Corticosteroid and nutrition induced muscle wasting in a rat model

8

Summary and general discussion

8.1. Introduction

The studies described in this thesis were designed to discriminate between the effects of corticosteroids and undernutrition on muscle functioning, as determined by muscle mass, morphology and muscle metabolism. Our aim was to investigate whether the metabolic pathways and the functional and structural consequences of muscle wasting are comparable between these two situations. The current literature on this particular subject reveals many unclarities and omissions. Firstly the results of our studies will be framed in perspective of the literature; thereafter the results regarding the differentiation between corticosteroids and undernutrition will be discussed.

8.2. Effects of undernutrition on muscle mass, morphology, contractility and metabolism

In all the studies reported in the present thesis, it was shown that undernutrition resulted in muscle mass reduction of both peripheral skeletal and respiratory muscles. However in contrast to diaphragm muscle wasting, wasting of peripheral skeletal muscles was less pronounced than body wasting. As mentioned in chapter 1, muscle wasting of the diaphragm was accompanied by an overall fibre type atrophy pattern (1-5), which was confirmed by our results in chapter 2. However in chapter 3, atrophy was limited to type IIx/b fibres under conditions of less severe and shorter undernutrition.
As earlier observed in *in vitro* diaphragm bundles after nutritional deprivation, existence of a decreased fatigability (1-4), an increased half-relaxation time (1) and a leftward shift of the force-frequency curve was confirmed in chapter 2 (1, 2). In addition, an increased time to peak tension was observed, which was not reported earlier. Considering the fact that the cross-sectional area of type I fibres is relatively increased in the diaphragm (chapter 2)(1), it is not surprising that *in vitro* diaphragm fatigability was decreased and that half-relaxation time and time to peak tension were increased. However, *in situ* muscle contractility of dorsiflexor muscles showed only a reduced external work as a consequence of the diminished muscle mass, while normalised for muscle mass, no changes were observed (chapter 4). This could imply different muscle atrophy patterns between the diaphragm muscle and the studied peripheral skeletal muscle. This is however not likely, since other studies have found similar effects of undernutrition on the diaphragm and peripheral skeletal muscles, like a generalised muscle fibre atrophy (1, 3). Therefore, other alterations have to be considered, as muscle metabolism and also motor unit characteristics.
In our studies muscle metabolism of both the diaphragm and peripheral skeletal muscles after chronic undernutrition was investigated. In chapter 4, it was confirmed that glycogen levels in the peripheral skeletal muscle after undernutrition are decreased (6). However, other studies also showed alterations in muscle enzyme activities involved in carbohydrate and β-oxidation (7, 8). In chapter 5 unchanged PFK, LDH, CS and HAD activities were found in the peripheral skeletal muscle. No muscle enzyme activities were measured in the diaphragm. Lactate concentrations were found to be unchanged in the diaphragm after undernutrition, while in the peripheral skeletal muscle an increased lactate level was observed (data not published), which was also reported by others (8, 9).
In addition, muscle energy status was studied. Earlier studies observed decreased PCr levels associated with maintained ATP levels in the peripheral skeletal muscle after undernutrition (8-10). In chapter 3 as well, a decreased energy metabolism in the diaphragm was found as shown by decreased PCr levels and total adenine nucleotides. However, a decreased diaphragmatic ATP concentration could also be demonstrated. This has not been reported before in the diaphragm. In addition, total creatine pool was maintained, suggesting the establishment of a new metabolic equilibrium, with lower ATP and PCr levels. This was accompanied by an increased creatine concentration. A similar tendency was also observed earlier in the gastrocnemius muscle (10). This increase could be caused by an increased Cr

