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Chronic treatment with the ß$_2$-adrenoceptor agonist pro-drug BRL-47672 impairs rat skeletal muscle function by inducing a comprehensive shift to a faster muscle phenotype

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Abstract

Discovering approaches to maintain or improve muscle function (fatigue resistance) in patients with cachexia, post-operative weakness and sarcopenia is of clinical importance. β_2-agonist treatment increases muscle mass yet alters fiber proportions, such that the net consequences on muscle function remains unclear. In the present study we focus on the contractile and metabolic consequences of chronic treatment with the β_2-agonist pro-drug BRL-47672 (BRL). Gastrocnemius-Plantaris-Soleus (GPS) muscles were harvested at rest and studied for fatigue characteristics during 4s and 20s of isometric stimulation (30 Hz, 10V, 200 ms) using the perfused hindlimb model. BRL treatment increased GPS mass by 21% ($P< 0.05$) while greater fatigue occurred during 20s of contraction (45% less work, $P<0.05$). Phenotypically, BRL resulted in 17% more Type IIb MyHC protein expression ($P<0.001$) and greater adenine nucleotide catabolism during 20s of contraction ($P< 0.05$). Chronic BRL treatment impaired maximal lipid oxidation capacity by 30% ($P<0.05$) and reduced GDH activity by 15% ($P< 0.05$). We conclude that β_2-agonist induced muscle hypertrophy may be clinically limited as impaired energy metabolism and function occur, presumably as a consequence of the shift in muscle phenotype

Decrements in skeletal muscle mass and functional capacity in response to cachexia, post-operative inactivity and sarcopenia (Busquets, et al., 2004;Ryall, et al., 2004) have a negative impact on quality of life.. The maintenance or, ideally, improvement of muscle strength and function (fatigue resistance) is therefore of clinical importance. Chronic administration of a ß$_2$-agonist, such as clenbuterol or fenoterol, undoubtedly induces muscle fiber hypertrophy in animal models (Cartana, et al., 1994;Emery, et al., 1984;Kim, et al., 1987;MacLennan and Edwards, 1989;Yang and McElligott, 1989), highlighting its potential for use in a clinical setting. However, chronic β_2-agonist treatment also results in impaired on cardiac function (Sleeper, et al., 2002), and a change in skeletal muscle fiber composition towards a faster phenotype (Dodd, et al., 1996;Jones, et al., 2004;Morton, et al., 1995;Rajab, et al., 2000;Ryall, et al., 2002;Ryall, et al., 2004;Timmons, 2002;Zeman, et al., 1988). For example, in young animals, the maturation-related increase in slow myosin heavy chain (MyHC) expression is attenuated following chronic β_2-agonist treatment, resulting in a greater fast muscle fiber population (Jones, et al., 2004;Timmons, 2002). Furthermore, it appears that this response is at least partly the result of a rapid β_2-agonist mediated increase in the expression of the "fast muscle" transcription factor MyoD (Jones, et al., 2004). This is in concordance with other studies demonstrating the development of fast muscle twitch characteristics *in vitro* (Ryall, et al., 2002;Ryall, et al., 2004) and an increase in muscle ATP content and cellular energy charge (Rajab, et al., 2000) as a result of chronic β_2-agonist treatment. Collectively, these findings point towards a global shift in muscle phenotype, not just a shift in contractile protein expression. Interestingly, the shorter muscle relaxation rates and greater muscle peak twitch forces observed following chronic administration of clenbuterol (Zeman, et al., 1988) can be reversed with concurrent administration of ß$_2$-antagonists (Morton, et al., 1995;Zeman, et al., 1988), demonstrating that the underlying control mechanism(s) relates directly to ß$_2$-agonism.

We have previously demonstrated that administration of the β_2-agonists pro-drug BRL-47672 (BRL), which is structurally similar to clenbuterol (Sillence, et al., 1995) and is metabolized *in vivo* to its active component, results in an attenuation of unwanted cardiovascular responses; a more favorable profile for use in a clinical setting compared with direct agonists (Jones, et al., 2004). The impact that BRL has on contractile protein expression, metabolic phenotype and function, has not been the subject of detailed scrutiny. Any deleterious alterations, even in the face of an improved cardiovascular side-effect profile, would have major implications for muscle function and therefore requires investigation prior to proceeding with such ideas beyond the pre-clinical laboratory. In rodent models, ß$_2$-agonist treatment has been reported to improve muscle function following hind limb unloading (Dodd and Koesterer, 2002), and more recently, to reverse age associated decline in muscle mass (Ryall, et al., 2002). These positive effects were noted, as function reflected the increase in total muscle mass, a parameter which may be most relevant for patients with extreme muscle wasting. We must also consider that the ß$_2$-agonist induced increase in fast MyHC expression, may promote fatigue development during sustained contraction, due to a limited inherent capacity for fast muscle fibers to maintain ATP turnover (Hultman, et al., 1991). The issue is further complicated by the observation that an increase in mitochondrial enzyme expression has been noted following chronic β_2-agonist treatment (Dodd, et al., 1996), which could be beneficial to function, during sustained contraction. In light of these considerations, and the potential clinical benefits from chronic β_2-agonist treatment, the current study investigated the effects of chronic treatment with BRL on muscle twitch characteristics and isometric tension development during sustained contraction. We determined muscle adenine nucleotide breakdown during contraction, as a measure of the ability of muscle to maintain ATP turnover during contraction. Finally, mitochondrial enzyme activities and the maximal

in vitro rate of mitochondrial ATP production were determined to inform us about changes in mitochondrial density and function, respectively.

