458

Lipid Composition Of Adipose Tissue

In

Human Progressive Muscular Dystrophy

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TABLE OF CONTENTS

C E R T I F I C A T E

The research work embodied in this thesis has been carried out in the School of Life Sciences, Jawaharlal Nehru University, New Delhi. This work is original and has not been submitted so far, in part or in full, for any other Degree or Diploma of any University.

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INTRODUCTION

It has been generally regarded that "Muscular dystrophy" is a genetically determined primary degenerative myopathy (Walton, 1964). The concept of exclusive involvement of muscle in muscular dystrophy has been seriously challenged (Harris, 1971; Dubowitz, 1971; Bradley, 1971; Rowland, 1974). Lately, theories have evolved implicating a primary defect in systems other than the skeletal muscle fibre i.e. primary neurogenic involvement, vascular involvement, alteration in biogenic amine metabolism, defect in connective tissue development and generalised membrane defect.

The histological changes in dystrophic muscle, such as, variation in fibre size, fibre splitting and internal nucleation of muscle fibres have been regarded as secondary effect due to repeated cycles of necrosis and regeneration. Accumulation of collagenous tissue has been also regarded in the same way (Cullen et al., 1980). The endomysial connective tissue becomes prominent in relatively early stages of the disease i.e., before the apparent onset of muscle degeneration (Bell, 1968). An aberration i.e., excessive production of collagen (Bourne, 1959; Thompson et al., 1977) or, formation of an immunochemically abnormal form of collagen around muscle fibres (Duance et al., 1980) has been reported in muscular dystrophy. Sweeny et al. (1981), suggested that failure in normal development of connective tissue in muscular dystrophy leads to functional ischemia which affects differentiation of

various cellular components of muscle (myofibres, vessels and nerves).

Pennington et al. (1966), observed the fatty acid composition of triacylglycerols from dystrophic human muscle to be similar to that of normal adipose tissue. According to them infiltration of fat cells in dystrophic muscle is contributed from adipose tissue. Later studies have also shown that the abnormal lipid composition of dystrophic muscle may be due to contamination of muscle cells with fat and connective tissue (Takagi et al., 1973; Pearce et al., 1981). In dystrophic mice, the free fatty acid content of pectoral and abdominal muscles, heart and liver shows an increase while, it is decreased in adipose tissue (Susheela et al., 1968). Seitz et al.(1969), have reported the importance of interistitial adipose tissue in muscle for lipid metabolism.

In vitro studies (Goyle et al., 1973), in subcutaneous fat and fascia from muscular dystrophy patients have shown poor growth. The Lipocytes show an intense reaction for glucose 6-phosphate dehydrogenase. The distribution pattern of triacylglycerol and phospholipid granules was same in fat and fascia cultures from normal and dystrophic patients except that the lipocytes from dystrophic cultures showed a less intensely stained granular triacylglycerol content. Adenylate cyclase activity was detected in certain parts of the plasma membrane of normal adipose tissue but it was absent in dystrophic mice, suggesting the abnormality in plasma membrane of dystrophic adipose tissue of mice (Susheela et al., 1973).

Vallynathan (1976), studied lipogenesis in muscle, liver and adipose tissue of normal and dystrophic chickens to find out whether the characteristic accumulation of lipids in dystrophic chicken muscle is a result of the altered metabolism of muscle itself or increased lipid synthesis elsewhere. On the basis of his experimental findings he suggested that the source of lipid which gets accumulated in dystrophic muscle is of hepatic origin. He reported that adipose tissue contributes little to overall increase of lipids in dystrophic muscle except, perhaps in advanced stages of dystrophy.

Barakat et al. (1977), reported that the rate of incorporation of ^{14}C glycerol-3-phosphate into glycerides of heart, muscle and adipose tissue is depressed in cardiomyopathic hamsters. Subsequently (1978), they observed that fatty acid synthesis is also depressed in liver and adipose tissue of hamsters due to decrease in the activity of several enzymes involved in lipogenesis. A slight increase in total phospholipid, sphingomyelin, phosphatidylserine content and a slight decrease in phosphatidylcholine, phosphatidylethanolamine content of adipose tissue microsomes in dystrophic hamster has been observed (Barakat et al., 1980). The size (wet weight, total protein, total cytochrome oxidase) of intrascapular adipose tissue has been reported to be reduced to about one half of normal in Cardiomyopathic hamsters (Himms-Hagen et al., 1980). Glucose transport and oxidation in adipose tissue of patients with myotonic dystrophy has been reported to be impaired (Mably et al., 1981).

Changes in lipid composition in the subcutaneous adipose tissue have been reported in two cases of Duchenne muscular dystrophy (Banerjee et al., 1983). The present work is an attempt to study the lipid composition of subcutaneous adipose tissue from various types of human progressive muscular dystrophies and neurogenic atrophy in light of the contemporary work on various other tissues i.e., muscle (Takagi et al., 1968; Kunze et al., 1970; Hughes, 1972; Hughes, 1973; Kunze et al., 1975), erythrocytes Kunze et al., 1973; Kalofoutis et al., 1977; Howland et al., 1977; Ruitenbeek et al., 1978), neural tissues (Kwok et al., 1978) and fibroblasts (Rounds et al., 1980), to elucidate the opinion with regard to aetiology of muscular dystrophy.

REVIEW OF LITERATURE

"Muscular dystrophy" is a hereditary disease characterized by progressive atrophy (wasting away) of muscles and increasing weakness. The term "Muscular dystrophy" was first used by Erb (1981), in historical context and is now applied by convention to a number of different muscle disorders. They have in common their hereditary nature, primary involvement of the voluntary muscles and tendency to progressive deterioration because of gradual and piecemeal muscle necrosis (Gardner-Medwin, 1980). The primary defect according to Rowland (1979b), lies in an abnormal gene which leads to deletion of a polypeptide chain or to a polypeptide chain so altered in amino acid composition that its function is deficient. The simultaneous de novo appearance of Duchenne mutation and X,1 rearrangement suggests possible site for Duchenne locus on the X chromosome short arm, (xp 21) (Lindenbaum et al., 1979; Greenstein et al., 1980; Jacobs et al., 1981). The exact pathogenesis of various types of muscular dystrophy still remains elusive. Different theories have been proposed to explain the pathogenesis of muscular dystrophy.

(a) Vascular Theory:

This theory suggests a defect in the microcirculation and biogenic amine metabolism of dystrophic muscle. Kure et al., (1930), were probably the first to suggest that the degenerative changes in dystrophic muscle were due to inadequate blood flow within muscle. Subsequently, Stern et al., (1956), reported

increased urinary excretion of epinephrine and nor epinephrine in DMD patients. Demos (1961), found that the circulation time from arm to tongue was slow in patients and carriers of DMD. Misra et al, (1965), reported increased concentration of serotonin in cerbrospinal fluid of DMD patients. However, observations of Stern et al. (1956) and Misra et al. (1965), have been disputed (Mendell et al. 1972a). Platelets from patients with Duchenne dystrophy but not from those with other neuromuscular disorders have been reported to show decreased rate of uptake of serotonin (Murphy et al.,1973).

Demos (1973), reported deficient oxidation of catecholamines by platelets specifically in DMD, implying abnormal control of small vessels. This observation was contradicted by Pacold (1975), who found no difference in platelet dihydroxyphemylalanine (DOPA) oxidase in normal and DMD boys or carrier mothers. Fluorescent histochemical technique showed lime green fluorescene suggesting abnormal catecholamines or related substances in groups of muscle fibres in DMD, but not in other neuromuscular disorders (Wright et al., 1973). These histological and histochemical observations can be reproduced in rats by injecting pargyline, a monoamine oxidase inhibitor (Yu et al., 1974).

Hathway et al.(1970), advocated the vascular hypothesis due to the presence of small groups of necrotic fibres early in the disease. Further, they observed focal necrosis in rabbits by microemobilization of muscles using small dextran particles injected into the femoral artery. Further, these lesions were produced in rats by combination of aortic ligature and small doses of vasoactive agents e.g. serotonin or noradrenaline (Mendell et al., 1971) leading to increased serum creatine kinase activity (Mendell et al., 1972 b and Silverman et al., 1976). Appenzeller et al. (1975), advanced a theory to reconcile the presumed vascular disorder with neurogenic theory, postulating that sympathetic nerves were defective in controlling muscle blood flow.

Several arguments have been advanced in opposition to the vascular theory. Measurements of muscle blood flow by several different methods have been reported to be normal (Emery et al., 1965; Kunze et al., 1973; Paulson et al., 1974; Braddy et al., 1975; Bradly 1977) and capillaries in nail beds were also not found abnormal (Dudley et al., 1964). No abnormality was reported by morphometric analysis of small blood vessels in muscle of dystrophic patients (Jerusalem et al., 1974 a, Musch et al., 1975). Histological pattern in muscle of chronically ischemic animals differ in important respect from the human disease (Karpati et al., 1974). This theory was further criticized because treatments

designed to improve circulation in DMD have been ineffective (Mendell et al., 1977).

(b) Neurogenic Theory:

The muscular dystrophies have long been regarded as primary degenerative disorders of skeletal muscle. The classical nerve-cross-union experiment of Buller et al.(1960), demonstrated that fast and slow muscles changed their contractile properties after cross-union of their respective nerves. The fast muscle thus became slow and the slow muscle fast. They postulated that the neural influence on muscle speed is not exerted by nerve impulse as such, but, a substance from the CNS passes down the axon of motoneurones, crosses the neuro-muscular junction and traverses the muscle fibres. Subsequent histochemical study revealed that enzymatic pattern of fibres within the muscle was changed following cross innervation (Dubowitz 1967). This suggests that neural influence which determines the contractile properties of fast and slow muscle also has a profound influence on the structure and metabolic activity of muscle fibres. Conard et al. (1962), reported enhanced transmission through neuromuscular junction in dystrophic mice in comparison to normal. He suggested changes at myoneural junction and terminal innervation in dystrophic muscle. Histochemical studies revealed marked reduction in the amount of available choline esterase activity at the motor end

plates of dystrophic mice muscle and thereby increased acetylcholine activity at the synaptic junction of the end plate resulting in the excitibility of dystrophic muscle (Glaser et al., 1967).

