4. DISCUSSION

Survival of an individual depends on the ability to adjust to drastic changes in the environment, and movement constitutes the major adjustment to changes. This is accomplished solely by the co-ordination of skeletal and muscular systems (see Antony and Thibodeu, 1983). Skeletal muscle is the major biochemical transducer that converts chemical energy into mechanical energy (see Rodwell, 1988). Skeletal muscle is greatly equipped with complex biochemical pathways in order to perform its various functions (see Guyton and Hall, 1996).

Skeletal muscles in general can be categorised on the basis of their location, biochemistry and functional ability (see Van de Graaf, 1988). Since skeletal muscles located in various regions perform different functions, it is natural to have variation in their biochemistry to suit their specific functions. Therefore, skeletal muscles from different regions were considered in the present study.

Temporals muscle which originates from the temporal fossa and inserted into coronoid process and medial surface of the ramus of the mandible, was found to have less enzyme activities than other skeletal muscles studied. Since this muscle is involved in the closure of jaw which may not need much metabolic energy (see Hebel and Stromberg, 1976), the activities of enzymes associated with turnover of active metabolites may be less than others involved in active mechanical functions. The observed high activities of various enzymes in masseter muscle may be attributed to its active function of mastication.
Triceps and biceps muscles are from brachial region which help in the extension of the elbow and flexion of the shoulder (see Van de Graaf, 1988). These muscles need constant supply of energy for contraction and relaxation of the forearm. Hence, the enzymes involved in the energy release in the form of ATP, specially TCA cycle enzymes, CPK and myokinase and ATP content are higher than temporalalis muscle, which is less active comparatively.

Gracilis is the muscle from the thigh. Its function is adduction of limb and flexion of the stifle. Vastus lateralis is one of the muscles of stifle joint which in co-ordination with two other muscles helps to extend the stifle and to draw the limb forward. This action needs continuous supply of energy therefore, activities of NAD-LDH, ICDH, SDH, MDH, CPK, myokinase and contents of pyruvate and ATP are high in these muscles (see Hebel and stromberg, 1976).

Gastrocnemius and soleus are the muscles of the tarsal joint (Edington et al., 1973). Hence, the activities of enzymes involved in energy release like CPK, myokinase and TCA cycle and ATP contents etc., are much higher in these muscles. Thus, it is obvious that depending upon the region where the skeletal muscles are located, their metabolic activities vary.

Skeletal muscles undergo adaptive changes in response to increased level of regular activity. A predominant adaptation is the variation in biochemistry of the muscle depending upon the functional activity (Holloszy and Both, 1976; Salmons and Henriksson, 1981). Skeletal muscles can be
biochemically categorised as oxidative, glycolytic and intermediate type (Peter et al., 1972).

The oxidative type of muscle is characterized by its red colour due to the presence of myoglobin pigment and high activity of oxidative metabolic pathways. It is aerobic with prolonged slow contraction rate, low myosin ATPase and low energy utilization (Takekura and Yoshioka, 1988). The glycolytic type of muscle is characterized by its white colour due to the absence of myoglobin pigment and low activity of oxidative metabolic pathways and is anaerobic with fast contraction rate, high myosin ATPase and thereby high energy utilization (Holloszy and Coyle, 1984). The intermediate type is a mixture of both glycolytic and oxidative type of muscles. In mammalian system, the functions are complex and vary from time to time and are not particularly defined as in other animals (see Banks et al., 1979). Therefore, a mixture of oxidative and glycolytic types are in existence.

In the present study also skeletal muscles from different regions of normal control rats showed variations in different parameters. As observed, the soleus and vastus lateralis muscles are of oxidative type and gastrocnemius and gracilis are of glycolytic type and other muscles namely triceps, biceps, masseter showed lower activity of all the enzyme parameters studied and are of intermediate type.

The relativity of oxidative and glycolytic metabolism in skeletal muscles is reflected in lactate and pyruvate (the end products of glycolytic pathway) contents and the corresponding changes in the activity of NAD and NADH
dependent LDH. Oxidative muscles have high pyruvate content and NAD-LDH activity, whereas glycolytic muscles have high lactate content and NADH-LDH enzyme activity. This is reflected in the present study wherein gastrocnemius and gracilis muscles have high lactate and NADH-LDH enzyme, depicting the glycolytic nature of these two muscles; soleus and vastus lateralis muscles have high pyruvate content and activities of NAD-LDH and TCA cycle enzymes ensuring high oxidative nature of these muscles. Other muscles have nearly equal amount of lactate and pyruvate and LDH enzymes suggesting the intermediate nature.

The presence of high SDH enzyme activity is considered as the marker for the oxidative capacity of the muscle (Benzi et al., 1975; Talesara and Narang, 1979). In the present study also, SDH activity was high in soleus and vastus lateralis muscles reflecting their oxidative capacity. Presence of high myosin ATPase activity reflects the glycolytic capacity of the skeletal muscle (Schantz et al., 1982; Roy et al., 1987) and therefore, from the results of the present study gastrocnemius and gracilis muscles are suggested as glycolytic types.

The oxidative and glycolytic capacity of the skeletal muscle actually reflects its functional ability (Desplanches et al., 1987), thereby it is expected that any variation in the functional capacity of the skeletal muscle is likely to alter the oxidative or glycolytic nature of the skeletal muscle. Hence, gracilis, gastrocnemius, soleus and vastus lateralis muscle from the hind limb region require constant supply of energy since both oxidative and glycolytic
metabolism leads ultimately to the release of energy in the form of ATP. Thus, any alteration in the functions of the skeletal muscles will also be reflected in their biochemistry as encountered in the present study as well.

