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Review Article

Muscle Plasticity and β_2-Adrenergic Receptors: Adaptive Responses of β_2-Adrenergic Receptor Expression to Muscle Hypertrophy and Atrophy

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We discuss the functional roles of β_2-adrenergic receptors in skeletal muscle hypertrophy and atrophy as well as the adaptive responses of β_2-adrenergic receptor expression to anabolic and catabolic conditions. β_2-Adrenergic receptor stimulation using anabolic drugs increases muscle mass by promoting muscle protein synthesis and/or attenuating protein degradation. These effects are prevented by the downregulation of the receptor. Endurance training improves oxidative performance partly by increasing β_2-adrenergic receptor density in exercise-recruited slow-twitch muscles. However, excessive stimulation of β_2-adrenergic receptors negates their beneficial effects. Although the preventive effects of β_2-adrenergic receptor stimulation on atrophy induced by muscle disuse and catabolic hormones or drugs are observed, these catabolic conditions decrease β_2-adrenergic receptor expression in slow-twitch muscles. These findings present evidence against the use of β_2-adrenergic agonists in therapy for muscle wasting and weakness. Thus, β_2-adrenergic receptors in the skeletal muscles play an important physiological role in the regulation of protein and energy balance.

1. Introduction

The skeletal muscle is the most abundant tissue in the human body comprising 40–50% of body mass. Skeletal muscle protein undergoes rapid turnover, which is regulated by the balance between the rates of protein synthesis and degradation. Physical activity (exercise training) and anabolic hormones and drugs (sports doping) increase muscle protein content. However, sarcopenia and muscle disuse (due to unloading, microgravity, or inactivity) and diseases decrease muscle protein content. The rate of protein synthesis is at least in part mediated by β_2-adrenergic receptors (β_2-ARs) in skeletal muscles in both anabolic and catabolic conditions.

ARs belong to the guanine nucleotide-binding G-protein-coupled receptor (GPCR) family. Skeletal muscle contains a significant proportion of β-ARs. The β_2 subtype is the most abundant, while ~7–10% of ARs are the β_1 subtype [1, 2]. Furthermore, β_2-AR is more dense in slow-twitch muscles than in fast-twitch muscles [3, 4]. However, the magnitude of anabolic responses to β_2-adrenergic agonists is greater in fast-twitch muscles than in slow-twitch muscles [5–8].

The family of β-ARs was originally believed to signal predominantly via coupling with a stimulatory guanine nucleotide-binding protein, Gα_s; however, recent studies revealed that both β_2- and β_3-ARs in skeletal muscle are also capable of coupling to an inhibitory guanine nucleotide-binding protein, Gα_i [9]. β_2-AR activates the Gα_s/adenylyl cyclase (AC)/cyclic adenosine monophosphate (cAMP)/cAMP-dependent protein kinase A (PKA) signaling