Methods

Experiment to Determine the Time course of Change in Muscle Mass and MyHC Expression During β_2-agonist Pro-drug Treatment

Sixty male Wistar rats (150 – 160g, Charles River, Margate, UK) were divided into two treatment groups. One group (n = 30) received daily subcutaneous injections of β_2-agonist pro-drug BRL at 900 μg kg^{-1} body mass for 1 day (n = 10), 4 weeks (n = 10) or 8 weeks (n = 10). The second group (n = 30) received daily subcutaneous injections of an iso-volume of saline for 1 day (n = 10), 4 weeks (n = 10) or 8 weeks (n = 10). The day following the final injection of either β_2-agonist or saline, the rats were anaesthetized (Inactin, 120 mg kg^{-1} body mass, i.p) and the gastrocnemius muscles were excised, frozen in liquid nitrogen and then weighed prior to subsequent separation and quantification of MyHC expression as described below. All experimental use of animals was approved by the UK Home Office and conducted in accordance with the laws governing the use of animals in research Act of 1986.

Myosin Heavy Chain Analysis

Crude myosin was prepared as described previously (Jones, et al., 2004). 0.1-0.5 μg of protein extract was electrophoretically separated using SDS PAGE (6%) gels containing 40% glycerol, and then silver-stained using BioRad silver-stain plus kit (BioRad, Hemel Hempstead, UK), as done previously (Jones, et al., 2004). Gels were then scanned and computerized densitometry was used to quantify band intensity for each MyHC isoform

which was expressed as a percent of the total MyHC content, and thus are representative as MyHC content as a proportion of the total expression.

Experiment to Determine the Effect of Chronic β_2-agonist Pro-drug Treatment on Muscle Function and Metabolism

Forty-one male Wistar rats (250 – 350 g, Charles River, Margate, UK) received daily subcutaneous injections of BRL (900 μg kg^{-1} body mass) or an iso-volume of saline for eight weeks. The day following the final injection, rats were terminally anesthetized to allow vascular isolation of the gastrocnemius-plantaris-soleus (GPS) muscle group. Animals then underwent 60 min of perfusion (Resting muscle: saline: n=8; BRL: n=6) or 60 min of perfusion followed immediately by isometric contraction at maximal intensity for either 4s (saline: n=8; BRL: n=6) or 20s (saline: n=7; BRL: n=6).

Hind limb Perfusion Model

We utilized our novel version (Baker, et al., 2005;Baker, et al., 2006) of the standard perfused rat hind limb preparation. Male Wistar rats were anaesthetized (Inactin, 120 mg kg^{-1} body mass, i.p), and the musculature of the left hind limb was exposed by removal of the skin from this region. The branches of the femoral artery and vein were ligated using silk ligatures or thermocautery up to the point where these vessels entered the GPS muscle complex. This ensured that blood flow to and from this muscle group was solely via the intact femoral artery and vein. The bicep femoris was then removed, and a length of thread was tied around the Achilles tendon and cut distal to the ligature. This resulted in the GPS muscle group remaining fixed to the limb on the dorsal side of the knee joint. The femoral artery and vein were then cannulated, and heparinized saline (10 U ml^{-1}) was slowly flushed through the vasculature of the GPS muscle group. An arterial cannula was then attached to a

primed perfusion system which was contained in an enclosed chamber which maintained an ambient temperature of 37°C, and the muscle group was perfused with previously prepared perfusion media (see below for details). The animal was then killed humanely (according to UK Home Office Guidelines) and placed ventral surface down to enable the tibia to be secured using a clamp fixed to a stereological frame, after which the thread from the Achilles tendon was attached to an isometric force transducer (Grass Instruments, Warwick, USA). Clamping the tibia facilitated the measurement of muscle force production during contraction by minimizing inertia generated by movement of the animal.

The perfusion media contained isolated porcine red blood cells suspended in a modified Krebs buffer containing 5 % bovine serum albumin, insulin (100 μU ml^{-1}) and 0.15 mM pyruvate (adjusted to pH 7.4, Hct 47% and 6 mM glucose). The GPS was perfused with the cell suspension for 60 min (15 ml min^{-1} $100g^{-1}$ of wet muscle) prior to undergoing assessment of single twitch characteristics, and then in a randomized order one of the following: (i) excision of the GPS muscle group, which was then immediately snap frozen in liquid nitrogen (rested baseline muscle), (ii) 4s , or (iii) 20s of maximal intensity tetanic isometric contraction (30 Hz, 200 ms, 10 V), achieved via direct electrical stimulation of the sciatic nerve using a hook electrode (Harvard Instruments, USA). Isometric tension was recorded throughout (Mac Lab 400, AD Instruments, Australia). Immediately following contraction, the GPS muscle group was rapidly excised and snap frozen in liquid nitrogen. The muscle group was subsequently weighed frozen and then stored in liquid nitrogen until further analysis was performed.

Muscle Metabolite Analysis

The GPS muscle group was crushed under liquid nitrogen and thoroughly mixed in order to create a homogenous representation of the whole GPS muscle group. An aliquot of this pool of crushed frozen tissue was then freeze dried overnight and stored at -80 °C. At a later date this freeze dried muscle was powdered and extracted (Harris, et al., 1974), and used for the determination of muscle adenine triphosphate (ATP), adenine diphosphate (ADP), adenine monophosphate (AMP), inosine monophosphate (IMP), inosine (INO), hypoxanthine (HYP), xanthine (XAN) and uric acid (UA) concentrations by HPLC (Idstrom, et al., 1990), and determination of muscle glycogen concentration using spectrophotometry (Harris, et al., 1974).