Apart from the enzymes involved in carbohydrate metabolism, certain muscle specific enzymes like CPK, myokinase and energy pack-ATP were also considered in the present study. No sexual dimorphism was reflected in enzymes of carbohydrate metabolism, as no significant difference in the enzyme activities was seen between male and female control rats. However, CPK, myokinase and ATP exhibited a sexual dimorphism. In general, males have larger muscle mass with greater strength than females (Schemmel et al., 1972; Eriksson et al., 1973; Perry et al., 1979). This gender difference may be attributed to variations in the action of sex steroids in skeletal muscles of males and females as shown in the present study. CPK and myokinase enzyme activities and ATP content in skeletal muscles of males were significantly higher than female rats. This gives a possible evidence for the gender difference. Amelink et al. (1990) also have reported higher efflux of creatine kinase enzyme in the soleus muscle of male than in female control rats, which also reflects gender difference in the enzyme activities. The myosin and actin protein contents of skeletal muscle of rats were also found to be more in males than in females (unpublished data). This would also add up to the plausible difference in muscle mass and strength between males and females.
Glycogen metabolism and glycolysis

Muscle is the major biochemical transducer that converts chemical energy into mechanical energy (see Ganong, 1995). ATP, the major chemical energy used during muscle contraction-relaxation activity (see Rodwell, 1988) is generated from various pathways of carbohydrate metabolism (Eisenberg and Hill, 1985). Glycogen is the chief source of reserved energy (Hirsh et al., 1989; Hargreaves et al., 1995) which acts as a readily available source of hexose units for glycolysis in the muscles (see Mayes, 1988).

Glucose transported into skeletal muscle is either stored as glycogen or undergoes glycolysis and be oxidized in the TCA cycle or released as lactate (Gollnick et al., 1972). Once glucose enters the muscle cell, it is phosphorylated to form glucose-6-phosphate and routed into two major pathways, glycogen synthesis or glycolysis. Glycolysis is the unique principal route for glucose metabolism in the skeletal muscle, since it utilizes oxygen (aerobic) when available and also it functions in the absence of oxygen (anaerobic) (see Mayes, 1988). Glycolysis has high metabolic importance since it has the ability to provide ATP in the absence of oxygen (see Stryer, 1995). The principal end products of glycolytic pathway are lactate and pyruvate which decide the shunting of glucose into TCA cycle (Spencer et al., 1991).

The key glycolytic enzymes like HK, PFK, G-3-PDH, PK and LDH (NAD and NADH dependent) are involved in the conversion of glucose into pyruvate (Williamson, 1966). The energy liberated from this pathway is utilized for the contractility of skeletal muscles (see Ganong, 1995). Glycogen synthetase is the
key enzyme involved in glycogen synthesis. Since glycogen is the chief source of reserved energy, the glycogen synthetic pathway is of prime importance. Glycogen phosphorylase is the key enzyme of glycogenolysis which plays an important role in glucose homeostasis (see Guyton and Hall, 1996).

Hormones are the major regulators of metabolic activities in skeletal muscle and sex steroids have an important role in this direction (Diamond et al., 1988; Koenig et al., 1980a,b). Therefore, metabolic activities of any tissue may change with experimental or pathological manipulation of hormones which have influence over its functions (Mainwaring and Mangan, 1973). Identification of specific receptors for testosterone and estradiol in the skeletal muscle of rats (Dube et al., 1976; Kreig, 1976; Snochowski et al., 1980; 1981) suggests that skeletal muscle is one of the targets for sex steroids. Therefore, the specific influence of testosterone and estradiol on skeletal muscle glycogen metabolism and glycolysis has been considered in the present study.

The decreased activities of all glycolytic and glycogenetic enzymes accompanied by increased activity of glycogenolytic enzymes observed in skeletal muscles and hepatic tissues of castrated rats and an opposite effect produced by testosterone supplementation shall suggest their dependency on gonadal hormones. Gonadectomy induced diminution in the activities of glycolytic and glycogenetic enzymes is also reported in uterus (de Asua et al., 1968; Singhal and Valadares, 1970), epididymis (Brooks, 1976), prostate gland (Arunakaran, 1985), cerebral hemisphere (Chainy and Kanungo, 1976), cardiac tissue (Mohamed, 1991) and skeletal muscles (Ramamani et al., 1991) of
rodents and bonnet monkeys. Thus, it is evident that deprivation of gondal hormones not only affects the sex organs but also other organs such as cardiac, skeletal and nervous tissues. On the otherhand, estradiol appears to have no significant impact on glycogenetic or glycolytic activities in skeletal muscles and liver of rats as estradiol treatment to orchidectomized rats did not produce any pronounced influence on enzymes of glycolytic, glycogenetic and glycogenolytic pathways or lactate, pyruvate and glycogen concentrations. A decrease in PK enzyme activity in gastrocnemius muscle due to castration was enhanced by testosterone treatment, whereas treatment of estradiol to castrated rats resulted in a significant decrease (Chainy and Kanungo, 1978). This inconsistency between the present study and that of Chainy and Kanungo (1978) in estradiol effect on male rats may be attributed to the variation in the dose and duration of estradiol treatments.

Only a few reports are available on the involvement of testosterone and estradiol in the regulation of skeletal muscle glucose homeostasis in rats. Decreased glycogen content and glycogen synthetase activity has been reported in the levator ani muscle of castrated rats which increased after testosterone administration (Bergamini et al., 1969). Enhanced glucose uptake in the levator ani muscle and extensor digitorum longus muscles was observed in testosterone treated rats suggesting enhanced glycogen synthesis (Max and Toop, 1983). Similarly Krotkiewski et al., (1980) showed a decrease in LDH enzyme activity in gastrocnemius muscle of orchidectomized rats which was reversed by testosterone. Further, a study on male bonnet monkeys also showed a similar effect wherein castration decreased the activity of all the
glycolytic enzymes of gastrocnemius muscle and was brought to normalcy by testosterone supplementation (Ramamani et al, 1991). Thus, testosterone appears to be the major regulator of carbohydrate metabolism in the skeletal muscles of males and it enhances glucose utilization on one hand and glycogenesis on the other hand to provide an optimum energy supply to meet the energy demand for enhanced skeletal muscular performance.

