Enzyme activity of rat tibialis anterior muscle differs between treatment with triamcinolone and prednisolone and nutritional deprivation

Abstract  The maximal activity of a selection of enzymes involved in muscle carbohydrate handling, citric acid cycle and fatty acyl β-oxidation were studied after treatment with the fluorinated corticosteroid triamcinolone and compared to a similar treatment of the non-fluorinated corticosteroid prednisolone in an equipotent anti-inflammatory dose. Furthermore, because triamcinolone causes loss of body mass and muscle wasting, the effects of triamcinolone were investigated relative to a control group, with the same loss of body mass, due to nutritional deprivation. The study was performed in male Wistar rats in the following treatment groups: TR, triamcinolone treatment (0.25 mg · kg⁻¹ · day⁻¹ for 2 weeks), which resulted in a reduction of body mass (24%); ND, nutritional deprivation (30% of normal daily food intake for 2 weeks) resulting in a similar (24%) decrease of body mass as TR; PR, prednisolone treatment (0.31 mg · kg⁻¹ · day⁻¹ for 2 weeks), with a 10% increase in body mass; FF, free-fed control group, with a 12% increase in body mass in 2 weeks. Compared to FF, TR induced an increase in phosphofructokinase (PFK) activity (P < 0.01), glycogen synthase [GS(i + d)] activity (P < 0.05) and glycogen content (P < 0.01) in the tibialis anterior muscle. The PR and ND caused no alterations in PFK or citrate synthase (CS) activity compared to FF. Compared to PR, TR induced an increase in PFK (P < 0.01), CS (P < 0.05) and GS(i + d) activity (P < 0.01). Both TR and PR caused an increased muscle glycogen content, being more pronounced in TR (P < 0.05). Compared to ND, TR induced an increased CS (P < 0.05) and GS(i + d) activity (P < 0.01) and glycogen content (P < 0.01). The ND resulted in a decreased glycogen content compared to FF (P < 0.05). None of the treatments affected the activity of glycogen phosphorylase, β-hydroxyacyl coenzyme A dehydrogenase and lactate dehydrogenase. It was concluded that corticosteroids led to an increased muscle glycogen content; however, the changes in the enzymes of carbohydrate metabolism were corticosteroid type specific and did not relate to undernutrition, which accompanied the triamcinolone treatment.

Key words  Prednisolone · Triamcinolone · Nutritional deprivation · Muscle enzyme activity · Glycogen

Introduction  Glucocorticoids have been commonly used as anti-inflammatory agents in the treatment of chronic obstructive pulmonary disease (COPD; Ferguson 1993). It has been found that patients with COPD often suffer from respiratory and peripheral skeletal muscle weakness (Gosselink et al. 1996; Hamilton et al. 1995; Tobin 1988). The cause of this muscle weakness in COPD is not yet clear, but among others, muscle wasting related to nutritional depletion (Engelen et al. 1994) and corticosteroids (Decramer et al. 1994) have been suggested as contributing factors. Corticosteroids can be fluorinated to increase their anti-inflammatory potency. However, it has been shown that this results in more severe systemic effects than non-fluorinated corticosteroids (Thalen et al. 1989). It has been demonstrated in studies on experimental animals that the fluorinated corticosteroid triamcinolone causes muscle wasting and predominantly type IIx/b...
atrophy (Dekhuijzen et al. 1995; Koerts-de Lang et al. 1998; Petrof et al. 1995). In addition to histochemical changes, triamcinolone exerts many alterations in muscle energy metabolism, which can also be associated with alterations in muscle function. It has been noticed that triamcinolone causes an increase in glycogen concentration in muscle but the exact mechanism of action of triamcinolone on muscle carbohydrate handling is still a matter of debate. Evidence for increased glycogenesis after triamcinolone treatment has been found, associated with increased lactate concentrations (Shoji et al. 1974). In addition, glutamine release of the rat hindquarter has been observed to be increased (Koerts-de Lang et al. 1995; Welbourne 1988), which implies protein catabolism. Furthermore, triamcinolone has even been shown to cause in vitro uncoupling of oxidative phosphorylation and respiration (Peter et al. 1970), which will probably be reflected in changed muscle enzyme capacities.

The question that remains to be addressed is whether changes in muscle metabolism after triamcinolone treatment are induced by

1. Muscle wasting, related to loss of body mass, or
2. Corticosteroid treatment per se, as studied in a prednisolone group.

We hypothesized that triamcinolone-induced muscle atrophy and increased glycogen content is associated with alterations in the activity of muscle enzymes, involved in carbohydrate and β-oxidation. In an earlier study we have demonstrated that a similar administration of the non-fluorinated corticosteroid prednisolone in an equivalent glucocorticoid dose (Goodman and Gillman 1990) did not affect muscle mass (Koerts-de Lang et al. 1997). Since triamcinolone has been shown to cause type IIx/b atrophy, a muscle with predominantly type II fibres was studied – the tibialis anterior muscle.

