COMPREHENSIVE EVALUATION OF NUTRITIONAL AND HEALTH STATUS OF SELECTED FEMALE ATHLETES IN COIMBATORE DISTRICT AND INTERVENTION

University Grants Commission

Minor Research Project

HOME SCIENCE

F.No. 4907/14

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SUMMARY

Proper nutrition is an important element of training to achieve maximum performances in athletics. Over the past century, there has been an exponential increase in the number of female athletes both for either competitive or recreational purpose. Besides many beneficial effects of exercise, they are more susceptible to disordered eating behaviour, amenorrhea and osteoporosis because of disordered eating pattern. Considering this, the study aimed to determine the prevalence rate of Female Athlete Triad. About 420 students were selected through purposive random sampling from different colleges in Coimbatore district. A self-administered questionnaire was used to assess their sports activity, eating disorder, menstrual history, dietary habits, attitude besides blood sample was drawn for biochemical investigation. The results of the anthropometric measurements indicated 15 per cent of the subjects were underweight whereas 68 per cent belonged to overweight and obese category. The subjects participated in a wide variety of sports events. One hundred and nine subjects (26 per cent) had disordered eating behaviour, 40 percent were into weight loss measures to meet the image requirement in sports, whereas 32 per cent do not have control over how much they eat. Eighty eight individuals (20.9 per cent) reported to have amenorrhea/ oligomenorrhea, 2.85 percent suffer from polycystic ovarian disease. Psychological score stated that they had a balanced state of mind. A positive correlation was found between menstruation and FSH/LH, prolactin significantly correlated with parameters as Body fat (R=0.45), Body Mass Index (R=0.44) and Weight (R=0.39). Female athletes are at considerable risk for the disordered eating and amenorrhea components of the triad. Intervention through education made them to understand the importance of nutrition on their well-being.

COMPREHENSIVE EVALUATION OF NUTRITIONAL AND HEALTH STATUS OF SELECTED FEMALE ATHLETES IN COIMBATORE DISTRICT AND INTERVENTION

1. INTRODUCTION

Nutrition is an important component of any physical fitness program. The main dietary goal for active individuals is to obtain adequate nutrition to optimize health and fitness or sports performance (Bearing, 2000). This is not only important to help to improve performance but also to promote healthy dietary practices in the long term (Jonnalagadda *et al*, 2001).

Over the last few decades social changes have fostered the development of a positive attitude towards female athletic activities and there has been a dramatic increase in the number of girls and women participating in all levels of sports competitions. For most individuals, this is a positive experience which provides improved physical fitness and better health (Drinkwater *et al*, 1984). Besides many beneficial effects of exercise, female athletes are susceptible to disordered eating behavior, amenorrhea and osteoporosis. The constellation of these three clinical conditions was defined as the Female Athlete Triad by the American College of Sports Medicine (Nattiv *et al*, 1994; Otis *et al*, 1997; Yeager *et al*, 1993).

Female athletes have special concern about their bodies when engaging in sports (Otis, 2000; Weinberg and Gould, 1995). The body image influences their choice and participation in sports (Collins and Kay, 2007; Hoffmann, 2007). Researchers argue that female athletes feel conflict about how participation in competitive sport and exercise affects their feminity (Coakley, 2009; Hargreaves, 2001; Weinberg and Gould, 1995).

Association of diminished Bone Mineral Density (BMD) to amenorrhea was described in female athletes in 1987 (Drinkwater *et al*, 1984) and Female Athlete Triad, characterized by the presence of an eating disorder, together with amenorrhea and osteoporosis, was first documented twenty years ago (Yeager *et al*, 1993).

The Female Athlete Triad was officially recognized in a position stand issued by a panel of experts conveyed by the American College of Sports Medicine (ACSM) in response to the increase in stress fracture rates, decreases in Bone Mineral Density (BMD) and

menstrual dysfunction in otherwise healthy female athletes (Otis *et al*, 1997). This syndrome is now recognized as a complex set of interrelationships between energy availability, menstrual status, and bone mineral density, which may have a variety of clinical manifestations, including eating disorders, functional hypothalamic amenorrhea and osteoporosis (Nattiv *et al*, 2007).

Disordered eating is commonly defined as an abnormal eating behavior and may involve behaviors such as restrictive eating, fasting, skipping meals, or use of diet pills, laxatives, diuretics, enemas or binge eating followed by purgative (Beals, 2003; Beals, 2006; Nattiv *et al*, 2007; Rumball and Lebrun, 2004; Waldrop, 2005). Disordered eating behaviors may result in low energy availability, which is the amount of energy remaining for bodily function after exercise training, calculated as energy intake minus exercise energy expenditure (Omey and Micheli, 1993; Rumball and Lebrun, 2004; Waldrop, 2005). Eating disorders (EDs) are psychiatric disorders with diagnostic criteria based on psychological, behavior and physiologic characteristics (American psychiatric association, 2000).

Athletes participating in disordered eating may exhibit compulsive behaviors including "ritualized eating, food restriction and obsessive training" (Kirchner & Cohen, 2002). Hausenblaus and Carron, (1999) identified four risk factors for disordered eating female athletes. These risk factors induced societal pressure to be thin; body type expectation for an athlete's; excessive exercise while training and psychological characteristics of athletes. Disordered behaviors have been related to abnormal changes in the menstrual cycle, such as increased time between cycles, or cessation of cycles (Ackard and Peterson, 2008; Byrne and Mc Lean, 2001; Depalma *et al*, 2002; Greydanus, 2002; Huston *et al*, 2000; Kotler *et al*, 2001; Lean and Barr, 2003; Nichols, 2006; Nichols, 2007). Menstrual abnormalities are estimated to affect 20% of exercising females, with prevalence reported as high as 44% in ballet dancers and 51% in endurance runners (Greydanus and Patel, 2002; Shangold *et al*, 1990; Smolak *et al*, 2000).

The monthly menstrual cycle is a complex interaction of the endocrine and reproductive systems. External stimuli affect the system through hormonal signals to the hypothalamus. The cessation of menses coincident with physical training has long been recognized (Otis, 1997). Irregular menstruation can be defined based on the number of menstrual cycle in one year (Cobb *et al*, 2003). Eumenorrhea is defined as regular cycles occurring at intervals between 21 and 35 days. In adolescents, the cycles range between 21

and 45 days (ACOG, 2006). Primary amenorrhea is defined as no menarche by age 15 years (American Society of Reproductive Medicine Practice Committee, 2008). Secondary amenorrhea refers to an absence of three consecutive cycles post menarche. Oligomenorrhea is defined as a cycle length greater than 45 days. Estimates of prevalence of menstrual disorders in athletes vary widely (Redman and Loucks, 2005). Subtle menstrual dysfunction, such as very bleeding, mildly extended menstrual interval and premenstrual and postmenstrual spotting may occur and may be underestimated by routine screening (Souza *et al*, 2010).

Abnormal levels of hormones (Ellison and Lager, 1986) Luteinizing hormone pulsatility, inadequate body fat stores, low energy availability and exercise stress may be etiological factors in menstrual disorders in athletes. Marked reduction in energy availability may disrupt the luteinizing hormone pulsatility by affecting the hypothalamic hormone gonadotropin-releasing hormone output (Sonntag and Ludwig, 2012) which subsequently alters the menstrual cycle. This is known as Functional Hypothalamic Amenorrhea (FHA). Rapid or significant fat mass reduction, even over as short as a 1 month period, may compromise menstrual function, Low energy availability alters levels of metabolic hormone and substrates, for example, insulin, cortisol, growth hormone, insulin like growth factor-1, 3,3,5-triiodothyroine, grehlin, leptin, peptide tyrosine, glucose, fatty acids and ketone (Wade and Jones, 2004).

Manore, (2002) has taken notice of the general occurrence of athlete under nutrition and has emphasized that intake deficiencies of B-group vitamins, iron and zinc can unfavorably influence reproductive function in active women. It is also known that the level of leptin, sometimes called a "satiety hormone", is also significantly lower in female athletes with amenorrhea than in eumenorrhoeic athletes. Leptin expression is believed to be a key metabolic mediator which may "translate" nutritional information about the body's energy stores and amount of body fat into endocrine responses and is responsible for creating and maintaining a functioning hypothalamic-pituitary axis. Leptin has been suggested as a link between nutritional status and the reproductive axis in women (Miles, 2001). Prolonged secondary "athletic" amenorrhea causes very unfavorable consequences, including decreases in Bone Mineral Density (BMD) in different areas of the skeletal system (Christo *et al*, 2008). Many studies have confirmed that female athletes with amenorrhea have significantly lower bone density, compared with eumenorrhoeic athletes (Christo *et al*, 2008; Rickenlund

et al, 2005). Changes in hormone regulation are one of the causes of the decrease in Bone Mineral Density (BMD) found with dysmenorrheic female athletes (Cobb *et al*, 2003).

Lo *et al* (2003) have reported that even after spontaneous resumption of normal menses, these athletes may never again obtain normal bone mass and are at a greater risk of premature osteoporosis. Furthermore, prolonged disorders in the menstrual cycle have negative effects on the lipid profile, and lead to atherosclerosis and cardiovascular diseases (Rickenlund *et al*, 2005).

Osteoporosis is a disease characterized by low bone mass and micro architectural deterioration of bone tissue leading to enhanced skeletal fragility and increased risk of fracture (Otis *et al*, 1997). According to O'Brien, (1996) about 30% of all women above 50 years are osteoporotic.

The third part of the Female Athlete Triad, osteopenia/osteoporosis, can occur when female athlete decrease nutrient intake and lose significant body fat. A significant reduction in body fat to unhealthy levels can alter hormone levels that affect bone mineral density. A reduction in nutrients that are necessary for bone health, such as calcium, vitamin D and magnesium, can also contribute to bone loss. Bone Mineral Density (BMD) is often measured by a DEXA scan. Bone Mineral Density (BMD) can be decreased in female exhibiting disordered eating dysmenorrheal tendencies. Bone Mineral Density was lower in the lumbar spine, hip and whole body in oligo and amenorrhea female athletes (Cobb *et al*, 2003).

Some suggested factors potentially responsible for Female Athlete Triad include the specific type and amount of high intensity training in young female athletes (especially when begun before puberty), reduced body weight, a lower percentage of fat tissue and psychological stress (Loucks *et al*, 2007; Nattiv *et al*, 2007). The findings indicated that in most of them disorder eating was observed along with menstrual dysfunction and low Bone Mineral Density but the studies on three Triad components simultaneously are limited and this problem has not been investigated intensively in Indian athletes. Considering this, the present study **"Comprehensive Evaluation of Nutritional and Health Status of Selected Female Athletes in Coimbatore District and Intervention"** was undertaken.

BROAD OBJECTIVE

To determine the prevalence rate and assess the major components of Female Athlete Triad and their relationship.

SPECIFIC OBJECTIVES

- To determine the prevalence rate of Female Athlete Triad.
- To examine the association between eating disorder, nutrition status and menstrual dysfunction.
- To test the psychological dimension of athletes.
- To investigate the correlation between hormones and various parameters.
- To make them understand about the impact of nutrition.

2. REVIEW OF LITERATURE

The review of literature pertaining to the study on **"Comprehensive Evaluation of Nutritional and Health Status of Selected Female Athletes in Coimbatore District and Intervention"** is reviewed under the following headings

2.1 Importance of Nutrition in sports

2.2 Micronutrient deficiency in women

2.3 Female Athlete Triad – a review

2.4 Eating disorder and its consequences in Athletes

2.5 Impact of sporting in menstruation

2.6 Bone Health and the Female Athlete Triad

2.1 Importance of Nutrition in Sports

Nutrition interacts not only with growth and development of young athletes, but also with recovery, performance, avoiding injury and problems that may arise as a result of nutritional deficiencies (Dunford, 2006; Rodriguez *et al*, 2009). For young people practicing sports, proper nutrition is also important to promote healthy dietary practices in the long term (Jeukndrup and Cronin, 2011).

Over the past twenty years, researchers have documented the benefits of nutrition related to exercise performance. In a joint position statement, the American College of Sports Medicine, American Dietetic Association and Dietitians of Canada reported that "Physical activity, athletic performance and recovery from exercise are enhanced by optimal nutrition"(ACSM, 2009). Physically active individuals might encounter numerous barriers regarding healthy eating, including deficits in nutrition knowledge, vegetarian or restricted dietary intake, or participation in excessive exercise (Bonci *et al*, 2008).

Participation of the girls in sports has increased significantly over the years, unfortunately, increased participation in women athletics came without an understanding of the specific needs of the female athlete. Physiologic difference in females, combined with internal demands and external pressures during sports activities, have led to health problems and injuries occurring uniquely in the female players (Dunn, 2007; Ziegler, 1988; Nattiv, 1977).

Kathe and Gabel, (2006) review addresses nutritional concerns of the female athlete, identification of those at risk, relationship of energy intake to menstrual irregularities and recently identified chronic disease associated with the Female Athlete Triad. Petrie *et al*

(2004) identified female distance runners as athletes at higher risk for mineral deficiencies (example: iron and calcium). Cupisti *et al* (2002) also found energy inadequacies, when dietary intakes of adolescent female athletes (n=60) were compared with recommended intake for Italian adolescent.

Female runners need to challenge their body composition further from their natural shape than males to achieve the leanness that is considered optimal for the discipline. Trying to eliminate body fat beyond the biological disposition can have direct negative effects as for example, disturbances of the adipose tissue secretome. Restricted energy, protein, carbohydrate and micronutrients intakes are other more subtle and more indirect consequences from nutritional strategies aiming at decreasing weight and body fat (Burke *et al*, 2007), which may finally have significant effects on health and physical performance (Nattiv *et al*, 2014).

2.2 Micronutrient deficiency in Women

Trace elements play a key role in the body's manufacture and use of energy, which is important not only for adequate physical performance (Anderson, 1991) but also for general health (Campbell and Anderson, 1987; McDonald and keen, 1988). Iron deficiency is widely reported in athletes; above all in women (Faber and Benade, 1991; Newhouse *et al*, 1993; Nuviala *et al*, 1996), it is of interest to focus on other trace elements such as zinc and copper. Athletes in general have increase losses of micronutrients through sweat and urine (Hralambie, 1981; Haymes, 1994) especially during prolonged activity (Lukaski, 1995) which when associated with low dietary intakes can reduce body stores of these micronutrients (Clarkson and Haymes, 1994), affecting sports performance (Campbell and Anderson, 1987; McDonald and Keen, 1988).

Cogswell *et al* (2009) reported that iron deficiency with anemia effects 3-5%, an iron deficiency with anemia (IDNA) nearly 16%, of premenopausal women. Changes in energy metabolism and physical work capacity have been described in humans and animals with iron depletion (Beard, 2001; Haas and Brownlie, 2001; Willis *et al*, 1987). Adolescent female athletes are pre-disposed to iron deficiency for two main reasons: Nutritional deficiency and increased hemolysis due to exercise (Brigham *et al*, 1993 and Clarkson *et al*, 1995).

Many studies suggest that elite female athletes may be at increased risk for iron deficiency (Clement and Asmundson, 1982; Ehn *et al*, 1980; Wishnitzer *et al*, 1983; Jacobs *et al*, 1972). Clement and Asmundson (1982) reported that 82% of female Canadian distance

runners were iron deficient, as estimated by serum ferritin levels, which are believed to accurately reflect the size of the body iron stores (Miller *et al*, 1972) Another report found that despite normal hemoglobin (Hb) and serum iron values, bone marrow showed either an absence or only traces of iron (Carmack *et al*, 1983). Several other investigators have confirmed this surprisingly high incidence of iron deficiency in active persons (Davies *et al*, 1982; Vorst *et al*, 1983).

Sixteen female collegiate rowers (10%) were identified as anemic (Hgb <12.0 g/dl). Using a sFerritin cutoff of <20.0 μg/L, 30% (n = 44) of the non-anemic rowers were identified as iron depleted without anemia and reported 2-km times nearly 21 s slower ($p < .004$) than rowers with normal iron status. Iron depleted rowers (sFerritin<20–25 μg/L) reported a decrease in performance time compared with those with normal iron stores (Dellavelle and Hass, 2011).

Reduction of iron deficiency is also aimed at reducing the risk of developing anemia and perhaps other performance-related problems (Bothwell *et al*, 1979). Rowland *et al* (1988) noted that 4-week oral iron treatment improved serum ferritin levels (8.7 to 26.6 μg/L) in non-anemic iron deficient runners. Schoene *et al* (1983) studied the effect of 2 weeks of iron therapy on exercise performance in trained, mildly iron deficient female athletes. They reported that performance was unchanged after therapy (Galan *et al*, 1992).

Athletes trained for endurance often have low blood hemoglobin levels. It may even be below the normal range (i.e. 13 to 14 g/100ml in men and 12g/100ml in women) and associated with low haematocrit values (Clarkson and Haymes, 1995; McDonald and Keen, 1988; Newhouse *et al*, 1988; Yoshimura, 1970; Inoue, 1980). De Wijn *et al* (1971) found that only 5 to 6% of the athletes on the Dutch national teams had hemoglobin values below 14 g/100ml (men) and 12 g/100ml (women) and that only 2 of them experienced true anemia.

Fedyeh *et al* (2008) stated zinc deficiency was found in 7.1% of participants. (n=480). In women with body weight between 50.1-60kg, zinc deficiency was less than patients with body weight of < 50kg ($p=0.04$). According to study conducted by Zahedah, zinc deficiency was 42.8% in teen girls and 49% in pregnant women (Di Mg *et al*, 1993). In study in Tehran, its prevalence was 65% in high school students (Mahmoodi, 1996).

In a survey of elite Germen athletes, no significant difference in serum zinc concentrations was found between athletes and sex matched control subjects (Haralambie, 1981). Among female marathon runner's plasma zinc concentrations were at the lower limit (Singh *et al*, 1990).

The clinical report by Liu *et al* (1983) suggested that magnesium deficiency can impair physical performance. A young adult female tennis player reported frequent episodes of muscle spasms associated with prolonged outdoor exercise. A diagnosis of magnesium deficiency was based on hypomagnesaemia (serum magnesium: 0.65 mmol/L; normal range: 0.8-1.20 mmol/L) in the presence of otherwise normal physical, neurological and blood biochemical examinations. Daily treatment with 500 mg of magnesium gluconate relieved the muscle spasms within a few days. Comparisons of dietary magnesium among male and female cross country skiers and control subjects found magnesium intake of 170-185% for athletes, as compared to 108-116% for the gender matched controls (Fogelholm *et al*, 1992).

Micronutrient deficiency may be aggravated in athletes who restrict their energy intakes to reduce body mass, as in the case of long distance runners (Clarkson, 1995) hence, there is a need to control micronutrient intake and nutritional state. Magnesium regulates membrane stability and neuromuscular, cardiovascular, immune and hormonal function (Lukaski, 1995) and is a critical cofactor in many metabolic reactions (Clarkson and Haymes, 1994). Physical exercise also seems to deplete magnesium, which together with a marginal intake, may impair energy metabolism efficiency and the capacity for physical work (McDonald and Keen, 1988; Rayssiguier *et al*, 1990). The study of these nutrients, apart from being rather incomplete, has been centered fundamentally on women runner (Deuster *et al*, 1989; Singh *et al*, 1990).