and ATP production from PCr in order to satisfy the need for ATP. In future research it would be interesting to compare muscle energy metabolism after undernutrition between the peripheral skeletal muscle and the diaphragm, since in contrast to the findings in the diaphragm muscle in chapter 2, ATP levels were never found to be decreased (8-10) and lactate levels were increased in peripheral skeletal muscles (8, 9).
In order to obtain more insight in muscle amino acid metabolism, muscle glutamine metabolism was studied in the peripheral skeletal muscle. This conditionally essential amino acid is an important nitrogen and ammonia carrier in interorgan metabolism (11). Little information is available on muscle glutamine metabolism after chronic undernutrition. However, an increased glutamine concentration in the gastrocnemius muscle was demonstrated after 2 days of undernutrition (12), and confirmed in chapter 6 of this thesis. Additionally, glutamine efflux from the hindquarter was studied in chapter 6 and found to be increased, as well as glutamine *de novo* production and membrane transport rates. However in the diaphragm, unchanged intramuscular glutamine levels were found after undernutrition and basal intramuscular glutamine levels were twice as high as in the gastrocnemius (data not published). This suggests different muscle glutamine metabolism between the diaphragm and the peripheral skeletal muscle. Further research on this phenomenon is needed.
It can be concluded from these studies that muscle wasting induced by undernutrition differs between the peripheral skeletal muscle and the diaphragm. More information could be obtained in future research, studying simultaneously diaphragm and peripheral skeletal muscle energy metabolism. However, it should be realised that amino acid and substrate fluxes cannot (yet) be studied across the diaphragm muscle.

8.3. Effects of corticosteroids on muscle mass, morphology, contractility and metabolism

Intramuscular *prednisolone* treatment in the studies of the present thesis did not result in a decrease in body and muscle mass (chapter 4). After intramuscular or intraperitoneal *prednisolone* administration no body or muscle mass reduction was reported earlier (13, 14). Reductions in body and muscle mass were however observed after subcutaneous *prednisolone* administration (15-17). The observed absence of muscle wasting after i.m. *prednisolone* treatment was accompanied by minor alterations in *in situ* peripheral skeletal muscle contractility, as an increased recovery during a rest period. This implies that muscle wasting is not related to muscle recovery.
More striking effects were found after *triamcinolone* treatment. It was clearly demonstrated that *triamcinolone* treatment resulted in loss of body and muscle mass. Strikingly, wasting of the tibialis anterior and the gastrocnemius muscle was more pronounced than body wasting. This was also found earlier after *triamcinolone* treatment in the gastrocnemius (13, 18). Diaphragm muscle mass was however decreased to a comparable extent than body mass (chapter 2+3) as observed earlier (1, 13, 19). In chapter 2, the effects of muscle mass reduction on muscle fibre atrophy was described. Atrophy of type IIa and IIx/b fibres in the diaphragm was found after 0.5 mg *triamcinolone* per kg body weight during 4 weeks. Other studies have reported only type IIx/b atrophy of the diaphragm after a similar treatment of *triamcinolone* of 6 weeks (1). Even after a shorter *triamcinolone* treatment with a higher dose, both type I and IIx/b fibre atrophy was observed (20). This variation in atrophy pattern may be partly caused by the complications of corticosteroid treatment. During our study and that of Dekhuijzen and colleagues (1), many *triamcinolone*-treated rats died, while Petrof and coworkers (20) prevented corticosteroid-related pneumonia and reported no deaths. It is likely that high dose *triamcinolone* treatment induced also infections (like pneumonia), which may also affect the muscle atrophy pattern. Anyway, it is clear that at least fibre IIx/b atrophy occurred after *triamcinolone* treatment. In chapter 3, we have found only type IIx/b atrophy in the diaphragm after *triamcinolone* treatment with a lower dose and of shorter duration than in chapter 2. This was also supported by earlier studies (1, 13, 21).

The observed *triamcinolone*-induced atrophy of both type IIa and IIx/b fibres resulted in a decreased *in vitro* diaphragm fatigability, an increased half-relaxation time as well as a leftward shift of the force-frequency relationship (chapter2), in accordance with earlier studies (1, 13, 21). This could be the consequence of a relative increase in cross-sectional area of type I fibres. In addition, *in situ* muscle function of peripheral skeletal muscles was studied. The absolute external work was reduced, caused by muscle mass loss. However, normalised for muscle mass, an increased external work was found. Furthermore, muscle fatigue was diminished, in line with diaphragm *in vitro* contractility and in contrast to hindlimb muscle fatigue after *prednisolone* treatment (17). As discussed earlier, this decreased fatigue could be caused by a relative increase of type I fibre cross-sectional area, since type I fibres are more fatigue resistant. Furthermore, during a 5 minute rest period in between the stimulation sessions, *triamcinolone* rats fully recovered. This phenomenon was also found for the *prednisolone* rats.

