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TOPICAL REVIEW

The beta$_2$-adrenergic receptor – a re-emerging target to combat obesity and induce leanness?

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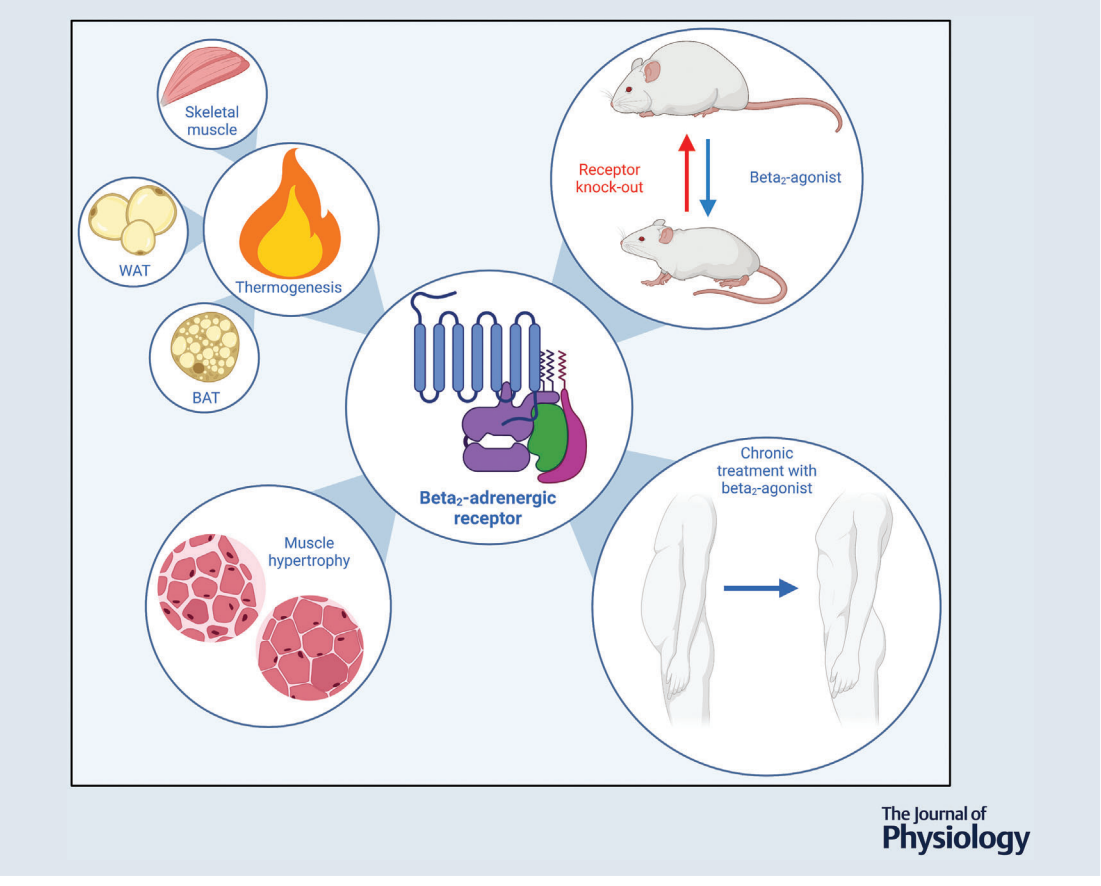

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Abstract Treatment of obesity with repurposed or novel drugs is an expanding research field. One approach is to target beta$_2$-adrenergic receptors because they regulate the metabolism and phenotype of adipose and skeletal muscle tissue. Several observations support a role for the beta$_2$-adrenergic receptor in obesity. Specific human beta$_2$-adrenergic receptor polymorphisms are associated with body composition and obesity, for which the Gln27Glu polymorphism is associated with obesity, while the Arg16Gly polymorphism is associated with lean mass in men and the development of obesity in specific populations. Individuals with obesity also have lower abundance of beta$_2$-adrenergic receptors in adipose tissue and are less sensitive to catecholamines. In addition, studies in livestock and rodents demonstrate that selective beta$_2$-agonists induce a so-called 'repartitioning effect' characterized by muscle accretion and reduced fat deposition. In humans, beta$_2$-agonists dose-dependently increase resting metabolic rate by 10–50%. And like that observed in other mammals, only a few weeks of treatment with beta$_2$-agonists increases muscle mass and reduces fat mass in young healthy individuals. Beta$_2$-agonists also exert beneficial effects on body composition when used concomitantly with training and act additively to increase muscle strength and mass during periods with resistance training. Thus, the beta$_2$-adrenergic receptor seems like an attractive target in the development of anti-obesity drugs. However, future studies need to verify the long-term efficacy and safety of beta$_2$-agonists in individuals with obesity, particularly in those with comorbidities.

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Abstract figure legend The beta$_2$-adrenergic receptor is a potential target to combat obesity because it regulates thermogenesis and the phenotype of highly metabolic tissues, such as skeletal muscle, white adipose tissue (WAT), and brown adipose tissue (BAT). Animal models underpin the beta$_2$-adrenergic receptor as integral in regulating body composition. Thus, beta$_2$-agonist-fed animals lose fat and gain muscle mass, and mice lacking the beta$_2$-adrenergic receptor have greater fat percentage than wild-type. Clinical trials also show that beta$_2$-agonists increase metabolic rate and promote leanness in humans. For these reasons, future studies should elucidate whether such effects can be harnessed in persons with obesity and comorbidities.

Introduction

With the increasing prevalence of obesity and its associated health consequences as well as the societal impact, strategies for its prevention and treatment are warranted (Bluher, 2019). Although it is well-established that a lifestyle consisting of regular exercise and a healthy diet counters the development of obesity, lifestyle interventions often fail to provide long-term changes in individuals with obesity (Bray & Wadden, 2015). Hence, treatment of obesity through pharmacological formulations targeting obesolytic pathways with both repurposed, older drugs, and novel experimental medications are expanding fields of research (Lund & Gillum, 2016; Bessesen & Van Gaal, 2018). Many drugs aim to increase thermogenesis or reduce energy intake, but the long-term weight loss achieved can be limited by counterregulatory mechanisms aiming to re-establish prior weight (Polidori *et al.* 2016; Schwartz *et al.* 2017; Bluher, 2019). Such limitations could be avoided if a drug not only induces a transient physiological response but also induces persisting adaptations.

One potential approach is to target beta-adrenergic receptors as they, for one, are involved in the regulation of metabolic rate and substrate utilization, and secondly, contribute to the regulation of the phenotype of adipose and skeletal muscle tissue (Astrup, 1995; Collins & Surwit, 2001; Hostrup *et al.* 2020). Beta-adrenergic receptors have had a historic appeal in drug development with applicability for obesity (Astrup, 1995; Collins & Surwit, 2001). This arose from the discovery that beta-adrenergic compounds increased metabolic rate and caused selective loss of white adipose stores in obese mice (Arch *et al.* 1984; Nagase *et al.* 1996). This is particularly true for the beta$_3$-adrenergic receptor subtype for which rodent studies highlight its applicability as a pharmacological target to induce weight loss via its role in regulating thermogenesis of brown adipose tissue (Danforth & Himms-Hagen, 1997; Cannon & Nedergaard, 2004). Compelling as this may seem, the implication for humans is limited by the fact that

brown adipose tissue is far less abundant in human adults than in rodents (Arch, 2011) and humans have a lower expression of beta$_3$-adrenergic receptors than rodents (Deng *et al.* 1996; Arch, 2011; Blondin *et al.* 2020). Indeed, studies administering beta$_3$-agonists to humans seldom demonstrate relevant clinical effects (Larsen *et al.* 2002; Redman *et al.* 2007; Arch, 2011). While a few studies show that the non-selective beta$_3$-agonist, mirabegron, induces thermogenesis in humans (Cypess *et al.* 2015; Blondin *et al.* 2020), a recent study indicated that this effect was mediated by off-target stimulation of beta$_2$-adrenergic receptors (Blondin *et al.* 2020).

Beta$_2$-adrenergic receptors are possibly a more applicable target to counter obesity than the beta$_3$-subtype because of their wider expression in the adipose and skeletal muscle tissue of humans (Elfellah *et al.* 1989; Deng *et al.* 1996; Blondin *et al.* 2020). This is supported by the elevated metabolic rate and muscle hypertrophic effects of beta$_2$-adrenergic stimulation (Hostrup *et al.* 2020) – effects that over time reduce fat mass and increase skeletal muscle mass (Acheson *et al.* 1988; Hostrup *et al.* 2015; Jessen *et al.* 2018). Compelling data from human genotyping also reveal specific beta$_2$-adrenergic receptor polymorphisms as determinants of body composition and obesity (Large *et al.* 1997; Ishiyama-Shigemoto *et al.* 1999; Mori *et al.* 1999; Jenkins *et al.* 2018). Thus, the beta$_2$-adrenergic receptor has become a re-emerging pharmaceutical target for leanness (Lee *et al.* 2013; Koziczak-Holbro *et al.* 2019; Blondin *et al.* 2020; Hostrup *et al.* 2020).

Herein, we discuss how beta$_2$-adrenergic receptors are involved in the aetiology of obesity and highlight the potential applicability, and pitfalls, of targeting beta$_2$-adrenergic receptors with selective agonists to induce leanness in humans.

The beta$_2$-adrenergic receptor as a target to induce leanness

Beta$_2$-adrenergic receptors are members of the G-protein-coupled superfamily and are widely distributed throughout the body (Bristow *et al.* 1982; Williams *et al.* 1984; Deng *et al.* 1996; Rasmussen *et al.* 2007; Ryall *et al.* 2010). While the cAMP-PKA-dependent pathway was for many years considered the main signalling pathway associated with the beta$_2$-adrenergic receptor, we now recognize that binding of an agonist to its receptor complex causes conformational heterogeneity, not only inducing cAMP-PKA-dependent signalling, but also activates G-protein-independent pathways (Shenoy *et al.* 2006; Sun *et al.* 2007). This allows the receptor to activate alternative signalling pathways, such as G_s, G_i, kinases and arrestins.

What makes the beta$_2$-adrenergic receptor an attractive target in obesity is its involvement in the regulation of metabolic rate, substrate utilization, and muscle hypertrophy (Koziczak-Holbro *et al.* 2019; Hostrup *et al.* 2020; Jessen *et al.* 2020). Targeting the receptor with selective beta$_2$-agonists dose-dependently increases metabolic rate by 10–50% and accentuates fat oxidation in humans at rest (Amoroso *et al.* 1993; Wilson *et al.* 1993; Beloka *et al.* 2011; Lee *et al.* 2013; Onslev *et al.* 2017; Jessen *et al.* 2020). With respect to its thermogenic actions, we consider skeletal muscle, brown adipose tissue and white adipose tissue to be of particular interest.