Awareness of a potential adverse effect of physical activity on copper status began with abstract describing decreases in blood biochemical indices of copper nurture among swimmers who increased their training (Dowdy *et al*, 1980). Both serum copper and ceruloplasmin concentrations decreased significantly from before to the end of a completive collegiate swimming season. In contrast, another report indicated no changes in either plasma copper or ceruloplamin concentrations in collegiate swimmers during the competitive season (Lukaski *et al*, 1983).

2.3 Female Athlete Triad – a review

The American College of Sports Medicine (ACSM) identified the Female Athlete Triad (FAT) as a health problem. Female Athlete Triad consists of disordered eating, amenorrhea and osteoporosis. Female Athlete Triad may occur because young women feel they have to maintain or achieve an unrealistically low body weight. Adolescent girls and women training in sports that emphasize low body weight or thin appearance are at a greater risk. Competing in some sports such as ballet, diving, figure skating, gymnastics, running and

swimming may make an athlete more susceptible to Female Athlete Triad (kirchner and Cohen, 2002). Athletes suffering from one component of the Triad should be screened for the others. Alone, or in combination, each component of Female Athlete Triad can decrease physical performance and cause morbidity and mortality (Gottschlich and Young, 2006).

The American College of Sports Medicine position statement on the Female Athlete Triad, listed a wide range of female athletes potentially affected by this syndrome. Individuals ranging from elite female competitors to non-athletic physically active female were identified as potential candidates developing conditions associated with the Female Athlete Triad (Otis *et al*, 1997).

Low energy availability may result when exercise energy expenditure increases more than energy intake, as may occur in endurance sports, but also appears when energy intake is reduced more than exercise energy expenditure (Loucks, 2007). Female athletes in sports such as gymnastics, ballet dancing, or figure skating, in which leanness and aesthetics are emphasized, fit into a risk profile for Female Athlete Triad and may develop poor nutritional behaviors such as food restriction, binging or purging, laxative, enema or diuretic abuse and excessive exercise, resulting in low energy availability (Marquez, 2008).

A substantial number of high school athletes (78%) and a surprising number of sedentary students (65%) suffer from one or more components of the Triad. Given the high prevalence of Triad characteristic in both the groups, education in the formative elementary school years has potential to prevent several of the components in both groups, therefore, improving health and averting long-term complication (Anne z Hoch *et al*, 2010).

Some female athletes do not consider training or exercise as sufficient to accomplish their idealized body shape or level of thinness. Therefore, a significant number of active females diet and use harmful weightless practices to meet their goals (Sundgot-Borgen, 1993; Smolak *et al*, 2000; Byrne and Mclean, 2001). These patterns may lead to under-nutrition, menstrual dysfunction and subsequent bone loss. Each portion of this Triad increases the chance of morbidity and mortality, but the dangers of the three together are synergistic (Otis *et al*, 1997).

A survey in southern California high school, athletes confirms that disorder eating, menstrual irregularity and osteopenia continue as challenges for active young women of 170 female athletes representing eight sports, Nichols *et al* (2006) found 18.2%, 23.5% and 21.8% of the athletes meet criteria for disorder eating, menstrual irregularity and low bone mass respectively.

Torstreit and Sundgot-Bargien, (2005) surveyed the total population elite female athletes in Norway (n=938), to answer the questions personal histories of diet, training, menstruation and oral contraptive use, weight control method, injuries, drive for thinness subscale of the eating disorder inventive who is at greater risk for the syndrome the elite athlete, non-athlete results revealed based on leanness, a higher percentage athletes competing in leanness sports (70.1%) were classified at risk for the syndrome as compared with non-leanness sports (55.3%; $p < 0.001$).

Athlete at risk for the Female Athlete Triad determined in a population consisting 84 collegiate athletes and 62 non-athletes. Both athletes and non-athletes, about 21% of subject reported either oligomenorrhea or amenorrhea; however, no difference in menstrual status exited between the two groups or between lean and non-lean sports athletes (Reinking and Alexander, 2005).

Female endurance athletes have particularly high prevalence of the Female Athlete Triad, which involves menstrual dysfunction, low Bone Mineral Density and dietary restriction/energy deprivation (Nattiv *et al*, 2007) all these may be risk factors for Stress fractures (Bennell *et al*, 1999; Maltheson *et al*, 1996; Nattiv, 2000).

Eating psychopathology was associated with increased risk of stress fracture in endurance athletes, but this may be mediated by menstrual dysfunction and compulsive exercise. Compulsive exercise, as well as amenorrhea, is independently related to stress fracture risk (Rachel *et al*, 2012).

Female athletes with amenorrhea or oligomenorrhea have reduced Bone Mineral Density (BMD) for their Age (Braam *et al*, 1988; Cobb *et al*, 2003; fox *et al*, 2003; Zanker *et al*, 2004). Athletic amenorrhea is strongly related to disordered eating and caloric restriction (Barchrch *et al*, 2003; Souza *et al*, 2005; Williams *et al*, 2001) and exogenous estrogens may be ineffective at improving Bone Mineral Density (BMD) in the absence of improved nutrition and weight gain (Williams *et al*, 2005; Fredericson *et al*, 2005; Zanker *et al*, 2004).

Prevalence of menstrual irregularities in female athletes range from 6% to 79% and vary with type of athlete and intensity of activity (warren and perlroth, 2001). Identified causes of amenorrhea include energy deficit, severe emotional stress and athletic training in which leanness is emphasized. Although early research indicated a relationship between body composition and amenorrhea, recent evidence support changes in energy balance and potentially the composition of calories as influences on menstrual irregularities in female athletes. A review by Stafford, (2005) of the altered hypothalamic-pituitary-ovarian axis and

its relationship to menstrual irregularities supports energy balance as the determining factor for menstrual dysfunction (Ellakim and Beyth, 2003).

A relationship between bone loss and both Disordered Eating (DE) and Menstrual Dysfunction (MD) has been reported in multiple athletic populations, including the high school athletic population (Barrack *et al*, 2008; Kelsey *et al*, 2007; Nichols *et al*, 2007; Rauh *et al*, 2007; Lawson *et al*, 2006; Trone *et al*, 2006). In adolescent female runners, Barrack *et al* (2013) concluded that the dietary restraint subscales of the Eating Disorder Examination Questionnaire (EDE-Q) may reflect the Disordered Eating behaviors most associated with negative bone health effects.

Rauh *et al* (2006) concluded that a lack of menses for 6 or more consecutive months in the past 12 months was significantly associated with stress fracture among female military recruits. Kelsey *et al* (2007) studied stress fractures among young female cross country runners and concluded that a history of menstrual irregularity was a potential predictor for stress fracture incidence, although the association was not statistically significant. In summary, female athletes with Disorder Eating or Menstrual Dysfunction may be at greater risk for bone loss, changes in Bone Mineral Density and stress fracture occurrence.

In the short term, detrimental health consequences include stress fractures, osteopenia, fatigue and infertility, as well as impaired endothelial function (Hoch *et al*, 2007). In the longer term, former athletes who have suffered from the Female Athlete Triad might be at increased risk of osteoporosis and cardiovascular disease (De Souza and Williams, 2004; Nattiv *et al*, 2007; Papanek, 2003).

The prevalence of the complete condition is relatively low, with only 1–5% of female exercisers presenting with all three components (Beals and Hill, 2006; Nichols *et al*, 2006; Torstveit and Sundgot-Borgen, 2005). However, one in four to one in five female athletes present with at least one component of the Female Athlete Triad (Beals and Hill, 2006; Nichols *et al*, 2006; Torstveit & Sundgot-Borgen, 2005), which places them at greater risk for developing the complete condition. Because treatment of the Female Athlete Triad (and more generally, treatment of disordered eating) is known to be challenging, emphasis should be placed on preventing the condition through education (Nattiv *et al*, 2007; Otis *et al*, 1997).

2.4 Eating disorder and its consequences in Athletes

Disordered eating behavior is characterized by disturbances in eating behavior, body image, emotions and relations. Anorexia nervosa (AN) is the extreme of restrictive eating

behavior in which an individual continues to starve and feel fat in spite of being 15% or more below an ideal body mass. Bulimic behavior refers to a cycle of food restriction or fasting followed by binging and Purging. Anorexia Nervosa and Bulimia Nervosa (BN) are clinical eating disorders (EDs) (American Psychiatric Association, 1994).

Complications of Bulimia Nervosa occur as a result of binge-eating and purging (Thompson and Tratter-Sherman, 1993). The loss of fluids and electrolytes during purging can lead to serious medical problems like dehydration, acid-base abnormalities and cardiac rhythm disturbances. Dehydration and electrolyte abnormalities decrease coordination, balance and muscle function. Therefore, the behavior is dangerous to their health and counterproductive to improving their athletic performance (Nielsen *et al*, 1998).

Many women athletes are engaging in disordered eating habits combined with athletic behavior due to a current emphasis on extreme thinness. Too much physical activity, without proper nutritional intake, may lead to the cessation of menses (amenorrhea) and a weakening of the skeletal system (Putukian, 2001).

Smolak *et al* (2000) reviled the Prevalence of Disorder Eating behavior and Eating Disorders among athletes have been estimated to range from 1–62% (Ruble *et al*, 1993). Disorder Eating and Eating Disorders are more frequent among female athletes competing in aesthetic and weight-class sports than among athletes competing in sports where leanness is considered less important (Sundgot-Borgen, 1993; Byrne and Mclean, 2001).

Psychological, biological and social factors are implicated in the development of Eating Disorders (Katrz, 1985; Garfinkel *et al*, 1987). Because of additional stress associated with the athletic environment female elite athletes appear to be more vulnerable to Eating Disorder than the general female population (Thompson and Tratter-Sherman, 1993; Sundgot-Borgen and Torstveit, 1998). Risk factors are restrained eating and training, frequent weight-cycling, early start of sport-specific training, personality factors, injury, overtraining and the impact of coaching behavior (Smolak *et al*, 2000; Sundgot-Borgen, 1994).

2.5 Impact of Sporting In Menstruation

Menstrual dysfunction seen in athletes is characterized by a significant decrease in reproductive hormones, especially estrogen and disruption of the normal menstrual cycle. Manore, (2002) found exercise-induced or athletic menstrual dysfunction (amenorrhea, oligomenorrhea, anovulation, luteal phase deficiency and delayed Menarche) is more common in active women and can significantly affect health and sport performance.

Sharma and Shukla, (1991) found the combined mean age at menarche for sports women is 13.56 years. Menarche was significantly delayed in those sportswomen who embarked on physical training activities before the onset of menstruation. The age at menarche may differ between participants and non-participants in sports. Malina *et al* (1973) found that menarche appears significantly later (13.58 years) in athletes than in non-athletes (12.23 years).

The physiological mechanisms leading to chronic energy deficit in female athletes are far from being elucidated but recent studies in exercising women with functional hypothalamic amenorrhea highlight the potential role of appetite-related hormones in the etiology of chronic energy deficiency and menstrual disturbance (Barrack *et al*, 2013). Similarly to patients with anorexia nervosa (Misra *et al*, 2006), exercising women with functional hypothalamic amenorrhea exhibit paradoxically elevated levels of the orexigenic hormone, ghrelin and the anorexigenic hormone, peptide YY, yielding a general anorexigenic effect (Leidy *et al*, 2003; Scheid *et al*, 2008). Furthermore, fasting Peptide YY concentrations have been negatively correlated with the rate of energy expenditure and positively correlated with a score of leanness in women ranked according to their level of physical activity and their menstrual status (Acker Man *et al*, 2013). High peptide YY, ghrelin concentrations and the subsequent suppressive effect on appetite, suggested that peptide YY is able to blunt the orexigenic effects of ghrelin and prevents compensatory increase in energy intake in exercising women with functional hypothalamic amenorrhea (Burows and Bird, 2000).

Dusek estimated the influence of intensive training on menstrual cycles in female athletes and revealed that menarche was significantly delayed in the athletes who started physical activities before the onset of menstruation (13.8+1.4vs. 12.6+1.0 years, $p<0.001$).

The prevalence of amenorrhea/oligomenorrhea is high in athletes. Athletes would be greatly benefited by greater general awareness about the complications of amenorrhea/oligomenorrhea. To increase awareness of exercise-associated menstrual cycle irregularities, it is necessary to design complete and comprehensive education programs for female athletes, their parents, their coaches and the relevant authorities (Dadgostar *et al*, 2009).

2.6 Bone Health and the Female Athlete Triad

Osteoporosis, which was once considered a disease of the elderly, is now prevalent in young women of any age whose Bone Mineral Density has fallen below a critical threshold (Snow-Harter, 1994). Various risk factors for osteoporosis have been postulated such as

menstrual cycle disturbances (cumming, 1996), diet (Zanker, 1999) and inactivity (Chilibeck, 1995). The research indicates that, although moderate exercise loads may benefit Bone Mineral Density (Okano, 1995), extreme loads may be detrimental to bone health in adults (Heinonen, 1995) and children (Grimston, 1993). Indeed, despite large volumes of weight bearing exercise, female endurance runners have been shown to have lower BMD that their sedentary counterparts (Drinkwater, 1996).

Data from numerous cross-sectional studies demonstrate a positive association between Bone Mineral Density (BMD) and physical activity (Barry and Kohrt, 2008; Beck and Snow, 2003; Nelson *et al*, 2004). Findings from intervention studies in premenopausal women indicate that young women who exercise continuously to increase bone mass compared to non-exercising controls (Wallace and Cumming, 2000; Kemper *et al*, 1999; Snow-Harter *et al*, 1992). In postmenopausal women, systematic reviews indicate that physical activity may slow the rate of bone loss on weight-bearing sites with an effect of approximately 1% per year (Wallace and Cumming, 2000; Wolff *et al*, 1999).

There is a marked variation in Bone Mineral Density (BMD) among women from different ethnic groups. Thus, women of European origin have been observed to have lower Bone Mineral Density (BMD) at different skeletal sites compared to their African-American counterparts (Cunty *et al*, 1995; Aloia *et al*, 1996) but a higher Bone Mineral Density than those of Far East Asian origin (Bachrach *et al*, 1999). Among environmental factors, Nutrition and Vitamin D status play a crucial role in acquisition of Bone Mineral Density (Marwaha and Sripathy, 2008; Rizzoli, 2008). Also, there is evidence to suggest that physical activity during adolescence and early adulthood is a key determinant of peak bone mass (Valimaki *et al*, 1994; Welton *et al*, 1994; Bass *et al*, 1998). A positive association between bone mineral status and daily participation in high-impact physical activity has also been reported (Gustavsson *et al*, 2003; Ginty *et al*, 2005).

The study on risk factors to stress fractures in military recruits identified irregular menstruation, low bone mineral density, leanness, low muscle size and strength, poor skeletal alignment and low physical fitness higher compared to those who did not have stress fracture (Cumming, 1996; Souza *et al*, 2005; Drinkwater *et al*, 1986; Fredricson and Kent, 2005). Kathryn and Madhumitamisra, (2015) emphasized on the prevention and treatment strategies for the Female Athlete Triad (i.e., the interrelationship of decreased energy availability, menstrual irregularity and low bone density).

Burrows *et al* (2003) determined a negative association between endurance running distance and lumbar spine and femoral neck Bone Mineral Density, with a positive

association between body mass and femoral neck and lumbar spine Bone Mineral Density. Cross sectional data have shown that athletes involved in weight bearing activities with such loading characteristics exhibit greater Bone Mineral Density compared with non-athletic controls (Heinonen *et al*, 1995; Heinonen *et al*, 1993; Pettersson *et al*, 2000; Robinson *et al*, 1995) and athletes involved in non-weight bearing sports with a lower degree of strain magnitude and rate (Heinonen *et al*, 1993; Mester *et al*, 1999; Taaffe *et al*, 1995).

3. METHODOLOGY

The Methodology pertaining to the study on **"Comprehensive Evaluation of Nutritional and Health Status of Selected Female Athletes in Coimbatore District and Intervention"** is presented under the following headings:

3.1 Selection of Samples and Formulation of Schedule

3.1.1 Selection of Area and Samples

3.1.2 Formulation of schedule

3.1.3 Conduct of survey

3.2 Data Collection

3.2.1 Demographical information

3.2.2 Anthropometric Assessment

3.2.2.1 Height

3.2.2.2 Body weight

3.2.2.3 Body Mass Index (BMI)

3.2.2.4 Waist and Hip Circumference

3.2.2.5 Waist hip ratio (WHR)

3.2.2.6 Body composition

3.2.3 Sports activity assessment

3.2.4 Eating disorder

3.2.5 Menstrual status

3.2.6 Dietary practice

3.2.7 Psychological Test

3.3 Bio-chemical investigation

3.3.1 Human Growth Hormone

3.3.2 Follicle Stimulating Hormone

3.3.3 Luteinizing Hormone

3.3.4 Prolactin

3.3.5 Thyroid Stimulating Hormone

3.3.6 Free tri iodo thyronine (FT_3) and free thyroxine (FT_4)

3.4 Statistical analysis

3.5 Intervention

3.1 Selection of Samples and Formulation of Schedule

3.1.1 Selection of Area and Samples

Coimbatore district was selected for the study because of easy proximity and familiarity. The population for the study comprised of female athletes from different colleges in Coimbatore. The research adopted the cross sectional study design. The variables were studied without manipulation or introducing any control group. Only those who expressed willingness to participate in the study were selected after obtaining approval by the Head of the Institution. Four hundred and twenty (N=420) sports women were selected through the purposive random sampling method.

The sports women engaged in various sports activity such as competing sports, weight lifting, endurance sports, group event, athletic event, etc were recruited based on the inclusion and exclusion criteria. Healthy female college going students (N=186) in the age group of 18-21 year, residing in New Delhi were evaluated for anthropometry, bio-chemistry, diet, physical activity and lifestyle (Raman *et al*, 2010). Female hockey players (n=30) from different college teams of Haryana in the age group of 17-23 years were selected for determining the nutritional knowledge and attitude towards healthy eating by Vinti Davay (2012). Likewise, the subjects selected for the study fell in the age group between 17-28 years of age and are into various kinds of sports. Exclusion criteria included non-metabolic disease, pregnancy, long term oral steroid use, history of thyroid disorder and tumor or cardio vascular disease.

3.1.2 Formulation of Schedule

A self-administered schedule was used to assess the health and nutritional status of sports women. A total number of 224 female athletes participated in eating attitudes test (EAT 40) and a self-administered questionnaire was used to assess disordered eating behavior and menstrual status respectively (Selma Arzu Vardar *et al*, 2005). Young athletes (n=84), aged 18-40 years from two different schools of medicine, situated in Italy and Serbia were tested for their nutritional knowledge and behavior using self- administered questionnaire (Gabriele trabucco *et al*, 2013). The components included in the schedule for the study compromised of:

- Demographic information
- Anthropometric assessment
- Sports activity
- Disorder eating
- Menstrual history

- Dietary practice
- Psychological test

The schedule was reviewed for content validity and for content clarity before administration and presented in Appendix-I.