The physiological or biological actions of a particular hormone vary depending upon the tissue (see Granner, 1988). It is also important to note whether the principal hormone or its active metabolite exerts its effect on a particular tissue. In this respect it is worthwhile to note that testosterone is not metabolized extensively in skeletal muscles except in levator ani muscle (Max and Toop, 1983). Further, it is well established that testosterone is the potent androgen which acts on skeletal muscles due to later’s limited capacity to convert testosterone into 5-α-DHT (Dionne et al., 1979a) and estrogens (Max and Knudsen, 1980). Therefore, it is suggested that the observed effects of testosterone in the present study is mainly due to its direct effect on skeletal muscles rather than its conversion into DHT or estradiol. Results of the present study suggest that testosterone is the predominant sex steroid to act on skeletal muscle glycogen synthesis and glucose utilization in male rats whereas, estradiol is the major regulator in the females.

The observed non-effectiveness of the given dose of estradiol on glycogen synthetic, glycogenolytic or glycolytic pathways in the skeletal muscles of male rats despite normal serum estradiol titre may be explained in two ways:
(i) inadequate concentration of estradiol in muscles of these rats. (ii) very low concentration of estradiol receptors in males. Moreover, it is an accepted fact that local concentration of any hormone present in its target organ, rather than the peripheral titre determines the biological activity (see Wilson and Foster, 1992). Probably, the bioavailability of estradiol in the skeletal muscles of those castrated rats supplemented estradiol is insufficient to evoke any change in glycogen metabolism and glycolysis. Even though specific receptors for estradiol have been identified in skeletal muscles of male rats (Dionne et al., 1979b; Snochowski et al., 1980; Dahlberg, 1982), no information is available on receptor quantity, to the best of investigator’s knowledge. Probably, skeletal muscles of male rats have less number of estradiol receptors with poor sensitivity. Data on this line may enlighten our knowledge in understanding the non-responsiveness in the skeletal muscles of male rats to estradiol.

It is well established that skeletal muscle is one of the target organs for estradiol in female rats (Mooradian et al., 1987) which has specific receptors for estradiol (Dahlberg, 1982; Gruber et al., 1982). Therefore, it is suggested that the observed changes in skeletal muscle glycogen metabolism and glycolysis of female rats treated with estradiol is the result of its direct stimulatory effect. Puah and Bailey (1985) reported increased glucose uptake and glycogen content in estradiol treated ovariectomized mice. Similar effect of estradiol was also reported in extensor digitorum longus muscle of female rats (Ahmed-Sorour and Bailey, 1981) and soleus muscle of female mice (Puah and Bailey, 1985). Therefore, it may be concluded that estradiol modulates skeletal muscle glycogen metabolism in female rats by augmenting glycogen-
esis and glycolysis on one side and inhibiting glycogenolysis on the other side to maintain an optimum amount of glycogen reserve in muscles.

The present study suggests that the effect of testosterone and estradiol on glycogenesis, glycolysis and glycogenolysis in the skeletal muscles is gender specific as their effects are confined to male and female rats, respectively. This gender specific effect may probably be due to the difference in the number or sensitivity of androgen and estrogen receptors in males and females. However, the observed increased activity of glycogen synthetase, LDH (NAD and NADH dependent) activities and glycogen, lactate and pyruvate contents of testosterone supplemented ovariectomized rats shall contradict this suggestion. Thereby, it is evident that testosterone exhibits differential role on different enzyme activities in female rats. An interesting observation is that the effect of estradiol was more pronounced in females when compared to that of males, whereas the effect of testosterone was marked in both the sexes. The data on serum testosterone indicate an elevated level in these ovariectomized rats suggesting that the high dose of testosterone employed is responsible for such significant stimulatory effect of testosterone than estradiol. Increased androgen receptors in the skeletal muscle of ovariectomized rats has also been reported treated with testosterone (Michel and Baulieu, 1980). Therefore, the high dose of testosterone employed, along with enhanced androgen receptors may be correlated to the stimulatory effect of testosterone on TCA cycle and glycogenic enzymes in female rats.
It may be noted that the different skeletal muscles studied have exhibited a similar pattern of response to testosterone or estradiol in both male and female rats except that temporalis muscle of female rats alone did not show any significant change under any experimental condition. This may be due to the absence or insufficient number of specific receptors for either testosterone or estradiol in the temporalis muscle of female rats.

Liver is the chief processing organ wherein all pathways of intermediary metabolism take place and is linked to the energy metabolism in other organs specially the muscles (see Kirchberger and Schwartz, 1990). Further, the major function of hepatic tissue is to provide glucose for skeletal muscles to be utilized as energy substrate for its mechanical activity or functions (Wasserman and Cherrington, 1991).

Glycogen synthesis in the hepatic tissue was also found to be stimulated by testosterone and estradiol in rats (Gustafsson et al., 1983). Decreased hepatic glycogen content and glycogen synthetase enzyme activity has also been reported in castrated rats and testosterone treatment increased the same (Ambadkar and Gangaramani, 1980). Castration induced decrease in glycogenesis, glycolysis and increase in glycogenolysis and their reversal by testosterone treatment were observed not only in the hepatic tissue but also in the skeletal muscle. Whereas, estradiol did not bring about any significant change in the glycogen metabolism of hepatic tissue or in the skeletal muscle of male rats. Thus, glycogenesis and glycolysis in the liver and skeletal muscle
appears to be regulated by testosterone in male rats and testosterone/estradiol in female rats in a similar fashion as that of the skeletal muscles.