Ferguson and coworkers (22) observed only in the diaphragm increased glycogen levels after cortisone treatment. However in this thesis, we found increased glycogen stores in the peripheral skeletal muscle both after *triamcinolone* and *prednisolone* treatment. This increased muscle glycogen concentration did not seem to be related to alterations in muscle function, except for the abovementioned increased muscle recovery. An increased glucose influx was observed in the hindquarter muscles after *triamcinolone* treatment in chapter 6, but data on glucose muscle influx after *prednisolone* treatment are not yet available. This increased glucose muscle influx is very surprisingly, since other studies have demonstrated a decreased glucose uptake by the muscle after dexamethasone treatment (23). However, the increased glucose muscle influx after *triamcinolone* administration could very well contribute to the increased muscle performance of the peripheral skeletal muscle. In addition, Lieu and colleagues (16) observed after *prednisolone* treatment, in the peripheral skeletal muscle a decreased PFK activity. However, we could not confirm changes in PFK activity after *prednisolone* treatment in the peripheral skeletal muscle. After *triamcinolone* treatment however, PFK activity was increased, in line with an increased glycolysis. In addition, oxidative capacity was also found to be increased after *triamcinolone* in contrast to *prednisolone*. We furthermore confirmed for the diaphragm after *triamcinolone* treatment, the earlier observed increased intramuscular pyruvate and lactate levels after corticosteroid treatment (22, 24), suggesting also in the diaphragm an increased glycolysis. The increased glycolysis could be related to the increased glucose influx.

To the best of our knowledge, no data are available in literature on diaphragm muscle energy metabolism after *triamcinolone* treatment. We found decreased levels of ATP, PCr and total adenine nucleotides and creatine pools (chapter 3), suggesting a metabolic impairment. This could be related to the observed increased glycolysis, but further research is needed.

As mentioned earlier, a disproportional loss of peripheral skeletal muscle mass to body mass was observed after *triamcinolone* treatment. Therefore muscle amino acid metabolism was studied in the peripheral skeletal muscle after *triamcinolone* treatment. We demonstrated an increased intramuscular glutamine concentration only after acute triamcinolone treatment (chapter 6), in contrast to earlier studies. A decreased intramuscular glutamine concentration was found after corticosteroid treatment in the peripheral skeletal muscle (24-27), except for the soleus (25). Intramuscular amino acid concentrations by themselves however, do not give any information about muscle uptake or release. Therefore, it is necessary to study amino acid fluxes. Increased glutamine and phenylalanine efflux from the hindquarter was observed in this thesis after chronic *triamcinolone* treatment. This observation is in line with previously reported data (24, 27). This indicates net muscle degradation and metabolic stress. These effluxes were accompanied by an increased glutamate influx, as well as a decreased BCAA influx. Glutamate is probably a precursor for glutamine production, since glutamine *de novo* production was found to be increased. The decreased BCAA influx implies no precursor activity of the BCAAs for glutamine production. In addition we

demonstrated increased glutamine membrane transport rates in the muscle after *triamcinolone* treatment. The latter finding as well as the decreased BCAA influx were not reported previously.
The action of corticosteroids is mediated by glucocorticoid receptors. Two functionally distinct receptor forms α and β have been demonstrated in human tissues (28). The β GR form is considered as a dominant negative inhibitor of the α form (28, 29). Several studies have shown that the α GR is downregulated in lung cells and peripheral lymphocytes after exposure to corticosteroids (30-32). However, little is known about the autoregulation of the β form. In rats, no information is available about the existence of the β GR in skeletal muscles. In chapter 7, the presence of both the α and β glucocorticoid receptor mRNA forms in the rat gastrocnemius muscle was described. These two GR forms were found to be downregulated in the muscle even after corticosteroid inhalation, suggesting a systemic effect in the peripheral skeletal muscle after this local corticosteroid administration. It is to be expected that similar effects could be found after systemic administration, but further research is needed to study GR mRNA expression under these conditions.
In conclusion, we observed different effects of *prednisolone* and *triamcinolone* in an equipotent dose and similar treatment, on muscle wasting, function and metabolism. Both corticosteroids were found to be associated with increased muscle glycogen concentrations and an increased recovery of the peripheral skeletal muscle in between two stimulation sessions. In addition, we observed no muscle wasting and alterations in peripheral skeletal muscle performance and metabolism after *prednisolone* treatment. *Triamcinolone* treatment however, was accompanied by muscle wasting of both the diaphragm and peripheral skeletal muscles, associated with type IIx/b atrophy of the diaphragm and a decreased fatigability of the diaphragm and peripheral skeletal muscles. In addition an increased glycolytic and oxidative capacity was found. Furthermore, decreased ATP, PCr and total adenine nucleotides and creatine pools were observed, suggesting metabolic impairment. Muscle protein metabolism was also disturbed, as indicated by an increased glutamine efflux from the muscle and an increased glutamine *de novo* production and membrane transport rates.