3.1.3 Conduct of Survey

The purpose of the study and procedures of the investigation was explained after establishing a good rapport with the participants. The self-administered questionnaire was distributed and each question was explained to obtain a reliable data.

3.2 Data Collection

The data was collected in relation to anthropometry, body composition, sports activity, eating disorder, dietary practice and psychological test.

3.2.1 Demographical information

Although the sample came from a narrow population, demographic variable were obtained, providing a description of the population. Upon entry into the study, subjects were asked to fill their Demographical information such as age, religion and family type in the schedule.

3.2.2 Anthropometric Assessment

Anthropometry is an important aspect in the assessment of the nutritional status as it involves a serious of measurements of the external morphology, which include evaluation of body circumferences, growth rates, body build analysis and body composition. The use of anthropometry in female athletes is used mainly to get information on current body composition of fat and to predict the malnutrition status. Evaluation of body composition yields information about the amount and distribution of fat.

Anthropometric techniques are used to measure the absolute and relative variability in size and shape of the human body. The measurements were performed with subjects in light clothing following standard procedures (Lee and Neiman, 2007).

The parameters measured were

- Height
- Body weight
- Body Mass Index
- Waist circumference
- Hip circumference

- Waist hip ratio
- Percent body fat

3.2.2.1 Height

Height is the distance from the crown of the head to the soles of the feet. Height is measured with the help of a scale drawn on the wall, height was recorded using a wall studio meter (Joshi, 2008). The students stood bare foot with minimal clothing, heels together and the head positioned so that the line of vision was perpendicular to the body, arms hanging freely by the side and the head, back and buttocks and heels in contact with the wall. A straight rod was brought onto the topmost point on the head with sufficient pressure to compress hair. Height was recorded to the nearest of 0.1 cm.

3.2.2.2 Body weight

Weight remains the best indicator of protein, calorie, over and under nutrition. Rate of weight change has a prognostic indication of the body's ability to survive stress. The easiest way of measuring weight is by an electronic balance. Weight should ideally be recorded after voiding, without shoes and with minimal clothing. Before weighing the scale should be calibrated to zero (Joshi, 2008). The students stood over the machine and the head positioned straight after which weight was noted.

3.2.2.3 Body Mass Index (BMI)

The most widely used height for weight index in adults is the Body Mass Index (BMI). This index describes the degree of body fatness. It assumes the weight, when corrected for height is positively correlated to the degree of adiposity.

Body mass index (kg/m^2)	**Nutritional status**
< 16	Severely malnourished
16.0-16.9	Moderately malnourished
17.0-18.0	Mildly malnourished
18.5-24.9	Normal
25.0-29.9	Overweight
30.0-34.9	Obese (class I)
35.0-39.0	Obese (class II)
>40	Obese (class III)

It is a measure of height versus weight using a metric calculation (Joshi, 2008). Body Mass Index (BMI) was calculated by dividing a person's weight in kilograms by the square of their height in meters.

3.2.2.4 Waist and Hip Circumference

Waist circumference measures the degree of weight distribution around the waist. Fat located in the abdominal area is associated with a greater health risk than fat in the gluteal femoral region. A waist circumference over 35 inches in women (> 88 cm) is considered high, placing individual at greater risk for disease (Heather Hedrick Fink *et al*, 2006). Waist circumference was measured in standing position using a flexible tap of the narrowest part of the waist. For individual, who did not have an obvious narrow part of waist on visual inspection, the measurement was taken in the waist at the midpoint between the iliac crest and the lower rib. The Hip measurement is taken around the hip and over the buttocks wherever the greatest girth is found.

3.2.2.5 Waist Hip Ratio (WHR)

Waist Hip Ratio is a fat distribution measure that compared abdominal circumference to hip girth. It gives an indication as to where fat deposition is occurring (i.e., upper body versus lower body). Waist Hip Ratio (WHR) was calculated by dividing the waist girth by hip girth (Heather Hedrick Fink *et al*, 2006).

3.2.2.6 Body composition

Body composition measurement is one of the factors in predicting optimal sports performance. Percent body fat is the percentage of total body weight that fat mass. The percentage body fat is the number that most athletes and their coaches and trainers use to determine whether the athlete is at an optimal body composition. Percent body fat combined with Body Mass Index (BMI) will give a better picture of overall health and ability to perform in the sport (Heather Hedrick Fink *et al*, 2006. The percentage of body fat to that of lean body mass was assessed with the "Omron fat analyzer''. The device is a portable one and was easily carried to the field visit without any discomfort. The manufactures instructions were followed during assessment.

After the assessment on anthropometric measurement, students were asked to fill their values in the schedule.

3.2.3 Sports activity assessment

Regular physical activity (PA) can alter the requirements for some micronutrients (Woolf and Manore, 2006). This makes it important to choose foods carefully, taking into account the quality and quantity of macronutrient intakes, since requirements can vary depending on the type of exercise performed (Rousseau *et al*, 2005). There is strong evidence that appropriate selection of nutrients, timing of intake and proper supplement choice are associated with optimal health and exercise performance (Rodriguez *et al*, 2009). Students activity in sports were assessed with the type of sports involved such as Athletic event, Group event, Hand ball, Kho-Kho, Volley ball, Power lifting, Badminton, Table tennis, Basketball and Hockey; number of years engaged in sports; session of practice and duration of practice in regular and event times.

3.2.4 Eating disorder

Disordered eating can eventually manifest itself into an actual eating disorder such as anorexia or bulimia, so early detection is vitally important. What many athletes do not understand is that disordered eating can lead to low energy availability which in turn can lead to the suppression of "physiological functions that are essential for growth, development and health" (IOC Medical Commission Working Group Women in Sport (IOC-MCWGWS), 2006). Disorder eating behavior questions comprised for subscales such as weight concern, shape concern, eating concern and dietary restraint. The questions included in the schedule were related to pathological eating behaviors (Bulimia and use of vomiting, laxatives, diuretics and diet pills to control body weight) attitudes and feelings.

3.2.5 Menstrual status

After completing the questions on eating disorder, the athlete completed a menstrual status and history questionnaire, which was derived from athlete pre-participation medical history form, developed to screen for the presence of Female Athlete Triad components (Van de Loo DA and Johnson, 1995). Menstrual status covered information such as age at menarche, between cycles gap, number of cycles in a year and clinical symptoms prominent during each menstrual cycle. Details on menstrual dysfunction as Polycystic Ovarian Disease (PCOD), awareness on PCOD and any use of pills taken were also recorded.

The criteria used for classifying athletes with menstrual irregularity were Primary Amenorrhea (menarche has not been occurred by age 14 in the absence of secondary sexual characteristics or by 16 years of age in the presence of normal growth and secondary sexual

characteristics), Secondary Amenorrhea (secondary amenorrhea described as absence of at least 3 to 6 consecutive menstrual cycle or four or fewer menstrual cycle per year), Oligomenorrhea (menstrual cycles fewer than 8 cycles per year or the duration of the cycle exceeds 35 days) and Late-onset menarche (menarche occurred after the age of 16) (Rumball and Lebrum, 2005). For analysis purpose, girls meeting any of these criteria were combined into a single (Oligomenorrhea/ Amenorrhea) group and girls having normal menses (Eumenorrheic). The questions regarding menstrual status were specifically checked in the case on any uncertainty and re-enquired.

3.2.6 Dietary practice

Dietary information regarding type of diet, carbohydrate loading, Fat restriction, Water and Electrolyte consumption, Special dietary guidelines followed and dietary supplements taken by the sports people was also collected through the schedule.

3.2.7 Psychological Test

Profile of mood state schedule was used to assess the Psychological behavior. Mood, as measured by the profile of Mood States questionnaire and resting salivary cortisol levels were examined in 14 female college swimmers during progressive increase and decrease in training volume and were compared to the same measures in college women indulging in eight activities (Parrick *et al,* 1989). After filling the schedule by participants, the schedule was scanned and controlled for any error or missing item. If found any, they were asked to fill the unanswered questions.

3.3 Bio-chemical investigation

Based on the data collected, using stratified random sampling, a sub sample was chosen for biochemical investigation. The criteria used for the selection process was based on Body Mass Index (BMI), use of pathogenic weight control method, self-reported eating disorder and menstrual dysfunction. Menstrual cycle problems result from the suppression of the pulsatile secretion of Hypothalamic Gonadotropin-Releasing Hormone (GnRH), which leads to a reduced secretion of Luteinizing Hormone (LH) and follicle stimulating hormone (FSH), thus preventing ovarian stimulation and causing a fall in the levels of estrogens and progesterone (Loucks, 2003). Various other hormones, such as Corticotrophin Releasing Hormone (CRH), Growth Hormone (GH), Insulin-Like Growth Factor (IGF)-1, thyroxin or melatonin could also play a role (Mendelssohn and Warren, 2010 and Lebrun, 2007). Among the selected 460 sample, about

twenty five blood samples were taken for estimating parameters that are indicators of Triad. Blood samples were taken in the morning (7.00-9.00 Am) before the first meal. The subjects were instructed to abstain from caffeine for 24 hours before the blood sampling and to refrain from performing strenuous exercise on the day of sampling. None of the girls in the study used oral contraceptives or any medication, which might alter the level of the evaluated parameters. On the basis of the Luteinizing Hormones (LH) and Follicle stimulating hormones (FSH) data, the presence of hypothalamus-pituitary insufficiency was estimated (Weimann, 2002). For this purpose, the Luteinizing Hormones (LH) results were divided by the Follicle stimulating hormones (FSH) results, with ratio less than 0.6 signifying hypothalamus-pituitary insufficiency (Weimann, 2002). Blood samples were collected from a peripheral vein in a resting state to determine the endocrine profile using standardized procedures. The hormones evaluated are as follows

- Human Growth Hormone (HGH)
- Follicle Stimulating Hormone (FSH)
- Luteinizing Hormone (LH)
- Prolactin
- Thyroid Stimulating Hormone (TSH)
- Free tri iodo thyroxine (FT_3)
- Free thyroxine (FT_4)

3.3.1 Human Growth Hormone

The rates of growth hormone secretion are influenced by exercise, stress hypoglycemia, estrogen, corticosteroids and others (whitely *et al*, 1994). Growth hormone (GH), an anabolic agent mediated by insulin like growth factor 1(IGF); thought to accelerate growth in children and improve protein synthesis in injuries (Krause's, 2008). Growth hormone was measured using Fully Automated Chemi Luminescent Immuno Assay. The detailed procedure is given in the Appendix-II.

3.3.2 Follicle Stimulating Hormone

Follicle stimulating hormone regulates development, growth, pubertal maturation and reproductive process of the body (Pierce and parson, 1981). Follicle stimulating hormone initiates follicular growth and influences immature ovarian follicles in the ovary (Fowler *et al*,

2003). Follicle stimulating hormone was estimated by Fully Automated Bidirectionally Interfaced Chemi Luminescent Immuno Assay. The procedure is given in the Appendix-III.

3.3.3 Luteinizing Hormone

An acute rise of Luteinizing hormones triggers ovulation and development of the corpus luteum. Luteinizing hormone is necessary to maintain luteal function for the first two weeks of the menstrual cycle (Guyton and Hall, 2006). Luteinizing hormones was measured by Fully Automated Bidirectionally Interfaced Chemi Luminescent Immuno Assay. The procedure followed is given in the Appendix-IV.

3.3.4 Prolactin

Prolactin, one of the hormones of the anterior pituitary gland that stimulates milk production by alveolar breast cells (Krause, 2008). Prolactin plays an essential role in metabolism, regulation of the immune system and pancreatic development (Bates and Riddle, 1935). Fully Automated Bidirectionally Interfaced Chemi Luminescent Immuno Assay method was use to assess the level of prolactin. The detailed procedure is given in the Appendix-V.

3.3.5 Thyroid Stimulating Hormone

Thyroid hormone imbalance has as effect on menstrual cycle. Thyroid stimulating hormone was measured by Ultra-Sensitive Sandwich Chemi Luminescent Immuno Assay. The procedure is given in the Appendix-VI.

3.3.6 Free tri iodo thyronine (FT_3) and free thyroxine (FT_4)

Competitive chemi luminescent immuno assay method was used to estimate free tri iodo thyroxine and free thyroxine. The procedure followed is given in the Appendix-VII.

3.4 Statistical analysis

Values were given as mean ± SD. Pearson's correlation coefficient was performed to evaluate relationship between nominal variables and students 't' test was used for determining the significant difference. Spearman's correlation coefficients were calculated in order to examine the relationship between the hormones and other parameters. A p value of below 0.05 was considered to be significant.

3.5 Intervention

Through pamphlet and oral discussion the subjects were educated about optimal health.

4. RESULTS AND DISCUSSION

The results and discussion pertaining to the study on **"Comprehensive Evaluation of Nutritional and Health Status of Selected Female Athletes in Coimbatore District and Intervention"** is discussed under the following heads:

4.1 Demographical Information

4.2 Anthropometric Assessment

4.2.1 Height and Weight

4.2.2 Body Mass Index

4.2.3 Waist and Hip circumference

4.2.4 Waist Hip ratio

4.2.5 Body Fat percent based on Omron Measurements

4.2.6 Correlation between Body Mass Index and Body Fat

4.2.7 Correlation between Age and Body Fat

4.3 Sports profile

4.3.1 Types of Sports

4.3.2 Number of Years in Sports

4.3.3 Practice Session

4.3.4 Duration of Practice

4.4 Eating Disorder

4.5 Menstrual History

4.5.1 Menstrual Status

4.5.2Comparison of general characteristic in Eumenorrhea and Amenorrhea or Oligomenorrhea group

4.5.3 Pain during Menstrual cycle

4.5.4 Feel during Menstrual cycle

4.5.6 Awareness regarding Polycystic Ovarian Disease

4.6 Dietary practice

4.6.1 Type of diet

4.6.2 Dietary Habits

4.6.3 Skipping of Meals

4.6.4 Preference for Junk Foods

4.6.5 Water Intake

4.6.6 Special Diet considerations of sports women

4.6.7 Form of Electrolyte Consumption

4.6.8 Special foods during practice

4.6.9 Diet consumption on the day before the event

4.6.10 During the event-diet consumption

4.6.11 Diet consumption on the day after the event

4.6.12 Foods Avoided

4.7 Psychological Test

4.7.1 Stress Level

4.7.2 Emotions related to Negative Feel

4.7.3 Feelings pertaining to Positive Attitude

4.8 Bio-chemical investigation

4.8.1 Luteinizing Hormone and Follicle Stimulating Hormone Ratio

4.8.2 Association between Human Growth Hormone (HGH) and Body weight

4.8.3 Correlation between Body weight and Thyroid Stimulating Hormone (TSH), Tri iodo thyronine (FT_3), Thyroxine (FT_4)

4.8.4 Relationship between Hormones, Body composition and Menstrual history

4.1 Demographical information

Age, religion, family type of the sports people are given in Table - I.

TABLE - I

Demographical information

Particulars	Number	Per cent
Age		
15-18	136	32
19-22	250	57
23-26	44	11
Religion		
Hindu	382	91
Christian	24	3
Muslim	14	6
Family type		
Joint	86	20
Nuclear	334	80

The selected sports people were in the age group of 15-18 (32 per cent), 19-22 (57 per cent) and 23-26 (11 per cent) years. Among the sports people, majority were Hindus (91 per cent), followed by Muslim (6 per cent) and Christians (3 per cent). The family type of subject showed 80 per cent of the sports people were from nuclear family background, while 20 per cent from joint family system. Kumudhini (2011) in her study stated that majority of sportswomen (75 per cent) were up to the age of 19 years, 23.33 per cent were found in 20-22 years of age group whereas only 1.67 per cent was found in the age group of 23-25 years, whereas in this study 57 per cent belonged to 19-22 years age group.

4.2 Anthropometric measurements

Anthropometric techniques are used to measure the absolute and relative variability in size and shape of human body. The use of anthropometry in female athlete is used mainly to get information on current body composition of fat and to predict the malnutrition status (Lee and Neiman, 2007). Results pertaining to the anthropometric measurements of the sports women are presented in the following tables.

4.2.1 Height and Weight

Table - II gives the height and weight of the sports women.

TABLE - II

Anthropometric measurements

Particulars	Numbers	Per cent
Height		
140-155 cm	104	25
155-170 cm	274	65
170-185 cm	42	10
Weight		
30-45kg	72	17
45-60kg	268	64
60-75 kg	64	15
75-90kg	16	4

Body weight is the most widely used and sensitive and simplest reproducible measurement for the evaluation of nutritional status of individuals. It indicates the body mass and is a composite of all body constituents like water, mineral, fat, protein and bone. It reflects more recent nutrition than height. Height is affected only by long-term nutritional deprivation; it is considered as an index of chronic or long duration malnutrition. Anthropometric data showed that 25 per cent had the mean height of 140-155 cm, 65 per cent ranged between 155-170 cm and 10 per cent were between 170-185 cm respectively. Similarly, Kumudhini (2011) reported that the majority of sports women in her study (75 per cent) ranged between 160-170 cm in height, only 8.33 per cent sports women were found to be above 170 cm. Majority of sports women (83.34 per cent) to have a body weight between 51-60 kg. In this study, about 64 per cent sports women were found in the body weight category 45-60 kg, while 17 per cent between 30-45 kg and 15 per cent between 60-75 kg. Nearly 4 per cent of the subjects were overweight (75-90 kg).

4.2.3 Body Mass Index

Body Mass Index (BMI), an important indicator of obesity prevalence in large population, generally reflects degree of fatness among individuals. Body Mass Index (BMI) can however over or underestimates adiposity depending upon certain circumstances. Body Mass Index (BMI) is a relative body weight assessment and widely accepted tool in determine obesity (Hickson *et al*, 1987).

Table - III gives the Body Mass Index (BMI) of the sports women.

TABLE - III

Body Mass Index

Body Mass Index	Per cent
<16	5
16-16.9	3
17-18.4	14
18.5-24.9	66
25-29.9	10
30-34.9	2
35-39.9	0.5
>40	0.5

Body Mass Index (BMI) value indicate that majority of the selected sports person had ideal Body Mass Index (66 per cent), 14 per cent were mildly malnourished, 3 per cent fall under moderately malnourished, 5 per cent were classified as severely malnourished, 10 per cent were overweight, only 2 per cent were found in obese (class I) category and about 0.5 per cent belonged to obese (class II and class III) categories of Body Mass Index (BMI). Wan Nudri *et al* (1996) identified most of the female athletes (19 subjects or 79 per cent) were classified as normal (BMI 18.6 - 23.8), 3 subjects (13 per cent) were classified as underweight (BMI <18.6), only 2 subject (8 per cent) were classified as overweight, while none were classified as obese.