Beta$_2$-adrenergic stimulation of skeletal muscle

Being the most abundant adrenergic receptor in human skeletal muscle (Elfellah *et al.* 1989), and accounting for approximately 90% of the beta-adrenergic receptor isoforms in rodent skeletal muscle (Williams *et al.* 1984; Jensen *et al.* 2002), the beta$_2$-subtype presents a particularly interesting target as skeletal muscle contributes to around 20% of basal metabolic rate (Stainsby & Lambert, 1979; Zurlo *et al.* 1990) and modest increases in skeletal muscle metabolism produce only a few adverse effects, equivalent to mild exercise. Stimulation of skeletal muscle beta$_2$-adrenergic receptors augments muscle metabolic rate by around 20–80% in humans (Simonsen *et al.* 1992; Onslev *et al.* 2019; 2021). This possibly involves multiple myocellular processes, including futile cycling and induction of various ATP-dependent processes (Wijers *et al.* 2009; Onslev *et al.* 2019; Hostrup *et al.* 2020). Beta$_2$-adrenergic stimulation induces repetitive leak and re-sequestering of Ca^{2+} from and into the sarcoplasmic reticulum – a process that is ATP-dependent via SERCA (Bakker *et al.* 1998; Andersson *et al.* 2012; Aschar-Sobbi *et al.* 2012; Akin *et al.* 2013; Hostrup *et al.* 2014b). The myoplasmic Ca^{2+} oscillations also cause repetitive contraction and relaxation of the myofibrils, which explains the tremors commonly associated with administration of beta$_2$-agonist. Other mechanisms augmenting muscle metabolic rate in response to beta$_2$-adrenergic stimulation include increased Na^+/K^+-ATPase activity (Whyte *et al.* 1987; Clausen & Nielsen, 2007) and induction of growth-promoting processes (Hostrup *et al.* 2020). The latter is particularly relevant because skeletal muscle mass is a determinant of basal metabolic rate (Zurlo *et al.* 1990). Beta$_2$-adrenergic stimulation also engages several metabolic pathways in human skeletal muscle, leading to an increase in glucose uptake and utilization (Onslev *et al.* 2019; 2021), lactate$^-$ formation (Kalsen *et al.* 2014; 2016a; 2016b; Onslev *et al.* 2021), protein turnover (Lee *et al.* 2015; Hostrup *et al.* 2018), and fatty acid release (Onslev *et al.* 2019). Hence, targeting beta$_2$-adrenergic receptors

of skeletal muscle could induce both acute and long-term effects of relevance to counter obesity.

Beta$_2$-adrenergic stimulation of brown adipocytes

Beta-adrenergic receptors of brown adipose tissue, the beta$_3$-subtype in particular, have for decades been an area of pharmaceutical drug development with application for obesity due to the finding that beta$_3$-agonists induce thermogenesis and weight loss in rodents (Weyer *et al.* 1999; Collins & Surwit, 2001). But while the beta$_3$-subtype is the dominant beta-adrenergic receptor in brown adipocytes of rodents, studies in humans indicate that the beta$_2$-subtype is not only predominant (accounting for around two-thirds of transcripted beta-adrenergic receptor isoforms) (Deng *et al.* 1996; Blondin *et al.* 2020) but is also involved in the energy-dissipating properties of brown adipocytes (Blondin *et al.* 2020). What is noteworthy in this regard is that the beta$_2$-adrenergic receptor is co-expressed with uncoupling protein 1 (UCP1), which possibly allows the receptor to readily convey an increase in mitochondrial leak respiration upon stimulation. Indeed, Blondin *et al.* observed that stimulation with the selective beta$_2$-agonist formoterol increased leak respiration of human brown adipocytes *in vitro*, which was negated by beta$_2$-blocker or siRNA knockdown of the receptor (Blondin *et al.* 2020).

Whether beta$_2$-adrenergic receptors in brown adipocytes contribute to the thermogenic actions of beta$_2$-agonists in humans remains to be explored *in vivo* but some findings do suggest a role. In the aforementioned study by Blondin *et al.* (2020), the non-selective beta$_3$-agonist mirabegron augmented resting energy expenditure in healthy lean young men by 12% and 17% at oral doses of 50 and 200 mg, respectively, but only the 200 mg dose caused an increase in glucose uptake and oxidative metabolism of supraclavicular brown adipose tissue (around a twofold increase) as assessed using tracers and positron emission tomography (Blondin *et al.* 2020). Given that only the 200 mg dose also induced systemic side effects normally associated with beta$_2$-adrenergic stimulation, including an increase of heart rate and lipolysis, along with the observation that brown adipose tissue specimens obtained from the deep neck region demonstrated an overwhelming expression of beta$_2$-adrenergic receptor mRNA and close to no expression of the beta$_3$-subtype, the authors attributed the thermogenic actions of mirabegron in brown adipose tissue to off-target stimulation of beta$_2$-adrenergic receptors as also confirmed in their human brown adipocyte *in vitro* model with formoterol (Blondin *et al.* 2020). On the other hand, the non-selective beta-agonist isoproterenol (24 ng $\times$ kg fat-free mass^{-1} $\times$ min^{-1}), which has affinity for both beta$_1$- and beta$_2$-adrenergic receptors, did not affect glucose uptake in brown adipose tissue of young healthy men despite elevating resting energy expenditure by 20% (Vosselman *et al.* 2012) – neither when administered alone nor when co-administered with acipimox, which is a potent inhibitor of lipolysis. However, when isoproterenol was co-administered with acipimox, resting energy expenditure only increased by 13%. This indicates that the lipolytic action of isoproterenol is important for its thermogenic effect – possibly via triglyceride–fatty acid cycling, in which the released fatty acids are re-esterified through energy-requiring processes rather than oxidized (Wolfe *et al.* 1987; 1990; Wijers *et al.* 2009). But whether this involves brown adipose tissue only or also skeletal muscle and white adipose tissue remains to be assessed in future studies using selective beta$_2$-agonists.

Beta$_2$-adrenergic stimulation of white adipocytes

Beta$_2$-adrenergic receptors are widely expressed in human white adipose tissue, accounting for 40–90% of the transcripted beta-isoforms (Lafontan & Berlan, 1993; Reynisdottir *et al.* 1994; Deng *et al.* 1996; Blondin *et al.* 2020) with a relative cell membrane concentration of 60–70% for the beta$_2$-subtype *vs.* 30–40% for the beta$_1$-subtype (Mauriege *et al.* 1988; Lonnqvist *et al.* 1992; Reynisdottir *et al.* 1994). These values differ considerably between individuals and body regions (Arner *et al.* 1990), and individuals with obesity, in particular, have a low abundance of the beta$_2$-subtype and are less catecholamine sensitive (Lonnqvist *et al.* 1992; Reynisdottir *et al.* 1994).

A key role of beta$_2$-adrenergic receptors in white adipocytes is to regulate the breakdown and release of fatty acids to the bloodstream (Holm, 2003; Fortier *et al.* 2004; Watt *et al.* 2006). What's more interesting, however, is the involvement of the beta$_2$-adrenergic receptor in inducing phenotypic changes of white adipocytes to present brown-like characteristics under periods of chronic stimulation of the receptor – a so-called 'browning' or 'beiging' (Ohyama *et al.* 2016). Rodents exposed to cold exhibit beiging of white adipose tissue, underpinned by upregulation of PR domain containing 16 (PRDM16), which is a central transcriptional regulator of brown and beige adipocyte development (Seale *et al.* 2008; Cohen *et al.* 2014), alongside an upregulation of UCP1 and mitochondrial oxidative proteins (Ohyama *et al.* 2016). Given that these adaptations can be abolished by co-treatment of beta$_2$-blocker or beta$_2$-adrenergic receptor knock-out indicates that beta$_2$-adrenergic signalling is key in promoting the phenotypic changes. Consistent with this, animal studies demonstrate that chronic beta-adrenergic stimulation mediates beiging of white adipose tissue (Cousin *et al.* 1992;

Miller *et al.* 2015). In addition to this effect, chronic treatment with beta$_2$-agonist induces global transcriptional changes of genes involved in lipid metabolism of white adipose tissue in pigs, including an upregulation of apolipoproteins (Zhang *et al.* 2007). Collectively, this suggests that white adipocytes change their metabolic phenotype and expression of apolipoproteins, and may also adopt brown-like characteristics during prolonged beta$_2$-adrenergic stimulation.

The effect of prolonged beta$_2$-adrenergic stimulation in the white adipose tissue of humans, however, is still not entirely clear due to the lack of clinical trials with selective beta$_2$-agonists. That said, some findings indicate that the beta-adrenergic receptors are involved in regulating the phenotype of white adipose tissue in humans. Patients with burn trauma and pheochromocytoma (adrenal gland tumour) secrete excessive amounts of catecholamines that activate beta-adrenergic receptors (Jeschke *et al.* 2008; Frontini *et al.* 2013; Sidossis *et al.* 2015) and markedly accentuate metabolic rate (McCullagh & Engel, 1942; Giantin *et al.* 1995), due at least in part to augmented triglyceride–fatty acid cycling (Wolfe *et al.* 1987). Because of the excessive thermogenesis and hence highly negative energy balance, these conditions lead to significant loss of both fat (Petrák *et al.* 2013; Porter *et al.* 2016b) and lean mass (Lee *et al.* 2019). What's notable is that these conditions have also been linked with trans-differentiation of white adipocytes to resemble 'brown-like' characteristics. In burn trauma patients, subcutaneous fat biopsy specimens show a higher abundance of UCP1, PRDM16 and mitochondrial oxidative proteins than the norm, as well as visible morphological re-arrangement (Frontini *et al.* 2013; Sidossis *et al.* 2015). In patients with pheochromocytoma, UCP1 rarely has measurable levels in subcutaneous white adipose tissue (Vergnes *et al.* 2016) but is markedly elevated in periadrenal and omental fat depots (Frontini *et al.* 2013). This likely relates to the higher concentrations of catecholamines that periadrenal and omental fat depots are exposed to than subcutaneous depots.

Although the above mechanisms may involve all beta-adrenergic receptor subtypes, the findings indicate that prolonged beta-adrenergic stimulation may induce phenotypic changes of white adipocytes in humans. The functional implication of such changes for the thermogenic capacity of white adipose tissue *in vivo*, however, is not completely clear (Porter, 2021) as the protein abundance levels outlined of, for example, UCP1 still only represent a fraction of those observed in brown adipocytes (Porter *et al.* 2016a; 2017). Nevertheless, white adipose tissue constitutes a significant proportion of body mass, which is why only minor changes in its thermogenic capacity induced by prolonged beta-adrenergic stimulation may be significant.

Skeletal muscle accounts for the bulk of the thermogenic response to beta$_2$-agonists

A considerable proportion of the thermogenic response to beta$_2$-agonists in humans appears to originate from skeletal muscle. Thus, the estimated increase of oxygen consumption ($\dot{V}O_2$) in tissues mainly consisting of muscle is around 0.5–1.2 ml $\times$ min^{-1} $\times$ kg lean mass^{-1} in humans administered selective beta$_2$-agonists (Onslev *et al.* 2019; 2021). This corresponds to an augmented skeletal muscle energy dissipation of around 20–80% when accounting for concomitant shifts in skeletal muscle substrate oxidation as assessed using the leg arteriovenous balance technique with respiratory quotient measurements (Onslev *et al.* 2019; 2021). Investigations with adrenaline yield similar results (Simonsen *et al.* 1992). Assuming a total muscle mass of 30 kg, Simonsen *et al.* (1992) estimated skeletal muscle to account for 40% or more of the thermogenic response to adrenaline in six females and one male subject at doses that increased metabolic rate by 18% and 25% (intravenous infusion of 0.2 and 0.4 nmol $\times$ kg^{-1} $\times$ min^{-1}). Estimates from our laboratory correspond with this, indicating that skeletal muscle accounts for 50% or more of the increase in whole-body energy expenditure in response to selective beta$_2$-agonists terbutaline and salbutamol (Onslev *et al.* 2019; 2021). For example, oral doses of salbutamol (24 mg) augmented leg $\dot{V}O_2$ by around 1.2 ml $\times$ min^{-1} $\times$ kg lean mass^{-1} in trained lean young men (Onslev *et al.* 2021). Considering the trained nature of these subjects, having a negligible amount of leg adipose tissue, and assuming a total muscle mass of 40 kg, which is a conservative estimate given their lean body mass of 61 kg, skeletal muscle constituted an increase in whole-body $\dot{V}O_2$ of approximately 50 ml $\times$ min^{-1} in response to salbutamol (Onslev *et al.* 2021) at a systemic salbutamol concentration estimated to increase resting whole-body $\dot{V}O_2$ by around 100 ml $\times$ min^{-1} and metabolic rate by around 25% when accounting for shifts in substrate oxidation. Hence, skeletal muscle likely accounts for the bulk of the thermogenic response to beta$_2$-adrenergic stimulation in humans. To what extent white and brown adipose tissue contributes to the beta$_2$-adrenergic-induced increase of metabolic rate remains elusive but is likely to contribute to some extent (Simonsen *et al.* 1992; 1993; Vosselman *et al.* 2012; Cypess *et al.* 2015; Blondin *et al.* 2020).