8.4. Differentiation between corticosteroids and undernutrition

During our first experiment it was shown that muscle wasting induced by triamcinolone treatment, was a consequence of an increased energy expenditure not adequately restored by dietary intake, which takes us to the basic difference between triamcinolone treatment and nutritional deprivation. Triamcinolone treatment showed acutely a temporarily anorexia accompanied by hypermetabolism, which was also found chronically. Therefore, one control group was matched for the temporarily diminished food intake and one for the diminished body mass. Unfortunately, peripheral skeletal muscle mass reduction was not proportional to body mass reduction and could not be controlled for.
After both triamcinolone treatment and undernutrition, diaphragm muscle mass was reduced proportional to body mass reduction. On the other hand, peripheral skeletal muscle wasting after triamcinolone treatment, was more pronounced than body wasting, indicating a pronounced catabolic response. During nutritional deprivation, relative sparing of the peripheral skeletal muscle was observed. This discrepancy in muscle wasting between these two treatments, is also reflected by different atrophy patterns of the diaphragm. Triamcinolone treatment caused type II fibre atrophy, while undernutrition caused a generalised atrophy of all fibre types. Both treatments resulted in a relative increase of type I fibre cross-sectional area of the diaphragm, which could be responsible for the decreased fatigability of the diaphragm bundle *in vitro*. Other diaphragm contractility characteristics were also comparable between triamcinolone treatment and nutritional deprivation, like an increased half-relaxation time and a leftward shift of the force-frequency relationship.

Contractility of dorsiflexor hindlimb muscles however, was not comparable between these two treatments as studied in chapter 4. Undernutrition showed a decreased fatigability and external work was actually not altered when normalised for muscle mass, while triamcinolone treatment showed an increased fatigue resistance and external work. Probably muscle structure alterations cannot explain the discrepancy in *in situ* muscle performance between these two treatments.

Metabolic differences after triamcinolone treatment and undernutrition have to be considered as well. As above mentioned, triamcinolone rats were hyperglycaemic and an increased glucose influx in the hindquarter muscles was observed. Undernutrition however, showed no alterations in glucose metabolism. It was even demonstrated that triamcinolone treatment was associated with increased intramuscular glycogen levels, while undernutrition was accompanied by decreased intramuscular glycogen concentrations. In chapter 5 it was shown that muscle enzyme activities of both glycolytic and oxidative pathways were increased after triamcinolone treatment in contrast to undernutrition. These alterations in muscle metabolism could very well explain the increased muscle performance per gram muscle after triamcinolone treatment.

The alterations in muscle energy metabolism seem comparable between triamcinolone treatment and undernutrition, as reflected by decreased ATP and PCr levels and total adenine nucleotides. However, the additionally reduced creatine pool and the increased pyruvate and lactate levels in the muscle after triamcinolone treatment in contrast to undernutrition, suggest other mechanisms for the decreased muscle energy status. The alterations in muscle metabolism after triamcinolone treatment could imply metabolic impairment, while those after undernutrition imply a new metabolic equilibrium with lower ATP and PCr levels.

Surprisingly, muscle glutamine metabolism was almost comparable after triamcinolone treatment and undernutrition. Muscle wasting was accompanied by an increased glutamine *de novo* synthesis, membrane transport rates and glutamine efflux. These alterations were however, more pronounced for the triamcinolone-treated rats.