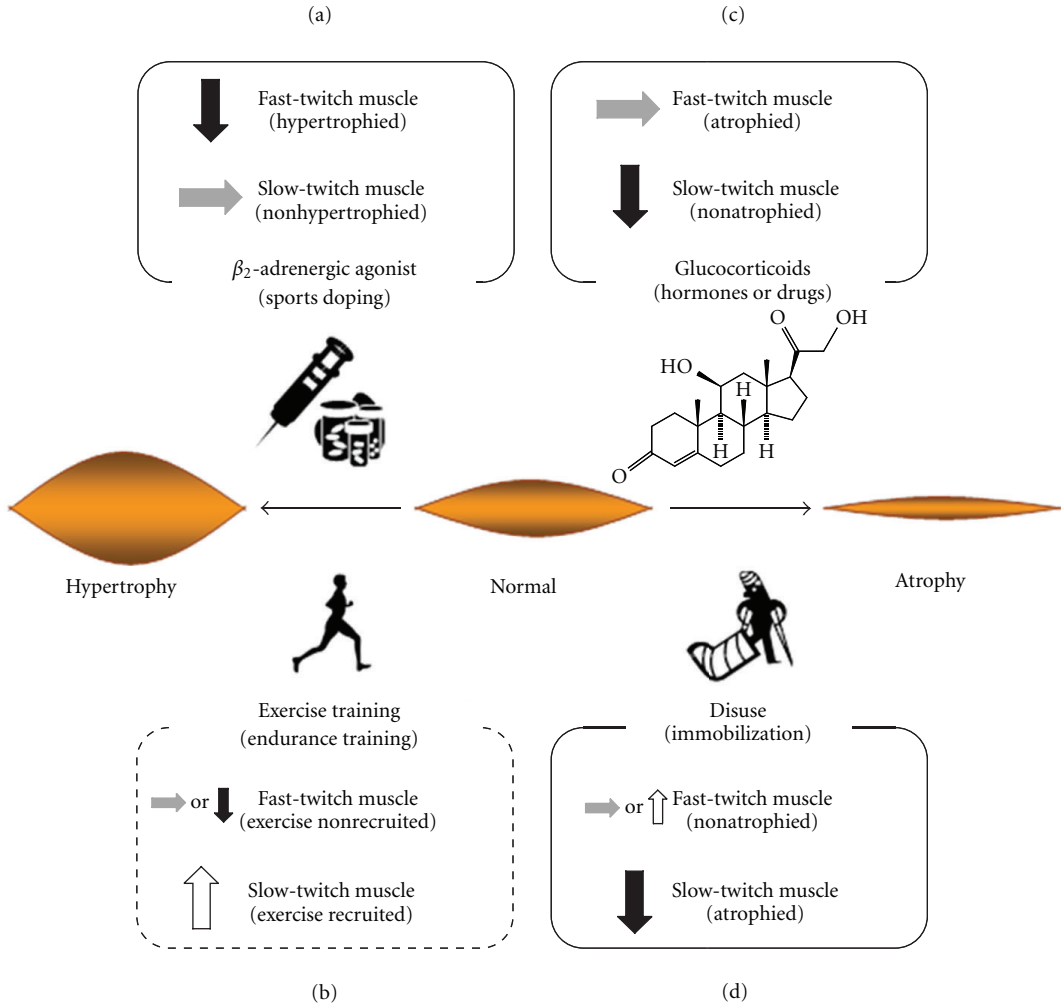

Figure 1: Changes in β_2-AR expression in hypertrophied and atrophied skeletal muscles. (a) β_2-AR stimulation using anabolic drugs downregulates β_2-AR expression in hypertrophied fast-twitch muscles but not in slow-twitch muscles [4, 7, 8, 14–17]. (b) Exercise training such as endurance training upregulates β_2-AR expression in exercise-recruited slow-twitch muscles, whereas no changes or downregulations are observed in fast-twitch muscles [18, 19], although muscle mass is not altered. However, although exercise training such as isometric strength training induces muscle hypertrophy, there is no insight regarding the effects of such exercise on β_2-AR expression. The differential effects of types of exercise training on physiological responses such as β_2-AR expression and muscle hypertrophy should be clarified in more detailed and are currently being investigated by our group. (c) Catabolic hormones or drugs such as glucocorticoids downregulate β_2-AR expression in nonatrophied slow-twitch muscles but not fast-twitch muscles [16, 20, 21]. (d) Muscle disuse downregulates β_2-AR expression in atrophied slow-twitch muscle, whereas no changes or upregulation of receptor expression are observed in fast-twitch muscles [14, 22]. Up arrow (open arrow): upregulation of β_2-AR expression; down arrow (filled arrow): downregulation of β_2-AR expression; lateral arrow (shade arrow): no change.

pathway. The signaling pathway is at least in part responsible for the anabolic response of skeletal muscle to β_2-AR stimulation. Further, in addition to the well-documented inhibition of AC activity [10], β_2-AR coupling to $G\alpha_i$ activates $G\alpha_s$-independent pathways [11].

β_2-AR has 7 transmembrane α helices forming 3 extracellular loops, including an NH_2 terminus and 3 intracellular loops that include a COOH terminus [12]. β_2-AR contains phosphorylation sites in the third intracellular loop and proximal cytoplasmic tail. Phosphorylation of these sites triggers the agonist-promoted desensitization, internalization, and degradation of the receptor [13]. These regulatory mechanisms contribute to maintaining agonist-induced β_2-AR responsiveness in various conditions.

The adaptive responses of β_2-AR expression to anabolic and catabolic conditions in skeletal muscles are shown in Figure 1. Understanding the correlation between changes in muscle mass and β_2-AR expression in several anabolic or catabolic conditions present scientific evidence to eradicate sports doping and identify novel approaches for attenuating

muscle atrophy concomitant with disuse and various diseases. This paper will discuss the effects of (1) pharmacological β_2-AR stimulation (sports doping), (2) muscle hypertrophy (exercise training), and (3) muscle atrophy (catabolic conditions and hormones) on β_2-AR expression in skeletal muscles.