As for a potential contribution from cardiac muscle, the non-selective beta-agonist isoproterenol, which also stimulates cardiac beta$_1$-adrenergic receptors to induce inotropy, has been shown to augment resting myocardial $\dot{V}O_2$ by around 10 ml $\times$ min^{-1} at a dose that increased heart rate from 55 to 107 bpm (Simonsen & Kjekshus, 1978). Considering the increase of 35–130 ml $\times$ min^{-1} in resting whole-body $\dot{V}O_2$ in humans administered selective beta$_2$-agonists at doses only increasing heart

rate by 10–25 bpm and with no significant changes in blood pressure (Amoroso *et al.* 1993; Wilson *et al.* 1993; Beloka *et al.* 2011; Lee *et al.* 2013; Hostrup *et al.* 2014a; Onslev *et al.* 2017), we consider the heart to constitute a minor fraction (<10%) of the thermogenic actions of beta$_2$-agonists in humans. This concurs with estimates by Lee *et al.* (2013) of the myocardium to account for only 2–5% of the increase in resting metabolic rate induced by formoterol at oral doses of 80–320 μg. Along these lines, Simonsen *et al.* (1992) estimated the heart to account for around 15% of the augmented metabolic rate in response to adrenaline, being a substance with greater effects on heart rate and systolic blood pressure (Stratton *et al.* 1985) than selective beta$_2$-agonists.

Accordingly, stimulation of beta$_2$-adrenergic receptors causes a profound thermogenic response, which is predominantly mediated by actions in skeletal muscle and secondarily in other tissues, including white and brown adipose tissue, as well as cardiac muscle (Fig. 1). Studies in subjects with markedly elevated catecholamines (e.g. patients with pheochromocytoma and burn victims) suggest that beta$_2$-adrenergic receptors also contribute to the regulation of the phenotype of human white adipocytes by promoting brown-like characteristics upon chronic stimulation. Thus there is a physiological rationale in targeting the beta$_2$-adrenergic receptor for the purpose of inducing leanness.

Beta$_2$-adrenergic receptor polymorphisms impact body composition

If the beta$_2$-adrenergic receptor is a relevant target in drug development with application for obesity, one expects genetic variability in the beta$_2$-adrenergic receptor to contribute to the aetiology of obesity. In the human beta$_2$-adrenergic receptor coding region, several single base substitutions have been identified (Takenaka *et al.* 2012) of which two polymorphisms are associated with body composition (Large *et al.* 1997; Jenkins *et al.* 2018) and obesity (Ishiyama-Shigemoto *et al.* 1999; Mori *et al.* 1999; Zhang *et al.* 2014) – one for which glycine substitutes arginine at amino acid 16 (Arg16Gly) and another for which glutamine substitutes glutamic acid at amino acid 27 (Gln27Glu).

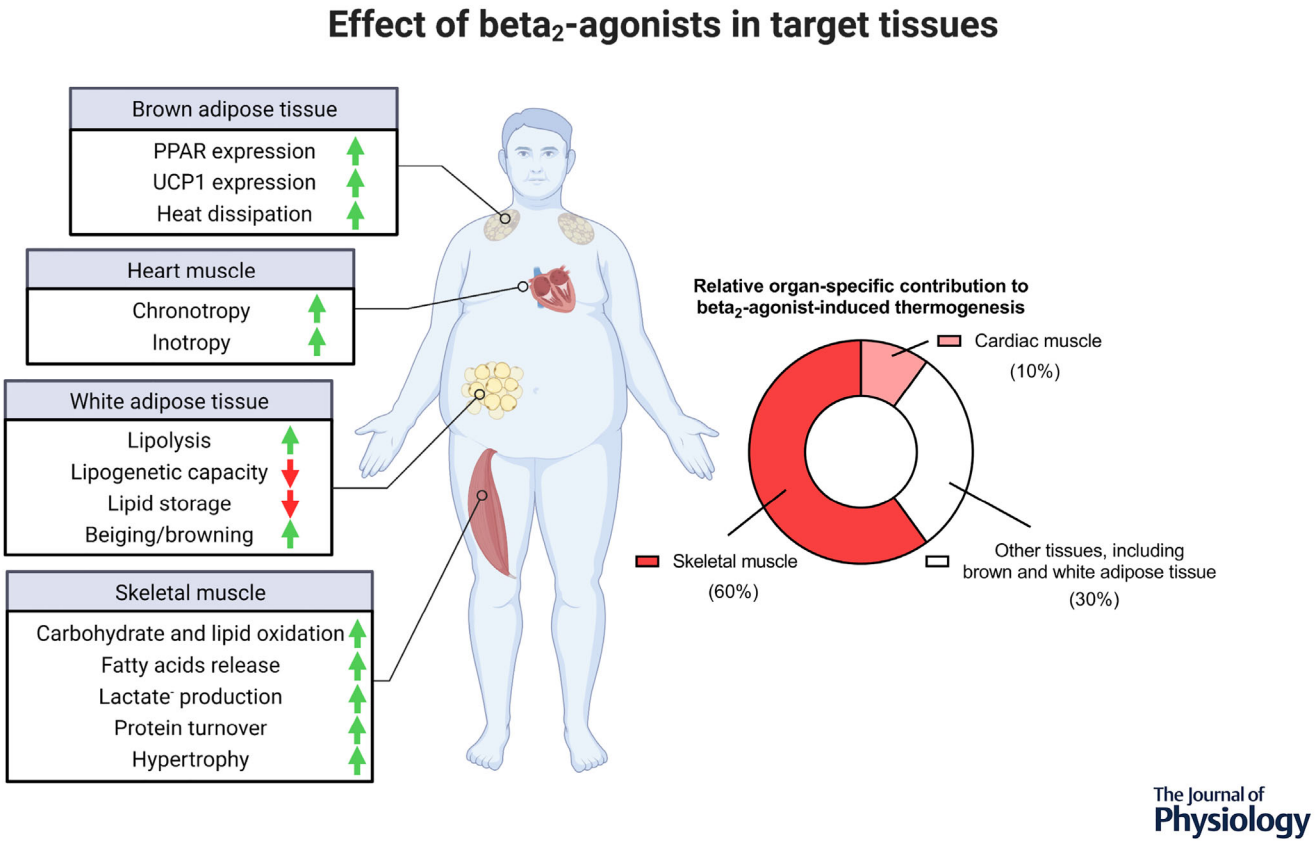

Figure 1. Effect of selective beta$_2$-agonists in relevant target tissues of relevance for its thermogenic actions
The donut chart shows the estimated organ-specific relative contribution to the thermogenic effect of beta$_2$-agonist in humans.

Well-trained healthy male carriers of the Arg16Gly polymorphism have around 12% higher lean mass index (i.e. lean mass relative to height) and 30% greater isometric muscle strength of the quadriceps if being homozygous for glycine (GlyGly) than being homozygous for arginine (ArgArg) (Jenkins *et al.* 2018), which suggest that the Arg16Gly polymorphism influences muscle mass. For fat mass, on the other hand, results are conflicting, as Japanese and Taiwanese female carriers of the Arg16Gly polymorphism being homozygous for ArgArg are more prone to become obese (Ishiyama-Shigemoto *et al.* 1999; Chou *et al.* 2012), whereas no such association has been observed in European females (Large *et al.* 1997; Oberkofler *et al.* 2000) or Japanese males (Hayakawa *et al.* 2000) nor in the aforementioned study in trained healthy males (Jenkins *et al.* 2018).

Individuals carrying the Gln27Glu polymorphism are more prone to develop overweight and obesity-related traits, such as insulin resistance, hypertension and hypertriglyceridaemia (Large *et al.* 1997; Ishiyama-Shigemoto *et al.* 1999; Mori *et al.* 1999; Lange *et al.* 2005; Hsiao & Lin, 2014; Leite *et al.* 2015). For example, Caucasian females homozygous for Gln27Glu had 20 kg greater fat mass and 50% larger adipocytes than females homozygous for GlnGln (Large *et al.* 1997). The Gln27Glu polymorphism has also been associated with obesity in Japanese males and females (Ishiyama-Shigemoto *et al.* 1999; Mori *et al.* 1999) but tends to be negatively associated with obesity in Swedish males (Hellstrom *et al.* 1999).

Although the exact reasons underlying the association between the Arg16Gly and Gln27Glu polymorphisms and body composition remain elusive, it is clear that both polymorphisms cause receptor variations that influence its function (Takenaka *et al.* 2012; Zhang *et al.* 2014). Thus, infusion of beta$_2$-agonist salbutamol increased resting energy expenditure by only $\approx$ 5% in male ArgArg carriers *vs.* an increase of $\approx$ 8% in GlyGly and $\approx$ 11% in GlyArg carriers (Oomen *et al.* 2005). Perhaps more notable was the finding of a fivefold greater receptor affinity for terbutaline in adipocytes isolated from subcutaneous abdominal fat biopsies in carriers of GlyGly than those carrying ArgGly and ArgArg, which indicates a greater sensitivity to beta$_2$-agonists in adipocytes with respect to their lipolytic action (Large *et al.* 1997). In addition, studies in adipose tissue cell lines show that haplotypes of the beta$_2$-adrenergic receptor gene predict sensitivity to terbutaline-induced lipolysis (Eriksson *et al.* 2004). Hence, it seems that variants in the beta$_2$-adrenergic receptor gene alter the functionality and sensitivity of the beta$_2$-adrenergic receptor, which may explain its association with body composition.

Collectively, these findings suggest that variability in the human beta$_2$-adrenergic receptor gene contributes to the regulation of body composition and aetiology of obesity, for which the Gln27Glu polymorphism is associated with obesity, whereas the Arg16Gly polymorphism impacts lean mass in men and the development of obesity in specific populations. This is further supported by beta$_2$-adrenergic receptor expression models for which receptor knock-out reduces the muscle mass of mice (Hinkle *et al.* 2002) while receptor-overexpression, incurred from adeno-associated virus-transfection, increases muscle mass (Hagg *et al.* 2016).