Mitochondrial ATP production rates

Approximately 50 mg of muscle was excised from the middle part of each soleus muscle undergoing perfusion only (i.e. resting), and, following rapid weighing, was used immediately for the bio-luminometric determination of ATP production (MAPR) rates in isolated mitochondria using a variety of substrates (Wibom, et al., 1990). Mitochondria were prepared by differential centrifugation following muscle homogenization in a Potter-Elvehjem glass homogenizer fitted with a Teflon pestle. This method is based on the reaction of ATP with firefly luciferase, providing a light signal proportional to the concentration of ATP in the solution. The following substrate concentrations (in the cuvette) were used to measure mitochondrial activity: 1) pyruvate 1 mM + palmitoyl L-carnitine 5 μM + α-ketoglutarate 10 mM + malate 1 mM, 2) glutamate 10 mM, 3) α-ketoglutarate 10 mM, 4) palmitoyl L-carnitine 5 μM + malate 1 mM, 5) pyruvate 1 mM+ malate 1mM, 6) succinate 20 mM and rotenone 0.1 mM, and 7) succinate 20 mM. The same mitochondrial suspension was used at a later date for the measurement of glutamate dehydrogenase (GluDH) activity. The

yield for mitochondria for each mitochondrial preparation was determined from the ratio between GluDH activity determined in the whole muscle homogenate and that determined in the mitochondrial suspension.

Enzyme Activities

The activities of muscle glutamate dehydrogenase (GDH) and citrate synthase (CS) were determined as markers for mitochondrial matrix, β hydroxy acyl-CoA dehydrogenase (HAD) was determined as an index of mitochondrial β oxidation, and glyceraldehyde 3 phosphate dehydrogenase (Gly3PDH) was determined as a measure of cytosolic based glycolysis. Frozen muscle (5-10 mg) was homogenised on ice in a solution containing KH_2PO_4 50 mM, EDTA 1mM and Triton X100 0.05 %. The muscle homogenates were then analysed for the enzymes activities outlined above as described by Opie and Newsholme (Opie and Newsholme, 1967) and Zammit & Newsholme (Zammit and Newsholme, 1976). Protein content in the mitochondrial suspension was determined by the Bradford method.

Statistics

Comparison of means was performed using two-way ANOVA with an LSD post-hoc test or Student's unpaired t-test where appropriate. Pearson's correlation co-efficient was used to determine any significant relationships. P values < 0.05 were deemed to be significant. Values in text, Tables and Figures represent mean $\pm$ SEM.

Results

Muscle Mass and MyHC Protein Expression

There was a 22% increase in wet GPS muscle mass following 8 weeks of BRL treatment compared with saline. (3.9 ± 0.1 vs 3.2 ± 0.1 g, respectively, $P<0.05$). Figure 1 shows MyHC protein expression (type I, IIa, IIx and IIb) in rat gastrocnemius muscle following 1 day, 4 weeks and 8 weeks administration of saline or BRL.

Saline administration did not alter type I MyHC protein expression over the course of the experiment. No difference in type I MyHC protein expression existed between saline and BRL treated groups after 1 day of administration. However, following 4 weeks of BRL administration the expression of type I MyHC protein had decreased by 50 % compared to 1 day ($P<0.01$) such that a clear difference was evident between treatment groups at this time point ($P<0.001$), which was maintained at the 8 week time point ($P<0.01$).

Type IIa MyHC protein expression did not differ between treatments after 1 day of administration (8.7 ± 0.7 vs 9.5 ± 0.3 %). After 4 weeks of saline treatment, an increase in IIa protein expression was observed (11.0 ± 0.9 % ($P<0.05$ compared with 1 day), which remained unchanged for the rest of the study. BRL treatment decreased type IIa protein expression to 7.8 ± 0.7 % after 4 weeks ($P<0.05$ compared to 1 day and saline treatment), which was also maintained for the remainder of the study (significantly lower than saline treated group $P<0.05$).

Type IIx MyHC protein expression was no different between treatment groups at day 1. Expression was unchanged in the saline treated group after 4 weeks, but a significant increase

was observed after 8 weeks ($P<0.05$ compared to 4 weeks; $P<0.001$ compared to 1 day) reflecting maturation related changes. Type IIx MyHC protein expression remained unchanged over the course of the study in the BRL treated group, such that expression was 25% less in this group compared to saline after 8 weeks ($P<0.001$).

Type IIb MyHC protein expression decreased in the saline treated group by 11% over the course of the study, the decline of which was similar in magnitude to the increase seen in type IIx MyHC protein expression (9%) in the same group. There was no difference in the type IIb MyHC protein expression between saline and BRL treated groups at 1 day, however, after 4 weeks, expression in the BRL treated group was ~ 10 % greater compared with the saline treated group ($P<0.01$), and increased further to ~ 17 % after 8 weeks administration ($P<0.001$).

Muscle function

There was no difference in twitch peak tension (pt) between treatments. Time to peak tension was 14% shorter following 8 weeks of BRL administration compared to saline ($P<0.001$, data not shown) Similarly, relaxation time, calculated as the time from peak tension to resting tension, was 20% lower following BRL administration compared to saline (Figure 2A). Figure 2B shows muscle tension development (expressed as a percent of initial peak tension) after 4 and 20 s of contraction following 8 weeks of saline or BRL administration. There was no difference in the percentage decline of force from peak tension between saline and BRL treatment groups after 4 seconds of contraction (39.5 ± 5.1 vs 36.7 ± 6.0 %, respectively). Muscle tension in the saline treated group was unchanged between 4 and 20s. BRL treatment however, caused a further 13 % reduction in force from 4 to 20s of contraction when compared to saline treatment ($P<0.05$, 37.0 ± 4.7 vs 50.4 ± 2.4 %, respectively). Figure 2C

shows the area under the tension x time curve in both treatment groups following 4 and 20 s of contraction, which represents the total amount of work performed. In line with Figure 2A, no difference in total work output was observed after 4s of contraction when comparing groups. However, by 20s BRL treatment resulted in 45% less work being performed when compared with the saline treated group ($P<0.05$).