2. Pharmacological Stimulation of β_2-AR

2.1. Muscle Hypertrophy and β_2-AR. A β_2-adrenergic agonist, clenbuterol [1-(4-amino-3,5-dichlorobenzyl)-2-(tert-butylamino) ethanol], is used as a nonsteroidal anabolic drug for sports doping. According to the recent World Anti-Doping Agency (WADA) documents, clenbuterol was the seventh most commonly used anabolic agent in 2009 (67 cases; 2.0% of all anabolic agents used).

Numerous studies have shown that the administration of β_2-adrenergic agonists induces muscle hypertrophy in many species [23–25]. Experiments using mice lacking β_1-AR, β_2-AR, or both demonstrate that β_2-adrenergic agonist-induced functions such as muscle hypertrophy are mediated by β_2-AR [26]. β_2-Adrenergic agonists promote muscle growth by increasing the rate of protein synthesis and/or decreasing protein degradation [23–25]. Furthermore, β_2-adrenergic agonists induce slow-to-fast [myosin heavy chain (MHC)I/β → MHCIIa → MHCIId/x → MHCIIb] transformation of muscle fibers.

The β_2-AR signaling pathway involves the agonist-dependent activation of Gα_s, which in turn activates AC, resulting in increased cAMP production. Cyclic AMP-activated PKA initiates the transcription of many target genes via the phosphorylation of cAMP-response-element-(CRE-) binding protein (CREB) or adaptor proteins such as CREB-binding protein (CBP) and p300, subsequently promoting protein synthesis [23]. While β_2-AR-mediated signaling was traditionally believed to involve selective coupling to Gα_s, recent studies revealed that β_2-AR exhibits dual coupling to both Gα_s and Gα_i in skeletal muscles [9, 23]. In addition to Gα_s, Gα_i-linked G$\beta\gamma$ subunits play an active role in various cell signaling processes such as the phosphoinositol 3 kinase (PI3 K)/protein kinase B (Akt)/mammalian target of rapamycin (mTOR)/p70S6 K and PI3 K/Akt/forkhead box-O (FOXO) pathways. These signaling pathways play important roles in β_2-adrenergic agonist-induced hypertrophy in skeletal muscles [23].

In addition to promoting protein synthesis, the hypertrophic response of skeletal muscles following β_2-adrenergic agonist administration is associated with decreased protein degradation. β_2-Adrenergic agonists attenuate protein degradation predominantly via Ca^{2+}-dependent proteolysis and the ATP/ubiquitin-dependent pathway [27–31]. However, there is little knowledge regarding the preventive effects of β_2-adrenergic agonists on the proteolysis system compared with the protein synthesis system.

The hypertrophic responses to β_2-adrenergic agonists are observed much frequently in fast-twitch muscle than in slow-twitch muscle. Our group previously demonstrated that clenbuterol administration ($1.0\,mg\cdot kg^{-1}\cdot day^{-1}$) to rats for 10 days increases the mass of fast-twitch (extensor digitorum longus: EDL) muscle without altering in slow-twitch (soleus) muscle [7, 8]; other groups also observed the same tendency [5, 6, 32–35]. However, the mechanisms of the fiber-type-dependent effects of β_2-adrenergic agonists on muscle hypertrophy remain unclear.

Pearen et al. [36, 37] and Kawasaki et al. [38] identified that β_2-AR activation increases the expression of the orphan nuclear receptor, NOR-1 (NR4A3), a negative regulatory factor of myostatin (a member of the transforming growth factor-β superfamily and a potent negative regulator of muscle mass), in fast-twitch muscles without altering that in slow-twitch muscles. Furthermore, Shi et al. [32] demonstrate the possibility that β_2-adrenergic agonist-induced fiber-type-dependent hypertrophy is in part due to the extracellular signal-regulated kinase (ERK)/mitogen activated protein kinase (MAPK) pathway. Moreover, the pharmacological inhibition of the PI3 K/Akt/mTOR signaling pathway revealed that the attenuation of the anabolic response to clenbuterol is greater in fast-twitch muscles than in slow-twitch muscles [30]. In addition to the protein synthesis system, Yimlamai et al. [35] found that clenbuterol inhibits ubiquitination more strongly in fast-twitch muscles than in slow-twitch muscles. Thus, β_2-AR-mediated signaling pathways tend to promote muscle hypertrophy to a greater extent in fast-twitch muscle than in slow-twitch muscle.