In summary, despite several similarities in muscle structure, function and metabolism after triamcinolone treatment and undernutrition, some alterations in muscle metabolism were triamcinolone specific. The most important differences were the increased glucose influx, increased glycogen levels and increased glycolytic and oxidative capacities after triamcinolone treatment. These observed differences are important to consider in the choice of adequate interventions to prevent muscle wasting induced by triamcinolone or undernutrition.

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Samenvatting

Veel chronisch zieke patiënten zoals die met chronisch hartfalen (CHF), nierfalen of obstructieve longziekten (COPD) zijn erg beperkt in hun dagelijks functioneren. Dit is niet alleen het gevolg van het slecht functioneren van het aangetaste orgaan maar spierzwakte speelt hierbij ook een belangrijke rol. Bovendien krijgen veel van deze patiënten chronisch corticosteroïden toegediend, een medicijn wat ontstekingsremmend werkt. Een van de bij-effecten van corticosteroïden is het optreden van spierzwakte. Verder vertonen veel van deze patiënten gewichtsverlies, wat ook kan bijdragen aan de spierzwakte. Aangezien chronisch corticosteroïdgebruik zelf kan leiden tot ondervoeding, is het relevant om deze twee factoren te bestuderen in relatie tot spierfunctie.

Spierfunctie wordt bepaald door de massa van de spier, de opbouw (morfologie) en door de biochemische processen die zich hierin afspelen (metabolisme). Al deze componenten zijn lastig in patiënten te bestuderen en daarom is gebruik gemaakt van een rattenmodel. Hierbij kunnen ook gemakkelijk interventies worden uitgevoerd en het verstorende effect van de ziekte kan worden uitgeschakeld. We hebben gekozen voor het gefluorideerde corticosteroïd triamcinolon. In dit corticosteroïd is een fluoridegroep ingebouwd, waardoor het een ontsteking beter kan remmen, maar bovendien ook meerdere bij-effecten veroorzaakt zoals spiermassaverlies. De ratten die met triamcinolon behandeld werden, werden vergeleken met een paarsgewijs-gevoede groep, ter correctie van de verminderde voedselinname. Vervolgens werd een paarsgewijze-gewichtsgroep geïntroduceerd, omdat het lichaamsgewicht van de met triamcinolon behandelde ratten sterker daalt dan alleen door de verminderde voedselinname verklaard kan worden. Tenslotte werd een controlegroep gebruikt met een normale voedselinname, die diende als referentie voor normale onbehandelde ratten. Het diafragma (de middenrifspier) is de belangrijkste ademhalingsspier. De functie van deze spier werd bestudeerd *in vitro* (buiten de rat) zoals beschreven in hoofdstuk 2. Een bundel van het diafragma werd opgehangen in een weefselbadje en gestimuleerd. Hierbij werd de spierfunctie van de verschillende groepen vergeleken. Het viel op dat de paarsgewijze-gewichtsgroep en de met triamcinolon behandelde groep tot dezelfde veranderingen in contractiliteit van het diafragma leidden, zoals een verminderde vermoeibaarheid van de spier en een verlenging van de half-relaxatietijd na stimulatie.

Een spierbundel is opgebouwd uit verschillende spiervezels die verschillende eigenschappen bezitten. Type I vezels kunnen bijvoorbeeld maar een kleine kracht opbouwen, maar zijn pas na lange tijd vermoeid. Type IIx/b vezels kunnen veel kracht opbouwen en zijn snel vermoeid, terwijl de eigenschappen van type IIa vezels ertussenin liggen. Wanneer de spiervezels gekleurd en dwars doorgesneden worden, kunnen de verschillende vezeltypes en hun oppervlakten worden bepaald. Dit werd gedaan in het diafragma van de verschillende groepen (hoofdstuk 2) en we vonden dat het oppervlakte van type IIa en IIx/b spiervezels afgenomen was (atrofie) in de met triamcinolon behandelde groep vergeleken met de controlegroep. De paarsgewijze-gewichtsgroep vertoonde een vergelijkbaar beeld maar vertoonde ook type I vezel atrofie. Na een behandeling met een lagere dosis triamcinolon vertoonde het diafragma alleen type IIx/b atrofie (hoofdstuk 3). Dit werd ook gevonden, hoewel in mindere mate, na ondervoeding in de paarsgewijze-gewichtsgroep. In geen enkele groep waren de spiervezels op een andere manier aangetast (myopathie) in het diafragma.