In addition to sex steroids, insulin (Del Prato et al., 1985; Shimazu, 1987; Kelly et al., 1990), glucocorticoids (David et al., 1970; Tan and Bonen, 1985; see Mayes, 1988), growth hormone (Ayling et al., 1989; Bak et al., 1991), thyroid hormones (Nwoye et al., 1982; Sugden et al., 1990) and catecholamines (Young et al., 1985; Raz et al., 1991) were found to play an important role in glycogenesis and glucose utilization in the skeletal muscles.

Skeletal muscle is a major site of insulin mediated glucose metabolism. Studies on rats suggest that insulin maintains glucose homeostasis by enhancing glycogen synthesis in skeletal muscles (Fell et al., 1982; Richter et al., 1984) with a concomitant decrease in glycogenolysis by inhibiting glycogen phosphorylase enzyme activity in hepatic tissue and skeletal muscle (Shimazu, 1987). Skeletal muscle glycolysis was also reported to be enhanced by insulin in human (Mandarino et al., 1987). The observed effects of testosterone and estradiol on glycogen synthesis and glucose utilization in skeletal muscles and liver are comparable to the reported effects of insulin. This may point out a possible mediatory effect of insulin on sex steroids induced changes in skeletal muscle glucose metabolism.

However, in the present study neither gonadectomy nor estradiol or testosterone administration caused any significant alteration in serum insulin titre. In this respect, it may be appropriate to note that testosterone/estradiol can influence the binding of insulin with its receptor in the skeletal muscles.
of rat (Holmang and Bjorntorp, 1992). Testosterone treatment to orchidectomized male rats increased the insulin sensitivity by enhancing the binding affinity of insulin to its receptors which was decreased due to castration (Holmang et al., 1990; Holmang and Bjorntorp, 1992). Since serum titres of insulin was not significantly altered by testosterone/estradiol treatment or gonadectomy, it is suggested that the insulin receptor sensitivity in the skeletal muscles might have been modified by testosterone/estradiol. Therefore, it is proposed that testosterone/estradiol have also influenced skeletal muscle glycogen synthesis and glycolysis, by facilitating the binding of insulin with its receptors, apart from their direct effects.

IGF-I has a close structural homology and has similar effects as that of insulin on skeletal muscle (Schoenle et al., 1982). IGF-I increased the rate of glucose transport and glycolysis in the soleus muscle of rats (Dimitriadis et al., 1990; 1992) and glycogen synthesis (Poggi et al., 1979; Dohm et al., 1990). IGF-I also enhanced glycolysis and lactate production in vitro in the soleus muscle of rats (Wegener et al., 1990). Treatment of IGF-I increased the rate of glucose utilization and glycogen synthesis in diaphragm and rectus muscles of rats (Zapf et al., 1986; Jacob et al., 1989).

From the above reports it is very clear that IGF-I mimicks almost all actions of insulin on skeletal muscle (Rinderknecht and Humbel, 1978). Since testosterone and estradiol in the present study exhibited similar effect as that of IGF-I, it may be worthwhile to note if any relationship exists between sex steroids and IGF-I. Since, it is shown that testosterone and estradiol increase
IGF-I level (Copeland et al., 1984; Keenan et al., 1993; Crawford and Handelsman, 1996), it is suggested that testosterone/estradiol enhance glycogenesis and glycolysis in skeletal muscles by enhancing IGF-I also.

The role of glucocorticoids in the regulation of glucose homeostasis is well established (see DeFronzo and Ferrannini, 1995). Glucocorticoids are reported to stimulate glycogenolysis in skeletal muscle and gluconeogenesis in the hepatic tissue of rats (Green et al., 1980). Dexamethasone treatment to normal rats inhibits glycogen synthetase activity and glycogen content and stimulates glycogen phosphorylase activity in gastrocnemius, soleus and plantaris muscles (Coderre et al., 1991). Corticosterone was found to decrease the rate of glycolysis in the soleus and extensor digitorum longus muscles in mice (Tan and Bonen, 1985). Hypercorticosteronism decreased glycolysis in the skeletal muscles of rats in vitro (Leighton et al., 1987). From the above, it is evident that glucocorticoids play an important role in the regulation of glucose homeostasis in the skeletal muscle and liver. The data obtained in the present study reveal that glucocorticoids have an opposite action to that of sex steroids i.e., it decreased glycolysis and enhanced glycogenolysis in skeletal muscles and hepatic tissue which is consistent with previous studies.

It may be of interest to note if any relationship exists between sex steroids and corticosterone. It is evident from the present study that gonadectomy enhanced serum corticosterone titres whereas, testosterone and estradiol treatments brought back the corticosterone titre to that of normals. Previous reports suggest that gonadectomy in male rats increased pituitary
and adrenal weight, adrenal RNA and DNA thereby increasing ACTH and corticosterone synthesis (Kitay, 1963). Whereas, testosterone replacement brought back all the above parameters to normalcy. Similarly in the present study, castration enhanced the serum corticosterone titres which was brought to normalcy on testosterone treatment.