2.2. Posttranslational Regulation of β_2-AR. As shown in Table 1, some reports focus on the responses of β_2-AR expression to β_2-AR stimulation in skeletal muscles [4, 7, 8, 14–17]. This is because β_2-AR functions such as muscle hypertrophy are maintained via receptor density, including synthesis and downregulation as well as receptor sensitivity, which includes receptor sensitization, desensitization, phosphorylation, and internalization [13, 39, 40].

The desensitization of β_2-AR is associated with receptor phosphorylation. McCormick et al. [41] demonstrate that fast-twitch fibers mainly express nonphosphorylated β_2-AR, whereas slow-twitch fibers predominantly express phosphorylated β_2-AR. Furthermore, treating muscle fibers with β_2-adrenergic agonists (e.g., clenbuterol, formoterol, and salbutamol) increases the phosphorylation of β_2-AR in slow-twitch fibers but not in fast-twitch fibers [41]. On the other hand, the receptor phosphorylation occurs via the actions of protein kinases (such as PKA) and/or GPCR kinase (GRK). Rat skeletal muscles contain predominantly GRK2 and GRK5; GRK protein is expressed more in fast-twitch muscles than in slow-twitch muscles. These expression levels in each type of muscle fiber are not altered by β_2-adrenergic agonist administration [42]. Thus, there is a negative correlation between the level of phosphorylated β_2-AR and receptor kinase. Therefore, further investigation is needed to reveal the detailed mechanism of β_2-AR phosphorylation.

Following β_2-AR phosphorylation, the receptor is internalized into the cytosol. The internalized β_2-AR is then degraded or dephosphorylated and subsequently recycled to the membrane [13, 43–45]. Prolonged administration of β_2-adrenergic agonists leads to the downregulation of β_2-AR density in skeletal muscles [15–17]. These posttranslational

Table 1: Responses of β_2-AR expression in skeletal muscle to anabolic and catabolic conditions.

Conditions	Species	β_2-AR		Other findings	References
		Protein	mRNA		
β_2-AR stimulation					
Fenoterol ($1.4\,mg \cdot kg^{-1} \cdot day^{-1}$, 4 weeks)	Rat	↓ (FT) → (ST)	n.d.		[4]
Clenbuterol ($1.0\,mg \cdot kg^{-1} \cdot day^{-1}$, 10 days)	Rat	n.d.	↓ (FT) → (ST)	β_1-AR mRNA ↓ (LV) β_2-AR mRNA ↓ (LV)	[7] [8]
Clenbuterol ($1.0\,mg \cdot kg^{-1} \cdot day^{-1}$, 10 days)	Rat	n.d.	↓ (FT) → (ST)	GR mRNA ↓ (FT) HuR mRNA ↓ (FT) AUF1 mRNA ↓ (FT) hnRNP A1 mRNA ↓ (FT)	
Fenoterol ($1.4\,mg \cdot kg^{-1} \cdot day^{-1}$, 2–7 days)	Rat	→ (FT, ST)	↓ (FT, ST)	$G\alpha_s$ content → (FT, ST) AC activity → (FT, ST)	[14]
Clenbuterol ($2.0\,mg \cdot kg^{-1} \cdot day^{-1}$, 18 days)	Rat	↓ (FT+ST)	n.d.		[15]
Clenbuterol ($4.0\,mg \cdot kg^{-1}$ of feed, 10 days)	Rat	↓ (FT)	n.d.	β_2-AR affinity → (FT)	[16]
Clenbuterol ($0.2\,mg \cdot kg^{-1} \cdot day^{-1}$, 7 days)	Rat	↓ (FT+ST)	n.d.		[17]
Clenbuterol (50 μM) Formoterol (100 μM) Salbutamol (500 μM)	Mouse (ex vivo)	Phosphorylated β_2-AR ↑ (ST), → (FT)	n.d.	cAMP concentration ↑ (FT, ST)	[41]
Endurance training					[18]
Treadmill (12 weeks)	Rat	↓ (FT)	n.d.	β_2-AR afflnity → AC activty ↓	
Treadmill (18 weeks)	Rat	→ (FT) ↑ (ST)	n.d.	$G\alpha_s$ content ↓ AC activity ↑ (FT, ST) β_2-AR density → (acute)	[19]
Catabolic conditions					[20]
Dexamethasone ($1.0\,mg \cdot kg^{-1} \cdot day^{-1}$, 10 days)	Rat	→ (FT, ST)	→ (FT) ↓ (ST)	GR mRNA ↓ (FT, ST) CREB mRNA ↓ (ST) AUF1 mRNA ↑(FT)	
Dexamethasone ($1.0\,mg \cdot kg^{-1} \cdot day^{-1}$, 10 days)	Rat	n.d.	→ (FT) ↓ (ST)	GR mRNA ↓ (FT, ST) β_1-AR mRNA ↑ (LV)	[21]
Dexamethasone ($0.2\,mg \cdot kg^{-1} \cdot day^{-1}$, 10 days)	Rat	→ (FT)	n.d.	β_2-AR affinity → (FT)	[16]
Casted-immobilization (10 days)	Rat	→ (FT, ST)	→ (FT) ↓ (ST)	GR mRNA ↓ (ST) GR protein ↓ (ST)	[22]
Aging	Rat	→ (FT, ST)	n.d.		[4]
Injury (bupivacaine injection)	Rat	↑ (FT) ↓ (ST)	↑ (FT) ↓ (ST)	$G\alpha_s$ content ↑ (FT), ↓ (ST) AC activity ↑ (FT, ST)	[14]