A period of treatment with beta$_2$-agonists induces leanness

Synthetically derived beta$_2$-agonists have been available for human use since the late 1960s when salbutamol (albuterol, US) was first marketed. Beta$_2$-agonists are characterized as either short- or long-acting, based on their duration of action, and as full or partial agonists based on whether they maximally activate the beta$_2$-adrenergic receptor or only produce submaximal activity even at saturating concentrations (Meltzer & Kemp, 1991; Rosenbaum *et al.* 2009; Baker, 2010; Jacobson *et al.* 2018; Hostrup *et al.* 2020). The molecular structure of beta$_2$-agonists is comparable to adrenaline but with some differences. Unlike adrenaline, beta$_2$-agonists are non-catechol compounds, meaning they are not substrates for catechol-*O*-methyltransferases, hence giving them a considerably longer elimination half-life and duration of action. Newer-generation beta$_2$-agonists have greater complexity and are more selective and effective due to distinct receptor–ligand interactions as illustrated for formoterol and vilanterol (Slack *et al.* 2013; Cameron *et al.* 2017). Characteristics of commonly prescribed beta$_2$-agonists are shown in Table 1.

While mainly developed to treat bronchoconstriction associated with asthma and other respiratory diseases, studies in the 1980s demonstrated that beta$_2$-agonists effectively reduced fat deposition and increased muscle mass of livestock – a so-called 'repartitioning effect' (Baker *et al.* 1984; Ricks *et al.* 1984). For example, lambs fed with clenbuterol 40 days before slaughter, exhibited a 48% increase of longissimus muscle cross-sectional area *vs.* only a 12% increase for controls, while subcutaneous fat thickness increased 88% in controls but remained unchanged in the clenbuterol-fed sheep (Hamby *et al.* 1986). These effects are dose-dependent and specific for the beta$_2$-adrenergic receptor, as co-administration of beta$_2$-blocker or receptor knock-out blunts the effect (Choo *et al.* 1992; Hinkle et al. 2002). Studies also revealed that beta$_2$-agonist-treated animals consumed less food during the first days of treatment, causing an anorectic effect (Bendotti *et al.* 1983; Benson *et al.* 1991). For instance, rats injected with salbutamol (10 mg $\times$ $kg_{b.w.}^{-1}$) consumed half as much as saline-treated rats during the first day of treatment but progressively returned

Table 1. Synthetically derived selective beta$_2$-agonists marketed for human use

Name	Type	Potency	Selectivity	Duration of action	Plasma elimination half-life	Generation
Arformoterol	Long-acting	Full	High	>12 h	26 h	3rd
Bambuterol	Long-acting	Partial	Moderate	24 h	2.5 h	1st
Clenbuterol	Long-acting	Full	Moderate	12 h	25–48 h	1st
Fenoterol	Short-acting	Full	Moderate	4–6 h	6–7 h	1st
Formoterol	Long-acting	Full	High	12 h	10 h	2nd
Indacaterol	Long-acting	Full	High	24 h	34–56 h	3rd
Levalbuterol	Short-acting	Partial	Moderate	5–6 h	3–4 h	1st
Olodaterol	Long-acting	Full	High	24 h	7–45 h	3rd
Procaterol	Short-acting	Partial	Moderate	4–8 h	4 h	2nd
Salbutamol	Short-acting	Partial	Moderate	4–6 h	5–6 h	1st
Salmeterol	Long-acting	Full	High	>12 h	11 h	2nd
Terbutaline	Short-acting	Partial	Moderate	4–6 h	3–4 h	1st
Vilanterol	Long-acting	Full	High	24 h	2.5 h	3rd

to an intake similar to controls after 4 days (Bendotti *et al.* 1983); an effect also observed in sheep and calves (Brockway *et al.* 1987). This likely involves activation of beta$_2$-adrenergic receptors in the central nervous system, as intracerebroventricular injection of beta-blocker propranolol reversed the effect of beta$_2$-agonist on food intake (Borsini *et al.* 1982). The anorectic effect and thermogenic action of beta$_2$-agonists (Brockway *et al.* 1987) therefore appear to explain the bulk of the considerable fat loss seen during the initial phase of treatment in animals (Shaw, 1993). Furthermore, in subcutaneous adipose tissue of clenbuterol-fed heifers, adipocytes were smaller and had decreased lipogenic activity (Miller *et al.* 1988), which suggests that depression of lipogenesis is a factor underlying the reduced fat deposition in beta$_2$-agonist-fed livestock. Given these findings, it is not surprising that beta$_2$-agonists have been used as feed additives to improve meat production efficiency for decades, predominantly in cattle, pigs and poultry – albeit being illegal nowadays in most modernized societies, including the European Union and North America (Centner *et al.* 2014).

In spite of the above findings and the repertoire of beta$_2$-agonists marketed, their application for weight loss has not been pursued in clinical trials to any substantial degree. In fact, only a few studies have investigated the effect of beta$_2$-agonists on body composition (Hostrup *et al.* 2020), but none had fat percentage or fat mass as the primary outcome nor did they enrol individuals with obesity (Table 2). One reason relates to the dosing regimens used in rodents and livestock that vastly exceed those considered safe for humans. Although beta$_2$-agonists clenbuterol and salbutamol are commonplace in the body-building milieu by individuals seeking leanness (Milano *et al.* 2018; Ip *et al.* 2019), not even doping fora recommend the clenbuterol and salbutamol doses used in animals (Hostrup *et al.* 2020) and several pitfalls need to be considered before prescribing individuals with chronic beta$_2$-agonist treatment, as discussed in later sections. Nevertheless, some promising human data are available that highlight the potential of beta$_2$-agonists at therapeutically safe doses to induce leanness.

Even at clinically relevant inhaled doses, such as those administered for asthma, beta$_2$-agonists increase resting metabolic rate by 10–15% (Amoroso *et al.* 1993; Wilson *et al.* 1993; Onslev *et al.* 2017), which is likely underestimated given the fact that beta$_2$-agonists also raise systemic lactate$^-$ levels, being indicative of anaerobic energy production in skeletal muscle (Kalsen *et al.* 2014; 2016a; 2016b; Onslev *et al.* 2019; 2021). While most metabolic studies with beta$_2$-agonists have been performed with non-obese individuals, a handful of studies indicate that their effects are lower in individuals with obesity. Thus, infusion of incremental salbutamol doses relative to fat-free mass induced a lower increase of resting energy expenditure adjusted for fat-free mass in individuals with obesity than lean individuals (Schiffelers *et al.* 2001). In females with obesity, interstitial glycerol concentrations were significantly lower during local subcutaneous adipose tissue interstitial administration of terbutaline than in lean females (Enoksson *et al.* 2000). Factors underlying the reduced metabolic impact of beta$_2$-agonists in obesity may include lower beta$_2$-adrenergic sensitivity, a lower density of surface beta$_2$-adrenoceptors, and receptor polymorphisms as already highlighted.

Despite the lower response to beta$_2$-agonists reported in individuals with obesity, the newer-generation beta$_2$-agonist, formoterol, was shown to augment energy

Table 2. Effect of daily treatment with beta$_2$-agonist on body composition in humans

				Intervention				Body composition changes		
Study	Subjects		Design	Activity	Drug	Route	Dose	Duration	Method	Effect *vs.* placebo
Acheson *et al.* (1988)	7 M	Lean	RCT	Habitual lifestyle	Terbutaline	Oral	7.5 mg twice daily	2 weeks	D_2O	+1.4 kg lean mass −1.4 kg fat mass
Martineau *et al.* (1992)	12 M	Lean	RCT	Habitual lifestyle	Salbutamol	Oral	8 mg twice daily	3 weeks	Skinfold and bioimpedance	No change
Maki *et al.* (1996)	7 M	Lean	Open-label	Habitual lifestyle	Salbutamol	Oral	8 mg twice daily	2 weeks	Bioimpedance	(−2.1% body fat)*
Le Panse *et al.* (2005)	15 M	Lean	RCT	Habitual lifestyle	Salbutamol	Oral	4 mg trice daily	3 weeks	DXA	No change
Le Panse *et al.* (2006)	14 F	Lean	RCT	Habitual lifestyle	Salbutamol	Oral	4 mg trice daily	4 weeks	DXA	No change
Hostrup *et al.* (2015)	9 M	Lean	RCT	Habitual lifestyle	Terbutaline	Oral	5 mg × 30 $kg_{b.w.}^{-1}$ twice daily	4 weeks	DXA	+1.7 kg lean mass −1.4 kg fat mass
Jessen et al. (2018)	13 M	Lean	RCT	Habitual lifestyle	Terbutaline	Inhalation	4 mg once daily	4 weeks	DXA	+1.0 kg lean mass
Jessen *et al.* (2018)	13 M	Lean	RCT	Resistance training	Terbutaline	Inhalation	4 mg once daily	4 weeks	DXA	+1.0 kg lean mass +1.0 kg body mass
Jessen *et al.* (2018)	12 M	Lean	RCT	Endurance training	Terbutaline	Inhalation	4 mg once daily	4 weeks	DXA	−1.9 kg body mass

RCT: Randomized placebo-controlled trial, DXA: dual X-ray absorptiometry.
*No placebo control.

expenditure by 12% at a relatively modest inhaled dose of 27 μg in overweight individuals (Onslev *et al.* 2017), which is remarkably similar to the increase of 13–17% observed in healthy non-obese individuals ingesting 80–160 μg orally (Lee *et al.* 2013). Bearing in mind the markedly higher systemic bioavailability of the inhaled route than oral (around 2–5 times higher) (Ward *et al.* 2000; Elers *et al.* 2012; Dyreborg *et al.* 2016), the systemic exposure to formoterol in these two studies seems fairly equivalent. In both studies, the greater energy expenditure was associated with a comparable accentuation of fat oxidation by around 30% (Lee *et al.* 2013; Onslev *et al.* 2017). Assuming a duration of action of 8 h with augmented fat oxidation of 16 mg $\times$ min^{-1}, Lee *et al.* speculated that formoterol could induce a 2.8 kg reduction in fat mass over 1 year at an oral dose of 160 μg/day. This, however, necessitates that the greater daily energy expenditure is not compensated for by a larger caloric intake. But while clinical trials on the effect of beta$_2$-agonists on satiety are scant, aforementioned studies in animals treated with beta$_2$-agonist reported a decreased food intake – albeit short-lived (Bendotti *et al.* 1983; Shaw, 1993). Suggestive of no significant compensation in food intake to the greater energy expenditure induced by beta$_2$-agonist in humans, daily treatment with high oral doses of terbutaline (5 mg $\times$ 30 kg$_{b.w.}^{-1}$ twice daily) for 4 weeks caused a 1.4 kg reduction of fat mass concomitant with a noticeable 1.7 kg gain of lean mass in healthy young lean men (Hostrup *et al.* 2015). Considering common caloric conversion estimates, this indicates that the subjects treated with terbutaline had a net negative energy balance of around 200 kcal/day during the intervention. In accordance, using respiratory chambers, Acheson *et al.* (1988) estimated a net negative fat balance of 115 kcal/day and a concomitant positive protein balance of 59 kcal/day during the last 2 days of a 2-week period with oral treatment of terbutaline at a slightly lower daily dose (7.5 mg twice daily) in lean young men. In that study, the 2-week treatment with terbutaline was associated with a 1.4 kg reduction of fat mass and a 1.4 kg increase of lean mass as determined by the double-labelled water (D_2O) technique (Acheson *et al.* 1988). Thus, during a relatively short period, terbutaline induces repartitioning in healthy non-obese individuals. Even at close-to-therapeutic inhaled doses of 4 mg/day for 4 weeks, terbutaline has been shown to increase lean mass by 1 kg though with only a small non-significant reduction of fat mass in lean young men (Jessen *et al.* 2018).