Ovariectomy caused a decrease in ACTH and corticosterone but estradiol replacement increased the same increased by estradiol replacement (Kitay, 1963; Kitay et al., 1965; Coyne and Kitay, 1969). In the present study an opposite trend was observed as ovariectomy increased corticosterone titres and estradiol restored it to normalcy. This may be due to alteration in corticosterone binding globulin. It was reported previously that high levels of corticosterone in ovariectomised rats treated with estradiol was also followed by increased circulatory levels of corticosterone binding globulin, thus reducing free, bioactive corticosterone (Gala and Westphal, 1965). Similar effect might have also been prevailed in the present study. Moreover, in the earlier studies the experimental design varies from that of the present study as they used Shermain strain rats and ovariectomy was performed at 30 days of age, and 6 weeks later a single injection of polyestradiol phosphate at a dose of 2mg/100g body weight was administered and 14 days after the animals were killed to do the assays (Kitay, 1963; Kitay et al., 1965). Whereas, in the present study ovariectomy was carried out in adult Wistar strain rats (90-100 days of age) and 30 days after ovariectomy the injection of 17-β estradiol was given at a dose of 5μg/100g body weight/day for 30 days. These differences in the animal strain, age, experimental duration and dose of hormone
administered might be the reason for the difference in the findings of the present study with that of others on serum corticosterone.

From the present study it is evident that testosterone and estradiol have opposite effects to that of corticosterone. Corticosterone was shown to decrease glycolysis and enhance glycogenolysis in skeletal muscle (Green et al., 1980; Tan and Bonen, 1985; Leighton et al., 1987). In the present study also castration enhanced serum corticosterone titres and decreased glycolysis and increased glycogenolysis in skeletal muscle and hepatic tissues and increased plasma glucose. Treatment of castrates with testosterone/estradiol reversed the same. Therefore, it is suggested that testosterone and estradiol modify muscle glycogenesis and glycolysis by interfering with the action of corticosterone also.

Growth hormone also is an important regulator of glucose homeostasis. Ayling et al. (1989) reported a decrease in LDH enzyme activity in rat soleus and extensor digitorum longus muscles in hypophysectomized rats which was reversed by growth hormone administration. Apart from this, growth hormone inhibits insulin-mediated activation of glycogen synthetase and glucose oxidation in the vastus lateralis muscle biopsy from normal human (Bak et al., 1991). Thus, it is seen that growth hormone enhances glycolysis and inhibits glycogenesis.

It may also be suggested that the effects of gonadal steroids on skeletal muscle metabolic activities may also be a result of altered growth hormone synthesis and secretion since growth hormone plays a pivotal role in enhancing skeletal muscle growth and metabolic activities. Testosterone treatment to
normal rats increased circulating growth hormone titres (Somana et al., 1978). Estrogens also stimulate growth hormone synthesis in female baboons (Copeland et al., 1984). Though, the growth hormone titre was not measured in the present study the available information suggests a possible involvement of sex steroids in the regulation of growth hormone synthesis. Further investigation on this line would enlighten this issue.

Thyroid hormones also stimulate the metabolic activities in skeletal muscles (Hasselgren et al., 1984; Schmidt et al., 1992). Sugden et al., (1990) reported increased glucose utilization and decreased glycogen content in the skeletal muscle of experimental hyperthyroid rats. Hyperthyroidism increased the rate of glycolysis and decreased glycogen synthesis in isolated rat soleus muscle in vitro (Leighton et al., 1990). Hypothyroidism decreased LDH and thereby lactate production in fast twitch skeletal muscle (extensor digitorum longus) and soleus muscle whereas, hyperthyroidism reversed the same (Nwoye et al., 1982).

Thus, from the above reports it is evident that thyroid hormones enhance glycolysis and glycogenolysis to provide more energy for skeletal muscular activity. It appears that thyroid hormones act similar to that of testosterone/estradiol in enhancing glycolysis but opposite in enhancing glycogenolysis in skeletal muscles and liver. The data on thyroid hormones reveal an unaltered status, suggesting that the effect of sex steroids on skeletal muscle glycolysis may be independent of thyroid hormones. Whether there is
any change in thyroid hormone action under altered gonadal steroid status, is not clear at present.

Catecholamines also play an important role in the regulation of glycogen metabolism in skeletal muscle and to maintain glucose homeostasis (Barker and Saito, 1981). It has been reported that epinephrine enhances glucose utilization and decreases glucose uptake in skeletal muscle, specially by enhancing glycolysis and glycogenolysis (Katz and Bogardus, 1991). Epinephrine was also found to enhance skeletal muscle glycolysis in human by accelerating the provision of substrates of glycolytic pathway (Raz et al., 1991). Epinephrine increased glycogen phosphorylase enzyme activity in the diaphragm and rectus femoris muscles of rats in vivo (Leonard, 1957). Epinephrine was also found to stimulate phosphorylase activity in epitrochlearis and soleus muscles of rats in vitro (Young et al., 1985). Raz et al. (1991) showed that epinephrine inhibits insulin-mediated glycogenesis in human skeletal muscles. It was also reported that glucocorticoids enhance epinephrine induced glycogenolysis in skeletal muscle of rats (Rizza et al., 1982). Therefore, the enhanced glycogenolysis in the skeletal muscles of castrated rats in the present study may also be due to the facilitatory influence of glucocorticoids on epinephrine action as there was a significant increase in corticosterone level in these rats. Castration induced increase in glycogen phosphorylase enzyme and blood glucose may also be due to the stimulatory effect of epinephrine on glycogen phosphorylase.
Testosterone (Kizer et al., 1974) and estradiol (Barden et al., 1982) were also suggested to play an important role in the regulation of epinephrine biosynthesis. Kizer et al. (1974) have reported that castration stepped up the biosynthesis of epinephrine by increasing the synthesis of epinephrine. Epinephrine was not assayed in the present study, but the available information suggests that testosterone/estradiol alter catecholamine status. Thus, it may be suggested that decreased glycolysis in the skeletal muscles of castrated rats of the present study may also be either due to the depletion of testosterone/estradiol or due to increased synthesis of catecholamines in castrated rats.