4.2.4 Waist and Hip circumference

Waist and Hip circumference measurements are given in the Table - IV.

TABLE - IV

Waist and Hip circumference

Particulars	Mean ± SD
Waist circumference (cm)	73 ± 17.07
Hip circumference (cm)	86 ± 11.96

The mean waist and hip circumference of athletes was 73 ± 17.07 cm and 86 ± 11.96 cm respectively.

4.2.5 Waist Hip ratio

Table - V present the Waist Hip ratio of the selected sports women.

TABLE - V

Waist Hip ratio

Waist Hip ratio	Numbers	Per cent
<0.75	80	19
0.75-0.79	126	30
0.8-0.85	82	20
>0.86	132	31

The Table shows that 31 per cent (132 subjects) of selected subjects were at higher health risk on basis of Waist Hip ratio (>0.86), 20 per cent (82 subjects) are at moderate risk (0.8 - 0.85), while 30 per cent (126 subjects) and 19 per cent (80 subjects) are with lower risk for diabetes and cardio vascular diseases.

4.2.6 Body Fat Percent based on Omron Measurements

The body fat percent of sports people presented in the Table - VI.

TABLE - VI

Body Fat Percent

Body Fat	Per cent
<19	15
19-22	17
22-25	18
25-30	29
>30	21

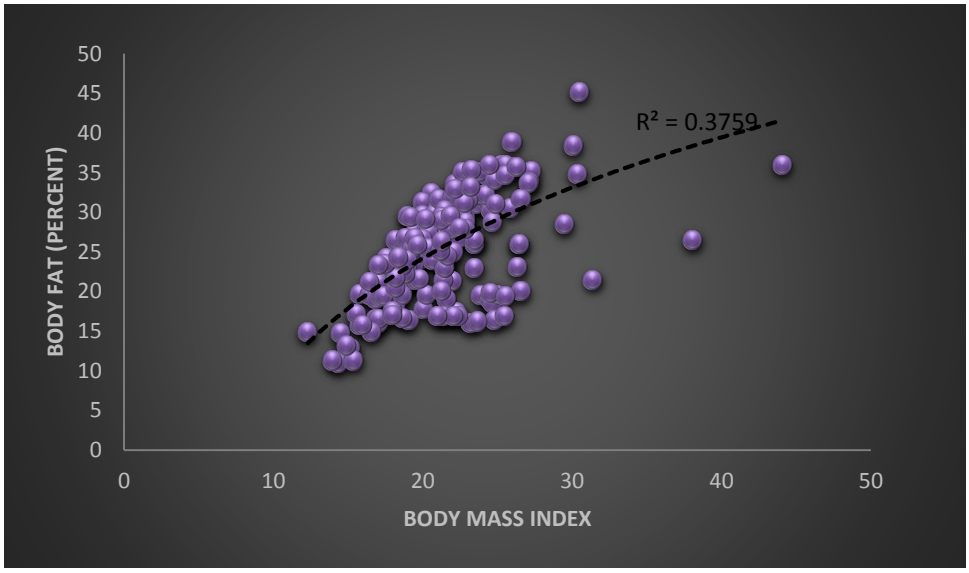
Based on the criteria by Janes, (2015), 21 per cent sport people had a very high body fat (>30 per cent) placing them in the obese category, 29 per cent were under high range (25-30 per cent), while 15 per cent was found to have body fat less than 19 per cent. Eighteen per cent were in acceptable range (22-25 per cent) and only 17 per cent were found (19-22 per cent) to have the body fat in ideal range. Neither too low body fat nor high fat can bring about imbalance in the homeostatic mechanism.

4.2.7 Correlation between Body Mass Index and Body Fat

Correlation between Body Mass Index and Body fat is given in the Figure - I.

FIGURE – I

Correlation between Body Mass Index and Body fat



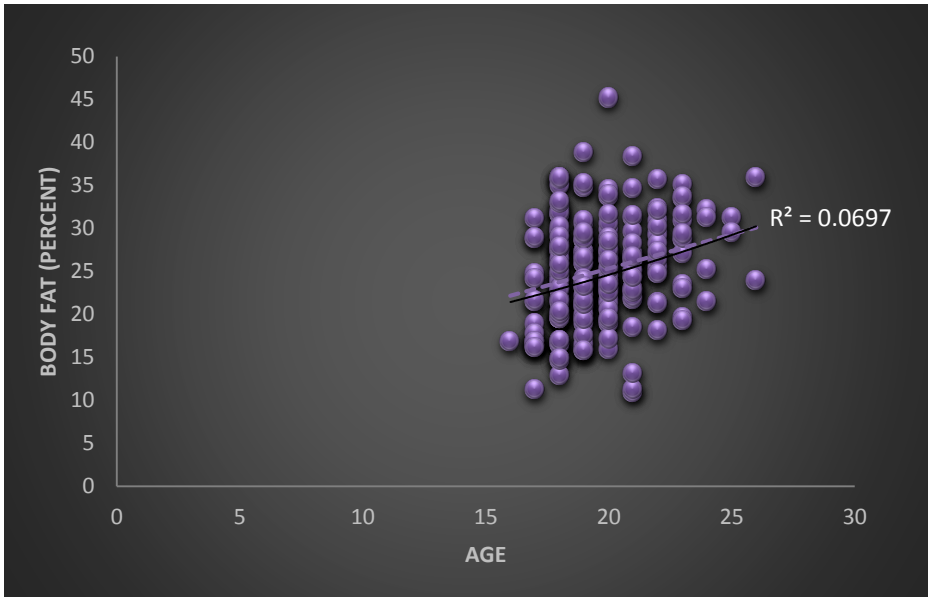
It is clear from the figure that a positive correlation exists between Body Mass Index (BMI) and Body Fat with R value of 0.5731, which mean there is a tendency for high (X variable) Body Mass Index (BMI) scores go with high (Y variable) Body fat per cent score. The P value <0.00001 indicates significant increase in Body Mass Index with Body fat. Chandrasekharan *et al* (2010) noted that Body Mass Index (BMI) was found to be highly correlated with Body Fat percentage (R = 0.73, p < 0.001) indicating statistical linear relationship between Body Fat percentage and Body Mass Index (BMI), as noted in the present research work.

4.2.8 Correlation between Age and Body Fat

Figure - II presents the correlation between Age and Body Fat.

FIGURE – II

Correlation between Age and Body Fat



The value of R is 0.256, even though a positive correlation was found between Age and Body fat, the relationship between the variable was weak because the obtained R value was closer to 0.1. Body Fat increased significantly with age with the P value of 0.000017.

4.3 Sports profile

Successful athletic performance is a combination of favorable genetics, desire, proper training and a sensible approach to Nutrition (Abood *et al*, 2006). From 1981 to 2004, female

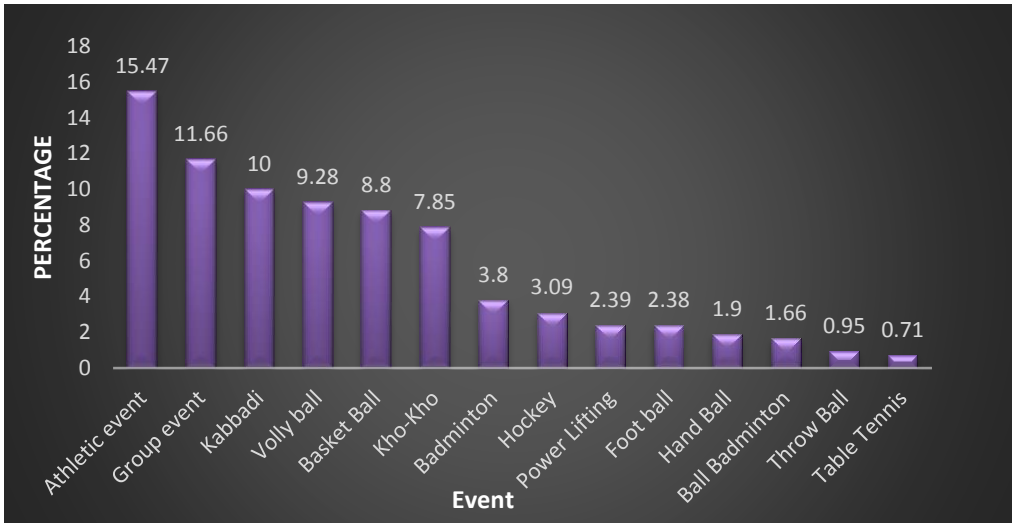
participation in collegiate had risen 137 per cent (Women's Sports Foundation, 2007). The following table gives the details on the sporting activity of the selected subjects.

4.3.1 Type of sports

The students of the study were involved in a wide range of sporting activity which is presented in the figure - III.

FIGURE - III

Type of sporting activity



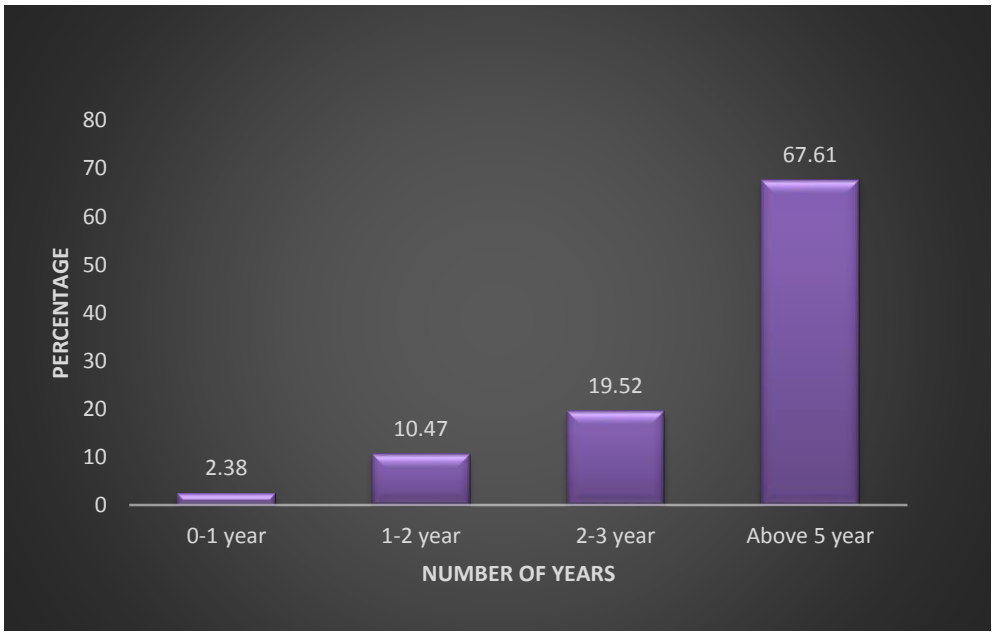
The Figure - III shows that majority (15.47 per cent) of sports people were involved in Athletic event, 11.66 per cent were Group Event players, 10 per cent played Kabaddi, 9.28 per cent involved in Volleyball, 8.80 per cent were Basketball players, 7.85 per cent played Kho-Kho, 3.80 per cent in Badminton, 3.09 per cent players were in Hockey, 2.38 per cent each in Football and Power lifting, 1.90 and 1.66 per cent were playing Handball and Ball badminton. Only a small per cent were in Throw ball (0.95 per cent) and Table tennis (0.71 per cent) respectively. Anne Z Hoch *et al* (2009) mentioned that in the sports team selected for the study, students belonged to categories such as track (N=24), cross–country (N=25), Volleyball (N=13), Basketball (N=14), soccer (N=24), Tennis (N=7), Swimming (N=17), Golf (N=3) and Softball (N=6). Among them, thirty four varsity athletes played one sport, thirty nine played two sports and seven played three sports. In our study through discussion, it was found that most of them confined themselves to one sport activity.

4.3.2 Number of years in sports

The number of years, the selected subjects engaged themselves in sports is presented in the figure – IV.

FIGURE - IV

Number of years in sports



The Figure shows that the majority of subjects (67.61 per cent) were engaged in sports for above 5 years, 19.52 per cent involved for 2-3 years, 10.47 per cent play sports for 1-2 years and 2.38 per cent have been playing for the past one year only.

4.3.3 Practice session of the selected players

Practice session of the sports people is given in the Table - VII.

TABLE - VII

Practice session

Practice	Per cent
Every day	72
Weekly twice	14
Weekly four times	7
Once in a month	7

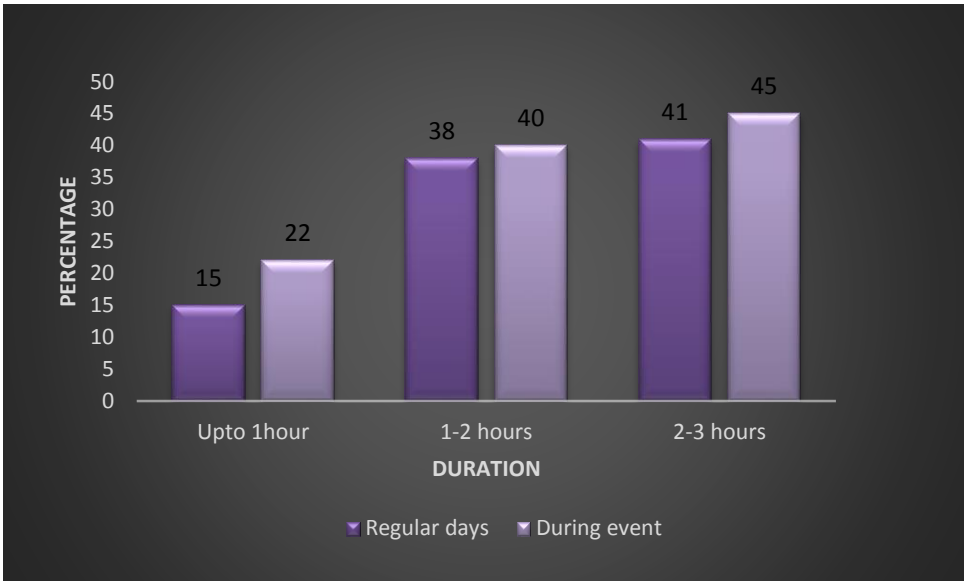
The Table - VII shows that 72 per cent of the sports people were doing practice every day, 14 per cent are involved twice in practice per week and 7 per cent each practice four times in a week and once in a month.

4.3.4 Duration of practice

Figure - V gives the duration of practice of sports people.

FIGURE - V

Duration of practice



It can be understood from figure –V, the duration of practice increased prior to event participation than regular session.

4.4 Eating Disorder questions

Coaches, parents and trainers may also suggest that a certain body type is advantageous and encourage change in the athlete's body size or shape to help them excel in their sport. In many sports, athletes and coaches believe excess weight inhibits speed, agility and endurance while increasing fatigue (krane *et al*, 2001). Disorder eating develops when athletes continue to strive for that "Ideal" at the expense to overall health and sport performance. As the obsession to achieve or maintain a certain ideal continues, athletes may develop eating disturbances that can lead to diagnosable eating disorders. Eating disorders have serious health consequences. Athletes with full-blown eating disorders often have decreased sport performance, are more likely to become injured and suffer emotional and psychological consequences that affect life functioning (Heather Hedrick Fink *et al*, 2006). Table - VIII gives the questions related to Eating Disorders.

TABLE - VIII

Eating Disorders of the selected subjects

Questions	Yes	No
Do you worry about your weight?	47	53
Do you limit the foods that you eat?	42	58
Do you try to lose weight to meet image requirement in your sports?	40	60
Does your weight affect the way you feel about yourself?	34	66
Do you lost control over how much you eat?	32	68
Do you make yourself vomit; use diuretics or laxatives after you eat?	8	92
Do you suffer from eating disorder?	26	74
Do you ever ate in secret?	26	74

The Table - VIII shows that 47 per cent worry about their weight or body composition, in which 42 per cent limit the food that they eat. Fourty per cent try to lose their weight to maintain the appearance, which is important in sports. About 34 per cent feel their weight affect the way the feel about themselves, 32 per cent do not have control over how much they eat. Nearly 8 per cent use diuretic or laxatives after food intake to avoid weight gain. Twenty six per cent in each suffer from eating disorder and subjects also mentioned that they ate in secret. Selma Arzu Vardar *et al* (2005) pointed that in high Eating Attitude Test-40 scores were reported by 37 athletes (16.8 per cent). Two of these athletes were diagnosed with having eating disorder, one of the athletes was diagnosed with anorexia nervosa and the other one had eating disorder. The remaining four athletes did not have the diagnostic criteria for anorexia nervosa, bulimia nervosa or Eating Disorder. Athletes with high Eating Attitude Test - 40 scores had higher body weight (59.8 ± 7.6) than those with normal Eating Attitude Test - 40 scores (56.5 ± 7.0 $p < 0.02$).

4.5 Menstrual history

Menstrual irregularity is a common condition among female athletes (De Souza *et al* (1998). Data collected in relation to menstrual history are presented in the following tables.

4.5.1 Menstrual Status

Table - IX gives the Menstrual status of sports people.

TABLE - IX

Menstrual status

Particulars	Per cent
Eumenorrhea (Normal cycle)	55.7
Secondary Amenorrhea (Absence of 3-6 consecutive menstrual cycle)	3.8
Oligomenorrhea (Cycle exceeds 35 days)	17.1
Late onset Menarche (Attaining puberty after the age 16)	3.1

The Table - IX indicates that 55.7 per cent had normal menstrual cycle. Eighty eight (20.9 per cent) individuals had Amenorrhea or Oligomenorrhea, in which Oligomenorrhea occurred in 17.1 per cent of participants. In 17 per cent, 2.38 per cent suffer from Polycystic Ovarian Disease. Secondary Amenorrhea was reported in 3.8 per cent (0.47 per cent suffer from Polycystic Ovarian Disease) and there was no case of primary Amenorrhea detected. Late onset menarche occurred in 3 per cent (26 subjects) of participants, of which 0.9 per cent was in the Amenorrhea/Oligomenorrhea group and 2.1 per cent in Eumenorrhea group. Selma Arzu Vardar *et al* (2005) reported that Amenorrhea was present in 22 (9.8 per cent) of all athletes. Of the 22 athletes assessed, two (0.9 per cent) reported primary Amenorrhea, while remaining 20 reported secondary Amenorrhea. Menstrual irregularities was reported by 43 (19.2 per cent) of the athletes. Eighty per cent of all participating athletes (N = 181) reported regular menstrual cycle during the past one year. Anne *et al* (2010) found that menstrual dysfunction was self-reported in 54 per cent of the athletes, 6 per cent reported primary Amenorrhea and 30 per cent had secondary Amenorrhea and 14 per cent had Oligomenorrhea. The present study results contra-indicated in relation to primary amenorrhea.

4.5.2 Comparison of parameters in Eumenorrhea and Amenorrhea / Oligomenorrhea group

Comparison of parameters like weight, Body Mass Index, Body fat and age at Menarche in Eumenorrhea and Amenorrhea/ Oligomenorrhea subject is presented in Table - X.