Muscle metabolites, energy charge and glycogen content

Table 1 shows muscle metabolites, energy charge and glycogen content at rest and after 4 and 20s of maximal intensity contraction following 8 weeks of saline or BRL treatment. Resting muscle ATP concentration was greater in the BRL treated group compared with saline ($P<0.05$), and resting AMP concentration was lower ($P<0.05$). These differences were reflected by a higher cellular energy charge (EC, ATP+0.5ADP/ATP+ADP+AMP) at rest in the BRL treated group compared to saline.

Muscle ATP concentration did not change from its resting value in the saline treated group during contraction (Table 1). However, muscle ATP was significantly lower (27.8 ± 0.5 $mmol \cdot kg^{-1}$ dry mass) by 20s of contraction in the BRL treated group ($P<0.05$). Muscle IMP concentration increased from rest in both treatment groups by 20s of contraction, however this increase was two fold greater in the BRL treated group compared with saline. No difference was observed between or within treated groups for muscle ADP, inosine, hypoxanthine and xanthine concentrations (Table 1). Figure 3A presents the sum of the products of muscle adenine nucleotide degradation at rest and following 4 and 20s of contraction in each treatment group. There was no difference between treatment groups at rest. Furthermore, contraction did not result in any change from rest in the saline treated group. However, BRL treatment was associated with a cumulative increase in adenine

nucleotide breakdown products after 4s ($P<0.05$) and 20s ($P<0.01$) of contraction (Fig. 3A). Figure 3B shows that a significant correlation existed between the magnitude of muscle fatigue development (% decline from peak tension) and accumulation of muscle adenine nucleotide breakdown products ($R^2 = 0.656$, $P<0.05$) over the course of 20s of contraction. Resting muscle glycogen content was 17% greater in the saline treated group (Table 1, $P<0.05$). However, the magnitude of glycogen degradation was no different between treatments being 31 $mmol \cdot kg^{-1}$ dm over the course of 20s of contraction.

Mitochondrial ATP production and Enzyme Activities

No significant difference exited between saline and BRL treated groups with respect to the mitochondrial yield (19.8±1.5 vs 23.8±2.5%, respectively; $P>0.05$). Figure 4A shows soleus muscle mitochondrial ATP production rates ($mmol \cdot min^{-1}\ kg^{-1}$ wet muscle at 25°C) in the presence of a mixture of pyruvate, palmitoyl-carnitine, α-ketoglutarate and malate (PPKM) and with each of the following: glutamate (Glut), α-ketoglutarate (Keto), palmitoyl L-carnitine and malate (Pal), pyruvate and malate (Pyr), succinate and rotenone (SuccRot) and succinate (Succ). The rate of mitochondrial ATP production in the presence of palmitoyl L-carnitine and malate was 30% lower following BRL treatment compared with saline (9.39±1.31 and 6.74±0.47 mmol ATP $min^{-1}\ kg^{-1}$ wet muscle, respectively; $P<0.05$). None of the remaining measurements of mitochondrial function were different between treatment groups.

Enzyme Activities

BRL treatment did not alter the activity of CS, HAD or Gly3PDH from that observed in the saline treated group. However, there was a significant reduction in the activity of GDH following 8 week of BRL treatment compared with saline (Fig 4B, $P<0.05$).

Discussion

Chronic ß$_2$-agonist administration increases muscle mass (Morton, et al., 1995). Furthermore, administration of a single i.p. bolus dose of BRL avoids much of the severe hemodynamic responses observed with clenbuterol (Jones, et al., 2004), presumably as BRL is a pro-drug of clenbuterol itself, thus rendering it a more favorable option for use in a clinical setting. However, BRL treatment is also associated with a shift in muscle fiber composition (greater faster contractile protein expression), giving it the potential to promote muscle fatigue development during sustained contraction and thereby of limited clinical use. In addition, it has been postulated that the altered muscle phenotype is secondary to the severe cardiovascular response (Rothwell, et al., 1987) such that the net impact of BRL on muscle function was unclear. The present study comprehensively determined the impact of chronic BRL treatment on muscle phenotype and contractile function in the rat. Collectively these observations provide insight into the long term viability of this therapeutic strategy.

The major findings of the present study were that although the 21% increase in muscle mass observed following 8 weeks of treatment with BRL was within the typical range of increase seen in rodents (Jones, et al., 2004;Zeman, et al., 1988), this was also accompanied by 17% greater Type IIb MyHC protein expression, a shorter rate of muscle twitch relaxation and almost 50% greater fatigue development during 20s of maximal isometric contraction. In accordance with these observations, muscle adenine nucleotide breakdown was greater during contraction following BRL. In addition, the maximal rate of mitochondrial ATP production from fat oxidation was reduced by 30%, as was muscle GDH activity (a marker of mitochondrial density) collectively indicating a transition in the ‘energy metabolism’ machinery. These observations indicate that BRL induced shift in contractile protein expression, but also a myriad of physiological and metabolic adaptations, not least changes in

rates of ATP turnover during contraction (Hultman, et al., 1991) and alterations in calcium re-uptake kinetics (Schertzer, et al., 2005). It would also seem clear that muscle phenotype alterations can occur in the face of blunted cardiovascular activation by the β_2-agonist, dissociating these two phenomena. It is logical to conclude therefore that whilst chronic β_2-agonist treatment can increase muscle mass, the greater fatigability that accompanies this response may limit the clinical usefulness of this strategy.