From the foregoing discussion it is suggested that sex steroids play an important role in the regulation of glycogenesis and glycolysis in the skeletal muscle and hepatic tissue by altering either the status of other hormones or by their direct effect.

**Enzymes of TCA cycle**

TCA cycle consists of a cycle of reactions in the mitochondria that brings about the catabolism of acetyl residues which on oxidation leads to the release of energy (see Ganong, 1995). The major function of citric acid cycle is to act as the common pathway for the oxidation of carbohydrate, lipids and proteins (see Ruderman et al., 1992). TCA cycle is considered to have high metabolic value since it produces large number of ATP molecules by the oxidation of glucose (see Mayes, 1988). The ATP generated by TCA cycle pathway is being
utilized by all tissues of the body. Skeletal muscle also utilizes ATP for enhancing muscle performance (see Guyton and Hall, 1996).

Deprivation of sex steroids in male and female rats, decreased the activity of key enzymes involved in TCA cycle. This suggests that sex steroids play an important role in the regulation of TCA cycle. Further, testosterone treatment to orchidectomized rats enhanced the activities of enzyme involved in TCA cycle in skeletal muscle and hepatic tissue, suggesting the dependency of these tissues on testosterone. It has been reported that testosterone enhances skeletal muscle energy metabolism in rats (Galbo, 1985). Thus, testosterone appears to be the predominant sex steroid to act on the skeletal muscles of male rats because only testosterone could enhance TCA cycle enzymes in males while estradiol did not bring about any profound influence on the same.

The non-responsiveness of the TCA cycle enzymes in males, to estradiol may be due to decrease in number/or sensitivity of estradiol receptors in skeletal muscles. Since, the dose of estradiol administered to castrates maintained normal serum titres, the dose of estradiol employed may not be considered as inadequate. Further, higher doses of estradiol was also found to elicit no significant response on these enzymes (Unpublished data). Therefore, the status of estradiol receptor concentration or receptor sensitivity in the skeletal muscle of male rats appears to be the valid explanation, for the observed blunted action of estradiol which needs confirmative studies.
Deprivation of sex steroids in female rats decreased the TCA cycle enzymes suggesting that sex steroids are essential for maintaining optimum activity of TCA cycle enzymes in skeletal muscles. Estradiol supplementation enhanced the TCA cycle enzymes in the skeletal muscles and hepatic tissue, suggesting the stimulatory role of estradiol in enhancing skeletal muscle TCA cycle enzymes thereby providing energy for enhancing skeletal muscle performance in females.

Unlike estradiol, the given dose of testosterone did not exhibit any sexual dimorphism since it stimulated TCA cycle enzymes in female rats also. However, a low dose of testosterone which maintained serum testosterone titres of ovariectomized rats just above normal female rats did not produce any obvious change in TCA cycle enzymes (Unpublished data). This shall point out that a high concentration of testosterone can evoke increase in TCA cycle enzymes in female rats. Probably, more of testosterone is converted into estradiol at the level of skeletal muscles. But the serum estradiol levels of ovariectomized rats treated with testosterone did not show any appreciable change. Therefore, it is suggested testosterone along with estradiol appears to have stimulatory effect on TCA cycle enzymes in the skeletal muscle of female rats provide extra energy to increased skeletal muscular activity. While the effect of estradiol is confined to females, testosterone in the given dose can influence the enzymes in females.
Muscle specific enzymes

CPK

CPK which catalyzes the phosphorylation of creatine to creatine phosphate (see Rodwell, 1988) is an important muscle specific enzyme with clinical utility in the detection of acute or chronic muscle disorders (see Ganong, 1995). CPK enzyme in the skeletal muscle is associated with energy production and muscle contraction (see Rodwell, 1988). CPK phosphorylates creatine at times when muscle is relaxed and also dephosphorylates creatine phosphate at times of need i.e., when the muscle is active (see Smith et al., 1988). Thus, CPK helps in the provision of energy for skeletal muscle contraction-relaxation cycle. CPK is bound to the sarcoplasmic reticulum intimately associated with myosin filaments. Creatine phosphate reacts with ADP by the action of CPK to form ATP and creatine. This ATP is used for ion transport and muscle contraction (Rubin et al., 1992).

Decreased CPK activity in gonadectomized rats of the present study indicates the existence of a definite relationship between gonadal hormones and CPK activity in skeletal muscle. Testosterone supplementation to castrated rats, increased CPK activity in all skeletal muscles studied. Thus, it is suggested that testosterone regulates CPK enzyme activity in the skeletal muscle of male rats. However, estradiol treatment to castrated male rats did not produce any profound alteration in the enzyme activity in any of the skeletal muscle studied, despite normal titre of serum estradiol. Even a higher dose of estradiol could not alter CPK enzyme activity in castrates (unpublished...
data). This again confirms the fact that the sensitivity of skeletal muscles of males to estradiol is low compared to testosterone.

As in the case of TCA cycle enzymes, testosterone treatment was able to induce CPK enzyme activity in both male and female rats and its effect on females was more pronounced than estradiol. This shall suggest that testosterone may be one of the skeletal muscle regulatory hormones involved in the generation of ATP, irrespective of sex. Whereas, the effect of estradiol exhibited sex dependency as it could enhance CPK activity only in females, without any effect in males.

Marchetti et al. (1984) reported that estrogen increases the CPK enzyme activity in the rat uterine tissue to provide energy for the extensive biochemical changes which enhanced cell growth. Similar influence of estradiol in female rats is also seen in the skeletal muscle CPK activity, in the present study. Similar to the skeletal muscles of the present study, testosterone augmented cardiac tissue CPK activity in gonadectomized male and female bonnet monkeys, whereas estradiol had stimulatory effect in females and selectively stimulated auricular enzyme activity in males (Mohamed, 1991). Therefore, it is suggested that in male rats in general, the sensitivity of skeletal muscle to estradiol is low, though cardiac tissue was able to respond to estradiol in male bonnet monkeys.