TABLE – X

Comparison of parameters in Eumenorrhea and Amenorrhea / Oligomenorrhea group

Particulars	Eumenorrhea	Amenorrhea/Oligomenorrhea
Weight	53 ± 9.80	55 ± 10.09
BMI	20 ± 3.77	22 ± 3.75
Body Fat	25 ± 5.76	25 ± 6.87
Age at menarche	13.6 ± 1.17	13.7 ± 1.30

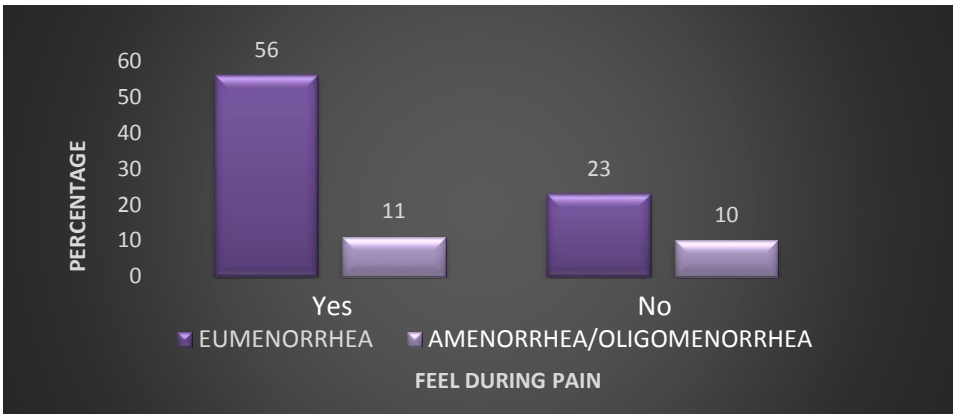
The mean weight in the Eumenorrhea group was 53 ± 9.80 and in the Amenorrhea/ Oligomenorrhea group was 55 ± 10.09, which was not statistically significant (t value = 0.9673, p = 0.3345). The mean Body Mass Index (BMI) was 20 ± 3.77 in Eumenorrhea group and 22 ± 3.75 in the Amenorrhea/ Oligomenorrhea group with a t value = 1.4025, p value = 0.1623, which was also not statistically significant as Mean weight. Body fat in the Eumenorrhea group was 25 ± 5.76 (Mean ± SD) and in the Amenorrhea/ Oligomenorrhea group were 25 ± 6.87 (Mean ± SD). No statistical difference was found between the group in body fat (p = 0.9528, t = 0.0593). The mean age at menarche in Eumenorrhea group was 13.6 ± 1.17 and in the Amenorrhea/ Oligomenorrhea group 13.7 ± 1.30, which was not statistically significant (p = 0.3818, t = 0.8764). Heleh Dadgostar *et al* (2009) revealed that the age ranged from 13 to 37 (Mean = 21.1, SD = 4.5) and the mean age of the Amenorrhea/ Oligomenorrhea group was 20.1 ± 4.4 and in the Eumenorrhea group (21.1 ± 4.5) was slightly different but was not statistically significant (T = test: t = 0.417, p = 0.677). The mean Body Mass Index (BMI) was 21.2 ± 2.9 (ranged 13.2 to 41.0), which was not statistically different between Amenorrhea/ Oligomenorrhea and Eumenorrhea group respectively.

4.5.3 Pain experienced during Menstrual Cycle

Pain experienced during Menstrual Cycle is given in the Figure - VI.

FIGURE - VI

Pain experienced during Menstrual Cycle



A total of 280 individuals reported pain during their menstrual cycle, 56 per cent were from Eumenorrhea group and 11 per cent from Amenorrhea/ Oligomenorrhea. Subjects in the Oligomenorrhea group were affected more (9 per cent) than secondary Amenorrhea group (2 per cent). The difference was statistically significant between Eumenorrhea and Amenorrhea/ Oligomenorrhea group with the p value of 0.0150 and t value = 2.4535.

4.5.4 Duration of pain during Menstrual Cycle

Table - XI gives the data on duration of pain during Menstrual Cycle.

TABLE - XI

Duration of Pain

Duration	Eumenorrhea (per cent)	Amenorrhea / Oligomenorrhea (per cent)
1 day	30.95	6.19
2 days	9.04	2.38
3 days	7.14	0.95
1-3 hours	5.71	0.47
4-6 hours	3.33	0.47

The Table-XI indicates that 30.95 per cent had pain lasting for about 1 day, 16.28 per cent it lasted for 2-3 days. In about 9.04 per cent, duration of pain was from 1-6 hours in the

Eumenorrhea group. In Amenorrhea / Oligomenorrhea group, 6 per cent of sports women reported pain lasting for 1 day and for remaining it was between 1-6 hours. When compared to Eumenorrhea group, less number of subjects experienced pain in Amenorrhea/ Oligomenorrhea group during their cycle.

4.5.5 Feel during Menstrual cycle

Table - XII gives the feel during menstrual cycle.

TABLE - XII

Feel during Menstrual cycle

Feelings	Eumenorrhea (per cent)	Amenorrhea/Oligomenorrhea (per cent)
Anxiety	8.57	2.38
Stress	20.00	7.61
Depression	15.20	6.66
Fatigue	23.33	4.28
Body cramps	20.00	6.66
None	18.57	3.33

From Table-XII, it is clear that 8.57 per cent of the subjects from Eumenorrhea group felt anxious whereas only 2.38 per cent from Amenorrhea/ Oligomenorrhea group. Stress level was higher in Eumenorrhea group (20 per cent) than Amenorrhea/ Oligomenorrhea group (7.61 per cent). Twenty three per cent experienced fatigue from Eumenorrhea group and 4.28 per cent from Oligomenorrhea group. Fifteen per cent in Eumenorrhea and only 6.66 per cent from Amenorrhea/ Oligomenorrhea group were depressed. Among the subjects in Eumenorrhea group 20 per cent reported body cramps, compared to only 6.66 per cent in Amenorrhea/ Oligomenorrhea group. The feelings were not influenced by menstrual cycle in 18.57 per cent and 3.33 per cent of the selected individuals in both the groups respectively.

4.5.6 Awareness regarding Polycystic Ovarian Disease

Table - XIII gives the Awareness regarding Polycystic Ovarian Disease by sports women.

TABLE - XIII

Awareness regarding Polycystic Ovarian Disease

Group	Per cent	
	Yes	No
Eumenorrhea	3.33	17.14
Amenorrhea/ Oligomenorrhea	18.5	60.95

A total per cent of 78.09 per cent from both the groups were not aware of the consequences of polycystic ovarian disease. Only about 3.33 per cent and 18.5 per cent had knowledge about this disease. This implies it is essential to create awareness about Polycystic Ovarian Disease, as the prevalence is increasing in the society at a significant rate.

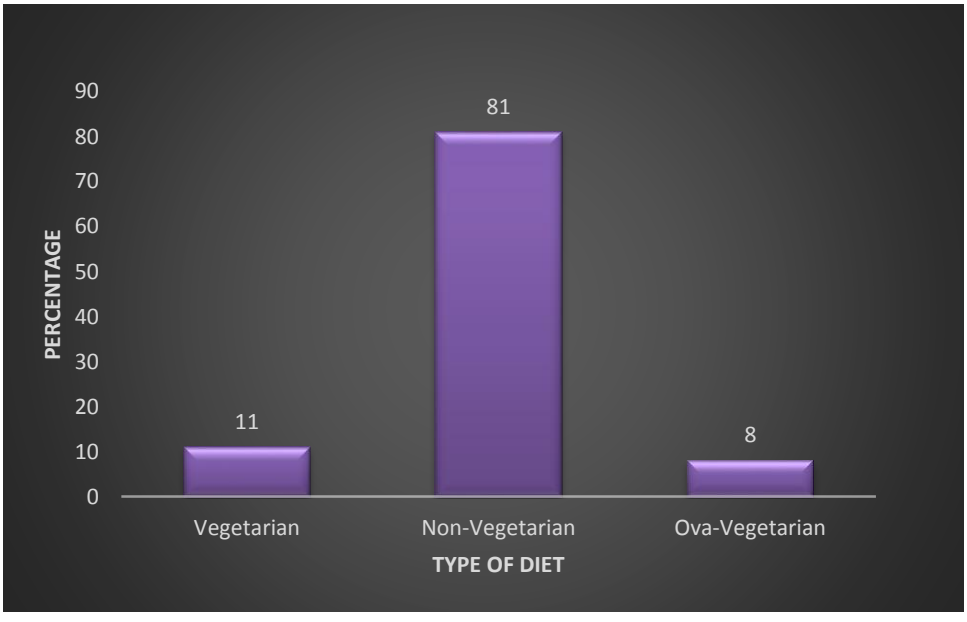
4.6 Dietary Practice

4.6.1 Type of Diet

Type of diet followed by sports people is presented in Figure -VII.

FIGURE - VII

Type of Diet



From the figure-VII, it is clear that the majority of sports people (81 per cent) were non-vegetarian, while 11 per cent of the selected sports people were vegetarian and the rest of the subject (8 per cent) were ova vegetarian. Kumudhini (2011) found that 85 per cent of sports women were consuming mixed type of diet and only 5 per cent sports women were consuming non vegetarian diet, contradicts the results of this study.

4.6.2 Dietary Habits

Dietary Habits of sports women are given in the Table - XIV.

TABLE - XIV

Dietary Habits

Questions	Always	Occasionally	Rarely
How often you skip your meal?	9.52	40.95	49.04
Carbohydrates loading before the event?	11.42	39.04	49.52
Restriction of fat intake?	12.85	38.09	49.04
Consumption of sports drinks?	7.61	32.85	55.71
Electrolytes consumption?	9.04	40.47	50.95

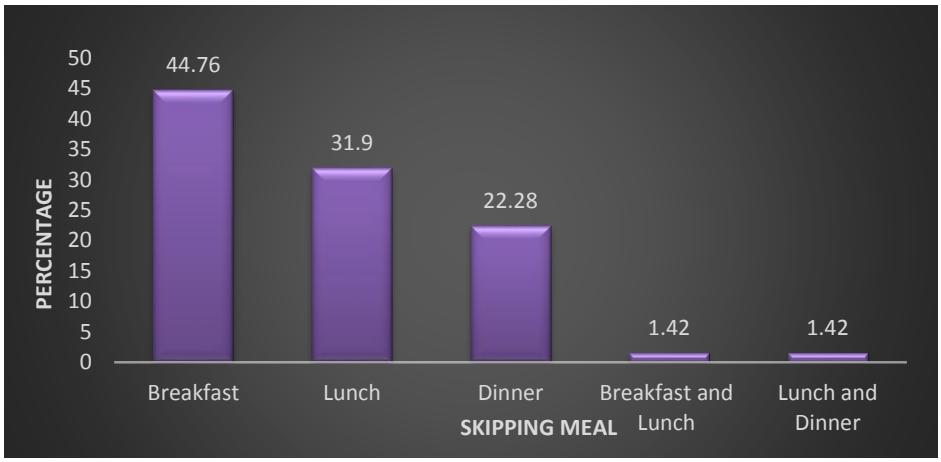
The Table shows that 40.95 per cent of selected sports people skip their meal occasionally, 9.52 per cent always and the rest of subject skip rarely. Only 11.42 per cent of sports person took high carbohydrate before the event, while 39.04 per cent followed it occasionally and 49.04 per cent did not load themselves with carbohydrates. Kumudhini (2011) mentioned that in her study majority of sports women (66.67 per cent) were consuming normal quantity of carbohydrates while 25 per cent less than normal and only 8.33 per cent sports women were consuming more than normal quantity of carbohydrates in their daily diet. In this study, nearly 12.85 per cent always restrict their fat intake while 38.09 per cent do it occasionally. About 7.61 per cent of the sports people consumed sports drink on regular basis while 55.71 per cent consumed rarely. The data regarding electrolytes consumption shows that 9.04 per cent of sports person took electrolytes while 50.95 per cent took rarely. Mahalakhsmi Sangeetha (2012) revealed that 36 per cent of the sports person always consumed electrolytes regularly while two per cent were consuming occasionally, as against 4 per cent in this study.

4.6.3 Skipping of Meals

Figure - VIII gives the skipping of meals in sports people.

FIGURE - VIII

Skipping of Meals



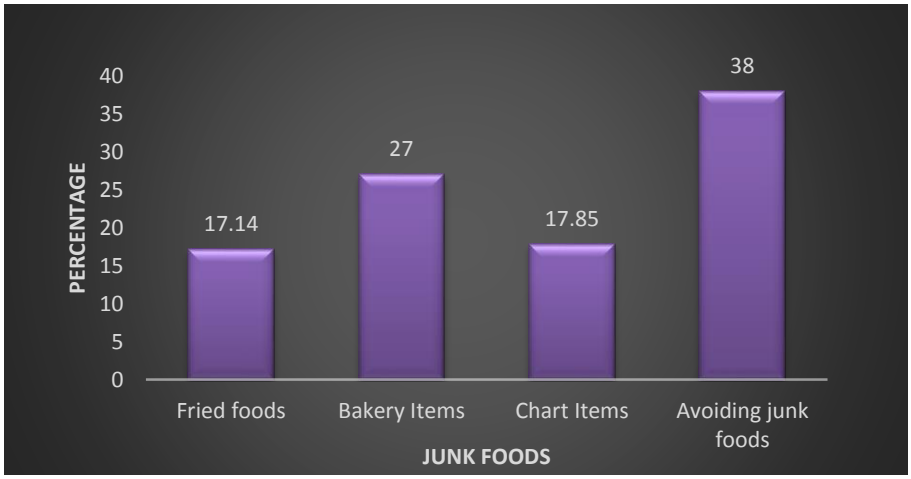
From the Figure-VIII it is clear that majority (44.76 per cent) of sports person had the habit of skipping their breakfast, 31.9 per cent skip their lunch and 22.28 per cent skip dinner. 1.42 per cent of sport people in each had the habit of skipping breakfast - lunch and lunch - dinner respectively. Mahalakshmi Sangeetha (2012) noted that 65 per cent of the sports person had the habit of skipping meal, of this 15 per cent skipped breakfast, 26 per cent lunch and 24 per cent dinner.

4.6.4 Preference for junk foods

Preference for junk foods by sports people is presented in the Figure - IX.

FIGURE - IX

Preference for junk foods by sports peoples



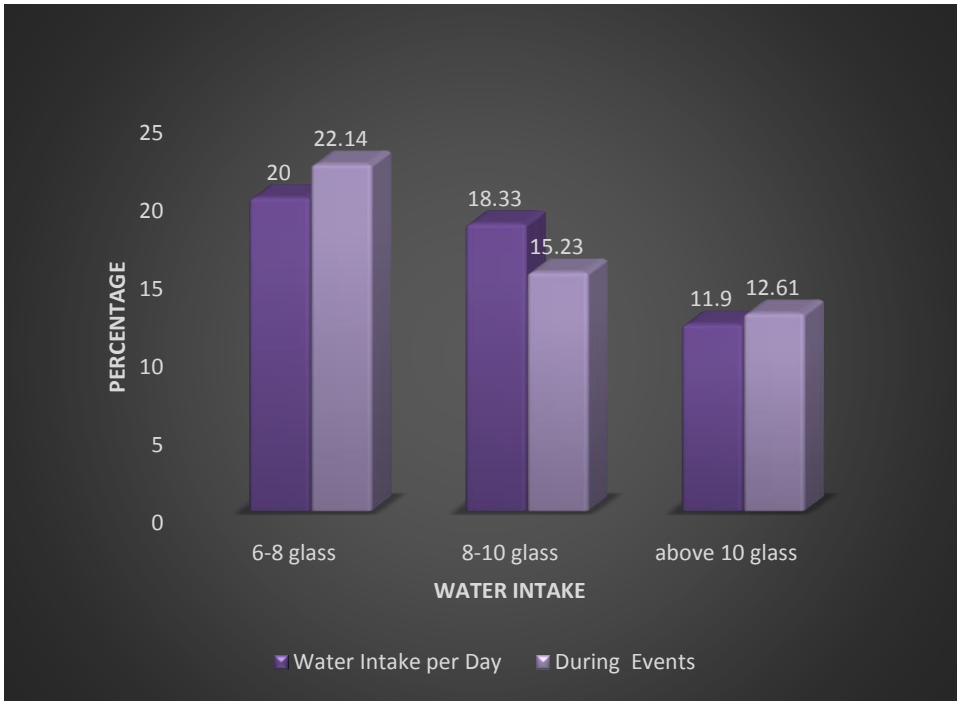
The Figure indicates that 27 per cent of the sports people preferred bakery items, 17.14 per cent consumed fried foods and 17.85 per cent subjects liked chart items. Thirty eight Per cent of sports women do not prefer junk foods. Mahalakhsmi Sangeetha (2012) stated that 54 per cent of the samples consumed bakery items and about 26 per cent sports person preferred fried foods.

4.6.5 Water Intake

Water consumption by sports people is given in the Figure - X.

FIGURE - X

Water Intake per day and during the event



The Figure shows that 6-8 glasses of water was consumed by 20 per cent during normal days and 22.14 per cent during events. About 18.33 and 15.23 per cent of the sports people consumed 8-10 glasses of water during normal and event days. 11.9 per cent consumed more than 10 glasses of water per day whereas 12.61 per cent of sports people consumed above 10 glasses during events. Mahalakhsmi Sangeetha (2012) mentioned that 59 per cent of sports person consumed more than 10 glass of water per day, 23 per cent consumed 8-10 glass and 18 per cent consumed 6-8 glass/day.

4.6.6 Special Diet considerations of sports women

Table - XV gives the special diet considerations of sports women.

TABLE - XV

Special Diet considerations of sports women

Questions	Per cent	
	Yes	No
Do you follow any special dietary guidelines to enhance your performance	24	76
Do to take any dietary supplements	35	65
Do you have any restricted foods	19	81

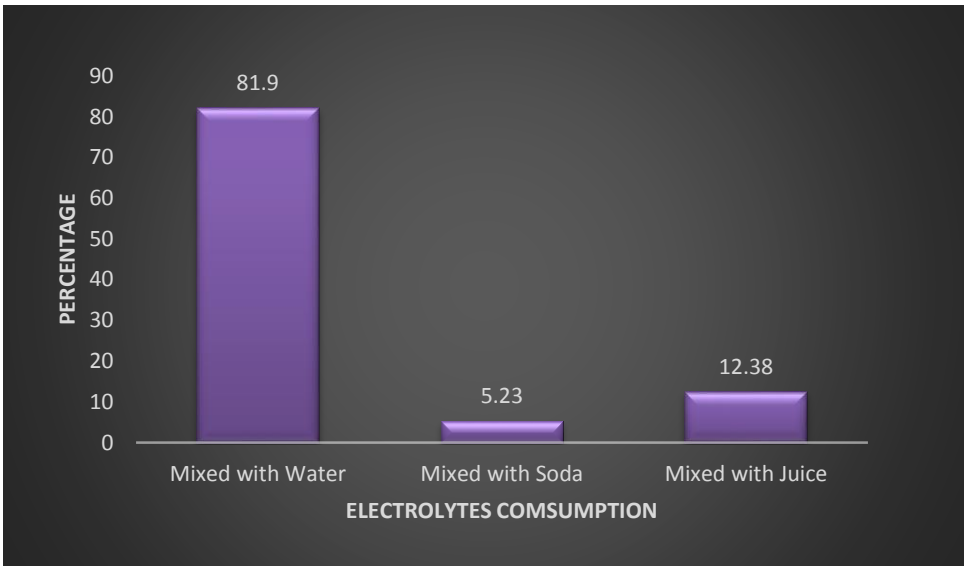
The Table shows that 24 per cent of sports people followed special dietary guidelines to enhance their performance whereas 76 per cent didn't follow any guidelines. Only about 35 per cent of the sports women included dietary supplements. Majority of the subjects did not have any food restrictions.

