information Questionnaire.

All subjects had acceptable plasma ascorbic acid levels (0.48 - 1.64). A-State and A-Trait scores, $\bar{X} = 1.55$ and $\bar{X} = 1.63$ respectively, indicated the majority of subjects to be little stressed. There were positive significant correlations between age and cortisol, A-State and A-Trait measures of STAI, nutritional scores from diet histories and plasma cortisol. Significant negative correlations were obtained

between month of pregnancy and plasma ascorbic acid levels, total ascorbic acid intake and A-State measurements of STAI, A-State measurements and income, A-State measurements and education, and A-State measurements and ascorbic intake as calculated from diet histories. There was no significant correlations between STAI, measurements and cortisol, plasma ascorbic acid and cortisol, and STAI measurements and plasma ascorbic acid. This study showed no conclusive evidence that ascorbic acid status or nutrient intake were affected by psychological stress.