FT, fast-twitch muscle; ST, slow-twitch muscle; LV, left ventricle muscle. Up arrow, increase; down arrow, decrease; lateral arrow, no change. n.d., no data.

regulations are advantageous for maintaining the rate of muscle protein synthesis and/or degradation.

2.3. Short-Term and Chronic Transcriptional Regulation of β_2-AR. β_2-AR synthesis, including transcription and subsequent translation, is required to restore transmembrane receptor density. The process of β_2-AR synthesis can be separated into 2 pathways: (1) the positive autoregulation of β_2-AR gene transcription via receptor-mediated elevation of cAMP concentration followed by the phosphorylation and activation of CREB [46, 47] and (2) the transactivation of the β_2-AR gene via interaction between hormones and the nuclear receptor complex and response elements on the β_2-AR promoter region [48]. In particular, the transcription of the β_2-AR gene and the subsequent mRNA expression via cAMP-mediated CRE activation increased in response to short-term β_2-adrenergic agonist exposure [46, 47]. Moreover, treatment with glucocorticoids or thyroid hormone transactivates the β_2-AR gene both in vitro and in vivo [48–51].

Our previous reports demonstrate that clenbuterol administration ($1.0\,mg \cdot kg^{-1} \cdot day^{-1}$) for 10 days to rats

decreases β_2-AR mRNA expression in the fast-twitch EDL muscle without altering that in the slow-twitch soleus muscle [7, 8]. Furthermore, the mRNA expression of glucocorticoid receptors (GRs) was also decreased with clenbuterol treatment in the EDL muscle but not in the soleus muscle [8]. Glucocorticoids and the GR complex activate the transcription of the β_2-AR gene via interaction with glucocorticoid response elements (GREs), consensus *cis*-acting DNA sequences (i.e., AGA ACA nnn TGT TCT) on its promoter regions [48], thus upregulating β_2-AR expression [16, 50, 51]. These findings corroborate our results that there is a positive correlation between the expression levels of β_2-AR and GR in skeletal muscles. Beitzel et al. [14] also report that administrating the β-adrenergic agonist, fenoterol ($1.4\,\text{mg}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$, i.p.), for 5 days decreases β_2-AR mRNA expression in the EDL and soleus muscles. Thus, in contrast to the transactivation of the β_2-AR gene and increase in the mRNA level in response to short-term agonist exposure, chronic β_2-adrenergic stimulation inhibits β_2-AR synthesis in skeletal muscles.

2.4. Posttranscriptional Regulation of β_2-AR. In addition to post-translational and transcriptional regulation, several groups focus on the posttranscriptional regulation of β_2-AR mRNA. β_2-AR mRNA contains an AU-rich element (ARE) within the 3′-untranslated region (3′-UTR) that can be recognized by several mRNA-binding proteins, including Hu antigen R (HuR), AU-rich element binding/degradation factor1 (AUF1), and heterogeneous nuclear ribonucleoprotein A1 (hnRNP A1) [52–55]. These factors play a role in the regulation of β_2-AR mRNA stability [52–55]. Our study demonstrates that clenbuterol-induced stimulation of β_2-AR decreases the mRNA expressions of these factors in the EDL but not in the soleus muscle [8], suggesting that the posttranscriptional process of β_2-AR synthesis requires the stability of its mRNA to be regulated.