Wilson and coworkers (1970) studied the regulation of acetylcholine esterase (AchE) activity during the development of normal and dystrophic chick muscle. They found that in normal embryonic chicken muscle, at least, three isoenzymes of AchE exist, and by two week after hatching, two of these disappear. On the other hand, all the three isoenzymes were present in both dystrophic chicken muscle and normal chicken muscle (denervated shortly after hatching). On the basis of these findings they postulated that nerves transfer specific inducer and repressor substances to the muscle where they regulate the muscle proteins. Thus, nerves innervating fast twich fibres of normal chicken produce an AchE repressor molecule which is lacking in the nerves from birds with muscular dystrophy. Based on a detailed study of motor unit population in normal and dystrophic mouse muscle, Harris et al.(1971) suggested primary abnormality of motor nerve in murine muscular dystrophy.

Mc Comas et al. (1970, 1971 a, and 1974) developed a new method for estimating the number of motor units in the muscle and correlated their results with isometric

twitch tension of the same muscle. A progressive reduction in the number of functioning motor units, without reduction in their size and a slowing of the nerve impulse conduction velocity in distal but, not the proximal portion of axon has been reported them. In 1971 b, they postulated the theory that muscular dystrophy is a chronic dysfunction of motor neurons, eventually leading to their physiological failure. According to them motor neurons are "Sick" in muscular dystrophy, which is characterized by "difficulty in maintaining satisfactory connection with muscle fibers". This difficulty is manifested by impaired neuromuscular transmission during maximal effort or repetitive nerve stimulation and by an inability to aquire previously denervated muscle fibres (Mc Comas et al., 1971 a). Sica et al. (1978) further provided evidence for abnormality involving motor neurons in DMD. They suggested that dystrophic process takes place in two stages, first during early embryoinc life involving faulty inductive actions of the neural tube upon mesoderm and upon itself. The neural consequences vary among individuals and are manifested as non-progressive mental retardation andEEG abnormalities. The second stage of DMD is loss of functioning motor units, which is associated with stricking reduction in the number of excitable muscle fibres and takes place in trunk and large limb muscle at 9-12 years of age.

A neuropathological study revealed significant histological changes in brain from cases of muscular dystrophy, suggesting a disorder of cortical development during fetal life with a disturbance of normal neuronal migration (Rosman 1966).

Cerebro-spinal fluid (C.S.F.) proteins were examined by isoelectric focusing and quantitative paper electrophoresis in muscular dystrophy patients. An increased level of β_1 globulin, found in all patients with myotonic dystrophy suggests either primary metabolic defect of nervous tissue or secondary changes in the protein metabolism of neurons or glial cells (Kjellin et al., 1976). Sensitization of lymphocytes from patients with muscular dystrophy by antigen from both muscle and nerve reflects possible degeneration in both muscle fibres and motor neurons (Caspary et al., 1971).

In tissue culture studies of normal and dystrophic human muscle Goyle et al. (1967 and 1968) and Kakulas (1968) showed marked differences between the myoblasts from normal and dystrophic human muscle cultures in vitro on the basis of morphology of cells, growth pattern and cytochemistry. On the other hand, no significant difference has been observed in their growth characteristics and morphological features (Bishop et al., 1971), histochemical profile of myotubes (Gallup et al., 1972 a) and RNA synthesis (Gallup et al.,1972 b). They suggest that some factor in vivo, possibly of a neural

or humoral nature is necessary to produce the dystrophic changes, and the absence of this factor *in vitro* would enable the muscle to revert to a normal state. Because dystrophic muscle appeared normal when transplanted to normal mice, Salafsky (1971) suggested that dystrophic mouse muscle is rendered defective by environment of dystrophic mouse.

Dubowitz (1979) supported the neurogenic theory of muscular dystrophy on the basis of intellectual retardation, presence of structural abnormalities in nervous system, as well as, electrophysiological and electroencephalographic abnormalities. Electron microspic studies of motor end plate also suggested muscular dystrophy as a neurogenic disorder (Harriman 1976).

Mc Comas "Sick motor nurrons" concept was challenged by Panayiotopoulos *et al*. (1974 and 1976). Their main objection was that the noise level of recording system (3-4 uV) might obscure motor unit action potentials (MUAPs) of small amplitude which are expected in myodystrophies. Further, modification of technique (i.e. superimposition of enlarged photographs of MUAP), which allows detection of small amplitude motor axon potential suggests a normal number of motor axon in DMD and LGD (Panayiotopoulos, 1974). Further studies of Panayiotopoulos *et al* (1977) suggested

that the muscle and nerve are independently affected by pleiotropic gene of disease. The muscle changes in dystrophia myotonica are due to combined lesion of these two systems with varied and unequal degree of participation from patient to patient. Morphometric study of motor end plate fine structure also does not provide evidence for a 'sick' motor neuron in DMD (Jerusalem et al., 1974 b) although, it does not exclude the possibility of neural influence on muscle. Mc Comas' theory could not account for the observed abnormalities in erythrocytes, cultured muscle or skin fibroblasts. This theory was further criticised since, incorporation study of ^{3}H-leucine into skeletal muscle showed a different protein synthesis pattern in dystrophic and denervated muscle (Monckton et al., 1976).

(C) Membrane Theory:

The clue for membrane theory came from general finding that the plasma concentration of enzymes that are normally confined to muscle cells were increased in muscular dystrophy suggesting defective structure of sarcolemma. This concept has been particularly associated with Duchenne type where the leakage is exceptionally high (Dreyfus et al., 1954 and Rowland 1976). According to Rowland (1979 b), the functional genetic fault of muscular dystrophy lies in an enzyme or structural protein which alters the composition and function of muscle surface membrane and thereby weakness and progressive

degeneration of skeletal muscle. Apart from a genetically determined defect in membrane structure, other possibilities according to Pennington (1981) are that membranes may be damaged by a circulating factor or may be affected by a disturbance in the metabolism of the fibres. An interference in energy supply to the membrane may also play an important role since it can increase enzyme efflux.

A number of workers have described focal areas of discontinuity in the muscle cell membrane in fibres that are otherwise normal or, show only minor degenerative changes in electron microscopic studies (Mokri et al., 1975, Schmalbruch 1975). Mokri et al. (1975) reported degenerative changes in the fibre beneath such focal plasma membrane defects. They were able to demonstrate abnormal permeability of the plasma membrane to horseradish peroxidase in the vicinity of such areas. Carpenter et al. (1979) on the basis of their electron microscopic observations suggested that necrosis of muscle cells is initiated by loss of plasma membrane followed after a short interval by Z-disc lysis and mitochondrial changes. They also suggested that small patches of membrane loss can be repaired by muscle fibre and that this may prevent necrosis.

Decreased activity of muscle adenylate cyclase has been observed in various muscle diseases (Susheela et al 1975). In muscle cells cultured from patients with

Duchenne dystrophy, adenylate cyclase showed an abnormally low response to catecholamines and to fluoride (Mawatari et al., 1976). A specific decrease in cyclic nucleotide phosphodiesterase in DMD muscle has been observed by Canal et al. (1975). Since, adenylate cyclase enzyme is found in plasma membranes, as well as, in sarcoplasmic reticulum of normal muscle (Rabile et al., 1978), any change in adenylate cyclase system in muscular dystrophy may be assumed to reflect membrane alterations.

Freeze fracture analysis revealed a decrease in sarcolemmal intramembranous particles both on the protoplasmic and extracellular faces of DMD muscle plasma membrane (Schotland et al., 1977 and 1981). Thompsom et al (1977) reported abnormal cell interaction in dystrophic muscle culture. The focal defects in the binding of concanavalin A to the plasma membrane of muscle fibres has also been observed in dystrophic patients (Bonilla et al., 1978). An abnormal permeability of high proportion of muscle fibres to dye procion yellow was observed in biopsies from DMD patients (Bradley et al., 1978). Molak et al.(1980) examined the space occupied by low and high molecular weight tracers in soleus and extensor digitorum longus (EDL) muscles of normal and dystrophic mice. It was found to be larger in dystrophic

than normal muscle suggesting a leaky membrane in dystrophic muscle.

It has been suggested that this structural defect at the cell surface membrane level (muscle fibre plasmalemma) permits egress of muscle enzymes including creatine kinase, as well as, ingress of harmful calcium rich extracellular fluid (Bodensteiner et al., 1978; Mokri et al., 1975; Wrogemann et al., 1976).

Excessive accumulation of Ca^{++} within the dystrophic muscle fibre leads to myofibrillar over-contracture and disassembly of myofibrills due to activation of Calcium activated neutral protease and various metabolic disturbances which culminate in the death of muscle fibre (Wrogemann et al., 1976; Oberc et al., 1977; Cullen et al., 1980).

Apart from the abnormalities reported in the muscle membrane there appears to be a generalized membrane defect in this disorder. Abnormalities of membrane have been reported in erythrocytes (Rowland et al., 1979 a), leucocytes (Scholte et al., 1980), monocytes (Moxley et al., 1981 and Pirro et al., 1982), lymphocytes (Pickard et al., 1978), fibroblast (Wyatt et al., 1977, Jones et al., 1979 and 1981) and adipose tissue (Mably et al., 1981).

Although, the evidences cited above provide a credible basis for the membrane theory but they could not

be confirmed because all the soluble enzymes or other muscle constituents were not increased in serum or decreased in muscle (Rowland et al., 1968). According to Rowland (1979 b), the serum enzyme abnormalities could be accounted for by muscle necrosis, and the increased muscle calcium content could be the result of cell damage and not the cause for it. He further found a problem with this theory because electrophysiological studies do not suggest a surface membrane abnormality.

Recently, it has been proposed that in muscular dystrophy the premature degeneration may be due to an aberration of programmed cell death (Webb 1974) or, due to alteration in the development of connective tissue (Sweeny et al., 1981).

Webb (1974) suggested that the basic defect in muscular dystrophy is due to a derangement in the normal process of 'muscle cell death' between week 10 and 16 of foetal life; which is a critical stage in development. According to him there are three possible ways of derangement in cell death process. First, the cell death 'Switch' may simply not be activated at the critical stage in muscle development (which is otherwise a normal process) resulting in imperfectly formed muscle fibres which die off prematurely. Second, death 'Switch' may not be activated at correct time i.e. instead of occuring between

week 10 and 16 of foetal life it is delayed until later in development or even into the postnatal period. Third, the cell death mechanism' may be triggered at the correct time but, failure occurs to turn off the process and muscle cell death continues until virtually all the muscle fibres have died.

