Lipid Changes in Neurogenic Muscular Atrophies

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Received April 23, 1974

Summary. Lipid composition of muscles of 14 patients with neurogenic muscular atrophies was determined, and compared statistically to that of 8 normal controls. The neurogenic muscular atrophies show no alteration in the lipid content, but single phospholipid fractions were modified. The lecithin content was significantly diminished in patients with neurogenic muscular atrophy, particularly in the chronic and infantile-juvenile forms, whereas sphingomyelin and phosphatidylserine were increased, but not significantly.

The lecithin decrease, described previously in progressive muscular dystrophy (Duchenne), does not appear to be specific but may simply indicate muscular degeneration.

Key words: Neuromuscular diseases — Muscle — Lipids — Phospholipids.

Phospholipid abnormalities, chiefly a decrease in lecithin content and an increase in sphingomyelin content, have been demonstrated in hereditary muscular dystrophy of the mouse [1, 2] and also in patients with Duchenne muscular dystrophy [3, 4]. It was also shown both in mouse [5] and man [6, 7] that the alterations in whole tissue phospholipid composition were due mainly to changes in a muscle microsome fraction, which is probably sarcoplasmic reticulum.

This lecithin decrease, together with changes in the fatty acid pattern [8], can be considered in connection with the phospholipid content and integrity of membranous structures of the muscle cell; in fact, a lecithin deficit in biomembranes in muscular dystrophy might be either a primary consequence of the enzyme genetic defect or the cause of the impaired calcium uptake by sarcoplasmic reticulum [3, 5, 7, 9]. However, because similar phospholipid changes occur in both human Duchenne and mouse dystrophy, they are clearly not specific but may indicate the presence of common pathological mechanisms in muscular degeneration. Consequently, it would be of interest to know whether such changes occur in other forms of human neuromuscular disease.
There have been few studies involving phospholipid analysis from neurogenic muscular atrophies and these have not demonstrated any significant abnormality [3, 8], although the number of patients investigated was too small for conclusions to be drawn.

The aim of the work reported here was to assess the lipid composition of muscles of patients with neurogenic muscular atrophy and to compare it statistically with that of normal human muscles.

**Methods**

**Selection of Patients**

14 male patients, aged between 10 and 50 years, with different clinical forms of neurogenic muscular atrophy were selected at the Neurological Clinic, University of Milan, according to clinical, histological and electromyographic parameters of intergroup and intragroup homogeneity. These patients were divided into four groups: those with chronic neurogenic amyotrophy (8 patients), infantile-juvenile neurogenic amyotrophy (3 patients), atrophic lateral sclerosis (2 patients) and Charcot-Marie-Tooth disease (1 patient). All patients were undergoing their first admission to hospital, and their disease was at an early stage, the chronic forms not being of longer duration than about 2 years. Thus, muscles selected for analysis were not affected seriously, so that contamination by lipid from other sources, such as fat cells or connective tissue, was minimal. This was confirmed by histological and histoenzymatic examination which showed that fatty infiltration was not marked. In addition, no patient had received drug treatment prior to investigation. Muscle used for analysis was removed from the patients by biopsy from deltoid muscle (8 patients) or quadriceps muscle (6 patients). Muscle specimens obtained in the course of routine orthopaedic operations from quadriceps muscle of 8 subjects free from any neurological or muscular abnormalities were used as controls.

**Chemical Analysis**

Muscle specimens (0.2—0.5 g) were homogenised in a Potter tube with physiological saline (10 ml NaCl 0.90%; 1 g tissue). Lipids were extracted in chloroform-methanol (2:1; v/v) according to the method of Folch et al. [10]. After separation of the organic phase at 4°C, this was evaporated, vacuum-packed, under nitrogen flow in a rotary evaporator (Rotavapor R. Bucki). The lipids thus obtained were dissolved in a known volume (1 ml/1 g tissue) of chloroform-methanol (2:1, v/v) and were then analysed using thin-layer chromatography (TLC) on silical gel H and magnesium acetate (5%) (Merck).

Monodimensional TLC was used for analysis of neutral lipids, the solvent system being hexane/ethyl ether/acetic acid (70/30/1 by vol.). The lipid spots were detected after exposure to iodine vapour, individually collected and dissolved in a known volume of isopropyl alcohol. After centrifugation (3000 rpm, 20 min), the upper phase was collected and lipids were determined by an Autoanalyser C. Erba (mod. C. L. A.) [11, 12]. The recovery was 95—100%. Two-dimensional TLC was used for analysis of phospholipids; the solvent mixture for the first run was chloroform/methanol/ammonia (65/40/7 by vol.), and for the second chloroform/acetone/methanol/acetic acid/water (5/2/1/1/0.5 by vol.), following the procedure of Rouser et al. [13, 14].