Studies with other beta$_2$-agonists are scarce – albeit a few studies with salbutamol have reported divergent results with respect to its effect on body composition (Table 2). Whether repartitioning effects are as evident in individuals with obesity remains to be assessed. Indeed, the attenuated metabolic response to beta$_2$-agonists in individuals with obesity, the insufficient knowledge of long-term compensatory mechanisms in caloric intake, and the potential progression of beta$_2$-adrenergic receptor down-regulation and desensitization with continued treatment make extrapolation from the short-term application of beta$_2$-agonists in lean individuals challenging.

Interaction between exercise and beta$_2$-agonists

Given that exercise is recommended in obesity, a key question is whether additive weight loss can be achieved with concomitant beta$_2$-agonist treatment when performing exercise. It is clear that the thermogenic effect of beta$_2$-agonists seen at rest declines with increasing exercise intensities (Beloka *et al.* 2011; Kalsen *et al.* 2014; Hostrup *et al.* 2014a; Onslev *et al.* 2017; 2019). For example, formoterol increased metabolic rate by 12% at rest but not during submaximal exercise (Onslev *et al.* 2017) with similar findings evident for terbutaline and salbutamol (Arlettaz *et al.* 2009; Beloka *et al.* 2011; Hostrup *et al.* 2014a). It should, however, be reiterated that the effect of beta$_2$-agonists on energy expenditure at rest and more so during exercise is likely underestimated by the lack of consideration of the contribution from anaerobic processes. Indeed, Kalsen *et al.* (2016a) reported an 11% greater anaerobic energy utilization with formoterol than placebo during maximal exercise. This is predominantly explained by a higher lactate$^-$ production in the contracting skeletal muscle, being reflected by 20–50% greater myocellular lactate$^-$ concentrations during cycling at intensities below and above $\dot{V}O_{2max}$ with prior beta$_2$-agonist intake (Kalsen *et al.* 2014; Hostrup *et al.* 2014b; Kalsen *et al.* 2016a). The greater muscle lactate$^-$ production likely explains the 1–4 mM higher systemic lactate$^-$ levels observed at rest, and up to 7 mM higher levels during exercise, commonly seen upon administration of beta$_2$-agonist (Kalsen *et al.* 2014; Hostrup *et al.* 2014b). Hence, estimates on the thermogenic actions of beta$_2$-agonists should include the contribution from anaerobic processes.

Another noticeable phenomenon is that the stimulation of fat oxidation with beta$_2$-agonist observed at rest shifts towards greater utilization of carbohydrates during exercise. Thus, formoterol induced a 38% greater fat oxidation than placebo at rest but a 15% higher carbohydrate oxidation than placebo during subsequent low-to-moderate intensity cycling (30–50% $\dot{V}O_{2max}$) (Onslev *et al.* 2017). Such a shift is likely attributed to a greater utilization of muscle glycogen stores when exercising with a prior intake of beta$_2$-agonist (Kalsen *et al.* 2014; Hostrup *et al.* 2014b; Onslev *et al.* 2021). For example, glycogen breakdown during short intense

exercise of around 2 min was almost 40% higher with terbutaline than placebo in highly trained men (Hostrup *et al.* 2014b). When exercise progresses for a longer duration, however, the effect of beta$_2$-agonist on carbohydrate oxidation appears to gradually diminish while the contribution from fat oxidation increases (Arlettaz *et al.* 2009; Kalsen *et al.* 2014); a phenomenon that may be due to the accentuated glycogenolysis with beta$_2$-agonist during the initial phase of exercise to expedite depletion of endogenous glycogen stores during later stages (Kalsen *et al.* 2014). Considering these observations, one could imagine a scenario where beta$_2$-agonists predominantly stimulate fat utilization at rest, shifting towards carbohydrate utilization in the initial phase of exercise, but as the exercise progresses and glycogen depletion ensues, this reverses towards greater fat utilization again (Fig. 2).

Long-term studies on the potential interaction between exercise training and beta$_2$-agonists with respect to body composition are limited and only one study has to our knowledge investigated the effect of combined beta$_2$-agonist treatment and exercise training on fat and lean mass in humans. Jessen *et al.* (2018) subjected active healthy non-obese men to 4 weeks of endurance or resistance training (three times per week) with and without daily treatment with terbutaline (4 mg/day). The endurance-training group receiving terbutaline experienced a weight loss of 1.9 kg compared with placebo, whereas the resistance-training group receiving terbutaline had a 1 kg greater gain of muscle mass than placebo but not a greater reduction of fat mass (Jessen *et al.* 2018). The latter observation corresponds with studies showing an additive effect of salbutamol on muscle power and hypertrophy in young men subjected to a period of resistance training (Caruso *et al.* 2005; Jessen *et al.* 2021). Hence, beta$_2$-agonists may exert some beneficial effects on body composition when taken concomitantly with training as demonstrated for terbutaline (Fig. 3). It is worth noting that the study by Jessen *et al.* (2018) did not manage dietary intake and was performed with lean young men. Therefore, the effect of training and concomitant beta$_2$-agonist treatment on body composition may be larger if under simultaneous hypocaloric diet management with sufficient protein. Additionally, it cannot be excluded that a larger intake of protein in the exercised group in Jessen *et al.* (2018) contributed to the skeletal muscle hypertrophy observed in the resistance-trained group compared with the endurance-trained group.

Pitfalls of beta$_2$-agonists as weight-loss treatment

Given their apparent repartitioning effects, one might be tempted to ask why beta$_2$-agonists are currently not marketed for weight loss? However, high standards of efficacy and safety are a prerequisite for substances to be used by otherwise healthy individuals and even more so by individuals with comorbidities. Beta$_2$-agonists have numerous side effects, including dizziness, tremor, headache, hypokalaemia, nausea, tachycardia, cardiac QT interval changes and nervousness (Phillips *et al.* 1980; Hochhaus & Möllmann, 1992; Johnson, 1995; Jacobson *et al.* 2018). Serious adverse effects, such as cardiac arrest and rhabdomyolysis, have been reported at very high doses (Spiller *et al.* 2013; Grimmer *et al.* 2016). Furthermore, beta$_2$-agonists are contraindicated in certain conditions.

A substantial proportion of individuals with obesity have or will develop type 2 diabetes mellitus. The application of beta$_2$-agonists for such individuals is complicated by their hyperinsulinaemic, hyperglycaemic and lipolytic actions that could interfere with regular diabetic treatment. And while data show that beta$_2$-agonists acutely augment glucose disposal in the skeletal muscle of humans (Onslev *et al.* 2019; 2021), this is not sufficient to counter the simultaneous

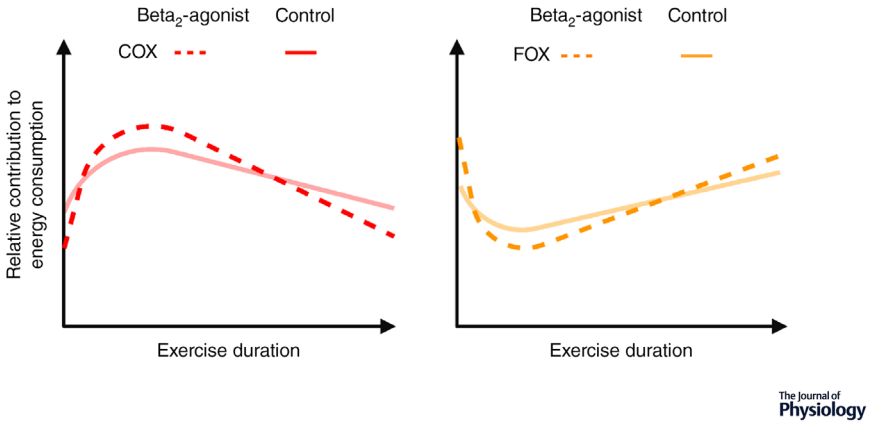

Figure 2. Relative contribution from carbohydrate (COX) and fat (FOX) oxidation to energy consumption during prolonged submaximal cycling with and without prior intake of beta$_2$-agonist in humans
Adapted from Arlettaz *et al.* (2009), Kalsen *et al.* (2014), Hostrup *et al.* (2014a) and Onslev *et al.* (2017).

hepatic glucose release that typically raises systemic blood glucose concentrations by 0.5–1.5 mM. The long-term effect of daily beta$_2$-agonist treatment on glycated haemoglobin (HbA1c) has not been thoroughly investigated in diabetics but repeated spikes in blood glucose incurred from daily beta$_2$-agonist treatment may be a concern. Treatment of type 2 diabetes mellitus also aims at delaying end-stage complications by managing cardiovascular risk factors (Davies *et al.* 2018). In this respect, the hyperlipidaemic actions of beta$_2$-agonists may be undesired. Notwithstanding, studies in rodent models of obesity and insulin resistance show that glucose tolerance can be improved by a period of treatment with beta$_2$-agonist (Pan *et al.* 2001; Kalinovich *et al.* 2020), highlighting an area to be pursued in future clinical trials.

Another concern relates to potential adverse cardiac effects, as daily treatment with beta$_2$-agonists at high doses induces cardiomyocyte hypertrophy, cardiac fibrosis and cardiomyocyte necrosis in rodents (Reeds *et al.* 1988; Gregorevic *et al.* 2005; Burniston *et al.* 2006). It should, however, be noted that these observations were reported with clenbuterol and fenoterol, which aside from the beta$_2$-adrenergic receptor also have significant affinity for the beta$_1$-subtype (Baker, 2010), and were administered at doses yielding a systemic exposure vastly exceeding that considered safe in humans (by at least 30 times and sometimes >1000 times). Nevertheless, epidemiological studies indicate that individuals on daily treatment with beta$_2$-agonists at high doses, such as in moderate-to-severe COPD, are at a higher risk of cardiovascular events (Au *et al.* 2003; Salpeter *et al.* 2004; Choudhary & Robinson, 2009). Hence, it seems apparent that high daily doses of currently marketed beta$_2$-agonists should be avoided in any potential treatment of obesity.

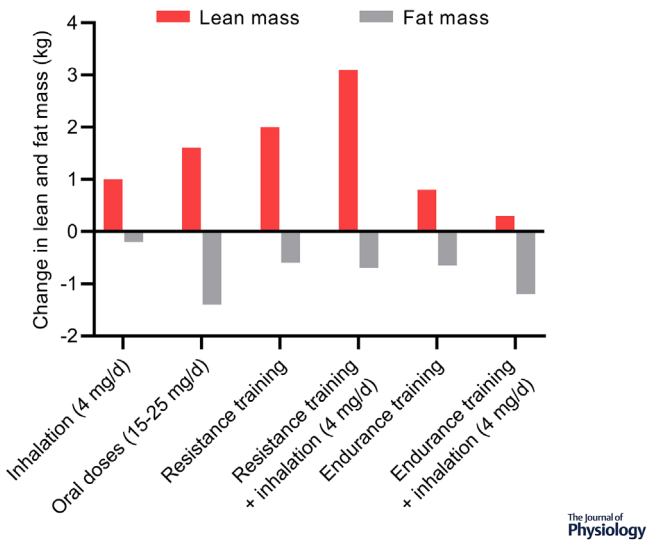

Figure 3. Change in body composition induced by a few weeks of daily treatment with the selective beta$_2$-agonist terbutaline with and without concomitant endurance- or resistance-based training three times weekly in healthy young men
Adapted from Acheson *et al.* (1988), Hostrup *et al.* (2015) and Jessen *et al.* (2018).