3. Exercise Training and β_2-AR

Strength-resistance training increases muscle mass [56], fiber cross-sectional area [57], protein and RNA contents [58], and the capacity to generate force [59]. In contrast to strength training, endurance training is characterized by increased mitochondrial mass [60], increased oxidative enzymes [61], decreased glycolytic enzymes [62], increased slow contractile and regulatory proteins [62], and decreased fast fiber area [63]. These findings suggest that the functional roles of β_2-AR in skeletal muscles differ with the type of exercise training.

3.1. Strength Exercise Training and β_2-AR. Mounier et al. [64] investigated the changes in the weight of the EDL muscle induced by clenbuterol administration, strength training, and a combination of both. They found that the effects of strength training and clenbuterol on muscle hypertrophy were not additive in fast-twitch muscles. Their report also demonstrates that the strength-training-induced enhancement of lactate dehydrogenase-specific activity is completely inhibited by clenbuterol administration, while the clenbuterol-induced decrease in monocarboxylate transporter1 mRNA expression is completely offset by strength training [64]. Thus, there are no synergetic effects of a combination of strength training and β_2-AR stimulation on muscle mass. Furthermore, strength training counteracts molecular modifications such as glycolytic control induced by chronic clenbuterol administration in fast-twitch muscles to some extent. However, our evidence regarding the synergistic effects of strength training and β_2-AR stimulation is insufficient because the experimental models of strength-trained animals are not fully established.

3.2. Endurance Exercise Training and β_2-AR. In contrast to strength training, β_2-AR stimulation affects endurance-training-induced modulations such as contractile activity [65], muscle fiber-type shift [65], metabolic enzyme activity [66], and insulin resistance [67, 68]. Lynch et al. [65] demonstrated that low-intensity endurance training prevents clenbuterol-induced slow-to-fast (type I fiber → type II fiber) fiber-type transformation in the EDL and soleus muscles, and thereby offsets the clenbuterol-induced decrease in Ca^{2+} sensitivity in fast-twitch fibers. These results suggest that endurance-training-heightened muscle aerobic capacity is attenuated by β_2-AR stimulation-induced muscle fiber-type transformations. Furthermore, pharmacological β-AR blockage diminishes the endurance-training-induced increase in citrate synthase activity in the fast-twitch plantaris muscle [66]. Moreover, clenbuterol administration prevents the endurance-training-induced improvement in insulin-stimulated glucose uptake and attenuates the increase in citrate synthase activity in the skeletal muscles of obese Zucker rats [67, 68]. These findings demonstrate that the endurance-training-induced increase in aerobic metabolism in skeletal muscles requires moderate but not excessive stimulation of β_2-AR.

Recently, Miura et al. [69] demonstrated that an increase in peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) mRNA in response to exercise is mediated by β_2-AR activation. Furthermore, the Ca^{2+}-signaling [70] and p38 MAPK pathways [71], which is downstream of β_2-AR, are activated in skeletal muscles in response to exercise, which regulates PGC-1α expression. Since PGC-1α promotes mitochondrial biogenesis [72], the exercise-induced activation of β_2-AR may in part enhance aerobic capacity by increasing PGC-1α expression. Thus, β_2-AR stimulation is essential for enhancing the effects of exercise training on muscle functions such as fiber-type shift as well as oxidative and anaerobic metabolism.

3.3. Response of β_2-AR Expression to Exercise Training. As mentioned above, the functional roles of β_2-AR during exercise training are physiologically important in skeletal muscles. Therefore, changes in the expression and sensitivity of β_2-AR should be important for the metabolic, anabolic, and catabolic adaptations of skeletal muscles during exercise training. Nevertheless, there is little information on the response of β_2-AR expression to exercise training in skeletal muscles. However, many studies demonstrate the effects of exercise training on β_2-AR expression in several tissues

and cell types such as myocardia [73, 74], adipocytes [75], and macrophages [76]. Barbier et al. [73] demonstrated that exercise training induces changes in the distribution of β_1-, β_2-, and β_3-AR densities in the rat left ventricle. In adipocytes, the exercise-induced trafficking of β_2-AR into the cell membrane from the cytosol is coupled with adipocytes' function to increase intracellular cAMP production [75]. Kizaki et al. [76] also found a reduction in the expression of β_2-AR mRNA in macrophages and highlight the significance of β_2-AR in the exercise training-induced improvement of macrophages' innate immune function. Thus, changes in β_2-AR expression play a role in physiological adaptations to exercise training in several tissues.