4.6.7 Form of Electrolyte consumption

Figure - XI presents the form in which electrolytes are consumed by sports people.

FIGURE - XI

Electrolyte consumption of sports people



Most of the sports women mix electrolytes with water (81.9 per cent), while 5.23 per cent with soda and 12.38 per cent with juice respectively. Mahalakhsmi Sangeetha (2012) reported that most of sports person consumed it with water while 2 per cent mixed with soda and the remaining in the powder form.

4.6.8 Special foods during practice

The special foods taken by the sports people apart from regular diet are listed in the Table - XVI.

TABLE - XVI

Special foods during practice

Special foods	Per cent
Pulses	2.38
soya	2.00
Egg	2.00
Nuts	1.42
Ragi	1.00
Sweets	1.00
Electrolytes	1.00
Juice	1.00
Fruits	1.00
Mutton	1.00
Fish	0.50

Protein foods such as pulses, soya, egg, mutton and fish were taken by 7.88 per cent of selected subjects. One per cent preferred ragi probably as it is rich in calcium. A small per cent went for sweets, probably considering that it may energies them. The liquid foods such as electrolytes, juices were preferred by 2 percent. Kumudhini (2011) revealed that 50 per cent sports women consumed extra Dhal, Rice, Roti and vegetable during participation in sports events, While 30 per cent sports women confined to regular diet. As much as 20 per cent took Roti and Vegetable, very few took Rice and Dhal as special foods.

4.6.9 Diet Consumption-the day before the event

Table - XVII gives the diet consumed on the day before Event.

TABLE - XVII

Diet consumption-Before the Event

Special foods	Per cent
Light foods	5.71
Fruits	5.71
Juice	4.76
Banana	2.38
Bread	1.42
Chocolates	1.00
Protein rich foods	0.50
CHO rich foods	0.50
Butter	0.50

It is seen from the table that 11.42 per cent each sports women consumed either light foods or fruits on the day before event, followed by 4.76 per cent of the subjects consumed juice, 2.38 per cent took banana, 1.42 per cent had bread and 1 per cent took chocolates. A small percentage of the selected sports women took foods rich in carbohydrate/protein/fat.

4.6.10 During the Event-Diet consumption

Table - XVIII presents the food type taken during the event.

TABLE - XVIII

During the Event-Diet consumption

Food type	Per cent
Normal foods	33.80
Light foods	21.42
Juice	14.76
Fruits	14.28
Electrolytes	9.04
Chocolates	5.71
Bread	3.33
Water	2.14
Biscuit	1.00
Nuts	0.50

Thirty three per cent of sports women consumed normal food during the event while 21.42 per cent consumed light foods. About 29.04 per cent of the sports women took either fruit juice or fruits. Electrolyte solution was consumed by 9.04 per cent, 5.71 per cent had chocolates and 3.33 per cent took bread. Among the selected subjects, some avoided food and carried themselves only with water. Biscuits and nuts were taken by 1 and 0.5 per cent respectively.

4.6.11 Diet consumption on the day after the Event

Table - XIX gives the diet consumed by sports people after the event.

TABLE - XIX

Diet consumption – After the Event

Food type	Per cent
Normal foods	51.42
Heavy foods	40
Juice	3.80
Light foods	3.33
Fruits	1.42

The Table clearly shows that majority (51.42 per cent) of sports women consumed only normal food on the day after the event. Fourty per cent sports women took heavy foods such as meat, fish and poultry. Juice, fruits and light foods were consumed by 3.80, 3.33 and 1.42 per cent respectively.

4.6.12 Foods Avoided

Table - XX gives the list of Foods Avoided by sports women.

TABLE - XX

Food Avoided

Food list	Per cent
Bitter gourd	2.85
Mutton	2.38
Chicken	2.38
Fatty foods	1.42
Oil foods	1.42
Oil foods	1.42
Brinjal	1.00
Pongal	1.00
Snacks	1.00
Fried foods	0.50
Beans	0.50
Ladies finger	0.50
Beetroot	0.50
Carrot	0.50
Radish	0.50
Sugar Rich foods	0.50
Junk foods	0.50
Onion	0.50
Parota	0.50
Cheese	0.23
Mushrooms	0.23

In the selected subjects, 2.85 per cent of sports peoples avoided bitter gourd, 2.38 per cent avoided mutton and chicken. Fatty and oily foods were avoided by 1.42 per cent. About 1 per cent of sports women avoided pongal, snacks and brinjal. Very few avoided onions, junk foods, parota, fried foods, beans, ladies finger, beetroot, carrot, radish and sugar rich foods. Kumudhini (2011) noted that 55 per cent sports women avoided spicy foods and 43.33 per cent avoided oily foods but here only a meagre per cent kept away from fried foods.

4.7 Psychological test

4.7.1 Stress Level

The mean score regarding various emotional states that contributes to stress level is presented in the Table - XXI.

TABLE - XXI

Stress Level

Emotional states	Mean ± SD
Tension	3.09 ± 1.12
Angry	3.06 ± 1.12
Guilty	3.33 ± 1.32
Annoyed	2.82 ± 1.12
Furious	3.03 ± 1.17
Anxious	3.37 ± 1.15

Total score = 5 (Score card: Not at all = 5; A little = 4; Moderately = 3; Quiet a bit = 2; Extremely = 1).

Subjects were able to have a better control over emotions such as tension (3.09 ± 1.12), angry (3.06 ± 1.12), guilty (3.33 ± 1.32), furious (3.03 ± 1.17) and anxious (3.37 ± 1.15) than compared to annoyed (2.82 ± 1.1) state.

4.7.2 Emotions related to Negative Feel

Table - XXII gives the emotions related to Negative Feel of the sports women.

TABLE - XXII

Emotions related to Negative Feel

Emotions	Mean ± SD
Unhappy	3.45 ± 1.10
Sadness	3.52 ± 1.12
Forgetful	3.23 ± 1.14
Nervous	3.28 ± 1.17
Hopeless	3.46 ± 1.62
Confused	3.21 ± 1.14

Total score = 5 (Score card: Not at all = 5; A little = 4; Moderately = 3; Quiet a bit = 2; Extremely = 1).

The subjects were having a balanced mind as feel they did not sense unhappiness, hopeless or confused often. They neither were absent minded nor nervous. There is evidence to state that endorphins secretion will be higher in physical active individuals. This can modulate the feelings to a positive level.

4.7.3 Feelings pertaining to Positive Attitude

Feelings pertaining to positive attitude of the sports person are listed in the Table - XXIII.

TABLE - XXIII

Positive Attitude Score

Feelings	Mean ± SD
Efficient	3.35 ± 1.14
Alert	3.29 ± 1.20
Cheerful	3.78 ± 1.12
Helpful	3.82 ± 1.24
Relaxed	3.36 ± 1.22
Energetic	3.60 ± 1.12
Friendly	4.04 ± 1.18

Total score = 5 (Score card: Not at all = 1; A little = 2; Moderately = 3; Quiet a bit = 4; Extremely = 5)

The selected individuals were in a positive frame of mind as they scored well during testing their attitudes. This implies that physical activity not only has an impact on physical health but as well as on mental health.

4.8 Bio-chemical investigation

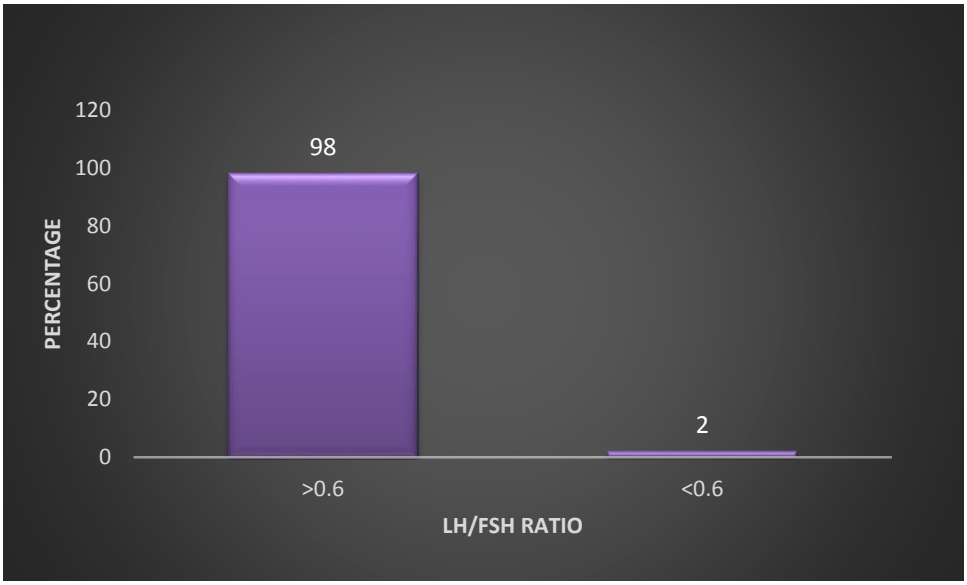
The change in body composition and energy metabolism associated with intense exercise may be responsible for a number of changes in endocrine function (Sari Eldelstein and Judith Shalin, 2010).

4.8.1 Luteinizing Hormone and Follicle Stimulating Hormone Ratio

Figure - XII gives the Luteinizing Hormone and Follicle Stimulating Hormone Ratio.

FIGURE - XII

Luteinizing Hormone and Follicle Stimulating Hormone Ratio



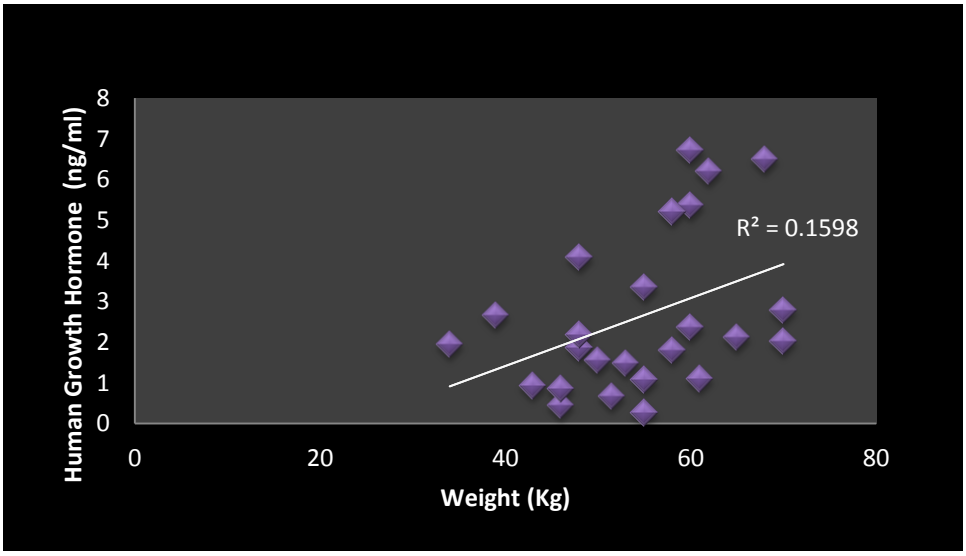
The figure-XII indicates that about 2 per cent of sports women suffer from hypothalamus pituitary insufficiency whereas 98 per cent of subjects are found to have normal pituitary integrity revealed from LH/FSH ratio.

4.8.2 Association between Human Growth Hormone (HGH) and Body weight

Figure - XIII presents the association between Human Growth Hormone and Body weight.

FIGURE - XIII

Association between Human Growth Hormone and Body weight



A positive correlation existed between the body weight and Human Growth Hormone with R = 0.3997, p value = 0.4775. The result was significant at $p<0.05$. Although technically a positive correlation was obtained, the relationships between the variables were weak.

4.8.3 Correlation between Body weight and Thyroid Stimulating Hormone (TSH), Tri iodo thyronine (FT_3), Thyroxine (FT_4)

Table - XXIV gives the correlation between Body weight and Thyroid Stimulating Hormone (TSH), Tri iodo thyronine (FT_3), Thyroxine (FT_4).

TABLE - XXIV

Correlation between Body weight and Thyroid Stimulating Hormone (TSH), Tri iodo thyronine (FT_3), Thyroxine (FT_4)

Hormone	R value	P value
Thyroid Stimulating Hormone (TSH)	0.3103	0.1314
Tri iodo thyronine (FT_3)	0.1516	0.4694
Thyroxine (FT_4)	- 0.1656	0.4305

The Table shows that body weight is positively correlated with Thyroid Stimulating Hormone (TSH) (R = 0.3103, p = 0.1314) and Tri iodo thyronine (FT_3) (R = 0.1516, p = 0.4694), but the difference was not statistically significant at $p < 0.05$. A negative correlation was found between body weight and Thyroxine (FT_4) with the R value = - 0.1656, p = 0.4305.

4.8.4 Relationship between Hormones, Body composition and Menstrual history

Relationship between Hormones, Body composition and Menstrual history is given in the Table-XXV.

TABLE-XXV

Relationship between selected hormones, body composition and menstrual history

Particulars	Follicle stimulating hormone (FSH)	Luteinizing hormone (LH)	Prolactin
Body fat (percent)	-0.31 (0.1295)	-0.14 (0.4974)	0.45 (0.0224)
Body Mass Index	-0.25 (0.2146)	-0.17 (0.4128)	0.44 (0.0274
Weight (kg)	-0.32 (0.1182)	-0.30 (0.1438)	0.39 (0.0537)
Age at Menarche (year)	-0.43 (0.0301)	-0.30 (0.1316)	-0.25 (0.215)
Menses past year (No)	0.32 (0.1111)	0.0991 (0.6373)	-0.0845 (0.6878)

In the relationship, hormone prolactin was significantly correlated with Body fat, Body mass index and Weight: Body fat (R = 0.45, p = 0.0224); Body Mass Index (R = 0.44, p = 0.0274) and weight (R = 0.39, p = 0.0537) respectively. Negative correlation was observed between Follicle stimulating hormones and Body fat (R= - 0.31, p = 0.1295); Body Mass Index (R = - 0.25, p = 0.2146); weight (R= - 0.32, p = 0.1182); Age at (R = - 0.43, p = 0.0301) and Luteinizing Hormone was also negatively correlated with Body fat (R = -0.14, p = 0.4974); Body Mass Index (R = - 0.17, p = 0.4128); weight (R = - 0.30, p = 0.1316); age at menarche (R = - 0.30, p = 0.1316). Menses past year (R = 0.0991, p = 0.6373) was positively correlated with Follicle Stimulating Hormone (R = 0.32, p = 0.1111) and Luteinizing Hormone (R = 0.0991, p = 0.6373) but a negative correlation existed between prolactin and age at menarche (R = - 0.256, p = 0.215); menses past year (No) (R= - 0.08, p = 0.6878). Similarly, Karolina (2011) found that Follicle stimulating hormones level were found to be positively correlated with the

mean skinfold measurement, energy per kg Free Fat Mass and intakes of protein, foliate, magnesium, iron and zinc. Positive correlations were observed between the level of Luteinizing Hormone and the intake of energy and most other nutrients (protein, fat, carbohydrates, vitamin D, magnesium, iron and zinc). A significant relationship was also found between the Luteinizing Hormone/Follicle stimulating Hormone ratio and intakes of energy, protein, carbohydrates and vitamin D. In this study, prolactin correlated well with parameters than other hormones.

5. SUMMARY AND CONCLUSION

Nutrition is an important component of any physical fitness program. The main dietary goal for active individuals is to obtain adequate nutrition to optimize health and fitness or sports performance (Bearing, 2000). This is not only important to help to improve performance but also to promote healthy dietary practices in the long term (Jonnalagadda*et al*, 2001).

Over the last few decades social changes have fostered the development of a positive attitude towards female athletic activities and there has been a dramatic increase in the number of girls and women participating in all levels of sports competitions. For most individuals, this is a positive experience which provides improved physical fitness and better health (Drinkwater *et al*, 1984). Besides many beneficial effects of exercise, female athletes are susceptible to disordered eating behavior, amenorrhea and osteoporosis. The constellation of these three clinical conditions was defined as the Female Athlete Triad by the American College of Sports Medicine (Nattiv*et al*, 1994; Otis *et al*, 1997; Yeager *et al*, 1993). This syndrome is now recognized as a complex set of interrelationships between energy availability, menstrual status and bone mineral density, which may have a variety of clinical manifestations, including eating disorders, functional hypothalamic amenorrhea and osteoporosis (Nattiv*et al*, 2007).

Some suggested factors potentially responsible for female athlete triad include the specific type and amount of high intensity training in young female athletes (especially when begun before puberty), reduced body weight, a lower percentage of fat tissue and psychological stress (Loucks *et al*, 2007; Nattiv *et al*, 2007). The findings indicated that in most of them disorder eating was observed along with menstrual dysfunction but the studies on Triad componentsare limited in Indian athletes. Considering this, the present study **"Comprehensive Evaluation of Nutritional and Health Status of Selected Female Athletes in Coimbatore District and Intervention"** was undertaken.

Broad objective

To determine the prevalence rate and assess the major components of Female Athlete Triad and their relationship.

Specific objectives

- To determine the prevalence rate of Female Athlete Triad.
- To examine the association between eating disorder, nutrition status and menstrual dysfunction.
- To test the psychological dimension of athletes.
- To investigate the correlation between hormones and various parameters.

Coimbatore district was selected for the study because of easy proximity and familiarity. The population for the study comprised of female athletes from different colleges in Coimbatore. The researcher adopted the cross sectional study design. The variables were studied without manipulation or introducing any control group. Only those who expressed willingness to participate in the study were selected after obtaining approval by the Head of the Institution. Four hundred and twenty (N=420) sports women were selected through the purposive random sampling method. The subjects selected for the study fell in the age group between 17-28 years of age and are into various kinds of sports. A self-administered schedule was used to assess the health and nutritional status of sports women. The components included in the schedule for the study compromised of:

- Demographic information
- Anthropometric assessment
- Sports activity
- Disorder eating
- Menstrual history
- Dietary practice
- Psychological test

The purpose of the study and procedures of the investigation was explained after establishing a good rapport with the participants. The self-administered questionnaire was distributed and each question was explained to obtain a reliable data. After filling the schedule by participants, the schedule was scanned and controlled for any error or missing item. If found any, they were asked to fill the unanswered questions.