Aside from these pitfalls, the application of beta$_2$-agonists to weight loss is limited in a few other populations. Individuals with asthma rely on inhaled beta$_2$-agonists as rescue medication because of their bronchodilatory effects. Overreliance on beta$_2$-agonists in asthma is ill-advised due to the risk of tolerance development (tachyphylaxis) and hence loss of the bronchodilator effect (Haney & Hancox, 2005; Jacobson *et al.* 2018). For example, the bronchodilator response to inhaled salbutamol, after exposure to a methacholine challenge (to induce bronchoconstriction), was already lowered by 28% 24 h after a single administration of formoterol and by 58% after 1–2 weeks' treatment with formoterol (Haney & Hancox, 2005). The tachyphylaxis incurred from chronic treatment is the reason why beta$_2$-agonists are usually prescribed along with an inhaled glucocorticoid, which counters desensitization of beta$_2$-adrenergic receptors and lessens the reliance on inhaled beta$_2$-agonist due to its anti-inflammatory actions in the airways (Jacobson *et al.* 2018). But even though glucocorticoids attenuate development of tachyphylaxis, daily treatment with beta$_2$-agonist at high doses likely increases the risk of asthma exacerbations due to tolerance. Lastly, in individuals with cardiovascular complications, beta$_2$-agonists can interact with common treatment regimes, including, but not limited to, beta-blockers.

Accordingly, several considerations should be made before initiating chronic treatment with beta$_2$-agonists, and safety studies are needed in relevant populations.

Conclusion and perspectives

Given the thermogenic actions and long-term changes in body composition associated with beta$_2$-adrenergic stimulation of skeletal muscle and white and brown adipose tissue, the beta$_2$-adrenergic receptor seems like an attractive target in the development of anti-obesity drugs. This is underpinned by the influence of specific receptor polymorphisms on body composition and by the apparent ability of selective beta$_2$-agonists to induce leanness in humans. Furthermore, prolonged activation of beta$_2$-adrenergic receptors may promote phenotypic changes of white adipocytes to potentially acquire a greater thermogenic capacity. Combined with the accretion of skeletal muscle mass, this could prove valuable in the long-term management of weight loss through persistent accentuation of metabolic rate.

An outstanding issue, however, is the fact that currently marketed beta$_2$-agonists can induce a number of side effects and may also interfere with common treatment regimens of classic comorbidities to obesity, such as type 2 diabetes and hyperlipidaemia, hence limiting their applicability for long-term treatment. And while the benefits of a persistent reduction of fat mass with a concomitant increase of muscle mass may outweigh the potential adverse effects, clinical trials are needed to assess the long-term safety and effect of beta$_2$-agonists on body composition and other clinically relevant markers in individuals with obesity, including glucose tolerance, blood lipid profile and blood pressure. Some promising studies in rodent models of obesity and diabetes do indeed highlight the potential of beta$_2$-agonists in reducing fat mass and improving glucose homeostasis and insulin sensitivity (Pan *et al.* 2001; Kalinovich *et al.* 2020), but these studies used older-generation beta$_2$-agonists, such as clenbuterol, at non-clinically applicable doses. For example, a recent study in mice hailed the efficacy of prolonged treatment with clenbuterol on glucose tolerance at what was claimed to be 'very low doses' and 'with therapeutic applicability'. This despite the fact that the intraperitoneally injected clenbuterol dose used of 25 $\mu g \times kg_{b.w.}^{-1} \times day^{-1}$ (Kalinovich *et al.* 2020) would yield a systemic exposure 80 times exceeding that recommended for humans (Maltin *et al.* 1993; Kamalakkannan *et al.* 2008; Koeberl *et al.* 2018). Therefore, we encourage research to be conducted with newer-generation and enantioselective beta$_2$-agonists because of their greater selectivity for the beta$_2$-adrenergic receptor and fewer cardiac side effects (Jacobson *et al.* 2018; Hostrup *et al.* 2019), and in doses more closely resembling those clinically relevant. Pharmaceutical development of novel beta$_2$-agonists that are more specific to skeletal muscle tissue is ongoing (Koziczak-Holbro *et al.* 2019) and could, in combination with exercise, diet or other treatment regimens, prove useful in weight-loss management.

References

Acheson KJ, Ravussin E, Schoeller DA, Christin L, Bourquin L, Baertschi P, Danforth E Jr. & Jequier E (1988). Two-week stimulation or blockade of the sympathetic nervous system in man: influence on body weight, body composition, and twenty four-hour energy expenditure. *Metabolism* **37**, 91–98.

Akin BL, Hurley TD, Chen Z & Jones LR (2013). The structural basis for phospholamban inhibition of the calcium pump in sarcoplasmic reticulum. *J Biol Chem* **288**, 30181–30191.

Amoroso P, Wilson SR, Moxham J & Ponte J (1993). Acute effects of inhaled salbutamol on the metabolic rate of normal subjects. *Thorax* **48**, 882–885.

Andersson DC, Betzenhauser MJ, Reiken S, Umanskaya A, Shiomi T & Marks AR (2012). Stress-induced increase in skeletal muscle force requires protein kinase A phosphorylation of the ryanodine receptor. *J Physiol* **590**, 6381–6387.

Arch JR (2011). Challenges in beta(3)-adrenoceptor agonist drug development. *Ther Adv Endocrinol Metab* **2**, 59–64.

Arch JR, Ainsworth AT, Cawthorne MA, Piercy V, Sennitt MV, Thody VE, Wilson C & Wilson S (1984). Atypical beta-adrenoceptor on brown adipocytes as target for anti-obesity drugs. *Nature* **309**, 163–165.

Arlettaz A, Le Panse B, Portier H, Lecoq AM, Thomasson R, De Ceaurriz J & Collomp K (2009). Salbutamol intake and substrate oxidation during submaximal exercise. *Eur J Appl Physiol* **105**, 207–213.

Arner P, Hellstrom L, Wahrenberg H & Bronnegard M (1990). Beta-adrenoceptor expression in human fat cells from different regions. *J Clin Invest* **86**, 1595–1600.

Aschar-Sobbi R, Emmett TL, Kargacin GJ & Kargacin ME (2012). Phospholamban phosphorylation increases the passive calcium leak from cardiac sarcoplasmic reticulum. *Pflugers Arch* **464**, 295–305.

Astrup A (1995). The sympathetic nervous system as a target for intervention in obesity. *Int J Obes Relat Metab Disord* **19**(Suppl 7), S24–S28.

Au DH, Udris EM, Fan VS, Curtis JR, McDonell MB & Fihn SD (2003). Risk of mortality and heart failure exacerbations associated with inhaled β-adrenoceptor agonists among patients with known left ventricular systolic dysfunctiona. *Chest* **123**, 1964–1969.

Baker JG (2010). The selectivity of beta-adrenoceptor agonists at human beta1-, beta2- and beta3-adrenoceptors. *Br J Pharmacol* **160**, 1048–1061.

Baker PK, Dalrymple RH, Ingle DL & Ricks CA (1984). Use of a β-adrenergic agonist to alter muscle and fat deposition in lambs. *J Anim Sci* **59**, 1256–1261.

Bakker AJ, Head SI, Wareham AC & Stephenson DG (1998). Effect of clenbuterol on sarcoplasmic reticulum function in single skinned mammalian skeletal muscle fibers. *Am J Physiol* **274**, C1718–C1726.

Beloka SP, Janssen C, Woff E, Brassine E, Deboeck G, Randria J, Philippart de Foy V, van de Borne P & Naeije R (2011). Effects of beta2-adrenergic stimulation on exercise capacity in normal subjects. *Eur J Appl Physiol* **111**, 2239–2247.

Bendotti C, Borsini F & Samanin R (1983). Studies on the mechanisms of tolerance to the anorectic effect of salbutamol in rats. *Eur J Pharmacol* **92**, 237–242.

Benson DW, Foley-Nelson T, Chance WT, Zhang F-S, James JH & Fischer JE (1991). Decreased myofibrillar protein breakdown following treatment with clenbuterol. *J Surg Res* **50**, 1–5.

Bessesen DH & Van Gaal LF (2018). Progress and challenges in anti-obesity pharmacotherapy. *The Lancet Diabetes & Endocrinology* **6**, 237–248.

Blondin DP, Nielsen S, Kuipers EN, Severinsen MC, Jensen VH, Miard S, Jespersen NZ, Kooijman S, Boon MR, Fortin M, Phoenix S, Frisch F, Guerin B, Turcotte EE, Haman F, Richard D, Picard F, Rensen PCN, Scheele C & Carpentier AC (2020). Human brown adipocyte thermogenesis is driven by beta2-AR stimulation. *Cell Metab* **32**, 287–300. e287.

Bluher M (2019). Obesity: global epidemiology and pathogenesis. *Nat Rev Endocrinol* **15**, 288–298.

Borsini F, Bendotti C, Thurlby P & Samanin R (1982). Evidence that systemically administered salbutamol reduces food intake in rats by acting on central beta-adrenergic sites. *Life Sci* **30**, 905–911.

Bray GA & Wadden TA (2015). Improving long-term weight loss maintenance: can we do it? *Obesity* **23**, 2–3.

Bristow MR, Ginsburg R, Minobe W, Cubicciotti RS, Sageman WS, Lurie K, Billingham ME, Harrison DC & Stinson EB (1982). Decreased catecholamine sensitivity and β-adrenergic-receptor density in failing human hearts. *N Engl J Med* **307**, 205–211.

Brockway JM, MacRae JC & Williams PE (1987). Side effects of clenbuterol as a repartitioning agent. *Vet Rec* **120**, 381–383.

Burniston JG, Tan LB & Goldspink DF (2006). Relative myotoxic and haemodynamic effects of the beta-agonists fenoterol and clenbuterol measured in conscious unrestrained rats. *Exp Physiol* **91**, 1041–1049.

Cameron RB, Peterson YK, Beeson CC & Schnellmann RG (2017). Structural and pharmacological basis for the induction of mitochondrial biogenesis by formoterol but not clenbuterol. *Sci Rep* **7**, 10578.

Cannon B & Nedergaard J (2004). Brown adipose tissue: function and physiological significance. *Physiol Rev* **84**, 277–359.

Caruso JF, Hamill JL & De Garmo N (2005). Oral albuterol dosing during the latter stages of a resistance exercise program. *J Strength Cond Res* **19**, 102–107.

Centner TJ, Alvey JC & Stelzleni AM (2014). Beta agonists in livestock feed: status, health concerns, and international trade. *J Anim Sci* **92**, 4234–4240.

Choo JJ, Horan MA, Little RA & Rothwell NJ (1992). Anabolic effects of clenbuterol on skeletal muscle are mediated by beta 2-adrenoceptor activation. *American Journal of Physiology-Endocrinology and Metabolism* **263**, E50–E56.

Chou YC, Tsai CN, Lee YS & Pei JS (2012). Association of adrenergic receptor gene polymorphisms with adolescent obesity in Taiwan. *Pediatr Int* **54**, 111–116.

Choudhary AS & Robinson GM (2009). Can β agonists cause dilated cardiomyopathy? *Heart, Lung and Circulation* **18**, 367–369.