Changes in contractile characteristics and biochemical properties following β_2-agonist treatment have previously been reported (Dodd, et al., 1996;Torgan, et al., 1993), however we have combined representative measurements of muscle function, metabolism and composition for the first time to comprehensively demonstrate that chronic β_2-agonist treatment shifts muscle in a global manner towards a faster phenotype. We have previously found that BRL induced shift in muscle phenotype may be linked to a rapid up regulation of MyoD, a myogenic transcription factor that predominates in fast muscle (Jones, et al., 2004). Ryall et al. (2002) demonstrated that 4 weeks treatment with the β_2-agonist, fenoterol, increased muscle fiber size but impaired EDL and soleus muscle function during recovery *in vitro* (Ryall, et al., 2002). The present study has been able to demonstrate that this impairment of muscle function persists in a setting, where oxygen supply is adequate and conditions are more similar to the *in vivo* scenario. Furthermore, because we have systematically characterized muscle metabolism during contraction, we have been able to provide insight as to the potential mechanism(s) by which chronic ß$_2$-agonist treatment accelerates fatigue. Specifically, we show that isometric tension development did not differ between treatment groups, during the initial 4s of contraction. However, after 20s of contraction there was a significant increase in fatigue development in the BRL treated group (Figure 2B), as evidenced by the 45 % reduction in the area under the time-tension curve

(Figure 2C). In accordance with these observations, muscle ATP concentration was maintained throughout contraction in the saline treated group, but declined in BRL treated group, and was paralleled by a sequential accumulation of the products of adenine nucleotide pool breakdown (Figure 3A). The loss of muscle adenine nucleotides during our study with BRL, presenting itself as an increase in muscle IMP, inosine, hypoxanthine and xanthine, has been closely implicated with fatigue development and is considered to reflect the inability of muscle to maintain ATP turnover during contraction, particularly during high intensity contraction in fast muscle fibers (Dudley and Terjung, 1985;Harris, et al., 1997;Meyer, et al., 1980;Sewell and Harris, 1992). When AMP is produced it is broken down to IMP in an effort to stabilize the cellular ATP/ADP ratio under conditions of cellular stress (Atkinson, 1970;Meyer and Terjung, 1980). Additionally, because AMP catabolism is regulated by AMP deaminase, which is activated by transient increases in muscle AMP and a decline in muscle pH (Dudley and Terjung, 1985;Wheeler and Lowenstein, 1979), it is plausible that AMP deaminase expression was increased by chronic ß$_2$-agonist treatment, particularly as its activity is typically greater in fast muscle fibers. In line with this proposed relationship between muscle adenine nucleotide loss and fatigue development, we observed a significant correlation between the extent of muscle tension loss over 20s of contraction and the magnitude of accumulation of the products of adenine nucleotide degradation (namely IMP, inosine, hypoxanthine, xanthine and uric acid; Figure 3B, R^2=0.656, P<0.05). This suggests that the global transition of skeletal muscle to a faster phenotype following BRL treatment is detrimental to muscle function, however some evidence suggests that β_2-agonist treatment in conjunction with aerobic training can counter these deleterious slow-fast fiber type transformations (Lynch, et al., 1996). The clinical usefulness of a β_2-agonist and exercise treatment regimen has yet to be elucidated.

In the present study we determined that muscle glycogen content was reduced in the basal state following 8 weeks of treatment with BRL when compared to saline treatment. We believe this response could have occurred as a result of chronic ß$_2$-adrenreceptor stimulation, resulting in muscle cyclic AMP concentration being chronically elevated and, in turn, activating glycogen phosphorylase and thereby glycogen degradation. This effect of adrenoreceptor agonism on muscle glycogen degradation has been shown to occur in resting human muscle (Chasiotis, et al., 1983) and in the rat (Chasiotis, 1985) following acute administration of adrenaline. Conversely, adrenoreceptor antagonists are known to inhibit muscle glycogen degradation during contraction (Van Baak, et al., 1995). However, because the rate of glycogenolysis over the 20s contraction was identical between treatment groups (1.57 vs 1.56 mmol glucosyl units s^{-1} kg^{-1} dm), this suggests that the glycogen availability was not limiting to muscle tension development during contraction. In summary, while 8 weeks of treatment with BRL resulted in a 21% increase in muscle mass, this was paralleled by phenotype changes that resulted in greater fatigue during 20s of maximal isometric contraction. The tight relationship between muscle adenine nucleotide breakdown and muscle function, coupled with a reduction in *in vitro* mitochondrial ATP production and mitochondrial density suggests that impaired energy metabolism may be central to the enhanced fatigue profile. Our findings suggest that while a strategy of chronic ß$_2$-agonist pro-drug administration may be helpful in reversing muscle atrophy models, the greater fatigability that accompanies such treatment complicate its clinical usefulness; particularly as elderly or frail individuals typically work close to the maximal limits of their muscle capacity (A.Young, 1987).

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Footnotes

* both authors contributed equally to the manuscript and should be considered as joint first authors

Legends for Figures

Figure 1.

