Understanding the non-thyroidal illness syndrome from in vivo and in vitro studies

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Skeletal muscle deiodinase type 2 regulation during illness in mice

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Abstract

We have previously shown that skeletal muscle deiodinase type 2 (D2) mRNA is upregulated in an animal model of acute illness. Human studies on the expression of muscle D2 during illness however report conflicting data. Therefore, we evaluated the expression of skeletal muscle D2 and D2-regulating factors in two mouse models of illness that differ in timing and severity of illness: 1) Turpentine induced inflammation 2) S. pneumoniae infection. During turpentine induced inflammation, D2 mRNA and activity increased compared to pair-fed controls, most prominently at day 1 en 2, whereas after S. pneumoniae infection D2 mRNA decreased. We evaluated the association of D2 expression with serum thyroid hormones, (de-) ubiquitinating enzymes USP33 and WSB-1, cytokine expression and activation of inflammatory pathways and cAMP pathway. During chronic inflammation the increased muscle D2 expression is associated with the activation of the cAMP pathway. The normalization of D2 five days after turpentine injection coincides with increased WSB-1 and TNFα expression. Muscle IL-1β expression correlated with decreased D2 mRNA expression after S. pneumoniae infection. In conclusion, muscle D2 expression is differentially regulated during illness, probably related to differences in the inflammatory response and type of pathology. D2 mRNA and activity increases in skeletal muscle during the acute phase of chronic inflammation compared to pair-fed controls probably due to activation of the cAMP pathway. In contrast, muscle D2 mRNA decreases 48h after a severe bacterial infection, which is associated with local IL-1β mRNA expression and might also be due to diminished food-intake.
Introduction

During illness, central and peripheral thyroid hormone metabolism changes profoundly. This is known as non thyroidal illness syndrome (NTIS). NTIS is characterized by decreased serum tri-iodothyronine (T₃) levels, while thyroid stimulating hormone (TSH) remains unchanged or even decreases. Furthermore, the expression of deiodinating enzymes changes in various tissues (Wiersinga 2005). Deiodinase type 2 (D2) is one of the three known deiodinases. It converts the prohormone T₄ into the active hormone T₃ by outer ring deiodination. D2 is expressed in brain, pituitary, skeletal muscle, brown adipose tissue and placenta and is present as an active dimer in the endoplasmic reticulum (Kohrle 2000; Bianco & Kim 2006).

Recently it has been shown that skeletal muscle D2 is involved in the peripheral production of T₃ under normal circumstances (Maia et al. 2005), which makes D2 a possible factor contributing to the low serum T₃ levels during illness. A study of Rodriguez-Perez et al indeed reports decreased muscle D2 mRNA and activity in septic patients (Rodriguez-Perez et al. 2008). In contrast, D2 mRNA and activity were upregulated in muscles of intensive care unit (ICU) -patients compared to healthy controls (Mebis et al. 2007), in line with our previous finding that muscle D2 mRNA is increased after LPS administration in mice (Kwakkel et al. 2008).

D2 expression is known to be influenced by thyroid hormone levels. T₃ downregulates D2 mRNA expression (Burmeister et al. 1997), and T₄ and rT₃ (the substrates of D2) increase D2 ubiquitination and subsequently proteasomal degradation, resulting in decreased D2 activity. The ubiquitin ligase adaptor WD repeat and SOCS Box-containing 1 (WSB-1) is involved in the ubiquitination process of D2, whereas ubiquitin specific peptidase 33 (USP33) is a de-ubiquitinating enzyme (Sagar et al. 2007).

cAMP activation stimulates D2 expression on mRNA and activity level. D2 mRNA is upregulated via the CREB responsive element present in the D2 promoter and cAMP inhibits WSB-1 mediated ubiquitination of D2 (Dentice et al. 2007; Bartha et al. 2000). Proinflammatory cytokines are also capable of affecting D2; Hosoi et al showed that Tumor Necrosis Factor (TNF)α reduced the forskolin-induced increase of D2 mRNA and activity in skeletal muscle cells (Hosoi et al. 1999). Proinflammatory cytokines exert their actions via specific signal transduction pathways, such as Nuclear Factor (NF)κB, Extracellular-signal Related Kinase (ERK)1/2 and activator protein (AP)-1. NFκB and AP-1 sites have been characterized in the D2 promoter (Zeold et al. 2006; Gereben & Salvatore 2005), suggesting that activation of these pathways results in changes in D2 expression.