### Methods

#### Animals

Male Wistar rats (n = 40) were treated during 2 weeks. The initial body mass of these animals, aged 12 weeks, ranged from 314 to 345 g. The animals were randomly allotted to four groups, a triamcinolone treated group (TR: 0.25 mg · kg⁻¹ · day⁻¹ triamcinolone acetate i.m., which had an ad libitum food intake, n = 10) and three reference groups. Because in previous studies the amount of muscle wasting after triamcinolone treatment has not been explained by the diminished food intake alone (Gardiner et al. 1980) but also by an increased energy expenditure (Koerts-de Lang et al. 1998), we did not introduce a pair-fed group, but introduced a nutritionally deprived group (ND: 0.05 ml · day⁻¹ saline i.m.), receiving a food intake of 30% of normal, calculated to result in a comparable reduction in body mass (n = 10). A prednisolone treated group was included which received an equipotent anti-inflammatory dose to triamcinolone (PR: 0.31 mg · kg⁻¹ · day⁻¹ prednisolone i.m.; n = 10), with an ad libitum food intake. Thirdly, a free-fed group (FF: 0.05 ml · day⁻¹ saline i.m.) was included which had an ad libitum food intake (n = 10). The corticosteroids dose chosen was comparable to that used in patients with COPD as maintenance therapy or during acute exacerbations.

The rats were anaesthetized with Nembutal (6 mg · 100 g⁻¹ body mass, i.p.) after short-term (< 20 s) CO₂ sedation. The tibialis anterior muscle was dissected, immediately weighed and freeze-clamped in liquid nitrogen and stored at −80°C until analysis. The muscle was homogenized (10% w/v) in SET-buffer by use of an Ultra-Turrax T25 tissue homogenizer (Janke and Kunkel, GmbH and Co., KG) for four 5-s intervals. At 0°C, the homogenization process was completed by treatment with ultrasonic sound (Sonoprep 150, MSE) for four 15-s intervals. Total protein was assayed from this homogenate using bichinonic acid as has been described earlier (Brown et al. 1989; Smith et al. 1985). For enzyme determination, the homogenate was centrifuged (4°C) for 10 min at 2100 g (3500 rpm) and supernatant was assayd for lactate dehydrogenase (LDH, Enzyme Commission no., EC 1.1.1.27; Bergmeyer 1974a), phosphofructokinase (PFK, EC 2.7.1.11; Ling et al. 1966), citrate synthase (CS, EC 4.1.3.7; Shepard and Garland 1969) and 3-hydroxacyl coenzyme-A dehydrogenase (HAD, EC 1.1.1.35; Bradshaw 1975) activities using a Cobas Bio centrifugal analyser (La Roche, Basel, Switzerland). Glycogen synthase (GS) activity was assayed on a centrifugal analyser (Cobas Fara, Hoffman La-Roche, Basel, Switzerland) in the muscle homogenate at 30°C. To discriminate between the active GS(i) part of GS and total GS activity GS(i + d), GS(i) activity was assayed in the absence of glucose-6-phosphate (G6P) while G6P (10 mmol · 1⁻¹) was added to the homogenate to assay total GS activity.

Uridine diphosphate (UDP)-glucose was used as starting reagents and formation of UDP was measured according to Danforth (1965). Glycogen phosphorylase (GP) activity was assayed according to Schreiber and Bowling (1990) on a centrifuge analyser and expressed as micromoles per minute per gram wet mass. Both the total GP(a + b) and active form GP(a) of GP were assessed. Part of the muscle was dissolved for 1 h in 1 mol · 1⁻¹ NaOH (37°C), precipitating glycogen with 96% ethanol (10 min at 80°C followed by overnight precipitation at 4°C). Subsequently the pellet was hydrolyzed using 1 mol · 1⁻¹ HCl at 100°C for 3 h after which HCl was neutralized with a KCl saturated KOH/TRIS (2.1 mol · 1⁻¹/ 0.12 mol · 1⁻¹) buffer. Glycogen derived glycosyl units were determined using a glucose kit (hexokinase method, Roche) for a centrifuge analyser. Glycogen was analysed on a centrifuge analyser (Cobas Fara, Hoffman La-Roche) and expressed as micromole glycosyl units per gram wet mass.

#### Statistical analysis

Differences between all groups were first detected with the Kruskall Wallis test and then assessed using the Mann-Whitney U-test, because of the non-normal distribution. Level of significance was determined as P equal to or less than 0.05. Analyses were performed using the SPSS/PC + (Wesssex, Inc, Winnetka, IL, USA) program.

#### Results

Animal food intake, body and muscle mass

Food intake of all the rats was comparable before the experimental period (± 21 g · day⁻¹). During treatment,