Percentage myosin heavy chain protein expression following 1 day, 4 weeks and 8 weeks of saline or BRL treatment. Filled bars = saline. Open bars = BRL.

* ($P<0.05$), **($P<0.01$), ***($P<0.001$) signify different from day 1 value within each treatment group; † ($P<0.05$) signifies different from 4 week value within each treatment group; ‡ ($P<0.05$), ‡‡ ($P<0.01$), ‡‡‡($P<0.001$) signify different from corresponding saline value. Data are mean ± SEM

Figure 2. Values are mean ± SEM. Filled bars = saline. Open bars = BRL.

A. Muscle twitch relaxation time (seconds) following 8 weeks of saline or BRL treatment. ‡ signifies different from corresponding saline value ($P<0.05$).

B. Muscle isometric tension development (expressed as % of initial peak tension) following 8 weeks of saline or BRL treatment. †signifies different from 4 second value within treatment group ($P<0.05$). ‡ signifies different from corresponding saline value ($P<0.05$).

C. Area under the tension x time curve during contraction following 8 weeks of saline or BRL treatment. †† signifies different from 4s value within treatment group ($P<0.01$). ‡ signifies different from corresponding saline value ($P<0.05$).

Figure 3.

A. The sum of muscle adenine nucleotide breakdown products ($mmol \cdot kg^{-1}$ dry muscle) measured at rest and after 4 and 20s of contraction following 8 weeks of treatment with saline or BRL. Filled bars = saline. Open bars = BRL. * and ** signify different from resting value within treatment group ($P<0.05$ and $P<0.01$, respectively). ‡ signifies different from corresponding saline value ($P<0.05$). Data are mean ± SEM

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B. The relationship between the magnitude of muscle fatigue development (% decline from peak tension) and the accumulation of muscle adenine nucleotide breakdown products over the course of 20s of isometric contraction following 8 weeks of saline or BRL treatment (R^2 = 0.656, $P<0.05$).

Figure 4.

Filled bars = saline. Open bars = BRL.

A. Soleus muscle mitochondrial ATP production rates (mmol min^{-1} kg^{-1} wet muscle at 25°C) in the presence of a mixture of pyruvate, palmitoyl-carnitine, α-ketoglutarate and malate (PPKM) and each of the following: glutamate (Glut), α-ketoglutarate (Keto), palmitoyl L-carnitine and malate (PalMal), pyruvate and malate (PyrMal), succinate and rotenone (SuccRot) and succinate (Succ). Values represent means ± SEM. (*n*=9). *Indicates different from saline ($P<0.05$).

B. Soleus muscle enzyme activity (μmol min^{-1} g^{-1} protein at 37°C). Glutamate dehydrogenase (GDH), citrate synthase (CS), β hydroxy acyl-CoA dehydrogenase (HAD) and glyceraldehyde 3 phosphate dehydrogenase (Gly3PDH). Values represent means ± SEM. (*n*=9). *Indicates different from saline ($P<0.05$).

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Table 1

Muscle adenine nucleotides and their breakdown products, cellular energy charge and muscle glycogen content determined at rest and after 4 and 20s of contraction following 8 weeks of treatment with saline or BRL treatment.

	Saline			**BRL-47672**		
	Rest *(n=7)*	***4 s*** *(n=8)*	***20 s*** *(n=8)*	***Rest*** *(n=6)*	***4 s*** *(n=6)*	***20 s*** *(n=6)*
ATP	27.7±0.6	28.2±0.5	27.3±0.6	29.8±0.4‡	28.9±0.6	27.8±0.5*
ADP	2.1±0.1	2.2±0.1	2.2±0.0	2.1±0.1	2.2±0.0	2.3±0.2
AMP	0.15±0.0	0.17±0.0	0.13±0.0	0.12±0.0‡	0.12±0.0	0.15±0.0
EC	0.959±0.0	0.959±0.0	0.959±0.0	0.963±0.0‡	0.961±0.0	0.957±0.0
IMP	0.42±0.03	0.47±0.04	1.14±0.20*†	0.34±0.03	0.62±0.11	1.85±0.37*†
INO	2.63±0.08	2.86±0.15	2.72±0.09	2.77±0.19	2.58±0.14	2.63±0.12
HYP	0.05±0.01	0.13±0.05	0.10±0.02	0.06±0.02	0.07±0.02	0.10±0.02
XAN	0.02±0.01	0.04±0.02	0.04±0.00	0.03±0.01	0.03±0.01	0.03±0.00
UA	2.15±0.52	2.26±0.34	1.21±0.16†	1.80±0.32	1.83±0.26	1.81±0.32
Gly	158.6±5.8	149.9±6.8*	127.3±4.2†*	135.1±4.9‡	121.2±2.4*‡	104.0±4.2‡†*

ATP = Adenosine Triphosphate, ADP = Adenosine diphosphate, AMP = adenosine monophosphate, EC = energy charge, IMP = inosine monophosphate, INO = inosine, HYP = hypoxanthine, XAN = xanthine, UA = uric acid, Gly = glycogen.

* signifies different from resting value within treatment group ($P<0.05$), † signifies different from 4 second value within treatment group ($P<0.05$), ‡ signifies different from corresponding saline value ($P<0.05$). Values represent mean ± SEM in $mmol \cdot kg^{-1}$ dry muscle (except EC which is arbitrary units).