Clausen T & Nielsen OB (2007). Potassium, Na+,K+-pumps and fatigue in rat muscle. *J Physiol* **584**, 295–304.

Cohen P, Levy Julia D, Zhang Y, Frontini A, Kolodin Dmitriy P, Svensson Katrin J, Lo James C, Zeng X, Ye L, Khandekar Melin J, Wu J, Gunawardana Subhadra C, Banks Alexander S, Camporez João Paulo G, Jurczak Michael J, Kajimura S, Piston David W, Mathis D, Cinti S, Shulman Gerald I, Seale P & Spiegelman Bruce M (2014). Ablation of PRDM16 and beige adipose causes metabolic dysfunction and a subcutaneous to visceral fat switch. *Cell* **156**, 304–316.

Collins S & Surwit RS (2001). The beta-adrenergic receptors and the control of adipose tissue metabolism and thermogenesis. *Recent Prog Horm Res* **56**, 309–328.

Cousin B, Cinti S, Morroni M, Raimbault S, Ricquier D, Penicaud L & Casteilla L (1992). Occurrence of brown adipocytes in rat white adipose tissue: molecular and morphological characterization. *J Cell Sci* **103**(Pt 4), 931–942.

Cypess AM, Weiner LS, Roberts-Toler C, Franquet Elia E, Kessler SH, Kahn PA, English J, Chatman K, Trauger SA, Doria A & Kolodny GM (2015). Activation of human brown adipose tissue by a beta3-adrenergic receptor agonist. *Cell Metab* **21**, 33–38.

Danforth E, Jr. & Himms-Hagen JH (1997). Obesity and diabetes and the beta-3 adrenergic receptor. *Eur J Endocrinol* **136**, 362–365.

Davies MJ, D'Alessio DA, Fradkin J, Kernan WN, Mathieu C, Mingrone G, Rossing P, Tsapas A, Wexler DJ & Buse JB (2018). Management of hyperglycemia in type 2 diabetes, 2018. a consensus report by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). *Diabetes Care* **41**, 2669–2701.

Deng C, Paoloni-Giacobino A, Kuehne F, Boss O, Revelli JP, Moinat M, Cawthorne MA, Muzzin P & Giacobino JP (1996). Respective degree of expression of beta 1-, beta 2- and beta 3-adrenoceptors in human brown and white adipose tissues. *Br J Pharmacol* **118**, 929–934.

Dyreborg A, Krogh N, Backer V, Rzeppa S, Hemmersbach P & Hostrup M (2016). Pharmacokinetics of oral and inhaled terbutaline after exercise in trained men. *Front Pharmacol* **7**, 150.

Elers J, Hostrup M, Pedersen L, Henninge J, Hemmersbach P, Dalhoff K & Backer V (2012). Urine and serum concentrations of inhaled and oral terbutaline. *Int J Sports Med* **33**, 1026–1033.

Elfellah MS, Dalling R, Kantola IM & Reid JL (1989). Beta-adrenoceptors and human skeletal muscle characterisation of receptor subtype and effect of age. *Br J Clin Pharmacol* **27**, 31–38.

Enoksson S, Talbot M, Rife F, Tamborlane WV, Sherwin RS & Caprio S (2000). Impaired in vivo stimulation of lipolysis in adipose tissue by selective beta2-adrenergic agonist in obese adolescent girls. *Diabetes* **49**, 2149–2153.

Eriksson P, Dahlman I, Ryden M, Hoffstedt J & Arner P (2004). Relationship between beta-2 adrenoceptor gene haplotypes and adipocyte lipolysis in women. *Int J Obes Relat Metab Disord* **28**, 185–190.

Fortier M, Wang SP, Mauriege P, Semache M, Mfuma L, Li H, Levy E, Richard D & Mitchell GA (2004). Hormone-sensitive lipase-independent adipocyte lipolysis during beta-adrenergic stimulation, fasting, and dietary fat loading. *Am J Physiol Endocrinol Metab* **287**, E282–E288.

Frontini A, Vitali A, Perugini J, Murano I, Romiti C, Ricquier D, Guerrieri M & Cinti S (2013). White-to-brown transdifferentiation of omental adipocytes in patients affected by pheochromocytoma. *Biochim Biophys Acta* **1831**, 950–959.

Giantin V, Ceccon A, Enzi G, Sergi G, Perini P, Bussolotto M, Schiavon M, Casadei A, Mazzoleni F, Nardo Sartori L & Zurlo F (1995). Heart rate and metabolic response to burn injury in humans. *Journal of Parenteral and Enteral Nutrition* **19**, 55–62.

Gregorevic P, Ryall JG, Plant DR, Sillence MN & Lynch GS (2005). Chronic beta-agonist administration affects cardiac function of adult but not old rats, independent of beta-adrenoceptor density. *Am J Physiol Heart Circ Physiol* **289**, H344–H349.

Grimmer NM, Gimbar RP, Bursua A & Patel M (2016). Rhabdomyolysis secondary to clenbuterol use and exercise. *J Emerg Med* **50**, e71–e74.

Hagg A, Colgan TD, Thomson RE, Qian H, Lynch GS & Gregorevic P (2016). Using AAV vectors expressing the beta2-adrenoceptor or associated Galpha proteins to modulate skeletal muscle mass and muscle fibre size. *Sci Rep* **6**, 23042.

Hamby PL, Stouffer JR & Smith SB (1986). Muscle metabolism and real-time ultrasound measurement of muscle and subcutaneous adipose tissue growth in lambs fed diets containing a beta-agonist1. *J Anim Sci* **63**, 1410–1417.

Haney S & Hancox RJ (2005). Tolerance to bronchodilation during treatment with long-acting beta-agonists, a randomised controlled trial. *Respir Res* **6**, 107.

Hayakawa T, Nagai Y, Kahara T, Yamashita H, Takamura T, Abe T, Nomura G & Kobayashi K (2000). Gln27Glu and Arg16Gly polymorphisms of the beta2-adrenergic receptor gene are not associated with obesity in Japanese men. *Metabolism* **49**, 1215–1218.

Hellstrom L, Large V, Reynisdottir S, Wahrenberg H & Arner P (1999). The different effects of a Gln27Glu beta 2-adrenoceptor gene polymorphism on obesity in males and in females. *J Intern Med* **245**, 253–259.

Hinkle RT, Hodge KM, Cody DB, Sheldon RJ, Kobilka BK & Isfort RJ (2002). Skeletal muscle hypertrophy and anti-atrophy effects of clenbuterol are mediated by the beta2-adrenergic receptor. *Muscle Nerve* **25**, 729–734.

Hochhaus G & Möllmann H (1992). Pharmacokinetic/pharmacodynamic characteristics of the beta-2-agonists terbutaline, salbutamol and fenoterol. *Int J Clin Pharmacol Ther Toxicol* **30**, 342–362.

Holm C (2003). Molecular mechanisms regulating hormone-sensitive lipase and lipolysis. *Biochem Soc Trans* **31**, 1120–1124.

Hostrup M, Jacobson GA, Jessen S & Lemminger AK (2020). Anabolic and lipolytic actions of beta2 -agonists in humans and antidoping challenges. *Drug Test Anal* **12**, 597–609.

Hostrup M, Kalsen A, Bangsbo J, Hemmersbach P, Karlsson S & Backer V (2014*a*). High-dose inhaled terbutaline increases muscle strength and enhances maximal sprint performance in trained men. *Eur J Appl Physiol* **114**, 2499–2508.

Hostrup M, Kalsen A, Onslev J, Jessen S, Haase C, Habib S, Ortenblad N, Backer V & Bangsbo J (2015). Mechanisms underlying enhancements in muscle force and power output during maximal cycle ergometer exercise induced by chronic beta2-adrenergic stimulation in men. *J Appl Physiol (1985)* **119**, 475–486.

Hostrup M, Kalsen A, Ortenblad N, Juel C, Morch K, Rzeppa S, Karlsson S, Backer V & Bangsbo J (2014*b*). beta2-adrenergic stimulation enhances Ca2+ release and contractile properties of skeletal muscles, and counteracts exercise-induced reductions in Na+-K+-ATPase Vmax in trained men. *J Physiol* **592**, 5445–5459.

Hostrup M, Narkowicz CK, Habib S, Nichols DS & Jacobson GA (2019). Beta2 -adrenergic ligand racemic formoterol exhibits enantioselective disposition in blood and skeletal muscle of humans, and elicits myocellular PKA signaling at therapeutic inhaled doses. *Drug Test Anal* **11**, 1048–1056.

Hostrup M, Reitelseder S, Jessen S, Kalsen A, Nyberg M, Egelund J, Kreiberg M, Kristensen CM, Thomassen M, Pilegaard H, Backer V, Jacobson GA, Holm L & Bangsbo J (2018). Beta2 -adrenoceptor agonist salbutamol increases protein turnover rates and alters signalling in skeletal muscle after resistance exercise in young men. *J Physiol* **596**, 4121–4139.

Hsiao TJ & Lin E (2014). Evaluation of the glutamine 27 glutamic acid polymorphism in the adrenoceptor beta2 surface gene on obesity and metabolic phenotypes in Taiwan. *J Investig Med* **62**, 310–315.

Ip EJ, Doroudgar S, Lau B & Barnett MJ (2019). Anabolic steroid users' misuse of non-traditional prescription drugs. *Research in Social and Administrative Pharmacy* **15**, 949–952.

Ishiyama-Shigemoto S, Yamada K, Yuan X, Ichikawa F & Nonaka K (1999). Association of polymorphisms in the beta2-adrenergic receptor gene with obesity, hypertriglyceridaemia, and diabetes mellitus. *Diabetologia* **42**, 98–101.

Jacobson GA, Raidal S, Hostrup M, Calzetta L, Wood-Baker R, Farber MO, Page CP & Walters EH (2018). Long-acting beta2-agonists in asthma: enantioselective safety studies are needed. *Drug Saf* **41**, 441–449.

Jenkins NDM, Colquhoun RJ, Tomko PM, Gradnigo T, Magrini MA, Muddle TWD, Fleming S, Ferrell M & El-Sohemy A (2018). Genetic variant in the beta2 -adrenergic receptor (Arg16Gly) influences fat-free mass, muscle strength and motor unit behaviour in young men. *Exp Physiol* **103**, 1645–1655.

Jensen J, Brennesvik EO, Bergersen H, Oseland H, Jebens E & Brors O (2002). Quantitative determination of cell surface beta-adrenoceptors in different rat skeletal muscles. *Pflugers Arch* **444**, 213–219.

Jeschke MG, Chinkes DL, Finnerty CC, Kulp G, Suman OE, Norbury WB, Branski LK, Gauglitz GG, Mlcak RP & Herndon DN (2008). Pathophysiologic response to severe burn injury. *Ann Surg* **248**, 387–401.

Jessen S, Onslev J, Lemminger A, Backer V, Bangsbo J & Hostrup M (2018). Hypertrophic effect of inhaled beta2 -agonist with and without concurrent exercise training: a randomized controlled trial. *Scand J Med Sci Sports* **28**, 2114–2122.

Jessen S, Reitelseder S, Kalsen A, Kreiberg M, Onslev J, Gad A, Ortenblad N, Backer V, Holm L, Bangsbo J & Hostrup M (2021). beta2-Adrenergic agonist salbutamol augments hypertrophy in MHCIIa fibers and sprint mean power output but not muscle force during 11 weeks of resistance training in young men. *J Appl Physiol (1985)* **130**, 617–626.