The aim of the present study was to evaluate the association between D2 expression and D2 regulating factors during illness. To this end we used two animal models that differ in acute phase response, timing and severity of illness. 1) Turpentine induced abscess in the hindlimb, a model of local chronic inflammation. 2) Streptococcus pneumoniae
infection, a lethal model that results in severe pneumoniae and sepsis. Muscle D2 expression and D2 regulatory factors were evaluated in both models.

**Materials & Methods**

**Animal experiments**

Female C57Bl6 mice (Harlan Spraque-Dawley, Horst, The Netherlands) were used at 6-12 weeks of age. The mice were kept in 12h light/dark cycles in a temperature controlled room. A week before the experiment the animals were housed in groups according to the experimental setup.

**Turpentine injection:** Local chronic inflammation was induced by s.c. injection of 100 µl steam-distilled turpentine in each hindlimb. Control mice received 100 µl saline in each hindlimb and were pair-fed, because the decreased food-intake 1 and 2 days after turpentine injection affects thyroid hormone metabolism. The pair fed control mice (5 mice per cage) received 5 g/cage at day 1, 5 g/cage at day 2, 8 g/cage at day 3, 12 g/cage at day 4 and 16 g/cage at day 5. Normal food-intake is approximately 20 g/cage. The mean decrease in weight was 9.3% (turpentine) and 9.4% (pair-fed controls) at day 1, 12.4% (turpentine) and 12.7% (pair-fed controls) at day 2; 2.3% (turpentine) and 8.6% (pair-fed controls) at day 5. At days 0, 1, 2 and 5 four to five mice per group were anaesthetized with isoflurane. Blood was taken by cardiac puncture and the mice were subsequently killed by cervical dislocation. Serum was stored at -20ºC until analysis. Turpentine injection resulted in a sterile abscess, infiltrating cells at the site of injection and a decrease in serum thyroid hormone levels as described before (Boelen et al. 2006a). Because the abscess and infiltrating cells in the hind-limb muscle tissue might influence the results, forelimb-muscle tissue was used for D2 analysis in this study. Muscle tissue was obtained and immediately stored in liquid nitrogen.

**S. Pneumoniae infection:** Acute pneumonia was induced as described before (Boelen et al. 2008b). Briefly, *S. pneumoniae* serotype 3 (American Type Culture Collection, Manassas, VA) were grown in Todd-Hewitt broth (Difco, Detroit, MI) at 37ºC, harvested at mid-logarithmic phase, and washed twice in sterile saline. Bacteria were then resuspended in sterile saline at a concentration of 5x10^4 Colony forming units (CFU)/50 µl. Mice (n=6) were lightly anesthetized by inhalation of isoflurane, and 50 µl containing 5x10^4 CFU was inoculated intranasally (i.n.). Control mice received 50 µl sterile saline i.n.. The amount of *S. pneumoniae* bacteria inoculated was determined by plating serial dilutions of the inoculum onto sheep-blood agar plates and incubated
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at 37°C and 5% CO₂. CFU’s were counted after 16h. Serum was stored at -20°C until analysis. Hind-limb muscle tissue was obtained after 48h and immediately stored in liquid nitrogen. Both studies were approved by the local animal welfare committee.

**Thyroid hormone levels**

Serum T₃ and T₄ were measured with in-house RIAs (Wiersinga & Chopra 1982). To prevent inter-assay variation (T₃: 6.2% and T₄: 7.3%), all samples of one experiment were measured within the same assay (intra-assay variability T₃: 3.6% and T₄: 6.6%).