Zoals eerder gezegd wordt de spierfunctie ook bepaald door de biochemische kenmerken in de spier. Energierijke fosfaten, zoals adenosine tri- en difosfaat (ATP en ADP) en creatine fosfaat (CrP), leveren energie voor spiercontractie. Na triamcinolonbehandeling en na ondervoeding vinden we een daling van ATP, ATP/ADP ratio, totale adenine nucleotiden,

CrP en CrP/Cr ratio in het diafragma (hoofdstuk 3). De met triamcinolon behandelde ratten hadden daarnaast ook nog een verlaagde totale creatinepool en een verhoogde concentratie van eindsubstraten van de glycolyse (pyruvaat en lactaat). Dit werd niet gevonden in de paarsgewijze-gewichtsgroep, maar deze had een verlaagde creatineconcentratie in het diafragma. Hieruit werd geconcludeerd dat deze twee groepen met behulp van verschillende metabole routes leiden tot vergelijkbare veranderingen op het niveau van energierijke fosfaten. De ondervoede groep leidt tot een verlaagde energiestatus in de spier maar herwint hierbij een nieuw metabool evenwicht, terwijl de met triamcinolon behandelde groep dit evenwicht niet kan vinden als gevolg van een verstoring tussen de glycolytische afbraakroute en het oxidatief metabolisme (de afbraakroute waar zuurstof voor nodig is).

Zoals gezegd is in hoofdstuk 2 de functie van het diafragma beschreven *in vitro*. In dit model is de spierfunctie voornamelijk afhankelijk van de substraten die in de spier (en het weefselbadje) aanwezig zijn. Hierbij speelt de spiervezelsamenstelling een belangrijke rol evenals de diffusie van substraten. *In situ* spierfunctie (in de levende rat) echter is ook nog afhankelijk van de bloedtoevoer en afvoer, die belangrijk is bij de aan- en afvoer van substraten en afvalprodukten. Daarom werd de spierfunctie in een rattendynamometer bestudeerd (hoofdstuk 4), wat fysiologisch meer overeenkomt met een normale spierfunctie. Hierin werden de achterpootspieren bestudeerd van ratten behandeld met triamcinolon en met prednisolon in een vergelijkbare ontstekingsremmende dosis. De met triamcinolon behandelde ratten werden daarnaast ook weer met een ondervoede groep vergeleken. Tijdens dit experiment werd een zenuw van de achterpootspieren gestimuleerd tijdens twee sessies van 60 contracties. Tijdens de eerste sessie daalde de externe arbeid van de met triamcinolon behandelde en de ondervoede ratten als gevolg van de daling in spiermassa. Wanneer de externe arbeid uitgedrukt werd per gram gestimuleerde spier, dan was de externe arbeid aan het begin van de sessie hoger voor de met triamcinolon behandelde ratten dan voor de met prednisolon behandelde ratten. Verder daalde de externe arbeid het minst (dus minder vermoeibaarheid werd geconstateerd) in de met triamcinolon behandelde ratten vergeleken met alle ander groepen. Prednisolonbehandeling en ondervoeding hadden geen effect op de externe arbeid (per gram gestimuleerde spier) tijdens de sessies. Het herstel in externe arbeid, tijdens de 5 minuten rust tussen de sessies in, was voor de met triamcinolon en prednisolon behandelde ratten nagenoeg volledig, in tegenstelling tot de ondervoede en controlegroep. Dit zou kunnen samenhangen met het verhoogde spierglycogeengehalte na triamcinolon of prednisolonbehandeling. Vervolgens werden de activiteiten van enkele spierenzymen bestudeerd (hoofdstuk 5). De fosfofructokinase-activiteit (PFK: een glycolytisch enzym) en de glycogeen synthetase (GS) activiteit waren verhoogd in de met triamcinolon behandelde groep, terwijl er geen veranderingen waren in spierenzymactiviteiten in de met prednisolon behandelde en de ondervoede groep. Vergeleken met prednisolonbehandeling en ondervoeding was ook de citraat-synthase-activiteit (CS: enzym uit de citroenzuurcyclus) in de met triamcinolon behandelde groep toegenomen. In geen enkele groep was de activiteit van glycogeen fosforylase (GP), 3-hydroxyacyl CoA dehydrogenase (HAD: enzym uit de vetzuur β-oxidatie) en lactaat dehydrogenase (LDH: vorming van melkzuur) veranderd. Hieruit werd geconcludeerd dat de veranderingen in spierenzymactiviteiten corticosteroïd-type specifiek zijn en onafhankelijk van de samenhangende ondervoedingstoestand.