Based on the data collected, using stratified random sampling, a sub sample was chosen for biochemical investigation. The criteria used for the selection process was based on Body

Mass Index (BMI), use of pathogenic weight control method, self-reported eating disorder and menstrual dysfunction.Blood samples were collected from a peripheral vein in a resting state to determine the endocrine profile using standardized procedures. The hormones evaluated are as follows

- Human Growth Hormone (HGH)
- Follicle Stimulating Hormone (FSH)
- Luteinizing Hormone (LH)
- Prolactin
- Thyroid Stimulating Hormone (TSH)
- Free tri iodo thyroxine (FT_3)
- Free thyroxine (FT_4)

Values were given as Mean±SD. Pearson's correlation coefficient was performed to evaluate relationship between nominal variables and students't' test was used for determining the significant difference.

The results obtained are as follows

Demographical information

- The selected sports people were in the age group of 15-18 (32 per cent), 19-22 (57 per cent) and 23-26 (11 per cent) years.
- Majority were Hindus (91 per cent), followed by Muslim (6 per cent) and Christians (3 per cent).
- Eighty per cent of the sports people were from nuclear family background, while 20 per cent from joint family system.

Anthropometric measurements

Height and Weight

- The mean height of 25 per cent subjects was 140-155 cm, 65 per cent ranged between 155-170 cm and 10 per cent were between170-185 cm respectively.
- About 64 per cent sports women were found to have body weight of about45-60 kg, while 17 per cent between 30-45 kg and 15 per cent between 60-75 kg. Nearly 4 per cent of the subjects were overweight (75-90 kg).

Body Mass Index

- About 66 per cent of the sports women had ideal Body Mass Index.
- Fourteen per cent and 3 per cent were mildly and moderately malnourished, 5 per cent were classified as severely malnourished.
- Based on Body Mass index, 10 per cent were overweight and only 2 per cent were found in obese (class I) category and about 0.5 per cent belonged tograde II and grade III obesity.

Waist and Hip circumference

- The mean waist and hip circumference of athletes was 73 ± 17.07 cm and 86 ± 11.96 cm respectively.

Waist Hip ratio

- Thirty one per cent (132 subjects) of subjects were at higher health risk on the basis of Waist Hip ratio (>0.86).
- Thirty per cent (126 subjects) and 19 per cent (80 subjects) were with lower risk for diabetes and cardio vascular diseases.

Body Fat Percent based on Omron Measurements

- Twenty one per cent sport people had a very high body fat (>30 per cent) placing them in the obese category.
- Eighteen per cent were in acceptable range (22-25 per cent) and only17 per cent were found (19-22 per cent) to have the body fat in ideal range.

Correlation between Body Mass Index and Body Fat

- A positive correlation exists between Body Mass Index (BMI) and Body Fat with R value of 0.5731. The P value <0.00001 indicates significant increase in Body Mass Index with Body fat

Correlation between Age and Body Fat

- The value of R is 0.256, even though a positive correlation was found between Age and Body fat, the relationship between the variable was weak because the obtained R value was closer to 0.1.

Sports profile

Type of sports

- Majority (15.47 per cent) of sports peoples were involved in Athletic event, 11.66 per cent were Group Event players, 10 per cent played Kabaddi, 9.28 per cent involved in Volleyball, 8.80 per cent were Basketball players, 7.85 per cent played Kho-Kho, 3.80 per cent in Badminton, 3.09 per cent players were in Hockey, 2.38 per cent each in Football and Power lifting, 1.90 and 1.66 per cent were playing Handball and Ball badminton. Only a small per cent were in Throw ball (0.95 per cent) and Table tennis (0.71 per cent) respectively.

Number of years in sports

- Sixty seven per cent were engaged in sports for above 5 years, 19.52 per cent involved for 2-3 years, 10.47 per cent for 1-2 years and2.38 per cent have been playing for the past one year only.

Practice session of the selected players

- Seventy two per cent of the sports people were doing practice every day, 14 per cent are involved twice in practice per week and 7 per cent each undertook practice session four times per week and once in a month.

Duration of practice

- Duration of practice increased prior to event participation than regular session.

Eating Disorder questions

- Forty seven per cent were concerned about their weight or body composition, in which 42 per cent are careful about food choices.
- Forty per cent are into losing weight to maintain the appearance, which is important in sports.
- About 34 per cent feel that weight affect the way they feel about themselves, 32 per cent do not have control over how much they eat.
- Nearly 8 per cent use diuretic or laxatives after they eat to avoid weight gain.
- Twenty six per cent suffer from eating disorders and subjects also mentioned that they ate in secret.

Menstrual history

Menstrual Status

- Fifty five per cent had normal menstrual cycle.
- Eighty eight (20.9 per cent) individuals had Amenorrhea or Oligomenorrhea, in which Oligomenorrhea occurred in17.1 per cent of participants(2.38 per cent suffer from Polycystic Ovarian Disease).
- Secondary Amenorrhea was reported in 3.8 per cent (0.47 per cent suffers from Polycystic Ovarian Disease) and there was no case of primary Amenorrhea detected.
- Late onset menarche occurred in 3 per cent (26 subjects) of participants.

Comparison of parameters in Eumenorrhea and Amenorrhea/ Oligomenorrhea group

- The mean weight in the Eumenorrhea group was 53 ± 9.80 and in the Amenorrhea/ Oligomenorrhea group was 55 ± 10.09, which was not statistically significant (t value = 0.9673, p = 0.3345).
- The mean Body Mass Index (BMI) was 20 ± 3.77 in Eumenorrhea group and 22 ± 3.75 in the Amenorrhea/ Oligomenorrhea group with at value = 1.4025, p value = 0.1623, which was also not statistically significant.
- Body fat in the Eumenorrhea group was 25 ± 5.76 (Mean ± SD) and in the Amenorrhea/ Oligomenorrhea group was 25 ± 6.87 (Mean ± SD). No statistical difference was found between the group in body fat (p = 0.9528,t = 0.0593).
- The mean age at menarche in Eumenorrhea group was 13.6 ± 1.17 and in the Amenorrhea/ Oligomenorrhea group being 13.7 ± 1.30, which was not statistically significant (p = 0.3818, t = 0.8764).

Pain experienced during Menstrual Cycle

- A total of 280 individuals reported pain during their menstrual cycle.
- The difference was statistically significant between Eumenorrhea and Amenorrhea/ Oligomenorrhea group with the p value of 0.0150 andt value = 2.4535.

Duration of pain during Menstrual Cycle

- Thirty one per cent had pain lasting for about 1 day, in 16.28 per cent it lasted for 2-3 days. In about 9.04 per cent, duration of pain was from 1-6 hours in the Eumenorrhea group.

- In Amenorrhea / Oligomenorrhea group, 6 per cent of sports women reported pain lasting for 1 day and for remaining it was between 1-6 hours.
- When compared to Eumenorrhea group, less number of subjects experienced pain in Amenorrhea/ Oligomenorrhea group during their cycle.

Feel during Menstrual cycle

- About 8.57 per cent of the subjects from Eumenorrhea group felt anxious whereas it was only 2.38 per cent from Amenorrhea/ Oligomenorrhea group.
- Stress level was higher in Eumenorrhea group (20 per cent) than Amenorrhea/ Oligomenorrhea group (7.61 per cent).
- Twenty three per cent experienced fatigue from Eumenorrhea group and 4.28 per cent from Oligomenorrhea group.
- Fifteen per cent in Eumenorrhea and only 6.66 per cent from Amenorrhea/ Oligomenorrhea group were depressed.
- Among the subjects in Eumenorrhea group, 20 per cent reported body cramps, compared to only 6.66 per cent in Amenorrhea/ Oligomenorrhea group.
- The feelings were not influenced by menstrual cycle in 18.57 per cent and 3.33 per cent of the selected individuals in both the groups respectively.

Awareness regarding Polycystic Ovarian Disease

- A total per cent of 78.09 per cent from both the groups were not aware of the consequences of polycystic ovarian disease.

Type of Diet

- Majority of sports people(81 per cent) were non-vegetarian, while11 per cent of the selected sports people were vegetarian and the rest of the subject (8 per cent) were ova vegetarian.

Dietary Habits

- Twenty per cent of selected sports people skip their meal occasionally, 9.52 per cent always and the rest of subject skip rarely.
- Only 11.42 per cent of sports women took high carbohydrate before the event, while 39.04 per cent followed it occasionally and 49.04 per cent did not load themselves with carbohydrates.

- Nearly 12.85 per cent always restrict their fat intake while 38.09 per cent do it occasionally.
- About 7.61 per cent of the sports people consumed sports drink on regular basis while 55.71 per cent consumed rarely.
- Regarding electrolytes consumption 9.04 per cent of sports person took electrolytes while 50.95 per cent took it rarely.

Skipping of Meals

- Majority (44.76 per cent) of sports person had the habit of skipping their breakfast, 31.9 per cent skip their lunch and 22.28 per cent skip dinner.
- One per cent of sport people in each had the habit of skipping breakfast - lunch and lunch – dinner respectively.

Preference for junk foods

- Twenty seven per cent of the sports people preferred bakery items, 17.14 per cent consumed fried foods and 17.85 per cent subjects liked chat items.
- Thirty eight Per cent of sports women do not prefer junk foods.

Water Intake

- Six - eight glasses of water was consumed by 20 per cent during normal days.
- About 18.33 and 15.23 per cent of the sports people consumed8-10 glasses of water during normal and event days respectively.
- Eleven per cent consumed more than 10 glasses of water per day whereas 12.61 per cent of sports people consumed above 10 glasses during events.

Special Diet considerations of sports women

- Twenty four per cent of sports people followed special dietary guidelines to enhance their performance.
- Only about 35 per cent of the sports women include dietary supplements.
- Nineteen per cent sports people follow food restrictions in every day's menu.

Form of Electrolyte consumption

- Most of the sports women mix electrolytes with water (81.9 per cent), while 5.23 per cent with soda and 12.38 per cent with juice respectively.

Special foods during practice

- Protein foods such as pulses, soya, egg, mutton and fish were taken by 7.88 per cent of selected subjects.
- A small per cent went for sweets, probably considering that it may energies them.
- The liquid foods such as electrolytes, juices were preferred by 2 percent.

Diet Consumption-the day before the event

- Eleven per cent sports women consumed either light foods or fruits on the day before the event, 4.76 per cent of the subjects consumed juice, 2.38 per cent took banana, 1.42 per cent had bread and 1 per cent took chocolates.
- A small percentage of the selected sports women took foods rich in carbohydrate/protein/fat.

During the Event-Diet consumption

- Thirty three per cent of sports women consumed normal food during the event while 21.42 per cent consumed light foods.
- About 29.04 per cent of the sports women took either fruit juice or fruits.
- Biscuits and nuts were taken by 1 and 0.5 per cent respectively.

Diet consumption on the day after the event

- Majority (51.42 per cent) of sports women consumed only normal food on the day after the event.
- Forty per cent sports women took heavy foods such as meat, fish and poultry.
- Juice, fruits and light foods were consumed by 3.80, 3.33 and 1.42 per cent respectively.

Foods Avoided

- Three per cent of sports peoples avoided bitter gourd, 2.38 per cent avoided mutton and chicken.
- Fatty and oily foods were avoided by 1.42 per cent. About 1 per cent of sports women avoided pongal, snacks and brinjal.
- Very few avoided onions, junk foods, parota, fried foods, beans, ladies finger, beetroot, carrot, radish and sugar rich foods.

Psychological test

Stress Level

- Subjects were able to have a better control over emotions such as tension (3.09 ± 1.12), angry (3.06 ± 1.12), guilty (3.33 ± 1.32), furious(3.03 ± 1.17) and anxious (3.37 ± 1.15) than compared to annoyed state.

Emotions related to Negative Feel

- The subjects were having a balanced mind as feel they did not sense unhappiness, hopeless or confused often. They neither were absent minded nor nervous.

Feelings pertaining To Positive Attitude

- The individuals were in a positive frame of mind as they scored well during testing their attitudes.

Bio-chemical investigation

Luteinizing Hormone and Follicle Stimulating Hormone Ratio

- About 2 per cent of sports women suffer from hypothalamus pituitary insufficiency whereas 98 per cent of subjects are found to have normal pituitary integrity revealed from LH/FSH ratio.

Association between Human Growth Hormone (HGH) and Body weight

- A positive correlation existed between the body weight and Human Growth Hormone with R = 0.3997, p value = 0.4775. The result was significant at $p<0.05$.

Correlation between Body weight and Thyroid Stimulating Hormone (TSH), Tri iodothyronine (FT_3), Thyroxine (FT_4)

- Weight is positively correlated with Thyroid StimulatingHormone (TSH) (R = 0.3103, p = 0.1314) and Tri iodothyronine (FT_3) (R = 0.1516, p = 0.4694), but the difference was not statistically significant atp < 0.05.
- A negative correlation was found between body weight and Thyroxine (FT_4) with the R value = - 0.1656, p = 0.4305.

Relationship between Hormones, Body composition and Menstrual history

- Hormone prolactin was significantly correlated with Body fat (R = 0.45, p = 0.0224); Body Mass Index (R = 0.44, p = 0.0274) and weight(R = 0.390, p= 0.05371) respectively.

- Negative correlation was observed between Follicle stimulating hormones and Body fat (R = -0.31, p=0.1295); Body Mass Index(R= -0.25,p=0.2146) and weight (R = -0.32, p= 0.1182) and Age at menarche (R = -0.43, p = 0.0301).
- Luteinizing Hormone was also negatively correlated with Body fat(R=-0.14, p=0.4974); Body Mass Index (R = -0.1713, p = 0.4128); weight(R= -0.30, p= 0.1316); age at menarche (R= -0.30, p= 0.1316).
- Menses past year (R=0.0991, p=0.6373) was positively correlated with Follicle Stimulating Hormone (R= -0.32, p=0.1111) and Luteinizing Hormone (R=0.0991, p=0.6373) but a negative correlation existed between prolactin and age at menarche (R= -0.256, p=0.215); menses past year (No) (R= -0.08, p=0.6878).
- Intervention through education made them to understand the importance of nutrition on their well-being.

CONCLUSION

In conclusion, the prevalence of disordered eating and amenorrhea was 25 per cent and 20.9 per cent respectively. Furthermore, this is the first study to report the prevalence rate of Polycystic Ovarian Disease (PCOD) in sports women in Coimbatore district. Education program helped to increase awareness on consequences of menstrual dysfunction, irregularities and eating disorders.

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APPENDIX – I

Schedule

Nutritional Assessment

Name	-
Age	-
D.O.B	-
Email	-
College	-
Religion	-
Family Type	-

Physical examination

Height	-
Weight	-
BP	-
BMI	-
Percent body fat	-
Waist circumference	-
Hip circumference	-
Waist Hip ratio	-

SPORTS ACTIVITIY

1. Types of sports involved

   a) Athletic event

   b) Group event

   c) Hand ball

   d) kho-kho

   e) Volley ball

   F) Others (mention it)

2. Number of years engaged in sports

   a) 0- 1 year

   b) 1- 2 years

   c) 3-5 years

   d) Above 5 years

3. Practicing session

   a) Everyday

   b) Weekly twice

   c) Weekly four times

   d) Others (mention it)

4. What is the duration of practice?

During Practice	During Events
a) Up to 1 hour	a) Up to 1 hour
b) 1-2 hours	b) 1-2 hours
c) 2-3 hours	c) 2-3 hours

WEIGHT CONSCIOUS

5. Do you worry about your weight or body composition?

   a) Yes b) No

6. Do you limit or carefully control the foods that you eat?

   a) Yes b) No

7. Do you try to lose weight to meet weight or image/appearance requirements in your sport?

   a) Yes b) No

8. Does your weight affect the way you feel about yourself?

   a) Yes b) No

9. Do you worry that you have lost control over how much you eat?

   a) Yes b) No

10. Do you make yourself vomit, use diuretics or laxatives after you eat?

    a) Yes b) No

11. Do you currently or have you ever suffered from an eating disorder?

    a) Yes b) No

12. Do you ever ate in secret?
    a) Yes b) No

MENSTRUAL CYCLE

13. What age was your first menstrual period?

14. Do you have regular menstrual cycles?

    a) Yes b) No

15. Do you experience menstrual irregularities?

    a) Yes b) No

16. What is your cyclic period?

    a) Once in a month
    b) Once in 45 days
    c) Once in 3 months
    d) Others (mention it)

17. Do you undergo pain during menstrual cycle?

    a) Yes b) No
    If yes, what is the duration of pain?

18. How many menstrual cycles have you had in the last year?

19. How do you feel during periods?
    a) Anxiety
    b) Stress
    c) Depressed
    d) Fatigue
    e) Body cramps
    f) None of the above

20. Are you aware of PCOD from any source like Magazines, Education and Medias?

    a) Yes b) No

21. Do you suffer from PCOD (poly cystic ovary disease)?

    a) Yes b) No
    (If yes, mention the pill taken)

DIET

22. Type of diet?

a) Vegetarian b) Non vegetarian c) Ova vegetarian

23. How often you skip your meal?

a) Always
b) Occasionally
c) Rarely

24. Skipping your meal
a) Breakfast
b) Lunch
c) Dinner

25. Preference for junk foods
a) Fried foods
b) Bakery items
c) Chat items
d) Others

26. Carbohydrate loading before event
d) Always
e) Occasionally
f) Rarely

27. Restriction of Fat Intake
a) Always
b) Occasionally
c) Rarely

28. Water Intake Per day
a) 6-8 glasses
b) 8-10 glasses
c) >10 glasses

29. During the event, the amount of water intake
a) 6-8 glasses
b) 8-10 glasses
c) >10 glasses

30. Consumption of Sports Drink
a) Always
b) Occasionally
c) Rarely
d)

31. Electrolyte consumption
    a) Always
    b) Occasionally
    c) Rarely
32. Mode of Consumption of electrolyte
    a) Mixed with water
    b) Mixed with soda
    c) Mixed with juice

33. Do you follow any special dietary guidelines to enhance your performance?

    a) Yes b) No

34. What is the type of diet you consume during the following?

DURING PRACTISE

DIET CONSUMPTION	

DURING THE EVENT

DURING	DIET CONSUMPTION
BEFORE THE EVENT	
DURING THE EVENT	
AFTER THE EVENT	

35. Do you take any dietary supplement (Eg: sports drinking, vitamins, protein, herbal products, etc)

    a) Yes b) No

36. Do you have any restricted foods?

    a) Yes b) No
    If yes (mention it)

PSYCHOLOGICAL TEST

How you have felt Feelings	Not at all	A little	Moderately	Quite a bit	Extremely
Tense					
Angry					
Unhappy					
Clear Headed					
Confused					
Friendly					
Sad					
Active					
Energetic					
Panicky					
Hopeless					
Relaxed					
Fatigued					
Helpful					
Annoyed					
Nervous					
Cheerful					
Anxious					
Alert					
Furious					
Efficient					
Forgetful					
Guilty					

APPENDIX-II

Human Growth Hormone

Principle

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for GH has been pre-coated onto a micro plate. Standards and samples are pipetted into the wells and any GH present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for GH is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of GH bound in the initial step. The color development is stopped and the intensity of the color is measured.