**RNA isolation and RT-PCR**

Muscle mRNA was isolated on the Magna Pure (Roche Molecular Biochemicals, Mannheim, Germany) using the Magna Pure LC mRNA tissue kit and appr. 25 mg of tissue. The protocol and buffers supplied with the kit were followed. cDNAsynthesis was performed using the First Strand cDNA Synthesis Kit for RT-PCR with oligo d(T) primers (Roche Molecular Biochemicals, Mannheim, Germany). Real Time PCR was performed using the Lightcycler (Roche Molecular Biochemicals, Mannheim, Germany). Lightcycler FastStart DNA MasterPlus SYBR Green I kit (Roche Molecular Biochemicals, Mannheim, Germany) was used, adding 50ng primers each (Biolegio, Nijmegen, The Netherlands). Primer pairs for hypoxanthine phosphoribosyl transferase (HPRT), IL-1β, TNFα and D2 and D3 were previously described (Sweet et al. 2001; Bouaboula et al. 1992; Kwakkel et al. 2008) (Boelen et al. 2004). We designed primer pairs for WSB-1 and USP33 (WSB-1-forward: 5’- GCC AGC CTT GCT GAT GAT A - 3’, WSB-1-Reverse: 5’- CCC AGC AGC TAA AAC ACT GC - 3’, USP33-forward: 5’- CTT TTC GAG GTT ATT CTC AGC AG - 3’, USP33-Reverse: 5’- GGC TCT TCC TCC ATT TCC AT - 3’). Primers were intron-spanning or genomic DNA contamination was tested using a cDNA synthesis reaction without the addition of Reverse Transcriptase. PCR programs were as follows: denaturation 10 min 95°C, 40-45 cycles of 0-10 sec 95°C, 10 sec annealing temperature, 15-20 sec 72°C. Annealing temperatures were: 54°C for HPRT, 55°C for D2, 62°C for TNFα and D3, WSB-1 and USP33 and 60°C for IL-1β. For quantification a standard curve was generated of a sequence-specific PCR-product ranging from 0.01 fg/μl until 100 fg/μl (measurements taken during the exponential phase of the amplification). Samples were corrected for their mRNA content using HPRT as a housekeeping gene. Samples were individually checked for their PCR-efficiency (Ramakers et al. 2003). The median of the efficiency was calculated for each assay, samples that differed more than 0.05 of the efficiency median value were not taken into account. Aberrant PCR-efficiencies occurred randomly and therefore did not bias the results.
Deiodinase activity

Muscle Deiodinase type 2 activity was measured as previously described (Mebis et al. 2007). Samples were homogenized on ice in 10 volumes of PED50 buffer (0.1M sodium phosphate, 2 mM EDTA and 50 mM DTT pH 7.2) using a Polytron (Kinematica, Luzern, Switzerland). Homogenates were used immediately. Protein concentration was measured with the Bio-Rad protein assay using bovine serum albumin (BSA) as the standard following the manufacturer’s instructions (Bio-Rad Laboratories, Veenendaal, The Netherlands). D2 activity was measured in duplicate, using 75 µl (≈150µg protein) homogenate incubated 4 hours at 37°C in a final volume of 0.15ml with 1 nM T4 or 500 nM T4 with the addition of approximately 2*10^5 cpm [3'5'-125I]T4 in PE/0.5% BSA. Reactions were stopped by adding 0.15 ml ice-cold ethanol. After centrifugation, 0.125 ml of the supernatant was added to 0.125 ml 0.02 M ammonium acetate (pH 4), and 0.1 ml of the mixture was applied to 4.6 x 250 mm Symmetry C18 column connected to a Waters HPLC system (Model 600E pump, Model 717 WISP autosampler, Waters, Etten-Leur, The Netherlands). Mobile phase A: 0.02M ammonium acetate (pH4.0), mobile phase B: acetonitril. The column was eluted with a linear gradient (28-42% B in 15 min) at a flow of 1.2 ml/min. The activity of T4 and T3 in the eluate was measured on-line using a Radiomatic Flow-one/Beta scintillation detector (Packard, Meriden, CT, USA). Incubation with 500 nM T4 saturates D2, therefore D2 activity measured with the incubation with 1nM T4 minus the incubation with 500 nM T4 represents true D2 activity. D2 activity was expressed as fmol generated T3 per minute per gram muscle tissue.

D3 : Muscle D3 activity was measured the same way as muscle D2 activity, with the following adaptations: Incubation for 4 hours at 37 ºC with 1 nM T3 or 500 nM T3 with the addition of approximately 2*10^5 cpm [3'5'-125I]T3 in PE. For each group, we included one 500 nM T3 incubation. Incubation with 500 nM T3 saturates D3, therefore D3 activity measured with the incubation with 1nM T3 minus the incubation with 500 nM T3 represents true D3 activity. D3 activity was expressed as fmol generated 3,3'T2 per minute per mg tissue.