Bourne et al., (1959) suggested that the fundamental defect in progressive muscular dystrophy may not be in muscle fibre but in connective tissue which supports it. Ionasescu et al.(1971, 1976 and 1977) found an over production of connective tissue with a concomitant decrease in muscle protein synthesis both by polyribosomes from skeletal muscle and by tissue culture of muscle tissue and skin fibroblasts from patients suffering from DMD. In tissue culture, it has been observed that dissociated muscle from patients with Duchenne and Becker muscular dystrophy forms unusual clusters of Sticky cells, which suggests abnormal collagen production (Thompson et al., 1977). Duance et al. (1980) on the basis of immunofluorescent technique reported excessive deposition of type III collagen in muscular dystrophy and suggested that collagen may play a role in the pathogenesis of muscular dystrophy.

Sweeny et al. (1981) gave the hypothesis for the role of connective tissue in the aetiology of muscular dystrophy. They suggested that if connective tissue has failed to

develop normally it would lead to a change in myoblast, the myofibre differentiation or within the elasticity of the tendons (probably a decrease) followed by a relaxation of muscle and concomitant atrophy. Further, the failure of this tissue would result in functional ischemia affecting differentiation of all cellular components of muscle (Myofibres, vessels and nerves). Probably, the lesion in connective tissue is due to failure in the development of the proper mature type of collagen. This in turn is a result of either, failure of fibroblasts to synthesize proper type of collagen or an abnormal synthesis of proteoglycans (Sweeny et al., 1981).

Changes in tissues other than muscle:

Morphological, biochemical and biophysical studies in DMD and other myopathies have revealed abnormalities in other systems, as well, in addition to muscle.

1. Erythrocytes:

Erythrocyte membrane has been widely studied because of its ready accessibility, easy sampling and easy uncontaminated membrane isolation, as a model membrane in muscular dystrophy.

Dramatic surface deformation of erythrocytes were observed by scanning electron microscopy (Matheson et al., 1975; Miller et al., 1975; Beyer et al., 1977; and Grassi et al., 1977) and light microscopy (Howells, 1976), in

muscular dystrophy. Increased osmotic fragility (Fisher et al., 1976; Somer et al., 1977), as well as, reduced deformibility (Lumb et al., 1975; Percy et al., 1975; Brain et al., 1978) have been reported in Duchenne erythrocytes. Biochemical investigations of the enzymatic and transport activity of $Na^+ - K^+$ - ATPase have provided conflicting results. According to Brown et al. (1967), ATPase activity of erythrocyte ghosts from muscular dystrophy patients was stimulated by ouabain which normally depresses the total ATPase activity by inhibiting Na^+, K^+-ATPase but, the contradictory results were found when Na^+, K^+ were taken at optimal concentration (Klassen et al., 1969; Souweine et al., 1978). This was supported by the work of Peter et al. (1969) and Siddique at al. (1977). They suggested that a plasma factor is responsible for the specific effects of ouabain. Hull et al. (1976) reported an alteration in normal ratio of sodium efflux to potassium influx which is ouabain sensitive process. Hodson et al.(1977) reported increased substrate affinity of Ca^{++} - ATPase of Duchenne erythrocyte ghosts while, Dise et al (1977) suggested that erythrocyte ghosts in this disease have an increased sensitivity to the influence of Ca^{++} ionophore. Plishker et al (1978) found increased rate of Ca^{++} uptake in erythrocytes from myotonic dystrophy patients. Alteration in the activity of adenylate cyclase

in response to catecholamines were reported in erythrocytes from patients with DMD (Mawatari et al., 1976).

In DMD, increased rate and degree of protein phosphorylation was observed in 220,000 dalton polypetide known as spectrin (Rose et al., 1976). In myotonic dystrophy, band III, which consists of different polypeptides, migrating in 95,000 dalton range demonstrated a decreased phosphorylation (Rose et al., 1975). On the basis of erythrocyte spectrin extractibility it was found that spectrin is more tightly associated with membrane in DMD patients compared to normal controls (Nagano et al., 1980; Tsuchia et al., 1981). Increased electrophoretic mobility of erythrocyte membrane has been observed in patients with DMD, Myotonic dystrophy, dystrophic mice and chicken (Bosman et al., 1976). Reduced uptake of adenine (Soloman et al., 1977) and increased resting ATP content (Danon et al., 1977) suggests abnormal adenine metabolism of erythrocytes in muscular dystrophy.

Butterfield et al. (1978) by their biophysical approach (ESR studies) reported increase in rigidity of erythrocyte plasma membrane in chicken muscular dystrophy. Further, it has been suggested that increased erythrocyte membrane fluidity occurs in human muscular dystrophy (Sato et al., 1978; Butterfield, 1977 and 1981).

2. Fibroblasts:

Abnormal collagen synthesis has been reported in cultures of Duchenne fibroblasts (Ionasescu et al., 1977). Wyatt et al. (1977) observed that cultured skin fibroblasts from DMD patients have characteristic cytoplasmic inclusions, that make these cells distinguishable from normal cultured fibroblasts. On the other hand, Cullen et al. (1977) were unable to detect such cytoplasmic inclusions in DMD patients. Goyle et al. (1973) also reported no difference in the growth pattern and enzymatic activity for succinic dehydrogenase and glucose 6-P dehydrogenase, as well as, distribution pattern of triacylglycerol and phospholipid in the fibroblasts from facia cultures of normal and dystrophic patients. The intracellular adhesiveness of skin fibroblasts from patients with DMD was reported to be reduced when compared to normal cells on the basis of their collison efficiencies (Jones et al., 1979, Jones et al., 1981). The size analysis of fibroblasts aggregates showed that the majority of dystrophic cells remain unaggregated but that a small number of very large aggregate was always formed. Normal cell suspensions rarely contained large aggregates but contained many intermediate size aggregates (Jones et al., 1981). They suggested these differences in adhesiveness and aggregate pattern due to some subtle alteration in the surface membrane of dystrophic cells. Qualitative and quantitative analysis of the staining

pattern revealed that cultured skin fibroblasts stain normally with concanvalin A in DMD (Newman 1982). Cerri et al. (1982) reported that in contrast to muscle and erythrocytes, fibroblast shows normal adenylate cyclase activity.

3. Lymphocytes:

Lymphocytes from patients with Duchenne, Becker, limb girdle, facioscapulohumeral (FSH) and congential muscular dystrophy have a decreased per centage of "Capping" (Aggregation of fluorescent antigen antibody complexes on the surface of B lymphocytes) due to altered membrane fluidity (Verrill et al., 1977 and Pickard et al., 1978).

4. Leucocytes:

Schotte et al. (1980) reported that in Duchenne patients 5'-nucleotidase, located on the surface of leucocytes showed a two fold increase. The activities of the other enzymes viz., (Monoamine oxidase, Palmitoyl co-A synthetase, phosphorylase 'a', phosphorylase 'a+b', Hexokinase Glocose 6-P dehydrogenase) were the same as in controls. Furthermore, the activity ratio between phosphorylase 'a+b' and 'a' was not changed, which indicates that the receptoradenylcyclase protein kinase system is intact. These workers also reported decreased phosphorylase activity in leucocytes of Duchenne carriers.

5. Monocytes:

Moxley et al. (1981) reported that monocytes from patients with myotonic dystrophy fail to demonstrate the normally observed qualitative increase in insulin binding affinity after oral glucose loading. Insulin binding to monocytes in DMD patients was found to be lower than in normal, but glucose metabolism was normal. Competition-inhibition studies showed that binding was lower in DMD patient than in controls at any given insulin concentration, thus suggesting that the insulin binding variation was due to a change in receptor concentration. This was confirmed by scatchard analysis of data, but the possiblity of genetically induced decreased receptor number is yet to be confirmed (Pirro et al., 1982).

6. Platelets:

Platelets from patients with DMD have been reported to show deficient oxidation of catcholamines (Demos 1973) but, this Pacold et al. (1975) observed no difference in platelet DOPA oxidase between normal, DMD boys or carrier mothers. Abnormal serotonin uptake was observed in platlets of DMD patients while, it was normal in other neuromuscular disorders (Murphy et al., 1973). In myotonic dystrophy, platelet aggregation displayed enhanced senitivity to adrenaline (Bousser et al., 1975).

7. Adipose Tissue:

In vitro studies of subcutaneous fat from normal and dystrophic patients showed no difference in the morphology of lipocytes but, the growth of cells was poor in the latter compared to the profuse growth in normal (Goyle et al., 1973). Cytochemical studies showed that succinic dehydrogenase activity in lipocytes of normal and dystrophic culture was similar. A significant difference was observed in the glucose 6-phosphate dehydrogenase activity. In 7 day old culture, no reaction for G-6-PDH was observed in normal subcutaneous fat while, in dystrophic cultures majority of cells showed faint reaction for the same enzyme. In older cultures (15 day, 21 day) of normal fat, only a few mono and binucleated cells were seen with uniformaly stained granules all over the cytoplasm. While, in older cultures of dystrophic fat the reaction was intense in all the cells (Goyle et al., 1973). In mouse adipose tissue, adenylate cyclase activity was observed in certain parts of the plasma membrane of normal but not in dystrophic (Susheela et al., 1973). Himms-Hagen et al. (1980) suggested a defect in control of the growth of brown adipose tissue in cardiomyopathic hamster. They reported that the size (Wet weight, total protein, total cytochrome oxidase) of intrascapular brown adipose tissue is reduced to about one-half of normal in the cardiomyopathic hamster (BIO 14.6). Mably et al. (1981)

reported impaired glucose transport and oxidation in adipose tissue of patients with myotonic dystrophy.

Lipid Composition and Metabolism Muscular Dystrophy:

Lipid composition and/or metabolism has been studied in various tissues including muscle, erythrocytes, neural tissues, fibroblast and adipose tissue in muscular dystrophy.

Muscle:

Total lipid and neutral lipids: Fatty infiltration has been reported in dystrophic muscle of mice (West et al., 1960). An increase in total lipid content has been found in mouse skeletal muscle (Kandutsch et al., 1958; Shull et al., 1958; Young et al., 1959 and Pearce et al., 1980); chicken fragmented sarcoplasmic reticulum (Hsu et al., 1971); and muscle (Chio et al., 1972); DMD muscle (Hughes., 1972); sarcoplasmic reticulum in different human neuromuscular disorders (Takagi et al., 1973) and mouse sarcolemma (Dekretser et al., 1977).