A few studies also report the effects of exercise training on β-AR in skeletal muscles [18, 19, 77, 78] (Table 1). Nieto et al. [18] demonstrate that β-AR density and $G\alpha_s$ content in the fast-twitch gastrocnemius muscle are significantly lower in endurance-exercised rats than in controls. They also reveal that exercise reduces receptor- and nonreceptor-mediated (i.e., pharmacological stimulation of AC by forskolin) AC activity in muscles [18]. However, Buckenmeyer et al. [19] report that endurance training increases β-AR density in slow-twitch muscles that are primarily recruited during endurance training, whereas β-AR density is not altered in fast-twitch muscles. Their report also demonstrates that receptor-mediated AC activity in slow-twitch muscles is increased by endurance training, and nonreceptor-mediated AC activity is increased by training in both fast- and slow-twitch muscles [19]. In contrast to chronic endurance training, the effects of acute exercise on β-AR density and AC activity in each type of muscle were not observed [19]. Therefore, endurance-exercise-training-induced changes in β_2-AR expression and signaling in slow-twitch muscle contributes to the adaptation of metabolic and anabolic capacities during exercise.

4. Muscle Atrophy and β_2-AR

4.1. Preventive Roles of β_2-AR in Disuse-Induced Muscle Atrophy. Muscle wasting and weakness are common in physiological and pathological conditions, including aging, cancer cachexia, sepsis, other forms of catabolic stress, denervation, disuse (e.g., unloading, inactivity, and microgravity), burns, human immunodeficiency virus-(HIV)-acquired immunodeficiency syndrome (AIDS), chronic kidney or heart failure, chronic obstructive pulmonary disease (COPD), and muscular dystrophies. For many of these conditions, the anabolic properties of β_2-adrenergic agonists provide therapeutic potential for attenuating or reversing muscle wasting, muscle fiber atrophy, and muscle weakness. These β_2-adrenergic agonists also have important clinical significance for enhancing muscle repair and restoring muscle function after muscle atrophy.

In particular, muscle disuse, which is mainly reflected by increased myofibrillar protein breakdown, causes a progressive decrease in muscle strength associated with a decreased cross-sectional area of muscle fibers. Therefore, preventing disuse-induced muscle atrophy is a problem requiring urgent attention and highlights β_2-AR as a target of pharmacological stimulation. Since 2000, many groups have focused on the preventive effects of β_2-adrenergic agonist on disuse-induced muscle atrophy [4, 34, 35, 79].

Yimlamai et al. [35] demonstrate that clenbuterol attenuates the hindlimb unweighting-induced atrophy and reduces ubiquitin conjugates only in fast-twitch plantaris and tibialis anterior muscles but not in the slow-twitch soleus muscle; this suggests that clenbuterol alleviates hindlimb unweighting-induced atrophy, particularly, in fast-twitch muscles at least in part through a muscle-specific inhibition of the ubiquitin-proteasome pathway. However, Stevens et al. [34] report that clenbuterol treatment accelerates hindlimb unweighting-induced slow-to-fast (MHCI/β → MHCIIa → MHCIId/x → MHCIIb) transformation in the soleus muscle. β_2-Adrenergic agonist also reverses muscle wasting and weakness in several conditions such as aging [4], muscular dystrophy [29], denervation [80], cancer cachexia [28], and myotoxic injury [81].

4.2. Preventive Roles of β_2-AR in Catabolic Hormone-Induced Muscle Atrophy. Prolonged muscle disuse and/or unloading increases the secretion of glucocorticoids, which promotes the catabolism of muscle proteins via the ubiquitin-proteasome pathway [82, 83]. Sepsis also elevates plasma glucocorticoids and adrenocorticotropic hormone (ACTH) levels [84]. Therefore, several studies focus on the counteractive effects of β_2-AR stimulation on glucocorticoid-induced muscle atrophy [16, 85]. Huang et al. [16] report that clenbuterol almost prevents the decrease in the weight of gastrocnemius/plantaris muscle bundles induced by dexamethasone, a synthetic glucocorticoid. Pellegrino et al. [85] demonstrate that concurrent treatment of clenbuterol with dexamethasone minimizes MHC-transformation-induced by clenbuterol (slow-to-fast) or dexamethasone (fast-to-slow) alone. Thus, β_2-AR stimulation plays an inhibitory role in muscle atrophy and weakness induced by catabolic diseases, mechanical unloading, catabolic hormones, and pharmacological agents.