Western Blotting

Homogenates prepared for deiodinase measurement were immediately 1:1 mixed with freshly prepared protein dilution buffer (250 mM sucrose, 10% glycerol, 2mM PMSF, 4 mM Na3VO4, 40 mM NaF, 2x Complete protease inhibitor cocktail (Roche Molecular Biochemicals, Mannheim, Germany). Protein content was measured and 25 µg was loaded on a 10% SDS-PAGE gel. Gels were blotted on Immobilon-P transfer membrane (Millipore, Bedford, MA, USA). Blots were blocked with 3% casein in TBS/T, for 1h at room temperature (RT). Primary antibodies were phospho-NFκB p65 (Ser536) (#3033), phospho-p44/p42 MAP kinase (Thr202/
Primary antibodies were incubated for 1h at RT followed by an overnight incubation at 4°C. Blots were washed 3 times 5 min with TBS/T. Following 1h incubation at RT with secondary antibody goat-anti-rabbit-HRP, blots were washed again and detected with Lumi-Lightplus chemiluminescent substrate (Roche Molecular Biochemicals). The emitted light was visualized and quantified on the Lumi-Imager (Roche Molecular Biochemicals). All antibodies were diluted 1:1000 in blocking buffer, except phospho-CREB, which is diluted 1:2000.

Statistics

Normal distribution of the data was tested using the Shapiro-Wilk test. Statistical significance between turpentine and control treatment were evaluated using two-way ANOVA with two grouping factors (time and treatment). When not normally distributed, data were ranked before performing ANOVA. P-values in the figures represent the significant effect of the treatment. To test pair-wise comparisons ANOVA was followed by students t-test when data was normally distributed or Mann-Whitney U tests when not normally distributed. Symbols in the figures represent the pair-wise P-values. P-values < 0.05 were considered statistically significant. Spearman rank correlation tests were performed to test correlations. All tests were performed using SPSS. (SPSS, Chicago, IL, USA)

Results

Muscle Deiodinase type 2 expression

Turpentine injection resulted in increased expression of muscle D2 mRNA and activity (P<0.01), compared to saline treated pair-fed controls, most prominent at day 1 and 2 after injection (fig1A). In saline treated pair-fed controls, D2 mRNA and activity decreased probably due to decreased food-intake. D2 activity in control groups was in some cases below detection limit. In contrast, muscle D2 mRNA decreased in muscle-tissue 48h after S.pneumoniae infection, compared to saline treated controls (P<0.01) (fig1B). Muscle D2 activity was around the detection limit (2 above and 4 below the detection limit in each group) in muscle tissue of S.pneumoniae infected and control mice.

Serum thyroid hormone levels

Serum T_4 and rT_3 levels decreased (P<0.01) 1, 2 and 5 days after turpentine injection, whereas serum T_3 did not change compared to pair-fed saline treated controls.
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Figure 1. A) Muscle D2 mRNA and activity expression 1, 2 and 5 days after Turpentine (●) or saline (□) injection, B) Muscle D2 mRNA expression 48h after *S. pneumoniae* infection (black bars) or saline-treated (white bars) controls. Mean values ± SEM (n=4/6) are shown. *P* values indicate differences between groups by non parametric ANOVA. Symbols indicate differences evaluated by Mann-Whitney U-tests; *P*≤0.05, **P**≤0.01.

(fig2A). Muscle D2 mRNA expression did not correlate to serum T3. Serum rT3 was associated with muscle D2 activity (r = -0.504, *P*<0.01), while serum T4 was not. After *S. pneumoniae* infection serum T4 decreased (*P*<0.01). Serum T3 and rT3 did not change (fig2B). Serum T3 was not related to muscle D2 mRNA expression.

Figure 2. Serum T4, T3 and rT3 levels A) 1, 2 and 5 days after Turpentine (●) or saline (□) injection, B) 48h after *S. pneumoniae* infection (black bars) or saline-treated (white bars) controls. Mean values ± SEM (n=4/6) are shown. *P* values indicate differences between groups by non parametric ANOVA. Symbols indicate differences evaluated by Mann-Whitney U-test; **P**≤0.01.
Muscle WSB-1 and USP33 mRNA expression

Ubiquitination promoting enzyme WSB-1 and de-ubiquitination enzyme USP33 mRNA expression were evaluated. Muscle WSB-1 mRNA increased significantly 5 days after turpentine injection (P<0.05), whereas USP33 mRNA did not change significantly (fig3A). WSB-1 and USP33 mRNA levels were not related to muscle D2 activity during turpentine induced inflammation. After S.pneumoniae infection muscle WSB-1 mRNA did not change whereas USP33 mRNA decreased compared to saline treated controls (fig3B).

**Figure 3.** Muscle WSB-1 and USP33 mRNA expression
A) 1, 2 and 5 days after Turpentine (●) or saline (□) injection, B) 48h after S.pneumoniae infection (black bars) or saline-treated (white bars) controls. Mean values ± SEM (n=4/6) are shown. P values indicate differences between groups by non parametric ANOVA. Symbols indicate differences evaluated by Mann-Whitney U-test; * P≤0.05, ** P≤0.01.