Triacylglycerol content is increased in dystrophic mice skeletal muscle (Young et al., 1959); chicken FSR (Hsu et al., 1971) and muscle (Chio et al., 1972); and mouse sarcolemma. (Dekretser et al., 1977). In dystrophic human muscle cultures, triacylglycerols occupy a larger area of cytoplasm, with the age of culture and in 4-week old cultures, the cytoplasm becomes loaded with triacylglycerol which

indicates that there is an intracellular increase in the lipid material (Goyle et al., 1973). Kunze (1973) found that incorporation of linoleic acid is decreased while, palmitic acid is increased in triacylglycerol of dystrophic human muscle. In vitro incorporation of acetate 2 ^{14}C into total lipids and triacylglycerol, and turnover rate of triacylglycerol was much greater in dystrophic than normal chicken muscle (Chio et al., 1972). West et al. (1977) reported 3-times greater incorporation of ^{14}C glucose into triacylglycerol and neutral lipid glycerol in DMD muscle than normal tissue. Inonasescu et al. (1981) also found significant increase in incorporation of ^{3}H glycerol in both fresh and cultured DMD and foetal muscle.

Pennington et al. (1966) and Pearce et al. (1981) reported that fatty acid profile of triacylglycerol is similar in dystrophic muscle and normal human adipose tissue, and suggested that the cause for increased lipid content in human dystrophic muscle is probably infiltration of fat. Jato-Rodriguez et al. (1974) suggested that the accumulation of triacylglycerol in dystrophic mice muscle is not related to increased synthesis from sn-glycerol 3P(viz., phosphatidic acid pathway) or decreased lipase action but, increased fatty acid synthesis combined with a decreased capacity to oxidise fatty acids (Lin et al., 1972); Jato-Rodriguez et al., 1972) is the main contributing factor for triacylglycerol accumulation.

Increase in cholesterol content has been reported in dystrophic mice muscle (Shull et al., 1958; Young et al., 1959; Owens et al. (1970), Chicken FSR (Hsu et al., 1971) and muscle (Chio et al. 1972); and DMD muscle (Hughes 1972 and 1973). Robinowitz (1960) reported increased cholesterol synthesis in dystrophic mice skeletal muscle.

Free fatty acids content increases in pectoral and abdominal muscle (Susheela et al., 1968 a), and Sarcolemma (Dekretser et al., 1977) of dystrophic mice while, its content remains unchanged in muscles of patients with different neuromuscular disorders (Susheela. 1968 b).

Phospholipids:

There is a controversy with regard to the change in phospholipid content in dystrophic muscle. No change in its content has been reported in dystrophic chicken FSR (Hsu et al., 1971); human muscle (Hughes 1972 and 1973); Sarcoplasmic reticulum (Takagi et al., 1973). In dystrophic muscle cultures a mild reaction for phospholipids has been reported both in nucleus and cytoplasm (Goyle et al., 1973). On the other hand, increased phospholipid content has been found in dystrophic chicken muscle (Chio et al., 1972), mouse sarcolemma (Dekretser et al., 1977) and muscle (Pearce et al., 1980).

Individual phospholipid components were found to be markedly affected in muscle from both, human and mouse muscular dystrophy. Hughes (1965) reported an increase in sphingomyelin and total plasmalogen, and decrease in phosphatidylcholine in dystrophic mouse muscle derived from both fore and hind limbs. Takagi *et al.* (1968) and Kunze *et al.* (1975) reported an increase in fatty acid 18:1 and decrease in phosphatidylcholine 18:2 in dystrophic human muscle. Owens *et al.* (1970) found major differences in phospholipid composition of dystrophic mouse muscle microsomes. The dystrophic samples showed a decrease in lecithin and phosphatidylcholine, and an increase in sphingomyelin, cardiolipin and ethanolamine compared to normal. Increase in sphingomyelin, phosphatidylserine, and decrease in phosphatidylcholine + lysophosphatidyl choline has been observed in dystrophic chicken (Hsu *et al.*, 1971). *In vivo* incorporation of acetate 2-^{14}C into phospholipids and turnover rate for latter was much greater in dystrophic than normal chicken muscle (Chio *et al.*, 1972). In DMD, both rectus abdominis and gastrocnemius muscle showed an increase in sphingomyelin, no change in cardiolipin, and decrease in lecithin and choline plasmalogen. The phospholipid composition in normal foetal muscle and from very young children is similar to DMD muscle (Hughes 1972,

Hughes 1973). He suggested that the lipid composition of human muscle changes after birth, and in DMD some of these changes are either delayed or fail to take place. Kunze (1973) found that phosphatidylcholine and phosphatidylethanolomine are decreased, whereas, lysophosphatidylcholine, sphingomyelin and fatty acids are increased in human progressive muscular dystrophy. He also reported that the incorporation of linoleic acid and palmitic acid in sphingomyelin and linoleic acid in phosphatidycholine is decreased in dystrophic human muscle compared to normal muscle and suggested that primary defect in progressive muscular dystrophy lies in the biosynthesis of phosphatidycholine.

Takagi et al. (1973) reported contamination of sarcotubular membrane preparations with membranes from fat and connective tissue. They suggested that the abnormalities in lipid composition i.e. decrease in phosphatidylcholine, phosphatidylinostiol and increase in sphingomyelin in sarcotubular membranes from dystrophic human muscle may be due to the increased amount of fat and connective tissue associated with the muscle. But, Dekretser et al. (1977) found significant increase in the amounts of phosphatidylcholine, phosphatidylethanolamine and lysophosphatidylcholine of purified sarcolemmal preparations from dystrophic mice, and after discussing the possibility of contamination with connective tissue,

concluded that this difference reflected changes inherent in the sarcolemma of dystrophic muscle.

Kowk et al. (1978) reported no significant difference in activity of enzymes cholinephosphotransferase, lysophospholipase, lysophosphatidylcholineacyltransferase in normal and dystrophic mouse muscle. Increased activities of two phospholipases A (5 folds) and phospholipase C(60%) were found in dystrophic mouse muscle compared to normal. Kunze et al. (1980) studied few enzymes of phospholipid metabolism in dystrophic human muscle but they could not get significant difference in activities, of CDP choline: diglyceride P-choline transferase, CDP choline: Ceramide P-Choline transferase enzymes.

Detailed analysis of fatty acid composition of individual phospholipids of normal and dystrophic mouse muscle revealed that both phosphatidylcholine and phosphatidylethanolamine showed decrease in fatty acid 16:0, while 18:0 and 18:1 increased significantly. In phosphatidylcholine there was increase in 18:2 and in phosphatidylethanolamine a significant decrease in 22:6 while sphingomyelin showed increase in 16:2, 23:0, 24:1 and decrease in 18:0, 18:1 (Pearce et al.,1980). They suggested that these changes may be result of altered turnover rate of phospholipids. Desai (1981) reported similarities in phospholipid composition of DMD and normal foetal muscle. According to him, this similarity may be

either, due to maturational defect or, genic depression which leads to expression of foetal biochemical characteristics in the adult DMD muscle or, it may be because of a selective loss of fibre type II in DMD muscle since, foetal muscle contains more or less type I muscle fibre. Inonasescu et al. (1981) reported significant increase of specific incorporation of (^{3}H) glycerol into phosphatidylcholine, phosphatidyl serine and phosphatidylinositol in both fresh and cultured DMD and foetal muscle and suggested that lipid alteration in dystrophic muscle might be related to defect in maturation. However Pearce et al (1981) suggested that increase in sphingomyelin in dystrophic muscle biopsies and the changes in the fatty acid composition of individual phospholipids may be accounted for by the increase amounts of fat and connective tissue which are found in dystrophic muscle samples.

Erythrocytes:

Normal phospholipid composition was reported in red cell membranes of myotonic patients. (Rose et al., 1973). Kunze et al. (1973) reported an increase in sphingomyelin in erythrocytes of DMD patient. Kalofoutis et al. (1977) found a decrease in phosphatidylcholine, increase in lysophosphatidylcholine, sphingomyelin and disphosphatidylglycerol in erythrocytes of DMD patients as compared to normal controls.

Howland et al. (1977) reported decreased level of fatty acid 16:1 in erythrocyte membranes from carriers and dystrophic patients but this finding was challenged for its methodology (Rao et al., 1978; Plishker et al., 1979). However Ruitenbeek (1978) reported decrease in fatty acid 16:1 in diacylglycerol of Duchenne, di and triacylglycerol of myotonic dystrophy patients. They reported increased content of fatty acid 18:1 and decreased content of 20:0 in lysophosphatidylcholine in Duchenne, In myotonic cases they reported five fold increase in the level of 22:4W6 in lysophosphatidylcholine and 19:0 level in triacylglycerol; decrease of 16:0 in phosphatidylserine and drastically lowered content of 16:1 in di and triacylglycerols. These findings was not similar to previous report (Kunze et al., 1975) especially with respect to their fatty acid pattern of sphingomyelin in both control and dystrophic patients.

Increased phospholipase activity was reported in erythrocytes from patients with DMD and Myotonic dystrophy (Iyer et al., 1976). The increased activity of this enzyme leads to membrane dysfunction due to increased synthesis of membrane disruptive lysophospholipids. The kinetic behaviour of enzyme protein kinase was reported to be altered in erythrocyte membrane of myotonic dystrophy patients. Its behaviour in normal erythrocyte membrane was found to be

similarly influenced by lipid extract from the serum of myotonic dystrophy patient suggesting dynamic interaction between serum and erythrocyte lipids, and role of lipid in influencing properties of erythrocyte membrane enzyme (Iyer et al., 1977).

Neural Tissues:

The turnover of phospholipids in neural tissues (viz., forebrain, spinal cord and sciatic nerve) and skeletal muscle of dystrophic mouse is faster compared to normal but it was not determined whether this was the result of an increased rate of synthesis and breakdown or an alteration in rate of exchange of precursors between blood and tissue (Kwok et al., 1976). They suggested that in dystrophic muscle, the alteration occurs in phospholipid metabolism in the nerve fibres and endings rather than muscle membrane. Lysophosphatidylcholine content increased in spinal cord while, sphingomyelin content decreased in sciatic nerve of dystrophic mice with respect to controls. In dystrophic mouse, phospholipase A activity increased by 50% in sciatic nerve, and Lysophosphatidylcholine acyltransferase activity increased in sciatic nerve and spinal cord by 50 to 100% over that of the controls. The forebrain and spinal cord from dystrophic mice had only 60% of lysophospholipase activities (Kwok et al., 1978). These anomalies provide further support for the involvement

of nervous tissues in the aetiology of mouse muscular dystrophy though, this may reflect a generalized membrane defect.