4.3. Response of β_2-AR Expression to Catabolic Hormones. Although the effectiveness of β_2-AR stimulation on muscle atrophy is well documented, catabolic condition-induced changes in the expression of β_2-AR in skeletal muscles are not fully understood. Understanding the responses of β_2-AR expression to muscle atrophy is required to establish treatments for muscle atrophy.

Table 1 shows the catabolic-condition-induced changes in β_2-AR expression in skeletal muscles. Our group investigated whether catabolic hormones or agents alter β_2-AR expression in skeletal muscles [20, 21]. Dexamethasone administration ($1.0\,mg\cdot kg^{-1}\cdot day^{-1}$) to rats for 10 days decreases the expression of β_2-AR mRNA in the soleus muscle without altering that in the EDL muscle, although the expression of β_2-AR protein in the EDL and soleus muscles is not altered [20, 21]. Dexamethasone also does not alter β_2-AR density in gastrocnemius/plantaris muscle bundles [16]. These phenomena are specifically observed in skeletal

muscles; meanwhile, glucocorticoids and the GR complex activate the transcription of β_2-AR gene in the human hepatoma cell line (HepG2) [48], subsequently leading to the upregulation of β_2-AR levels in DDT_1 MF-2 smooth muscle cells [50] and lung tissue [16, 51]. Furthermore, dexamethasone decreases the expression of GR mRNA in the soleus muscle [20, 21]. Dexamethasone also decreases and increases the expression of CREB mRNA, a transcription factor of the β_2-AR gene [46, 47], in the soleus and EDL muscles, respectively [20]. These findings suggest that the dexamethasone-induced decrease in the expression of β_2-AR mRNA in the slow-twitch soleus muscle is associated with transcriptional regulations.

4.4. Response of β_2-AR Expression to Muscle Disuse. The effects of physiological and pathological catabolic-condition-induced muscle atrophy on β_2-AR expression have also been studied (Table 1) [4, 14, 22]. Our recent investigation demonstrates that casted immobilization (knee and foot arthrodesis) for 10 days markedly induced atrophy in the soleus muscle, whereas it decreased the expression of β_2-AR mRNA [22]. Decreased GR mRNA and protein expression was also detected in the soleus muscle [22]. These results suggest that casted immobilization decreases the expression of β_2-AR mRNA in slow-twitch muscles via the downregulation of GR levels and subsequent glucocorticoid signals. On the other hand, Ryall et al. [4] demonstrate that aging-induced muscle wasting is observed in the EDL and soleus muscles, although there are no age-associated changes in β_2-AR density in these muscles. Furthermore, in the regeneration process from muscle injury induced by bupivacaine injection, β_2-AR density and mRNA expression as well as $G\alpha_s$ content are decreased in the soleus but increased in the EDL muscle [14]. Thus, the effects of catabolic conditions such as disuse, aging, and injury on β_2-AR expression are different from and/or dependent on the conditions, especially in fast-twitch muscles, whereas decreasing tendencies are observed in slow-twitch muscles.

Both pharmacological and mechanical studies indicate that the preventive effects of β_2-AR stimulation on muscle atrophy and weakness are limited by decreased β_2-AR synthesis and subsequently decreased density. In order to use β_2-adrenergic agonists as a therapeutic agent for muscle wasting, further studies are necessary to obtain detailed evidence regarding the responses of β_2-AR expression and function to muscle atrophy.

5. Conclusions

In this paper, we discussed adaptive responses of β_2-AR expression in skeletal muscles to β_2-adrenergic agonist treatment, exercise training, muscle disuse, and glucocorticoid treatment. This paper also outlined the functional roles of β_2-AR in skeletal muscles. Skeletal muscle partly requires β_2-AR activation for hypertrophy, regeneration, and atrophy prevention; however, its functions and responsiveness must be adaptively regulated by the receptor itself via downregulation, synthesis, and desensitization. New insight in the form of scientific evidence is needed to eradicate sports doping and to identify new therapeutic targets for attenuating muscle atrophy induced by physiological and pathological conditions.

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