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Figure 1

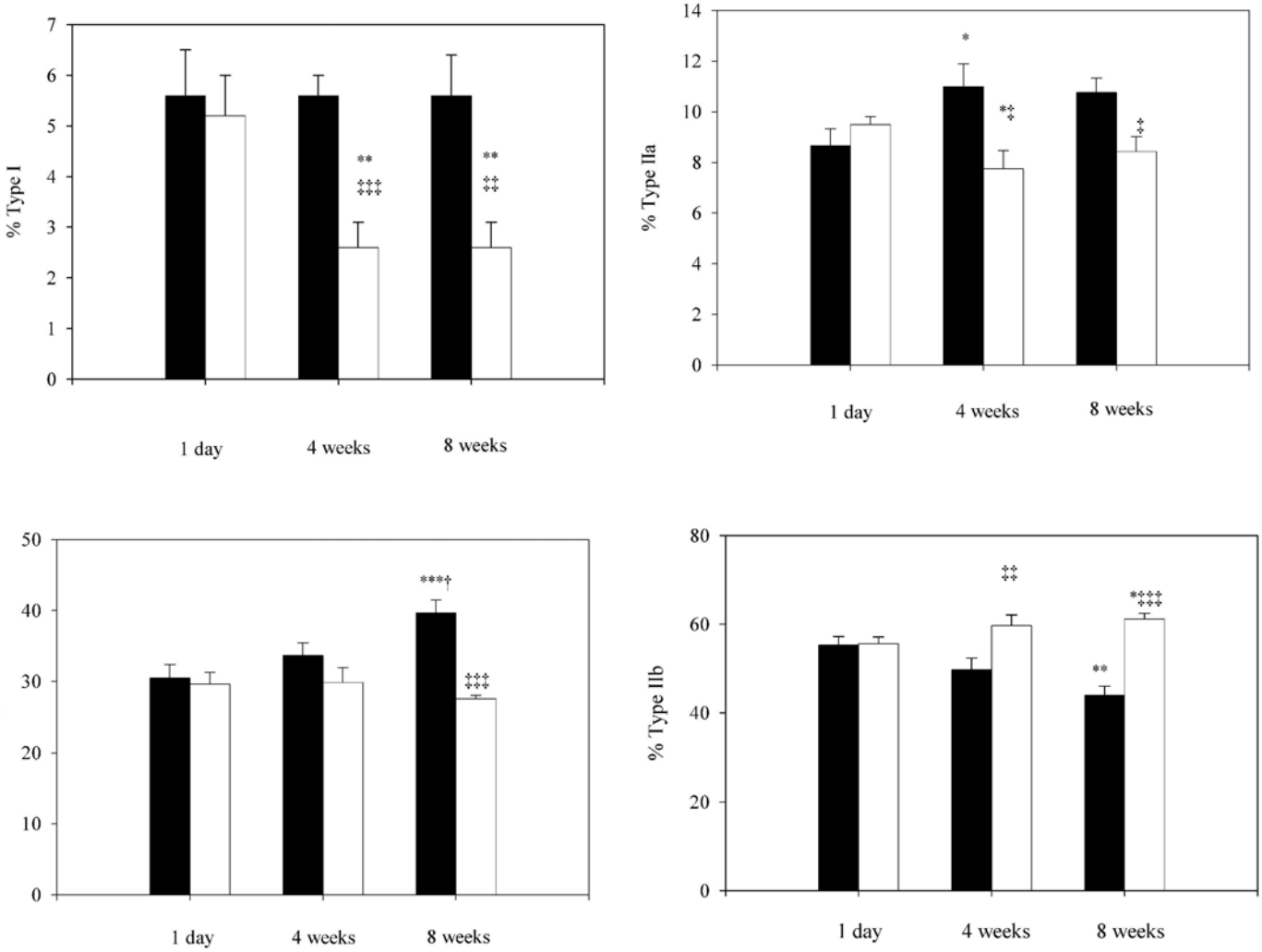

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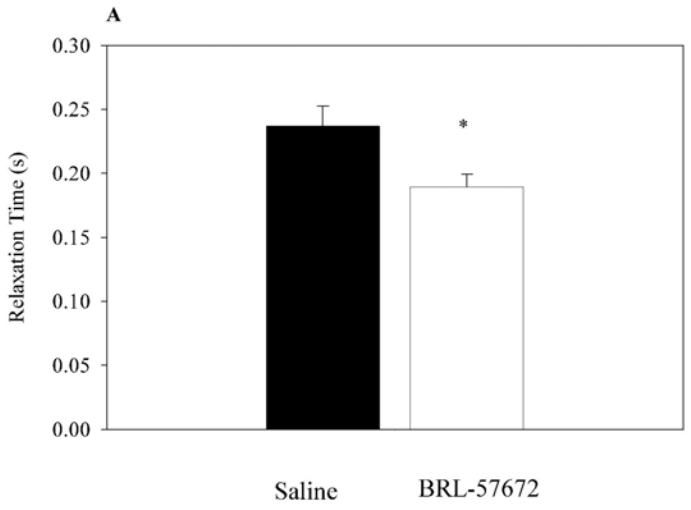
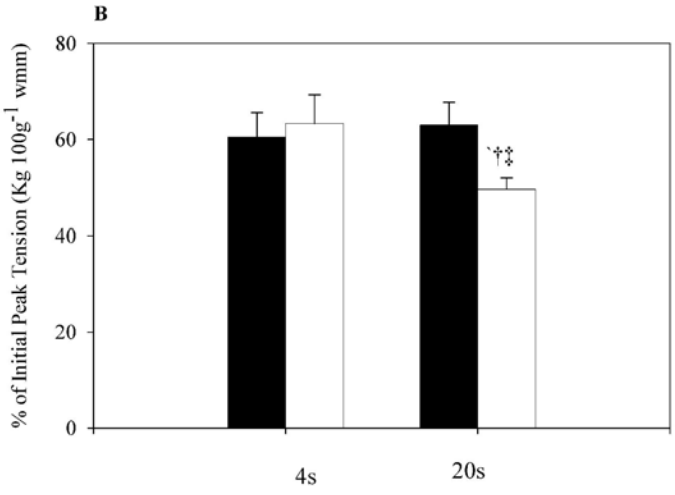
Figure 2