Procedure

Bring all reagents and samples to room temperature before use. It is recommended that all standards, samples, and controls be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess micro plate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 µL of Assay Diluent RD1-57 to each well.
4. Add 50 µL of Standard, control, or sample per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or auto washer. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 200 µL of GH Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.

7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
9. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a micro plate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

Calculation

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.). Create a standard curve by reducing the data using computer software capable of generating a log/log curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on a log/log graph. The data may be linearized by plotting the log of the GH concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis. If the samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

APPENDIX-III

Follicle Stimulating Hormone

Principle

The FSH Quantitative Test Kit is based on the principle of a solid phase enzyme-linked immune sorbent assay. The assay system utilizes a polyclonal anti-FSH antibody for solid phase (micro titer wells) immobilization and a mouse monoclonal anti-FSH antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the antibodies, resulting in FSH molecules being sandwiched between the solid phase and enzyme-linked antibodies. After 60 minute incubation at room temperature, the wells are washed with water to remove unbound labeled antibodies. A solution of TMB is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of 2N HCl, and the color is changed to yellow and measured spectrophotometrically at 450 nm. The concentration of FSH is directly proportional to the color intensity of the test sample.

Procedure

1. Secure the desired number of coated wells in the holder.
2. Dispense 50µl of standard, specimens, and controls into appropriate wells.
3. Dispense 100µl of Enzyme Conjugate Reagent into each well.
4. Thoroughly mix for 30 seconds. It is very important to have completed mixing in this setup.
5. Incubate at room temperature (18-22^{o}C) for 60 minutes.
6. Remove the incubation mixture by flicking plate content into a waste container.
7. Rinse and flick the micro titer wells 5 times with washing buffer.
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 100µl of TMB solution into each well. Gently mix for 5 seconds.
10. Incubate at room temperature in the dark for 20 minutes.
11. Stop the reaction by adding 100µl of Stop Solution to each well.
12. Gently mix for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.

13. Read optical density at 450nm with a micro titer reader within 30 minutes.

Important Note:

The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

Calculation

Calculate the mean absorbance value (A450) for each set of reference standards, specimens, controls and patient samples. Constructed a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in mIU/ml on graph paper, with absorbance values on the vertical or Y axis and concentrations on the horizontal or X axis. Use the mean absorbance values for each specimen to determine the corresponding concentration of FSH in mIU/ ml from the standard curve.

APPENDIX – IV

Luteinizing Hormone

Principle

The LH Quantitative Test Kit is based on a solid phase enzyme-linked immune sorbent assay. The assay system utilizes one anti-LH antibody for solid phase (micro titer wells) immobilization and another mouse monoclonal anti-LH antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the antibodies, resulting in the LH molecules being sandwiched between the solid phase and enzyme-linked antibodies. After 60 minute incubation at room temperature, the wells are washed with water to remove unbound labeled antibodies. A solution of TMB is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of 2N HCl, and the color is changed to yellow and measured spectrophotometrically at 450 nm. The concentration of LH is directly proportional to the color intensity of the test sample.

Procedure

1. Secure the desired number of coated wells in the holder. Make data sheet with sample identification.
2. Dispense 50µl of standard, specimens, and controls into appropriate wells.
3. Dispense 100µl of Enzyme Conjugate Reagent into each well.
4. Thoroughly mix for 30 seconds. It is very important to have completed mixing in this setup.
5. Incubate at room temperature (18-22°C) for 60 minutes.
6. Remove the incubation mixture by flicking plate content into sink.
7. Rinse and flick the micro titer wells 5 times with washing buffer (1X)
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 100µl of TMB solution into each well. Gently mix for 5 seconds.
10. Incubate at room temperature for 20 minutes.
11. Stop the reaction by adding 100µl of Stop Solution to each well.
12. Gently mix for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.

13. Read optical density at 450nm with a micro titer well reader within 30 minutes.

Important Note:

The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

Calculation

Calculate the mean absorbance value (A450) for each set of reference standards, specimens, controls and patient samples. Constructed a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in mIU/ml on graph paper, with absorbance values on the vertical or Y-axis and concentrations on the horizontal or X-axis. Use the mean absorbance values for each specimen to determine the corresponding concentration of LH in mIU/ml from the standard curve.

APPENDIX – V

Prolactin

Principle

The Prolactin Quantitative Test Kit is based on a solid phase enzyme-linked immune sorbent assay. The assay system utilizes one anti-prolactin antibody for solid phase (micro titer wells) immobilization and another mouse monoclonal anti-prolactin antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the antibodies, resulting in the prolactin molecules being sandwiched between the solid phase and enzyme-linked antibodies. After 60 minute incubation at room temperature, the wells are washed with water to remove unbound labeled antibodies. A solution of TMB is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of 2N HCl, and the color is changed to yellow and measured spectrophotometrically at 450 nm. The concentration of prolactin is directly proportional to the color intensity of the test sample.

Procedure

1. Secure the desired number of coated wells in the holder. Make data sheet with sample identification.
2. Dispense 50µl of standard, specimens, and controls into appropriate wells.
3. Dispense 100µl of Enzyme Conjugate Reagent into each well.
4. Thoroughly mix for 10 seconds. It is very important to have completed mixing in this setup.
5. Incubate at room temperature (18-22°C) for 60 minutes.
6. Remove the incubation mixture by flicking plate content into sink.
7. Rinse and flick the microtiter wells 5 times with washing buffer (1X).
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 100µl of TMB solution into each well. Gently mix for 5 seconds.
10. Incubate at room temperature for 20 minutes.
11. Stop the reaction by adding 100 µl of Stop Solution to each well.
12. Gently mix for 5 seconds. It is important to make sure that all the blue color changes to yellow color completely.

13. Read optical density at 450nm with a microtiter well reader.

Important Note:

The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

Calculation

Calculate the mean absorbance value (A450) for each set of reference standards, specimens, controls and patient samples. Constructed a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in ng/ml on graph paper, with absorbance values on the vertical or Y axis and concentrations on the horizontal or X axis. Use the mean absorbance values for each specimen to determine the corresponding concentration of prolactin in ng/ml from the standard curve.

APPENDIX – VI

Thyroid Stimulating Hormone

Principle

The TSH EIA test is based on the principle of a solid phase enzyme-linked immune sorbent assay. The assay system utilizes a unique monoclonal antibody directed against a distinct antigenic determinant on the intact TSH molecule. Mouse monoclonal anti-TSH antibody is used for solid phase (micro titer wells) immobilization and a goat anti-TSH antibody is in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the two antibodies, resulting in the TSH molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a 60 minute incubation at room temperature, the wells are washed with water to remove unbound labeled antibodies. A solution of TMB is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of 2N HCI, and the color is changed to yellow and measured spectrophotometrically at 450 nm. The concentration of TSH is directly proportional to the color intensity of the test sample.

Procedure

1. Secure the desired number of coated wells in the holder.
2. Dispense 50JlI of standards, specimens, and controls into appropriate wells.
3. Dispense 100JlI of Enzyme Conjugate Reagent into each well.
4. Thoroughly mix (or 30 seconds. It is very important to have completed mixing in this step.
5. Incubate at room temperature (18-22°C) for about 60 minutes.
6. Remove the incubation mixture by flicking plate contents into a waste container.
7. Rinse and flick the microtiter wells 5 times with washing buffer (IX).
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 100µl of TMB solution into each well Gentiy mix for 5 seconds.
10. Incubate at room temperature for 20 minutes.

II. Stop the reaction by adding 100µl of Stop Solution to each well.

12. Gently mix for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
13. Read optical density at 450nm with a micro titer well reader.

Important Note:

The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

Calculation

Calculate the mean absorbance value (A_{450}) for each set *of* reference standards, specimens, controls and patient samples. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in µIU/ml on graph paper, with absorbance values on the vertical or Y axis and concentrations on the horizontal or X axis. Use the mean absorbance values for each specimen to determine the corresponding concentration of TSH in µU/ml from the standard curve.

APPENDIX – VII

Free tri iodo Thyronine ($FT_{3)}$

Principle

Competitive Enzyme Immunoassay – Analog Method for Free T_3

The essential reagents required for a solid phase enzyme immunoassay include immobilized T_3 antibody, enzyme-T_3 conjugate and native free T_3 antigen. The enzyme-T_3 conjugate should have no measurable binding to serum proteins especially TBG and albumin. The method achieves this goal. Upon mixing immobilized antibody, enzyme-T_3 conjugate and a serum containing the native free T_3 antigen, a competition reaction results between the native free T_3 and the enzyme-T_3 conjugate for a limited number of insolubulized binding sites. The interaction is illustrated by the followed equation:

$Ab_{c.w.}$ = Monospecific Immobilized Antibody (Constant Quantity)

Ag = Native Free Antigen (Variable Quantity)

^{Enz}Ag = Enzyme-antigen Conjugate (Constant Quantity)

$AgAb_{c.w.}$ = Antigen-Antibody Complex

$^{Enz}Ag\ Ab_{c.w.}$ = Enzyme-antigen Conjugate -Antibody Complex

K_a = Rate Constant of Association

K_{-a} = Rate Constant of Disassociation

$K = K_a / K_{-a}$ = Equilibrium Constant

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is inversely proportional to the native free antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

Procedure

Before proceeding with the assay, bring all reagents, serum, references and controls to room temperature 20 - 27°C

1. Format the micro plate wells for each serum reference, control and patient specimen to be assayed in duplicate. Replaced any unused micro well strips back into the aluminum bag, seal and store at 2 -8°C.

2. Pipette 0.050 ml (50 µL) of the appropriate serum reference, control or specimen into the assigned well.

3. Add 0.100 ml (100µl) of FT_3-enzyme reagent solution to all wells.

4. Swirl the micro plate gently for 20-30 seconds to mix and cover.

5. Incubate 60 minutes at room temperature.

6. Discard the contents of the micro plate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.

7. Add 300µl of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.

8. Add 0.100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section) always add reagents in the same order to minimize reaction time differences between wells.

DO NOT SHAKE PLATE AFTER SUBSTRATE ADDITION

9. Incubate for 15 minutes at room temperature

10. Add 0.050ml (50 µL) of stop solution to each well and gently mix for 15-20 seconds. Always add reagents in the same order to minimize reaction time differences between wells.

11. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a micro plate reader. The results should be read within 30 minutes of adding the stop solution.

Calculation

A dose response curve is used to ascertain the concentration of free tri iodo thyronine in unknown specimens.

1. Record the absorbance obtained from the printout of the micro plate reader.

2. Plot the absorbance for each duplicate serum reference versus the corresponding FT_3 concentration in pg/ml on linear graph paper (do not average the duplicates of the serum references before plotting).

3. Draw the best fit curve through the plotted points.

4. To determine the concentration of FT_3 for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve and read the concentration in pg/ml from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated) In the following example, the average absorbance (1.855) (intersects the standard curve at (2.1pg/ml) FT3 concentration

APPENDIX – VIII

Free Thyroxine (FT_4)

Principle

Competitive enzyme immunoassay - Analog method for free T_4

The essential reagents required for a solid phase enzyme immunoassay include immobilized antibody, enzyme antigen conjugate and native antigen. Upon mixing immobilized antibody, enzyme-antigen conjugate and a serum containing the native free antigen, a competition reaction results between the native free antigen and the enzyme antigen conjugate for a limited number of insolubilized binding sites. The interaction is illustrated by the followed equation:

$Ab_{C.W.}$ = Mono specific Immobilized Antibody (Constant Quantity)

Ag = Native Antigen (Variable Quantity)

^{Enz}Ag = Enzyme-antigen Conjugate (Constant Quantity)

$AgAb_{C.W.}$ = Antigen-Antibody Complex

$^{Enz}Ag\ Ab_{C.W.}$ = Enzyme-antigen Conjugate -Antibody Complex

K_a = Rate Constant of Association

K_{-a} = Rate Constant of Disassociation

K = Ka / K-a = Equilibrium Constant

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is inversely proportional to the native free antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

Procedure

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20-27°C).

1. Format the micro plate wells for each serum reference, control and patient specimen to be assayed in duplicate. Replace any unused micro well strips back into the aluminum bag, seal and store at 2-8°C

2. Pipette 0.050 ml (50µl) of the appropriate serum reference, control or specimen into the assigned well.

3. Add 0.100 ml (100µl) of fT4-Enzyme Reagent to all wells.

4. Swirl the micro plate gently for 20-30 seconds to mix and cover.

5. Incubate 60 minutes at room temperature.

6. Discard the contents of the micro plate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.

7. Add 300µl of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.

8. Add 0.100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section). Always add reagents in the same order to minimize reaction time differences between wells.

DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION

9. Incubate at room temperature for fifteen (15) minutes.

10. Add 0.050ml (50µl) of stop solution to each well and gently mix for 15-20 seconds. Always add reagents in the same order to minimize reaction time differences between wells.

11. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a micro plate reader. The results should be read within thirty (30) minutes of adding the stop solution.

Calculation

A dose response curve is used to ascertain the concentration of free T4 in unknown specimens.

1. Record the absorbance obtained from the printout of the micro plate reader.

2. Plot the absorbance for each duplicate serum reference versus the corresponding Cortisol concentration in µg/dl on linear graph paper (Don't average the duplicates of the serum references before plotting).

3. Connect the points with a best –fit curve.

4. To determine the concentration of cortisol for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in μg/dl) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (1.071) intersects the dose response curve at (1.65ng/dl) Free T4 concentration.

Note 1: Computer data reduction software designed for (ELISA) assays may also be used for the data reduction.

Duplicates of the unknown may be averaged as indicated.

Note 2: DAI can assist the laboratory in the purchase and implementation of equipment/software to measure and interpret (ELISA) data.

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IMPORTANCE OF NUTRIENT INTAKE FOR FEMALE ATHLETES:

- Increased amount of protein - to maintain muscle strength
- Carbohydrate load - for instant energy and to decreased fatigue
- Essential fatty acids - for regular biological functions.
- Water intake - to meet the loss due to exercise
- Water along with electrolytes for homeostasis.
- Iron – supports protein functions and enzymes.
- Calcium – bone health
- Vitamin – D – absorption of calcium

ADD THESE TO YOUR MEAL PLATE

- ✓ Green Leafy Vegetables– Spinach, Agathi, Drumstick Leaves, Amaranth.
- ✓ Lemon, orange, red grapes, gooseberry (Amla), papaya, guava and pomegranate
- ✓ Sesame seeds and flax seeds.
- ✓ Nuts and other seeds.
- ✓ Lean meat cuts
- ✓ Tofu
- ✓ Soy beans
- ✓ Peas
- ✓ Colored fruits and vegetables
- ✓ Mushrooms
- ✓ Fish and fish oils.

Pamphlet for Nutrition Education on

Comprehensive Evaluation of Nutritional and Health Status of Selected Female Athletes in Coimbatore District and Intervention

University Grants Commission

Minor Research Project

HOME SCIENCE

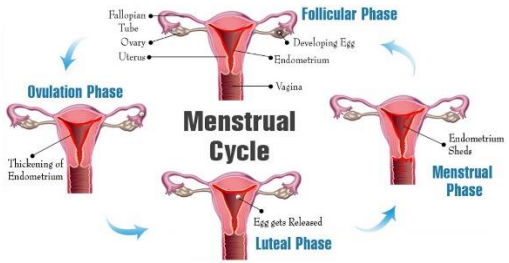
Principal investigator

Dr. L. Uthira
Associate Professor
Department of Nutrition and Dietetics
PSG College of Arts and Science
Coimbatore

PSG COLLEGE OF ARTS AND SCIENCE
An Autonomous College – Affiliated to Bharathiar University
Accredited with A Grade by NAAC (3rd cycle)
College with Potential for Excellence (Status awarded by the UGC)
Star College Status awarded by DBT – MST
An ISO 9001:2008 Certified Institution
Coimbatore – 641014.

MENSTRUAL CYCLE

- Menstrual cycle or mensuration is body's change that occurs for preparing pregnancy.
- Normal menstrual cycle occurs at every 28 days.
- It varies from women to women.
- It is hormonal and ovarian changes, with ovulation, bleeding, periods pain etc…,



MENSTRUAL CONDITIONS:

1) **Eumenorrhea** – it is normal or regualr menstrual cycle
2) **Oligomenorrhea** – it is irregular periods (cycle occuring at a interval of more than 35 days)
3) **Amenorrhea** – it is the absence menstrual cycle.
4) **Polymenorrhea** – menstrual cycle occuring less than 21 days.

Causes for Oligomenorrhea:

- Heavy exercise
- Insufficient food intake
- Lack of nutrients
- Medication
- Anorexia nervosa
- Diabetes & Thyroid disorders

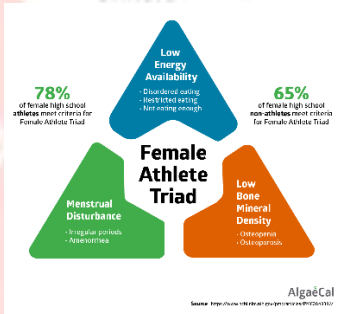
Causes for Amenorrhea:

- Very low body fat (<17%)
- Very high body fat (>40%)
- Excessive exercise
- PCOS(PolycysticOvarian Syndrome)
- Emotional stress
- Menopause

Causes for Polymenorrhea:

- Hyperpituitarism
- Malnutrition
- Tumor in uterus
- STD's (sexually transmitted diseases)
- Injury in uterus.

FEMALE ATHLETE TRIAD



Female athelete traid invloving three components:

- Eating disorders
- Menstrual dysfuntion
- Decreased bone mineral density

Causes:

- Intense workouts (sports / exercise)
- Gymnastics
- Excessive or very less food intake
- Intense weight loss
- Stress fractures
- Self induced vomitting

RELATIONSHIP BETWEEN ESTROGEN AND BONE:

- Estrogen hormone is invloved in menstrual cycle
- It prevents loss of bone mass.
- During menstrual disorders, estrogen level decreases and results in reduced bone mass
- Estrogen levels are indirectly affected by intense exercise and reduced food intake.

COMPLICATIONS OFATHLETE TRIAD:

- Reduced bone mass
- Decreased bone strength
- Osteoporosis
- PCOS
- Infertility ot delayed pregnancy
- Miscarriage
- Oligomenorrhea
- Amenorrhea
- Polymenorrhea