Muscle cytokine expression

Muscle TNFα mRNA increased day 5 after turpentine injection compared to saline treated, pair-fed controls whereas muscle IL-1β did not (fig4A). Muscle IL-1β or TNFα cytokine expression is not related to D2 mRNA expression. After S. pneumoniae infection muscle IL-1β mRNA increased (P<0.01) (fig4B) and was negatively correlated to D2 mRNA expression (r = -0.691, P<0.05). Muscle TNFα mRNA expression did not change significantly after S.pneumoniae infection and was not correlated to muscle D2 mRNA expression.

Activation of signalling pathways

Phosphorylated NFkB, c-jun, ERK1/2 and CREB were evaluated in muscle tissue by Western Blotting. After turpentine injection CREB was highly phosphorylated at day 1 and 2 (fig 5A) while NFkB (p65) and ERK1/2 did not differ compared to saline treated pair-fed controls. The phosphorylation of CREB coincides with
the observed increase in D2 expression after turpentine injection. No difference was observed in phosphorylation of CREB, NFκB or ERK1/2 after *S. pneumoniae* infection compared to saline treated controls (fig5B). Phosphorylated c-jun was not detectable in muscle tissue (data not shown).

**Muscle Deiodinase type 3 expression**

Turpentine injection resulted in increased expression of muscle D3 mRNA and activity (P<0.05), compared to saline treated pair-fed controls, most prominent
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at day 1 and 2 after injection (fig6A). D3 activity in control groups was in most cases below detection limit. After \textit{S.pneumoniae} infection muscle D3 mRNA did not change. Muscle D3 activity was below the detection limit in the saline controls, whereas 3 samples were positive after \textit{S.pneumoniae} infection. However, the observed difference was not significantly different ($P=0.056$).

**Discussion**

We studied the association between muscle D2 expression and D2 regulating factors in two different animal models of illness: turpentine-induced abscess in the hindlimb and \textit{S.pneumoniae} infection. The animal models used differ in timing of the acute phase response and severity of illness. After turpentine injection in the hindlimb, an abscess is formed in the first two days, causing serious discomfort, fever and decreased food-intake. Serum IL-6 and IL-1 are high during this early phase, whereas serum TNF\textgreek{a} does not play a role. Five days after turpentine serum IL-1 and IL-6 decrease, temperature and food-intake return to normal and mice recover, although liver IL-1\textgreek{b} mRNA increases which is characteristic for the development of a systemic acute phase response (Boelen et al. 1996; Boelen et al. 2005; Elhija et al. 2006; Leon 2002).