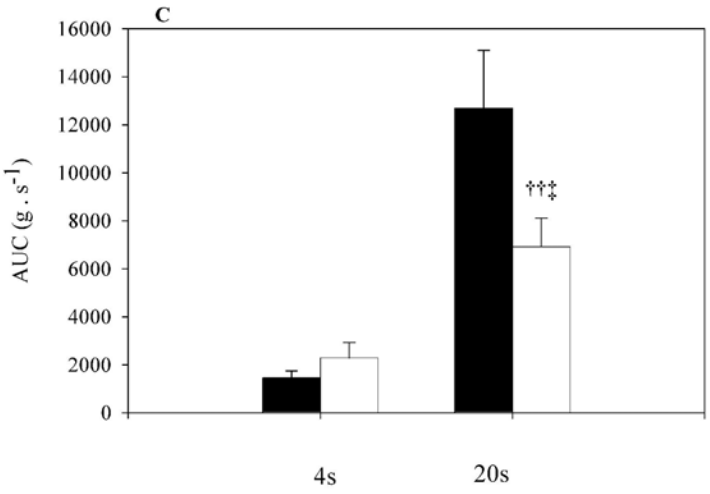


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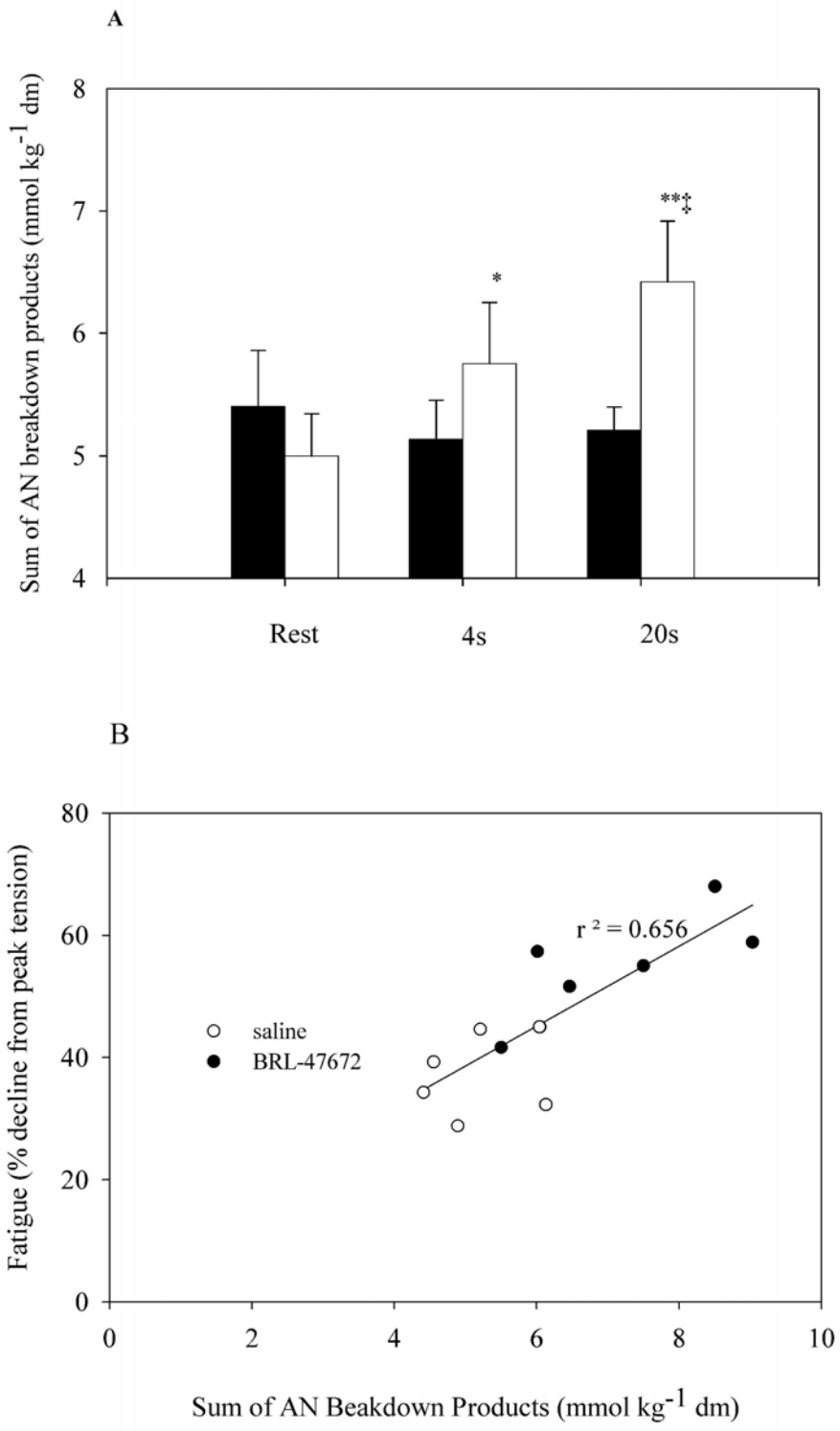
Figure 3



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