**Table 1:** Sequences of PCR primers used for amplification and sequencing

| Gene | Forward primer (5'/3') | Reverse primer (5'/3/) | Ref. |
|---|---|---|---|
| TNF-α | TGTGTTGTCGTCCTTCCTGCAAC | CTTGTAGGTGCCCAGGAGAG | 5 |
| IL-6 | ACAGACAGCCACTCACCTCTTC | TGCCTCTTTGCTGCTTTCACAC | * |
| IGF-I | GCTTTTGTGATTTCTTGAAGGTGA | GAAGGTGAGCAGGCACGACAGC | 17 |
| MGF | CGAAGTCTCAGAGAAGGAAAGG | ACAGGTAACTCGTGCAGAGC | 15 |
| MyoD | TGCCACAACGGACGACTTC | CGGTCCAGGTCTTCGAA | 24 |
| GAPDH | GAAGGTGAAGGTCGGAGT | CATGGGTGGAATCATATTGGA | 27 |

Definition of abbreviations: *TNF-α: tumor necrosis factor-alpha; IL-6: interleukin 6; IGF-I: insulin-like growth factor-I (IGF-IEabc); MGF: mechano growth factor (IGF-IEc); MyoD: myogenic differentiation factor D and GAPDH: glyceraldehyde-3-phosphate dehydrogenase. * see section of quantitative real-time PCR for information on primer design.*

## *Real time PCR and Western blotting*

### *RNA extraction*

Total RNA was extracted from 30 mg muscle biopsies using an RNeasy Fibrous Tissue (Qiagen, West Sussex, UK). To eliminate residual genomic DNA, the RNA samples were treated with DNAse I. Subsequently, total RNA was quantified (260 nm) and adjusted to a concentration of 1µg/µl.

### *Reverse transcription*

The cDNA was synthesized using 1 µg of total RNA from each sample, using a SuperScript First–Strand Synthesis System (Invitrogen, Carlsbad, CA) for RT-PCR according to the manufacturer's instructions.

### *Quantitative real-time PCR*

Two microliters of each cDNA sample were used as template for the amplification reaction. Each reaction included 1 x SyBR greener PCR Super Mix Universal (Invitrogen, Carlsbad, CA), 200 nM Forward and Reverse primers, whilst the PCR conditions were according to the manufacturer's instructions. Primer sequences for TNF-α, IGF-I total (IGF-IEabc) and MGF (IGF-IEc), MyoD, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) are given in Table 1. IL-6 (accession No: NM_0006000) primers were designed using the http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgiand, http://biotools.idtna.com/biotools/mfold.asp web sides. PCR amplifications were performed in triplicates in a Chromo4 Detector and PTC-200 Peltier Thermal Cycler and analysed with Opticon software 2.03 (MJ Research, Massachusetts, USA). The threshold cycle ($C_T$ value) was chosen as the first amplification cycle giving a signal above background. To calculate the relative quantity of the respective genes, the $\Delta\Delta C_T$ method was used. [26] GADPH mRNA expression was used for normalization. [27]

***Muscle protein immunoblotting***

Vastus lateralis muscle biopsies stored at -80ºC were homogenized in 10 volumes (wt/vol) of a lysis buffer containing 1% Triton-X, 1% sodium dodecyl sulphate (SDS), 150 mM NACl, 1 mM EDTA, 0.1 mM EGTA, and protein inhibitors (10µg/ml aprotinin, 10 µg/ml pepstatin, and 20 mM PMSF). [26] Protein concentration was determined by using DC protein assay (BioRad, Hercules, CA). Samples were subjected to SDS polyacrylamide gel electrophoresis (SDS-PAGE), followed by transfer to a polyvinylidene fluoride membrane (PVDF) (Millipore Corp. Bedford, MA). Immunoblotting was carried out by using primary antibodies raised against the MyoD (1:200, Santa Cruz Biotechnology, Santa Cruz, CA), IGF-I (1:200, Santa Cruz Biotechnology, Santa Cruz, CA), and TNF-a (1:1000, Santa Cruz Biotechnology, Santa Cruz, CA). To validate equal protein loading among the various lanes, PVDF memebranes were striped and reprobed with a monoclonal anti-sarcomeric α-actinin antibody (1:1000, Sigma-Aldrich, St. Louis, MO). [28] All bands were visualized using Chemiluminescent Substrate (Pierce, Rockford, IL). Data was digitalised and quantified by densitometric analysis.