Fibroblasts:

Cytochemical studies (Goyle et al., 1973) showed that the distribution pattern of triacylglycerol and phospholipid granules in fascia cultures from normal and dystrophic patients was similar. Rounds et al. (1980) reported that concanavalin A induced increase in phosphatidate radioactivity (label) and decrease in phosphatidyl inositol label was significantly greater in Duchenne fibroblasts. When compared to normal. They suggested a diminished rate of phosphatidylinositol synthesis from phosphatidate in Duchenne skin fibroblasts.

Adipose Tissue:

In vitro studies of Goyle et al. (1973) revealed that the distribution pattern of triacylglycerol and phospholipid granules was same in fat cultures from normal and dystrophic patients except that the lipocytes from dystrophic cultures showed a less intensely stained granular triacylglycerol content in older cultures.

Barakat et al. (1977), reported that the rate of incorporation of ^{14}C glycerol-3 phosphate into glycerides of adipose tissue is depressed in cardiomyopathic hamsters. Subsequently (1978), they observed that fatty acid synthesis

is also depressed in adipose tissue of hamsters due to decrease in the activity of serveral enzymes involved in lipogenesis. A slight increase in total phospholipid, sphingomyelin, phosphatidylserine content and a slight decrease in phosphatidylcholine, phosphatidylethanolamine content of adipose tissue microsomes in dystrophic hamsters has been observed (Barakat et al., 1980).

Recently, Banerjee et al., 1983) reported that neutral lipid as well as cholesterol are elevated, phospholipid is decreased and free fatty acids remain within the normal range in adipose tissue of DMD patients. An increase in sphingomyelin and phosphatidyl ethanolamine has been found. They also reported alterations in the fatty acid profile of triacylglycerol and phospholipid components.

MATERIALS AND METHODS

Subcutaneous adipose tissue (Table-I) was obtained by surgical biopsy from eight cases of neuromuscular disorders. Normal samples of adipose tissue were obtained from patients with no clinical sign of muscle disease in course of routine surgical operations. Each tissue sample was collected in normal saline, washed twice with the same to remove extraneous blood and its wet weight was recorded.

Extraction of Lipids:

The extraction procedure (Misra, 1968), based on the method of Folch *et al.* (1957) was followed:

Each tissue sample was ground separately with mortar and pestle in chloroform - methanol (2:1 v/v) containing the antioxidant BHT (2, 4-di-tert-butyl-p-cresol 5 mg/100 ml). The slurry was transferred to 10 volumes of chloroform-methanol (2:1 v/v). The mixture was allowed to stand with occasional shaking at room temperature for 4-6 hours, and filtered. The residue was re-extracted with 10 volumes of fresh chloroform - methanol (2:1 v/v), and allowed to stand for 2 hours at room temperature and filtered. The two filtrates were combined and evaporated at 45-50°C till dryness under a stream of nitrogen. The dried residue was dissolved in

TABLE - I

CLINICAL FEATURES

Case No.	Name/OPD No.	Age (Years)/ Sex	Clinical Diagnosis	Age of onset (Years)	Duration (Years)	Muscle Power
1-6	Normal	20 F, 30 F, 64 F, 25 M, 28 M, 54 M.				
7	A.S. 1530/82	8 M	Duchenne dystrophy	3	5	+++, ++++
8	S.D. 2663/82	16 M	Becker dystrophy	11	5	+++, ++++
9	J. 1760/82	23 M	Limb-girdle dystrophy	19	4	+++, ++++
10	G. 2136/82	28 M	Limb-girdle dystrophy	18	10	N.D.
11	A.C. 2310/82	40 M	Limb-girdle dystrophy	26	24	N.D.
12	S.M. 32420	16 F	Myopathy	14	2	N.D.
13	P.K. 2452/82	28 M	Peroneal muscular atrophy	26½	1½	++++
14	F. 19688	32 F	Peroneal muscular atrophy	20	12	N.D.

N.D. = Not done.

chloroform - methanol (2:1 v/v) solvent mixture containing 4% water (v/v) and evaporated under a stream of nitrogen at 45-50°C till dryness. This step was repeated twice to break proteo-lipid bonds. The final dried residue was dissolved in chloroform-methanol (2:1 v/v) and layered with 1/5 volume of normal saline; mixed and allowed to stand at room temperature till the separation of two phases. The upper aqueous phase was removed with the help of pasture pipette. Small amount of anhydrous sodium sulfite was added to the latter to remove any traces of moisture. It was filtered and reevaporated under a stream of nitrogen at 45-50°C. Dried lipid residue was finally dissolved in a known volume of chloroform.

Silicic acid chromatography (Pearce et al., 1980);

Silica gel (BDH, Poole England, mesh size 60-120) was rinsed with methanol followed by diethyl ether prior to activation at 150°C for 16 hours (Owens et al., 1970). A slurry of 4 gm silica gel in chloroform was used to pack the column, and washed with 15 ml chloroform. The lipid extract dissolved in 5 ml chloroform was applied to the column and the neutral lipid fraction was eluted with 100 ml chloroform (containing 4 mg BHT). Pigments which may interfere during thin layer chromatography of phospholipids were eluted with 50 ml acetone and then phospholipids were eluted with 100 ml methanol containing

4 mg BHT. The eluates were evaporated to dryness under a stream of nitrogen at 45-50°C. The dried residue was dissolved in a known volume of chloroform.

Thin layer chromatography:

Thin layer chromatography plates were prepared 0.3 mm thick and activated at 110°C for one and half hours before use. Samples and authentic standard were streaked on the plates and allowed to run in appropriate solvents. Lipids were detected by exposure to iodine vapours.

(a) Phospholipids: Phospholipids were separated by one dimensional thin layer chromatography on silica gel G (E. Merck, Darmstadt) plates, thickness 0.3 mm, using chloroform, Methanol, Acetic acid, water 65:43:1:3 (Owens, 1966) as solvent. The separated fractions, phosphatidylcholine, Phosphatidylethanolamine and sphingomyelin were identified by comparison with cochromatographed authentic standards (Sigma).

(b) Neutral Lipids: Neutral lipids were separated by one dimensional, two step thin layer chromatography (Misra, 1968) on silica gel plates thickness 0.3 mm with Hexane - Diethylether-Acetic acid. Plates were run upto 7.5 cm in solvent system I : Hexane, Diethylether, Acetic

acid 90:10:1. The plates were removed, air dried and allowed to run in the same direction in solvent system-II: Hexane, Diethelether and Acetic Acid (60:40:1) upto 15 cm. Plates were removed and separated fractions, monoacylglycerols, diacylglycerols, free fatty acids and triacylglycerols were identified by comparison with authentic standards. These spots were scrapped and eluted according to method of Skipiski et al. (1968).

Triacylglycerols, free fatty acids and diacylglycerols were eluted by addition of 5 ml of diethyl ether, to test tubes containing silica gel with adhered lipids. The test tubes were shaken at room temperature for 10 minutes and centrifuged for 15 minutes at 1500 rpm. Diethyl ether was removed with the help of pasture pipette and the elution step was repeated twice with fresh 5 ml diethylether. All these eluates were combined and concentrated to dryness.

Monoacylglycerols were eluted by addition of 5 ml chloroform - methanol 4:1 (v/v) to test tubes containing silicagel with adhered lipids. Test tubes were shaken in a water bath at 40°C for 10 minutes and centrifuged at 1500 rpm. Eluates were removed with pasture pipette. The elution procedure was repeated twice. The three eluates were combined and concentrated to dryness.

Estimations:

1. Total lipid: The total lipid was estimated by the method of Frings et al. (1970) based on sulfophosphovanillin reaction.

Reagents: (i) Conc.H_2So_4

(ii) Vanillin 0.6% (W/v)

(iii) Phosphovanillin reagent: 400 ml of cone. phosphoric acid was added with constant stirring to 100 ml of 0.6% vannillin solution and stored in a brown bottle at room temperature.

(iv) Working standard of 20 mg/ml (1g olive oil/50 ml absolute alchohol) was prepared and stored at 4 °C.

Procedure:

0.1 ml of lipid extract was diluted to 1.0 ml with absolute alcohol 2.0 ml of conc. H_2SO_4 was added to it and mixed well. The tubes were heated for 10 minutes in a boiling water bath; cooled in cold water bath for 5 minutes and 0.1 ml of this mixture was transferred to another tube. 0.1 ml of conc H_2SO_4 was poured into a tube labelled blank. To all the tubes 5 ml of phosphovanillin reagent was added, mixed well and incubated for 15 minutes at 37°C. Tubes were cooled at

room temperature for 5 minutes and absorbance was read in Carl Zeiss spectrophotometer (PMQ II) at 540 mμ in additional 5 minutes using silica cells with 1 cm light path.

Phospholipids:

Phospholipids were estimated by the method of Bartlett (1959) as modified by Parker et al. (1965):

Reagents: 1. 60% Perchloric acid (AR)

2. 0.4% Ammonium molybdate

3. 1 amino - 2 naphthol sulphonic acid reagent (ANSA):- 0.5 g of 1-amino 2 naphthol sulphonic acid was added to 200 ml of freshly prepared 15% solution of sodium metabisulfite. 1.0 gm of anhydrous sodium sulfite was added to it, mixed, filtered and stored for a maximum period of one week.

Standard inorganic phosphate solution (8 μg inorganic phosphorus/ml): The stock solution consisted of 3.51 gm $KH_2 Po_4$ dissolved in 100 ml distilled water. The working standard solution was prepared by diluting 0.1 ml of stock solution to 100 ml.

Procedure:

Each outlined spot containing phospholipid viz. phosphotidylcholine, phosphatidylethanolamine, sphingomylein (corresponding to authentic standards) and adjacent area of

blank silica gel was scrapped separately into test tubes. Some amount of silica gel was added to test tubes containing standard inorganic phosphorus solution (0.05 ml - 0.4 ml). For total phospholipid estimation, 0.2 ml of phospholipid fraction per samples and 0.05 ml - 0.4 ml of standard inorganic solution was taken without silica gel. To each tube, 1.0 ml of 60% perchloric acid (AR) was added. These were placed in a heating block for digestion till the whole solution became clear (about 2 hours).