Estradiol treatment to ovariectomized rats caused the efflux of creatine kinase enzyme in the soleus muscle of females but not in the soleus muscle of male rats treated with estradiol (Amelink et al., 1990). In the present study
also estradiol stimulated CPK enzyme activity in the skeletal muscle of female rats only. This may suggest that estradiol does not play a significant role in the regulation of CPK enzyme activity in males, whereas in females it is stimulatory. Therefore, it is suggested that estradiol exhibits sexual dimorphism in the skeletal muscle energy metabolism.

It is obvious from the present study that testosterone is the effective sex steroid in regulating CPK activity than estradiol in male rats whereas in females, both testosterone and estradiol are effective. Thus, it is interesting to note that testosterone specifically stimulated CPK enzyme activity in all the skeletal muscles studied.

It has long been reported that male and female athletes take anabolic steroids to enhance their performance (Catlin and Hatton, 1990). In this context it may be suggested that testosterone acts as the predominant sex steroid which enhances skeletal muscle activity in both males and females by augmenting the supply of metabolic energy through enhanced CPK enzyme activity.

It has been reported that there is larger efflux of CPK enzyme in the skeletal muscles of males after exercise than that of females (Shumate et al., 1979; Rogers et al., 1985; Driessen-Kletter et al., 1987; Noakes, 1987). In mouse, increased contractile activity was accompanied by a marked increase in CPK enzyme efflux in soleus and extensor digitorum longus muscles in vitro (Jones et al., 1983; Takagi et al., 1984). It was further reported that exercise induced an increase in creatine kinase release in rat skeletal muscle (Amelink
and Bar, 1986; Amelink et al., 1988; Bar et al., 1988). From the above reports it is evident that increased muscular activity leads to an increased energy expenditure. To compensate the energy expenditure, more of CPK enzyme is released so as to provide energy for working muscles. Thus, it is suggested that CPK is an important enzyme which provides energy for enhancing skeletal muscular activity and testosterone/estradiol play an important role in the regulation of CPK enzyme activity.

**Myokinase**

Myokinase is a muscle specific enzyme which catalyzes the formation of one ATP molecule and one AMP molecule from two ADP molecules. This reaction is coupled with the hydrolysis of ATP by myosin ATPase during muscle contraction (Hellsten-Westing et al., 1993). High rate of ATP utilization during high intensity exercise demands a rapid rate of rephosphorylation that may exceed the maximum capacity of the muscle. Under these conditions the ATP/ADP ratio is kept high through myokinase reaction that involves the generation of ATP from two ADP molecules (Thorstensson et al., 1975; Nevill et al., 1989; Tullson and Terjung, 1991). Myokinase is one of the source from which skeletal muscle derives energy for enhancing contractile activity. As in the case of CPK enzyme, gonadectomy decreased the enzyme activity in all the skeletal muscles studied and treatment of testosterone to gonadectomized rats stimulated the enzyme activity. This shall suggest that androgens are the major regulators of the myokinase enzyme activity in the skeletal muscle.
Estradiol administration to orchidectomized rats brought about no profound alteration in the activity of myokinase in any of the skeletal muscle studied. This may be due to decreased receptor availability or decreased receptor sensitivity as discussed earlier. From the serum levels it is evident that normal physiological level of estradiol is maintained in orchidectomized rats given estradiol at the given dose. Probably, further investigation on higher doses of estradiol would give evidence for the dose-dependent response of skeletal muscle to estradiol in male rats. In females, both testosterone and estradiol were found to be stimulatory but testosterone predominated the effect of estradiol.

Adequate information is not available on the influence of hormones on myokinase enzyme activity. Future indepth studies may be required to explain the specific effect of hormones in the regulation of myokinase enzyme activity. Nevertheless, the present study clearly shows a stimulatory effect of sex steroids, and androgens are suggested as the major stimulant of skeletal muscle myokinase activity. Probably, this is one of the mechanisms by which androgens bring about their anabolic effect on skeletal muscles. Estradiol appears to have a sexual dimorphism in its effect as it could not evoke any change in the enzyme activity in males.

**Myosin ATPase**

ATPase is an important enzyme which catalyzes the hydrolysis of terminal phosphate bond of ATP which releases large amount of energy for enhancing the activity in any cell (see Rodwell, 1988). Myosin ATPase is the
muscle specific enzyme wherein the myosin protein of muscle itself possess the characteristics of ATPase enzyme (see Stryer, 1995). Myosin ATPase is categorised into three forms, viz. $\text{Na}^+\text{-K}^+$, $\text{Ca}^{2+}$ and $\text{Mg}^{2+}$ dependent.

All these myosin ATPases play a key regulatory role in the contraction-relaxation cycle of skeletal muscles (Clausen et al., 1987). Therefore, myosin ATPase was taken into consideration in the present study.

Deprivation of gonadal steroids due to gonadectomy caused a decrease in all the myosin ATPases in both male and female rats which shows the influence of sex steroids in the regulation of myosin ATPase enzyme activities. Testosterone replacement enhanced and estradiol replacement did not show any significant alteration in the enzyme activities in males. It was also reported from our laboratory that testosterone enhances ATPases activity in the skeletal muscle in castrated male bonnet monkeys (Ramamani, 1989). Thus, it is suggested that testosterone activates skeletal muscle myosin ATPases for enhancing muscle performance.