Tot nu toe hebben we alleen het koolhydratenmetabolisme en de energierijke fosfaten bestudeerd. Een spier bestaat grotendeels uit eiwit, en we verwachten ook veranderingen op eiwitniveau na triamcinolontoediening. Het metabolisme van glutamine, een belangrijk aminozuur tijdens stresssituaties, werd bestudeerd in de achterpootspier (hoofdstuk 6). Hierbij werd kortdurende (3 dagen) triamcinolonbehandeling, waarbij ook een verminderde voedselinname meespeelt, vergeleken met langdurige behandeling (14 dagen). Drie dagen

triamcinolontoediening leidde tot verhoogde glutamineconcentraties in de achterpootspier van de rat, en een verminderde in- en uitbouw van glutamine in spiereiwit. Chronische triamcinolontoediening leidde tot een verhoogde afgifte van glutamine uit de achterpootspier, een verhoogde glutamineproductie en verhoogde snelheden van transport van glutamine in en uit de spier. Chronische ondervoeding resulteerde in dezelfde veranderingen, maar in een mindere mate.

In de voorafgaande studies zijn de corticosteroïden telkens toegediend in de spier. Dit betekent dat ze in de bloedbaan terechtkomen en systemisch effecten kunnen veroorzaken in het hele lichaam. Bij patiënten worden corticosteroïden vaak locaal toegediend, bijvoorbeeld in de vorm van inhalatiesteroïden bij patiënten met ademhalingsstoornissen.
De glucocorticoïd receptor (GR) speelt een centrale rol tijdens de werking van corticosteroïden. In verschillende humane weefsels is messenger RNA van twee functioneel verschillende glucocorticoïd receptors, α en β gevonden. In de rat is de aanwezigheid van de β GR vorm in skeletspieren nog niet beschreven. Daarom hebben wij in de rat de expressie van de α en de β GR vorm in een perifere skeletspier bestudeerd, ook om na te gaan of er systemische effecten na corticosteroïd inhalatie optraden (hoofdstuk 7). Zowel het mRNA van de α als de β GR vorm werd gevonden in deze spier. Tenslotte worden de belangrijkste resultaten van de studies samengevat en bediscussieerd in hoofdstuk 8.

In het algemeen zijn er redelijk wat overeenkomstige effecten op de spier gevonden na triamcinolonbehandeling en na ondervoeding, waaruit geconcludeerd zou kunnen worden dat ondervoeding deels verantwoordelijk is voor de veranderingen na triamcinolonbehandeling. Er zijn echter aanwijzingen gevonden dat deze effecten door verschillende mechanismen veroorzaakt worden. Na triamcinolonbehandeling is er sprake van een verhoogde glucoseopname door de spier en verhoogde glycogeenconcentraties in de spier. Zowel de glycolytische als de oxidatieve capaciteit van de spier is verhoogd, maar de verhoogde lactaat- en pyruvaatconcentraties duiden op een verstoring tussen deze twee afbraakroutes. Prednisolonbehandeling leidt in tegenstelling tot triamcinolonbehandeling niet tot spiermassaverlies en metabole veranderingen. Om spierzwakte te voorkomen bij patiënten die chronisch behandeld worden met corticosteroïden, is het van belang om de oorzaak van deze spierzwakte te achterhalen en eventueel rekening te houden met het type corticosteroïd.