In contrast to turpentine induced inflammation, \textit{S.pneumoniae} infection is lethal (Knapp et al. 2004). \textit{S.pneumoniae} infection results in severe bronchopneumonia within 24 hours and 48 hours after inoculation mice become septic (Boelen et al. 2008a). Serum IL-1 and IL-6 increase rapidly after infection, whereas TNF\textgreek{a} is produced at a later stage (Bergeron et al. 1998). The difference in cytokine expression, acute
phase response and severity of illness between these two animal models suggests differential regulation of D2 expression. During turpentine induced inflammation, D2 mRNA and activity increased compared to pair-fed controls, most prominently at day 1 en 2, whereas after *S. pneumoniae* infection D2 mRNA decreased. The D2 increase compared to pair-fed controls during turpentine induced inflammation is in line with increased D2 expression recently observed in skeletal muscle of ICU patients and in LPS treated mice (Mebis *et al.* 2007; Kwakkel *et al.* 2008), while decreased muscle D2 mRNA expression after *S. pneumoniae* infection corresponds with previously reported D2 decrease in muscle tissue of septic patients (Rodriguez-Perez *et al.* 2008). Although it might be possible that the observed differences in D2 expression during illness in our animal-models result from the different muscle origins, this seems unlikely because the D2 increase observed previously after LPS administration (Kwakkel *et al.* 2008) is similar in both muscle types (J. Kwakkel, unpublished observation). D2 mRNA and activity is regulated by thyroid hormones; D2 mRNA and activity increases during hypothyroidism, while hyperthyroidism results in decreased D2 mRNA expression and activity, due to transcriptional (T3) and translational (T4 and rT3) regulation. Thyroid hormone metabolism during illness however, differs from normal regulation (Wiersinga 2005). In both our animal models, no correlation between serum T3 and T4 levels and muscle D2 expression was observed. This is reminiscent to the T4-independent D2 activity increase in the mediobasal hypothalamus induced by LPS administration (Fekete *et al.* 2005). Factors involved in (de-)ubiquitination of D2 in our study did not correlate with D2 activity, although the observed increase in WSB1 mRNA 5 days after turpentine coincides with a normalisation of D2 activity. Inflammatory cytokines have profound effects on peripheral and central thyroid hormone metabolism (Boelen *et al.* 2006b). We studied muscle IL-1β and TNFα mRNA expression and the activation of three inflammatory pathways in muscle tissue. After turpentine injection IL-1β and TNFα mRNA expression tended to increase after 5 days, which is in accordance with the declining muscle D2 expression 5 days after turpentine injection as TNFα decreases muscle D2 mRNA expression in vitro (Hosoi *et al.* 1999). Furthermore, we observed increased IL-1β mRNA expression in muscle tissue of *S. pneumoniae* infected mice, which was inversely correlated to D2 mRNA expression. However, the observed alterations in D2 expression appeared not to be mediated via activation of the inflammatory pathways NFκB, ERK1/2 or AP-1. The decreased D2 expression after *S. pneumoniae* infection might also be due to diminished food-intake, as D2 expression decreases after fasting in healthy humans (Heemstra *et al.* 2009). In addition, D2 mRNA expression also decreased in the pair-fed controls of the turpentine experiment. cAMP activation is a potent stimulator of D2, both pre- and posttranscriptional (Hosoi *et al.* 1999; Bartha *et al.* 2000; Dentice *et al.* 2007). We evaluated phosphorylated CREB expression in muscle tissue as a marker for cAMP activation. CREB was
highly phosphorylated in muscle tissue within 48 hours after turpentine injection, which coincides with the marked increase of D2 expression compared to pair-fed controls, suggesting a dominant role of the cAMP pathway in the observed D2 increase.

Increased D2 activity theoretically results in increased T3 production, which is not reflected in increased serum T3 concentrations, suggesting that D2 contribution to systemic T3 levels is quantitatively limited. To evaluate the physiological implications of the observed D2 alterations during illness, muscle D3 expression was evaluated. After S. pneumoniae infection, D3 mRNA did not change, while D3 activity tended to increase, however the activity measured was very low. In contrast, muscle D3 mRNA and activity increased after turpentine injection compared to pair-fed controls. The increase of muscle D3 during illness has previously been reported (Rodriguez-Perez et al. 2008).

During turpentine induced inflammation the increase in muscle D2 and D3 compared to pair-fed controls coincides with the increased body temperature and decreased food-intake observed during this phase of inflammation (Leon 2002). Cooper et al showed increased β-adrenergic dependent oxygen-consumption in the first 36 hours after turpentine injection (Cooper & Rothwell 1991). Sympathetic stimulation of cultured human skeletal muscle cells results in activation of the cAMP pathway and in upregulation of D2 in muscle cells (Hosoi et al. 1999), subsequently followed by increased mitochondrial activity and thus increased oxygen-consumption (Watanabe et al. 2006).

Because both the measured D2 and D3 activity are very low, a local effect in the skeletal muscle seems more likely than an effect on serum thyroid hormone levels. The simultaneous upregulation of D2 and D3 theoretically only leads to the formation of 3,3'-T2, which might be able to activate the mitochondrial enzyme cytochrome c oxidase (Goglia et al. 1994) (Lanni et al. 1994) thereby also increasing oxygen consumption. A shortage of 3,3'-T2 might result in mitochondrial dysfunction, which is thought to play a role in the pathogenesis of sepsis (Fredriksson et al. 2006; Zang et al. 2007). Adding exogenous cytochrome c oxidase improves cardiomyocyte mitochondrial function in an animal model of sepsis (Levy & Deutschman 2007).

These speculations need further investigation in order to consider the role of D2 and D3 in muscle tissue as a regulator of muscle mitochondrial activity during illness.

In conclusion; muscle D2 expression is differentially regulated during illness, probably related to differences in the inflammatory response and type of pathology. D2 mRNA and activity increase in skeletal muscle during the acute phase of chronic inflammation compared to pair-fed controls probably due to activation of the cAMP pathway. In contrast, muscle D2 mRNA decreases 48h after a severe bacterial infection, which is associated with local IL-1β mRNA expression and might also be due to diminished food-intake. The observed alterations in muscle
D2 and D3 might result in changes in local 3,3’-T₂ concentrations and thereby affecting mitochondrial activity.

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