In females, both testosterone and estradiol were found to stimulate all the myosin ATPases. Testosterone does not exhibit any sexual dimorphism whereas estradiol does so. This shows that testosterone is also a regulating factor for enhancing skeletal muscle energy metabolism in female rats since almost all the enzymatic pathways involved in energy release were found to be stimulated by testosterone apart from estradiol in female rats. It is also suggested that estradiol in the given dose may not have any significant role in
the skeletal muscles of males. Probably, an increase in the dose of estradiol will be effective which need to be investigated.

Skeletal muscle myosin ATPase enzyme activity as well as the number of Na\(^+\)-K\(^+\) pumps are regulated by wide range of hormonal factors (Clausen, 1996). One of the major hormonal factors determining the concentration of Na\(^+\)-K\(^+\) ATPase in skeletal muscle is the thyroid hormone (Clausen and Everts, 1989). It is reported that during high-frequency stimulation, skeletal muscles make use of maximum number of Na\(^+\)-K\(^+\) pumps which explains that contractile activity is an important drive for the synthesis of Na\(^+\)-K\(^+\) pumps and activation of Na\(^+\)-K\(^+\)ATPase, and this is enhanced by thyroid hormones (Clausen, 1996).

Hypothyroidism induced decrease in Na\(^+\)-K\(^+\)ATPase in skeletal muscle of mouse, guinea pig and human was reversed in hyperthyroid patients (Clausen, 1986; Clausen and Nielson, 1994). Studies on rat soleus muscle suggested that hypothyroidism decreases both Ca\(^{2+}\) and Mg\(^{2+}\) dependent ATPases and hyperthyroidism increases the same (Nwoye et al., 1982).

From the above reports, it is evident that thyroid hormones regulate ATPases in the skeletal muscles. In the present study there was no obvious change in thyroid hormone status. The impact of sex steroids on thyroid receptors, if any is not known. Similarly, testosterone and estradiol were found to enhance skeletal muscle ATPases in the present investigation. This indicates that testosterone/estradiol action on skeletal muscle ATPases is similar to the effect of thyroid hormones. Thus, it is suggested that
testosterone/estradiol synergise the action of thyroid hormones, apart from their direct effect.

**ATP**

ATP is the energy pack which is required by each and every cell at all times of work (Hellsten-Westing et al., 1993). ATP is also required constantly for contraction-relaxation cycle of skeletal muscle. ATP in the skeletal muscle is generated by glycolysis, oxidative phosphorylation, creatine phosphate or by the catalyzing action of myokinase (Karlsson and Saltin, 1970). With these facts in mind ATP content in the skeletal muscles from different regions were taken into consideration in the present study.

Gonadectomy induced decrease in ATP contents in the skeletal muscles reveals that gonadal hormones play an important role in the regulation of ATP formation or utilization. In males, testosterone treatment to orchidectomized rats induced a significant increase in ATP contents whereas estradiol did not produce any significant change. This may suggest the dependency in the skeletal muscle ATP synthesis of male rats, on testosterone. As discussed earlier, the ineffectiveness of estradiol in male rats may be due to decreased number of estradiol receptors or decreased sensitivity of receptors in the skeletal muscles of male rats, as even a high dose of estradiol was ineffective (unpublished data).

In females, both testosterone and estradiol caused a significant rise in ATP content but the effect of testosterone was more pronounced than estradiol
in females. This may indicate that testosterone specifically influences ATP production in various skeletal muscles studied in a dose-dependent manner. The data on CPK and myokinase also correlate with this predominant effect of testosterone in muscle ATP synthesis. Skeletal muscle is a dynamic tissue which requires continuous supply of energy and it is supplied by ATP. Thus, constant provision of ATP is important to enhance skeletal muscle energy metabolism. Hence, it is suggested that testosterone is an important factor which stimulates ATP synthesis in the skeletal muscles of female rats. The stimulatory effect of testosterone on muscle CPK, myokinase and ATP in females was specifically higher than estradiol. Since the dominant effect of testosterone over estradiol is confined to these specific parameters, it is suggested that these parameters are most sensitive to testosterone than estradiol in female rats.

Exhaustive exercise in human was reported to decrease ATP levels in the skeletal muscle (Cheetham et al., 1986; McCartney et al., 1986). It was also reported that increased skeletal muscle activity led to a decrease in ATP content due to increased energy expenditure (Sahlin et al., 1978; Greenhaff et al., 1992). Therefore, it is suggested that increased skeletal muscle activity leads to increased energy utilization. In order to compensate for the energy utilized, continuous supply of energy in the form of ATP must be available in the skeletal muscle. It is evident from the present study that testosterone enhances ATP synthesis in the skeletal muscle. Thus, testosterone is suggested as the major sex steroid which plays an important role in the provision of ATP for enhancing skeletal muscle activity.
In general, the present study suggests that sex steroids have a stimulatory effect on skeletal muscle energy metabolism. Testosterone in males and estradiol in females stimulate glucose utilization by skeletal muscles by augmenting glycolytic enzymes.

Though a similar trend exists in the regulation of TCA cycle and muscle specific enzymes involved in ATP production and glycogen synthesis, testosterone in the given dose appears to have a potent stimulating effect than estradiol in females.

Estradiol in the given dose has no appreciable effect on skeletal muscle energy metabolism in males and was effective only in females.

From the data obtained in the present study it may be derived that testosterone is a major stimulant of skeletal muscle energy metabolism in males and both testosterone and estradiol are effective in the skeletal muscle energy metabolism in females.

In the light of available information on the hormonal control of skeletal muscle energy metabolism it is suggested that sex steroids may either act directly through their specific receptors or indirectly by modifying the actions of other hormones involved in this process. Further studies on the interaction of sex steroid and other hormone regulators of skeletal muscle metabolism and in vitro studies on isolated skeletal muscle cells with sex steroids and other hormones which basically regulate skeletal muscle energy metabolism may enlighten the problem further.