After digestion, enough water was added to give a volume of 3.6 ml (including 1.0 ml perchloric acid) and 6 ml of 0.4% ammonium molybdate was added to it. The contents of all tubes were mixed on a vortex mixer and 0.4 ml of 1 amino-2 naphthol sulfonic acid was added and mixed well. The tubes were placed in a boiling water bath for 10 minutes, removed and shaken well. The tubes containing silica gel were then centrifuged at 300 g for 40 minutes. Colorimetric determinations were performed on a Carl Zeiss spectrophotometer (Model PMQ II) at a wavelength of 820 mμ using silica cells with 1 cm light path.

Neutral Lipid:

Total neutral lipid was determined as the difference between total lipid and total phospholipid (Dekretser et al., 1977).

Acylglycerols:

Total acyl glycerols were determined as the difference between neutral lipid and sum of total cholesterol and free-fatty acids (De Kretser et al., 1977). Mono, di and triacylglycerols were assayed by estimating their glycerol content (Van Handel et al., 1957).

Reagents:

1. Alcoholic KoH 0.4%
2. Sulfuric Acid 0.2 N
3. Sodium Metaperiodate (0.05 M)
4. Sodium Sulfite (20%)
5. Chromotropic Acid (Sodium salt) (0.22 4%) (4, 5 dihydroxy-2, 7-napthalene disulfonic acid).
6. Standard solution: Mono, di-and tri-plarnitate (10 mg/ml).

Procedure:

0.5 ml of alcoholic KoH was added to each tube containing residues of Mono, di and tri acylglycerols and kept at 60-70°C for 15 minutes followed by addition of 0.5 ml of 0.2 N H_2 So_4. All the tubes were placed in a boiling water bath to remove alcohol.

To each tube, 0.1 ml of periodate solution was added. After 10 minutes, oxidation was stopped by the addition of 0.2 ml of sodium sulfite. 5 ml of chromotrophic acid reagent was added to each tube, mixed and kept at 100°C

water bath for half an hour in absence of excessive light. Tubes were cooled and the optical density was determined at 570 mu wavelength.

Cholesterol (Zlatkis et al., 1953):

Reagents:

1. Standard cholesterol solution (1 mg/ml) in glacial acetic acid.
2. Ferric chloride solution: 1 gm of ferric chloride (reagent grade) was dissolved in 10 ml of 100% glacial acetic acid.
3. Color reagent : 2 ml of ferric chloride solution was diluted to 200 ml with concentrated sulfuric acid.

Procedure:

0.2 ml of neutral lipid extract was evaporated to dryness and then diluted with glacial acetic acid to 3 ml. Similarly, 0.1 ml to 0.5 ml of standard cholesterol solution was diluted to 3 ml. To each tube, 1.0 ml of water was added and mixed throughly. The blank contained 3 ml glacial acetic acid and 1 ml distilled water. Finally, 2.0 ml of color reagent was added from the side of each test tube and mixed thoroughly. The tubes were brought to room temperature and the absorbance was read in Carl Zeiss spectrophotometer

at 560 mμ wavelength using silica cells with 1 cm. light path.

Free Fatty Acids(Duncombe 1963):

Reagents:

1. Copper reagent : It was prepared by mixing 9 volumes of 1 M-triethanolamine, 1 volume of 1 N-acetic acid and 10 volumes of 6.45% (W/v) $Cu(No_3)_2 . 3H_2O$. It was stored at 4°C and used within a week.

2. Diethyldithio Carbamate reagent: It was prepared by dissolving 100 mg of sodium di thiocarbamate in 100 ml of butane 2 - Ol; stored at 4°C and used within a week.

3. Standard Fatty acid: (100 μ mole palmitic acid/ml chloroform).

Procedure:

The residue of free fatty acid extract and standards of free fatty acid (10-100 μM) was diluted to 5 ml with chloroform. 2.5 ml of copper reagent was added to each tube. The blank contained 5 ml chloroform and 2.5 ml copper reagent. The tubes were shaken vigorously for 2 minutes and the two phases were allowed to separate. The aqueous phase was removed with the help of a pasture pipette. A portion(3.0 ml) of the chloroform solution was

pipetted into another tube (avoiding contact with the traces of copper containing aqueous phase) and 0.5 ml of the diethylthiocarbamate reagent was added to it and mixed well. The absorbance was read at 440 mμ wavelength using silica cells with 1 cm light path.

RESULTS

The lipid composition of human adipose tissue from normal and neuromuscular disorders has been studied. The percentage difference in the composition of lipid in various neuromuscular disorders is based on the mean value for normal.

Total Lipid (Table-II):

The total lipid content in the normal human adipose tissue shows a range from 462.7 to 698.2 mg/g wet weight. In dystrophic human adipose tissue, the total lipid content is found to be higher by 29% in Duchenne muscular dystrophy (DMD), 56% in Becker dystrophy (BD) and 40% in limb-girdle dystrophy (LGD). In a patient with myopathy of unknown origin (Case No. 12) the total lipid content shows an increase by 36%. In two cases of peroneal muscular atrophy (PMA), it is higher by 24% in Case No. 14 and is within the normal range in Case No. 13.

(A) Total Neutral Lipid (Table-II):

The content of total neutral lipid in adipose tissue of muscular dystrophy patients is higher by 29% in DMD, 56% in BD, 40% in LGD and 36% in myopathy. In PMA, it is higher

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DMD - Duchenne muscular dystrophy
BD - Becker dystrophy
LGD - Limb-girdle dystrophy
PMA - Peroneal muscular atrophy

TABLE - II

GROSS LIPID COMPOSITION OF ADIPOSE TISSUE IN NEUROMUSCULAR DISORDERS

Case No.	Patients (Age/Sex)	Total lipid mg/g wet weight	Neutral lipid mg/g wet weight	Phospholipid mg/g wet weight
Normal				
1.	20 F	698.2	697.492	0.708
2.	30 F	550.6	550.226	0.374
3.	64 F	462.7	462.014	0.686
4.	25 M	611.1	610.570	0.530
5.	28 M	545.6	544.464	1.136
6.	54 M	566.0	565.315	0.685
Mean(6)		572.4	571.680	0.596*
Duchenne dystrophy				
7.	8 M	739.0	737.970	1.030
Becker dystrophy				
8.	16 M	894.6	893.535	1.065
Limb-girdle dystrophy				
9.	23 M	777.8	776.698	1.102
10.	28 M	780.3	779.484	0.816
11.	40 M	853.8	852.844	0.956
Mean(3)		803.9	803.000	0.958
Myopathy				
12.	16 F	776.5	775.510	0.990
Peroneal muscular atrophy				
13.	28 M	692.0	691.026	0.974
14.	32 F	709.3	708.314	0.986

* Mean (5)

by 24% in Case No. 14, while it is within the normal range in Case No. 13.

Neutral Lipid Composition (Table-III):

Thin layer chromatography of neutral lipid fractions gave triacylglycerol as the major class. Faint bands corresponding to monoacylglycerol, diacylglycerol, free cholesterol and free fatty acids were obtained. The recovery of acylglycerols was about 80%. The band for esterified cholesterol could not be detected.

(a) Cholesterol: The normal human adipose tissue shows cholesterol content ranging from 0.7566 to 2.8970 mg/g wet weight. The content of cholesterol in dystrophy is higher by 352% in DMD, 472% in BD, 738% in LGD and 375% in myopathy. In PMA, it is higher by 352% in Case No. 13 and 435% in Case No. 14.

(b) Free Fatty Acids: The free fatty acid level in normal adipose tissue shows a range between 0.01 to 0.07 mg/g wet weight. In dystrophic human adipose tissue its content is higher by 201% in DMD, 435% in LGD and 1462% in myopathy. In PMA, free fatty acid level is high by 291% in Case No. 13 and 268% in Case No. 14, while it is within the normal range in BD.

(c) Total Acylglycerols: The range of total acylglycerol content in normal adipose tissue is 461 to 697 mg/g wet

TABLE - III

NEUTRAL LIPID COMPOSITION

Case No.	Patients (Age/Sex)	Cholesterol mg/g wet weight	Free-fatty acids mg/g wet weight	Total acyl glycerols mg/g wet weight
Normal				
1.	20 F	0.9143	0.0129	696.5648
2.	30 F	0.8116	0.0426	549.3718
3.	64 F	1.1533	0.0322	460.8285
4.	25 M	1.9100	0.0368	608.6232
5.	28 M	2.8970	0.0795	541.4875
6.	54 M	0.7566	0.0105	564.5479
Mean(6)		1.4070	0.0357	570.2372
Duchenne dystrophy				
7.	8 M	6.3543	0.1077	731.5080
Becker dystrophy				
8.	16 M	8.0533	0.0388	885.4429
Limb-girdle dystrophy				
9.	23 M	8.2112	0.1812	768.3056
10.	28 M	17.3155	0.1144	762.0541
11.	40 M	9.8364	0.2777	842.7299
Mean(3)		11.7877	0.1911	791.0298
Myopathy				
12.	16 F	6.6881	0.5583	768.2636
Peroneal muscular atrophy				
13.	28 M	6.3543	0.1397	684.5320
14.	32 F	7.5317	0.1315	700.6508

weight. In adipose tissue from dystrophic cases, it is higher by 28% in DMD, 55% in BD, 39% in LGD and 35% in myopathy. In PMA, it is within the normal range.

Acylglycerol Composition (Table-IV): The triacylglycerol forms the major part of total acylglycerols. The content of mono and diacylglycerols are comparatively insignificant both in normal and diseased conditions. Triacylglycerol shows an increase over normal by 28% in DMD, 55% in BD, 39% in LGD and 35% in myopathy. In PMA, it shows an increase by 23% in Case No. 14, and is within the normal range in Case No. 13.

(B) Total Phospholipid Content (Table-II):

Total phospholipid content shows a range of 0.374 to 0.708 mg/g wet weight in normal adipose tissue except case No. 5, which shows a high phospholipid content (1.136 mg/g wet weight). The percentage difference between normal and diseased cases has been worked out excluding the value for Case No. 5. There is an increase of 73% in DMD, 79% in BD, 61% in LGD and 66% in myopathy. In PMA, phospholipid shows an increase by 63% in Case No. 13 and 65% in Case No.14.

Phospholipid Composition (Table-V): Thin layer chromatography of phospholipid fractions gave three major classes viz., sphingomyelin, phosphatidylcholine and phosphatidylethanolamine. The recovery of lipid phosphorus was about 89%.

TABLE - IV

ACYLGLYCEROL COMPOSITION

Case No.	Patients (Age/Sex)	Monoacyl-glycerol mg/g wet weight	Diacyl-glycerol mg/g wet weight	Triacyl-glycerol mg/g wet weight
Normal				
1.	20 F	0.22	0.77	555.6
2.	30 F	0.18	0.62	438.4
3.	64 F	0.09	0.50	367.4
4.	25 M	0.19	0.67	485.1
5.	28 M	0.17	0.48	432.2
6.	54 M	0.18	0.63	450.2
Mean(6)		0.17	0.61	454.8
Duchenne dystrophy				
7.	8 M	0.23	0.81	582.9
Becker dystrophy				
8.	16 M	0.51	0.98	706.5
Limb-girdle dystrophy				
9.	23 M	0.24	0.85	614.5
10.	28 M	0.49	0.60	602.4
11.	40 M	0.27	1.20	672.4
Mean(3)		0.33	0.88	629.7
Myopathy				
12.	16 F	0.16	0.76	614.6
Peroneal muscular atrophy				
13.	28 M	0.23	1.10	446.1
14.	32 F	0.32	0.85	559.3

(a) Sphingomyelin: In normal human adipose tissue it constitutes 16.9 to 18% of total lipid phosphorus. There is an increase of sphingomyelin content by 31% in DMD, 29% in BD, 12% in LGD, 2.8% in myopathy and 2.3% in PMA.

(b) Phosphatidylcholine: It constitutes 40.6 to 42.3% of total lipid phosphorus in normal human adipose tissue. The phosphatidylcholine shows a decrease by 15% in DMD, 14% in BD, and 3% in LGD. In case of myopathy and PMA, its value is within the normal range.

(c) Phosphatidylethanolamine: The phosphatidylethanolamine constitutes 28.8 to 31.5% of total lipid phosphorus in normal human adipose tissue. Increased content of phosphatidylcholine has been found only in DMD (7%). In other cases, it is within the normal range.

Cholesterol/Phospholipid Molar Ratio (Table-VI):

In normal adipose tissue, the cholesterol/phospholipid molar ratio shows a range of 0.0886 to 0.2888. It is high in dystrophy, by 199% in DMD, 266% in BD, 528% in LGD and 227% in myopathy. In PMA, the ratio is increased by 216% in Case No. 13 and 270% in Case No. 14.

TABLE - V

PHOSHPHOLIPID COMPOSITION

Case No.	Patients (Age/Sex)	Sphingomyelin	Phosphatidyl-choline	Phosphatidyl-ethanolamine
Normal				
1.	20 F	17.8	40.6	30.5
2.	30 F	18.0	41.2	28.8
3.	64 F	16.9	40.8	31.5
4.	25 M	17.5	40.8	30.7
5.	28 M	17.2	42.3	29.5
6.	54 M	17.8	41.5	29.7
Mean(6)		17.5	41.2	30.1
Duchenne dystrophy				
7.	8 M	22.9	35.0	32.2
Becker dystrophy				
8.	16 M	22.5	35.6	31.5
Limb-girdle dystrophy				
9.	23 M	19.2	40.1	30.5
10.	28 M	19.4	40.2	30.4
11.	40 M	20.0	39.4	30.0
Mean(3)		19.5	39.9	30.3
Myopathy				
12.	16 F	18.0	41.1	29.8
Peroneal muscular atrophy				
13.	28 M	17.9	40.8	30.1
14.	32 F	17.9	41.5	29.9

Phospholipid contents are expressed as percentage of total lipid phosphorus.

TABLE - VI

CHOLESTEROL/PHOSPHOLIPID MOLAR RATIO OF ADIPOSE TISSUE IN NEUROMUSCULAR DISORDERS

Case No.	Patients (Age/Sex)	Cholesterol µmole/g wet weight	Phospholipid µ mole/g wet weight	Cholesterol/ Phospholipid ratio
Normal				
1.	20 F	2.3660	22.8616	0.1034
2.	30 F	2.1017	12.0698	0.1741
3.	64 F	2.9845	22.1588	0.1346
4.	25 M	4.9420	17.1066	0.2888
5.	28 M	7.4939	36.6481	0.2044
6.	54 M	1.9588	22.1057	0.0886
Mean(6)				0.1656
Duchenne dystrophy				
7.	8 M	16.4363	33.2250	0.4946
Becker dystrophy				
8.	16 M	20.8310	34.3700	0.6059
Limb-girdle dystrophy				
9.	23 M	21.2395	35.5500	0.5974
10.	28 M	44.7891	26.3250	1.7013
11.	40 M	25.4433	30.8500	0.8247
Mean(3)				1.0411
Myopathy				
12.	16 F	17.2997	31.9250	0.5418
Peroneal muscular atrophy				
13.	28 M	16.4363	31.4250	0.5230
14.	32 F	19.4818	31.8000	0.6126

DISCUSSION

Previous workers using human adipose tissue have recommended surgical removal rather than needle biopsy to minimize trauma to the tissue, as well as, need to assay the biopsy immediately after excision (Gries et al., 1967). Both of these recommendations have been followed in the present study. It has been suggested that within an individual of a given species, adipose tissue appears to be quite homogeneous and only minor differences, if any, are observed from one site to another (Hirch et al., 1960; Kingsbury et al., 1961; Jeanrenaud 1965). In the present study, control samples were obtained from abdominal region while, experimental samples were obtained from thigh region during normal course of muscle biopsy. There is no marked difference in the fatty acid pattern of adipose tissue between older and younger subjects (Hirsch et al., 1960); except in newly born, premature term infants. Furthermore, controlled dietary manipulations in adult life give rise to slow changes in adipose tissue. However, in most adult animals there seems to be some regulation of appetite so that lipid content of the body does not change rapidly (White et al., 1973).

Total Lipid:

In the present study, the range of total lipid content observed in normal human adipose tissue (463 to 698 mg/g wet weight) is in conformity with the earlier reports (Pawanet al.,

1960, Gellhorn et al., 1961, Kingsbury et al., 1961 and Jeanrenaud 1965). Recently (Pearce et al., 1981) reported much higher value (880 ± 56 mg/g wet weight) for total lipid content in normal human adipose tissue.

There is no report on the chemical composition of adipose tissue from various types of human neuromuscular disorders except in DMD by Banerjee et al.,(1983). In muscular dystrophy, the total lipid content (Table-II) shows an increase in DMD, BD and LGD. In LGD, an increase in total lipid is comparable with the duration of disease.

Total lipid increase has been reported in mouse muscle (Shull et al., 1958, Pearce et al., 1980), mouse sarcolemma (Dekretser et al., 1977), Chicken fragmented sarcoplasmic reticulum (HSU et al., 1971) and muscle (Chio et al., 1972). A similar increase in total lipid has been reported by Takagi et al.(1973) in sarcoplasmic reticulum in Becker dystrophy, limb-girdle dystrophy and neurogenic atrophy. In DMD, the total lipid content is normal in three out of four cases which shows a two fold increase. In the present study, in DMD, total lipid content shows an increase by 1.3 fold. In addition to muscle, an increase in lipogenesis has also been reported in other tissues of dystrophic mice eg. liver, kidney, spleen and skin (Robinowitz, 1960) and liver of dystrophic chicken (Vallynathan, 1976). Contrary to these reports,

lipogenesis has been reported to be depressed in liver and adipose tissue of cardimyopathic hamasters (Barakat et al., 1977 and Barakat et al., 1978).

Neurogenic involvement of muscular dystrophy is well documented (Dubowitz, 1971 and 1979). In the present study, out of two cases of PMA, Case No. 14 shows an increase in total lipid content by 24% compared to normal. It has been suggested that interruption in the orthosympathetic innervation of adipose tissue due to lesion in neural tissue, reduces lipolysis and enhances lipogenesis resulting in an increase of adipose tissue mass (Lefebvre et al., 1973).

Neutral Lipids:

The percentage of total neutral lipid content in normal adipose tissue (Table-II) is in conformity with earlier reports (Jeanrenaud et al., 1965 and White et al., 1973). In dystrophic human adipose tissue an increase in neutral lipid content is observed to be directly related to increase in triacylglycerol content (Table-VII). A similar increase in acylglycerols by 168% in dystrophic mice skeletal muscle (young et al., 1959), 6 fold increase in mouse sarcolemma (Dekretser et al., 1977), two fold increase in chicken fragmented sarcoplasmic reticulum (Hsu et al., 1971) and 15 fold increase in chicken muscle (Chio et al., 1972) have been reported. The mechanism for

increased lipid accumulation mainly in form of triacylglycerol in dystrophic muscle remains unexplained unless, it is related to the altered metabolism of muscle favouring lipogenesis (Lin et al., 1972) or, impaired ability of muscle mitochondria to oxidize fatty acids (Lin et al., 1972, Jato Rodriguez et al., 1972). Since, in man a negative correlation between the serum triacylglycerol level and adipose lipoprotein lipase activity has been found (Persson 1973), the increase in triacylglycerol content in serum from progressive muscular dystrophy patients (Kunze 1973) may lead to decrease in lipolysis and there by increase in triacylglycerol content in adipose tissue of these patients. It is well established that lipolysis in fat cells is regulated by C-AMP system and in human adipose tissue it has been shown that the rate of lipolysis is dependent on the C-AMP level (Arner et al., 1980). Thus abnormal lipid metabolism of various tissues in muscular dystrophy may be correlated with abnormal adenylate cyclase activity, as has been observed in dystrophic human muscle (Susheela et al., 1975, Mawatari et al., 1976), mice adipose tissue (Susheela et al., 1973) and erythrocyte membrane (Mawatari et al., 1976).

Cholesterol:

The results for cholesterol value in normal human adipose tissue (Table-III) correspond to the earlier reports

on adipose tissue from human (Khan et al., 1963 and Cherayil et al., 1981) and rat (Farakas et al., 1973). The non-saponifiable fraction in human adipose tissue is higher (1.9 - 13 mg) (Kingsbury et al., 1961 and Jeanrenaud et al.,1965) as compared to total cholesterol. In the present study band for esterified cholesterol could not be detected. Similar observation has been reported in normal rat (Farakas et al., 1973) and human (Cherayil et al., 1981) adipose tissue.

An increase in Cholesterol content (Table-III) has been observed in all cases of dystrophic and atrophic human adipose tissue under study. A similar increase in cholesterol content has been reported in adipose tissue (Banerjee et al., 1983) and muscle (Hughes 1972 and Hughes 1973) of DMD patients, as well as, in dystrophic mouse skeletal muscle (Shull et al., 1958, Young et al., 1959 and ownes et al., 1970) and dystrophic chicken FSR (Hsu et al., 1971).

Cholesterol synthesis in normal human adipose tissue is inversely related to plasma cholesterol concentration (Kovanen et al., 1977). The increased cholesterol content observed in the present study is perhaps due to its fall in plasma concentration in dystrophic patients (Kunze 1973) or, it is due to increased cholesterol synthesis as also

observed earlier in dystrophic mice viz., brain, liver, kidney, spleen, skin and muscle (Ribinowitz 1960).

Free Fatty Acids:

In dystrophic mice, free fatty acid content has been reported to be increased in pectoral and abdominal muscles, heart, liver and brain (Susheela et al., 1968a) and sarcolemma (Dekretser et al., 1977). It also shows an increase in human serum in muscular dystrophy (Kunze 1973). On the other hand, according to Susheela (1968 b) free fatty acid content is decreased in dystrophic mice adipose tissue while it is normal in dystrophic human muscle. In present study, free fatty acid content shows an increase in adipose tissue from patients of different neuromuscular disorders except in Case No. 8 (Table-III) where its content is normal. Banerjee et al (1983) reported that free fatty acids remains with in the normal range in adipose tissue of DMD patients.

Phospholipids:

There is a controversy with regard to the phospholipid content in various tissues in muscular dystrophy. Increased phospholipid content has been found in dystrophic chicken muscle (Chio et al., 1972), mouse sarcolemma Dekretser et al., 1977), plasma (Kwok et al., 1978) and muscle (Pearce et al., 1980) and in adipose tissue microsomes of dystrophic hamster (Barakat et al., 1980). On

the other hand, no change in total phospholipid content has been found in dystrophic chicken FSR (HSU et al., 1971), human muscle (Hughes 1972 and 1973), mouse fore brain and spinal cord while, it is reported to be decreased in mouse sciatic nerve (Kwok et al., 1978).

Banerjee et al., (1983) have shown a decrease in the phospholipid content in DMD. In the present study, phospholipid content has been found to be high in all the cases of dystrophy including DMD. Phospholipid composition of normal human adipose tissue (Table-V) is comparable to earlier reports on adipose tissue of human (Pearce et al., 1981), mouse (Spencer et al., 1962) and Pig, Beef and rat (Grigor et al., 1972).

Sphingomyelin shows an increase and phosphatidylcholine decreases in DMD, BD and LGD while, phosphatidylethanolamine increases only in DMD. In other neuromuscular disorders the changes are less apparent. This finding is similar to earlier reports that sphingomyelin and phosphatidylethanolamine increase while, phosphatidylcholine decreases in mouse muscle (Hughes 1965, Hughes 1972, owens et al., 1970, Pearce et al., 1980), chicken muscle (Chio et al., 1972), chicken FSR (Hsu et al., 1971), Duchenne erythrocytes (Kunze et al., 1973, Kalofoutis et al., 1977), DMD muscle (Pearce et al., 1981) and adipose tissue (Banerjee et al., 1983).

A slight increase in sphingomyelin and a slight decrease in phosphatidylcholine and phosphatidylethanolamine content of adipose tissue in dystrophic hamaster has also been reported (Barakat et al., 1980). On the other hand, it has been reported that sphingomyelin decreases in brain, sciatic nerve, plasma and sarcolemma of dystrophic mice while increases in spinal cord. Phosphatidylcholine increases in all these tissues while, phosphatidylethanolamine increases in brain, sciatic nerve and in sarcolemma, and decreases in spinal cord, as well as, in plasma (Dekretser et al., 1977) and Kwok et al., 1978).

This altered composition of phospholipids may be correlated with the well known membrane abnormality in these disorders (Rowland 1979 a), since many properties of membrane functions are thought to depend upon the structure, organisation and fluidity of membrane lipids (Lucy 1980). Phosphatidylcholine is important not only as structural component but also plays a significant role in function of some membrane bound enzymes (Sandermann 1978). It has been shown that many membrane bound enzymes require phospholipid for full enzymatic activity (Jain et al., 1980); and that for some enzymes the polar head group structure may be more important for activity than hydrophobic portion of amphiphile (Rice et al., 1979 and Dean et al., 1981). Difference in the head group composition of phospholipid bilayers can

change the fluidity (Schinitzky et al., 1974) and Hirata et al., 1978) of membrane.

Very little attention has been paid towards adipocyte membrane of dystrophic animals and patients. Mably et al., (1981) reported abnormal glucose transport (i.e. altered membrane) in adipocytes of myotonic patients. Since, phospholipids are mainly found in membranes, their alteration would reflect a membrane defect.

Cholesterol/Phospholipidmolar Ratio:

The cholesterol/Phospholipid molar ratio in normal human adipose tissue (Table-VI) is comparable to its molar ratio in mitochondria, microsomes and plasma membrane of rat adipose tissue (Farakas et al., 1973).

In the present study, this ratio has been found to be high in dystrophic and atrophic human adipose tissue. High cholesterol/phospholipid molar ratio is due to a large increase in cholesterol content. Its higher ratio in membrane gives structural stability and rigidity (Farakas et al., 1973). The presence of cholesterol can inhibit the interaction of many proteins with phospholipids and these inhibitory effects of cholesterol are the result of decreased molecular motion of acyl chains, which makes the deformation or penetration of the bilayer by proteins energetically unfavourable (Papahadjopoulos 1976).

High cholesterol/phospholipid molar ratio of adipose tissue in dystrophic patients thus apparently suggests increased rigidity of adipocyte membrane. This finding is in contrast to the earlier report of increased fluidity in dystrophic erythrocyte membrane (Butter field 1977, Sato et al. 1978, Butterfield 1981), but may be paralleled with increased rigidity of erythrocyte plasma membrane in chicken muscular dystrophy (Butterfield 1978) and reduced deformibility of Duchenne erythrocytes (Lumb et al., 1975), Percy et al., 1975 and Brain et al., 1978).

SUMMARY

The lipid composition of subcutaneous adipose tissue from cases of human progressive muscular dystrophy and peroneal muscular atrophy have been studied. (Table-VII).

Lipids were extracted from biopsies of adipose tissue with chloroform : methanol 2:1. They were subjected to silicic acid column chromatography to separate phospholipid and neutral lipid. These fractions were further fractionated by thin layer chromatography into their respective components. Each component was quantitatively assayed.

Total lipid content of adipose tissue was high in all the cases of progressive muscular dystrpphy as a result of a large increase in neutral lipid and a smaller increase in total phospholipid. Further analysis of neutral lipid fraction showed that triacylglycerol which forms major fraction of lipid in both normal and diseased human adipose tissue is the main contributing factor for increase of total lipid in adipose tissue of these patients. The relative composition of phospholipid fraction showed an increase in sphingomyelin and phosphatidylethanolamine content while, phosphatidylcholine was decreased. In one case of 'myopathy'(Case No. 12) of unknown aetiology, the total lipid content was high and the relative composition of phospholipid fraction, was within the normal range. In

peroneal muscular atrophy, total lipid content and relative composition of phospholipid fraction were within the normal range, while, the total phospholipid content was high.

Cholesterol/Phospholipid molar ratio was found to be high in adipose tissue in all the cases under study.

The possible significance of these changes in lipid composition in neuromuscular diseases has been discussed in relation to abnormality in membrane, as well as, lipid metabolism in light of the contemporary research findings.

It is premised that in muscular dystrophy, alterations in lipid composition and metabolism of various tissue systems suggest that the defect lies at the gene level which affects various systems of the body while, muscle is only a symptomatic tissue of this disorder.

TABLE - VII

LIPID COMPOSITION OF ADIPOSE TISSUE IN NEUROMUSCULAR DISORDERS

LIPID COMPOSITION (Mg/g wet wt)	NORMAL		CLINICAL CASES					
	(Mean-6)	Range	Duchenne-dystrophy	Becker dystrophy	Limb-girdle dystrophy (mean -3)	Myopathy	Peroneal muscular atrophy	
							Case No. 13	Case No. 14
Total lipid	572.4000	462.7000-698.2000	739.0000	894.6000	803.9600	776.5000	692.0000	709.3000
I. Neutral lipid	571.6800	462.0140-697.4920	737.9700	893.5350	803.0000	775.5100	691.0260	708.3140
(A) Total acyl-glycerols	570.2372	460.8285-696.5648	731.5080	885.4429	791.0298	768.2636	684.5320	700.6508
(a) Monoacyl-glycerol	0.1700	0.0900-0.2200	0.2300	0.5100	0.3300	0.1600	0.2300	0.3200
(b) Diacyl-glycerol	0.6100	0.4800-0.7700	0.8100	0.9800	0.8800	0.7600	1.1000	0.8500
(c) Triacyl-glycerol	454.8000	367.4000-556.6000	582.9000	706.5000	629.7600	614.6000	446.1000	559.3000
(B) Cholesterol	1.4070	0.7566-2.8970	6.3543	8.0533	11.7877	6.6881	6.3543	7.5317
(C) Free-fatty acids	0.0358	0.0105-0.0795	0.1077	0.0388	0.1911	0.5583	0.1397	0.1315
II. Phospholipid (n = 5)	0.5966	0.3740-0.7080	1.0300	1.0650	0.9580	0.9900	0.9740	0.9860
(As % of total lipid phosphorus)								
(a) Sphingomyelin	17.5000	16.9000-18.0000	22.9000	22.5000	19.5000	18.0000	17.9000	17.9000
(b) Phosphatidyl-choline	41.2000	40.6000-42.3000	35.0000	35.6000	39.9000	41.1000	40.8000	41.5000
(c) Phosphatidyl-ethanolamine	30.1000	28.8000-31.5000	32.2000	31.5000	30.3000	29.8000	30.1000	29.9000
III. Cholesterol/ Phospholipid-molar ratio.	0.1657	0.0886-0.2888	0.4946	0.6059	1.0411	0.5418	0.5230	0.6126

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