Jessen S, Solheim SA, Jacobson GA, Eibye K, Bangsbo J, Nordsborg NB & Hostrup M (2020). Beta2 -adrenergic agonist clenbuterol increases energy expenditure and fat oxidation, and induces mTOR phosphorylation in skeletal muscle of young healthy men. *Drug Test Anal* **12**, 610–618.

Johnson M (1995). Pharmacology of long-acting beta-agonists. *Ann Allergy Asthma Immunol* **75**, 177–179.

Kalinovich A, Dehvari N, Aslund A, van Beek S, Halleskog C, Olsen J, Forsberg E, Zacharewicz E, Schaart G, Rinde M, Sandstrom A, Berlin R, Ostenson CG, Hoeks J & Bengtsson T (2020). Treatment with a beta-2-adrenoceptor agonist stimulates glucose uptake in skeletal muscle and improves glucose homeostasis, insulin resistance and hepatic steatosis in mice with diet-induced obesity. *Diabetologia* **63**, 1603–1615.

Kalsen A, Hostrup M, Backer V & Bangsbo J (2016*a*). Effect of formoterol, a long-acting beta2-adrenergic agonist, on muscle strength and power output, metabolism, and fatigue during maximal sprinting in men. *Am J Physiol Regul Integr Comp Physiol* **310**, R1312–R1321.

Kalsen A, Hostrup M, Karlsson S, Hemmersbach P, Bangsbo J & Backer V (2014). Effect of inhaled terbutaline on substrate utilization and 300-kcal time trial performance. *J Appl Physiol (1985)* **117**, 1180–1187.

Kalsen A, Hostrup M, Soderlund K, Karlsson S, Backer V & Bangsbo J (2016*b*). Inhaled Beta2-agonist increases power output and glycolysis during sprinting in men. *Med Sci Sports Exerc* **48**, 39–48.

Kamalakkannan G, Petrilli CM, George I, LaManca J, McLaughlin BT, Shane E, Mancini DM & Maybaum S (2008). Clenbuterol increases lean muscle mass but not endurance in patients with chronic heart failure. *J Heart Lung Transpl* **27**, 457–461.

Koeberl DD, Case LE, Smith EC, Walters C, Han SO, Li Y, Chen W, Hornik CP, Huffman KM, Kraus WE, Thurberg BL, Corcoran DL, Bali D, Bursac N & Kishnani PS (2018). Correction of biochemical abnormalities and improved muscle function in a phase i/ii clinical trial of clenbuterol in pompe disease. *Mol Ther* **26**, 2304–2314.

Koziczak-Holbro M, Rigel DF, Dumotier B, Sykes DA, Tsao J, Nguyen NH, Bosch J, Jourdain M, Flotte L, Adachi Y, Kiffe M, Azria M, Fairhurst RA, Charlton SJ, Richardson BP, Lach-Trifilieff E, Glass DJ, Ullrich T & Hatakeyama S (2019). Pharmacological characterization of a novel 5-hydroxybenzothiazolone-derived beta 2-adrenoceptor agonist with functional selectivity for anabolic effects on skeletal muscle resulting in a wider cardiovascular safety window in preclinical studies. *J Pharmacol Exp Ther* **369**, 188–199.

Lafontan M & Berlan M (1993). Fat cell adrenergic receptors and the control of white and brown fat cell function. *J Lipid Res* **34**, 1057–1091.

Lange LA, Norris JM, Langefeld CD, Nicklas BJ, Wagenknecht LE, Saad MF & Bowden DW (2005). Association of adipose tissue deposition and beta-2 adrenergic receptor variants: the IRAS family study. *Int J Obes (Lond)* **29**, 449–457.

Large V, Hellstrom L, Reynisdottir S, Lonnqvist F, Eriksson P, Lannfelt L & Arner P (1997). Human beta-2 adrenoceptor gene polymorphisms are highly frequent in obesity and associate with altered adipocyte beta-2 adrenoceptor function. *J Clin Invest* **100**, 3005–3013.

Larsen TM, Toubro S, van Baak MA, Gottesdiener KM, Larson P, Saris WH & Astrup A (2002). Effect of a 28-d treatment with L-796568, a novel beta(3)-adrenergic receptor agonist, on energy expenditure and body composition in obese men. *Am J Clin Nutr* **76**, 780–788.

Lee P, Birzniece V, Umpleby AM, Poljak A & Ho KK (2015). Formoterol, a highly beta2-selective agonist, induces gender-dimorphic whole body leucine metabolism in humans. *Metabolism* **64**, 506–512.

Lee P, Day RO, Greenfield JR & Ho KK (2013). Formoterol, a highly beta2-selective agonist, increases energy expenditure and fat utilisation in men. *Int J Obes (Lond)* **37**, 593–597.

Lee SH, Kwak MK, Ahn SH, Kim H, Cho YY, Suh S, Song KH, Koh JM, Kim JH & Kim BJ (2019). Change of skeletal muscle mass in patients with pheochromocytoma. *J Bone Miner Metab* **37**, 694–702.

Leite N, Lazarotto L, Milano GE, Titski AC, Consentino CL, de Mattos F, de Andrade FA & Furtado-Alle L (2015). [Beta 2-adrenergic receptor gene association with overweight and asthma in children and adolescents and its relationship with physical fitness]. *Rev Paul Pediatr* **33**, 381–386.

Lonnqvist F, Wahrenberg H, Hellstrom L, Reynisdottir S & Arner P (1992). Lipolytic catecholamine resistance due to decreased beta 2-adrenoceptor expression in fat cells. *J Clin Invest* **90**, 2175–2186.

Lund J & Gillum MP (2016). Towards leanness by 'feeding' a novel thermogenic pathway? *Trends in Endocrinology & Metabolism* **27**, 529–530.

Maltin CA, Delday MI, Watson JS, Heys SD, Nevison IM, Ritchie IK & Gibson PH (1993). Clenbuterol, a beta-adrenoceptor agonist, increases relative muscle strength in orthopaedic patients. *Clin Sci (Lond)* **84**, 651–654.

Martineau L, Horan MA, Rothwell NJ, & Little RA (1992). Salbutamol, a β_2-adrenoceptor agonist, increases skeletal muscle strength in young men. *Clin Sci* **83**, 615–621.

Mauriege P, De Pergola G, Berlan M & Lafontan M (1988). Human fat cell beta-adrenergic receptors: beta-agonist-dependent lipolytic responses and characterization of beta-adrenergic binding sites on human fat cell membranes with highly selective beta 1-antagonists. *J Lipid Res* **29**, 587–601.

McCullagh EP & Engel WJ (1942). Pheochromocytoma with hypermetabolism: report of two cases. *Ann Surg* **116**, 61–75.

Meltzer DL & Kemp JP (1991). Beta 2-agonists: pharmacology and recent developments. *J Asthma* **28**, 179–186.

Milano G, Chiappini S, Mattioli F, Martelli A & Schifano F (2018). β-2 agonists as misusing drugs? assessment of both clenbuterol- and salbutamol-related european medicines agency pharmacovigilance database reports. *Basic Clin Pharmacol Toxicol* **123**, 182–187.

Miller CN, Yang JY, England E, Yin A, Baile CA & Rayalam S (2015). Isoproterenol increases uncoupling, glycolysis, and markers of beiging in mature 3t3-l1 adipocytes. *PLoS One* **10**, e0138344.

Miller MF, Garcia DK, Coleman ME, Ekeren PA, Lunt DK, Wagner KA, Procknor M, Welsh TH, Jr. & Smith SB (1988). Adipose tissue, longissimus muscle and anterior pituitary growth and function in clenbuterol-fed heifers. *J Anim Sci* **66**, 12–20.

Mori Y, Kim-Motoyama H, Ito Y, Katakura T, Yasuda K, Ishiyama-Shigemoto S, Yamada K, Akanuma Y, Ohashi Y, Kimura S, Yazaki Y & Kadowaki T (1999). The Gln27Glu beta2-adrenergic receptor variant is associated with obesity due to subcutaneous fat accumulation in Japanese men. *Biochem Biophys Res Commun* **258**, 138–140.

Nagase I, Yoshida T, Kumamoto K, Umekawa T, Sakane N, Nikami H, Kawada T & Saito M (1996). Expression of uncoupling protein in skeletal muscle and white fat of obese mice treated with thermogenic beta 3-adrenergic agonist. *J Clin Invest* **97**, 2898–2904.

Oberkofler H, Esterbauer H, Hell E, Krempler F & Patsch W (2000). The Gln27Glu polymorphism in the beta2-adrenergic receptor gene is not associated with morbid obesity in Austrian women. *Int J Obes Relat Metab Disord* **24**, 388–390.

Ohyama K, Nogusa Y, Shinoda K, Suzuki K, Bannai M & Kajimura S (2016). A synergistic antiobesity effect by a combination of capsinoids and cold temperature through promoting beige adipocyte biogenesis. *Diabetes* **65**, 1410–1423.

Onslev J, Jacobson G, Narkowicz C, Backer V, Kalsen A, Kreiberg M, Jessen S, Bangsbo J & Hostrup M (2017). Beta2-adrenergic stimulation increases energy expenditure at rest, but not during submaximal exercise in active overweight men. *Eur J Appl Physiol* **117**, 1907–1915.

Onslev J, Jensen J, Bangsbo J, Wojtaszewski J & Hostrup M (2019). beta2-agonist induces net leg glucose uptake and free fatty acid release at rest but not during exercise in young men. *J Clin Endocrinol Metab* **104**, 647–657.

Onslev J, Thomassen M, Wojtaszewski J, Bangsbo J & Hostrup M (2021). Beta2-adrenoceptor agonist salbutamol increases leg glucose uptake and metabolic rate but not muscle glycogen resynthesis in recovery from resistance exercise of the quadriceps in lean young men. J Clin Endocrinol Metab. Ahead of print. https://doi.org/10.1210/clinem/dgab752.

Oomen JM, van Rossum CT, Hoebee B, Saris WH & van Baak MA (2005). beta2-adrenergic receptor polymorphisms and salbutamol-stimulated energy expenditure. *J Clin Endocrinol Metab* **90**, 2301–2307.

Pan SJ, Hancock J, Ding Z, Fogt D, Lee M & Ivy JL (2001). Effects of clenbuterol on insulin resistance in conscious obese Zucker rats. *American Journal of Physiology-Endocrinology and Metabolism* **280**, E554–E561.

Petrák O, Haluzíková D, Kaválková P, Štrauch B, Rosa J, Holaj R, Brabcová Vránková A, Michalský D, Haluzík M, Zelinka T & Widimský J Jr(2013). Changes in energy metabolism in pheochromocytoma. *The Journal of Clinical Endocrinology & Metabolism* **98**, 1651–1658.

Phillips P, Vedig A, Jones P, Chapman M, Collins M, Edwards J, Smeaton T & Duncan B (1980). Metabolic and cardiovascular side effects of the beta 2-adrenoceptor agonists salbutamol and rimiterol. *Br J Clin Pharmacol* **9**, 483–491.

Polidori D, Sanghvi A, Seeley RJ & Hall KD (2016). How strongly does appetite counter weight loss? quantification of the feedback control of human energy intake. *Obesity* **24**, 2289–2295.

Porter C (2017). Quantification of UCP1 function in human brown adipose tissue. *Adipocyte* **6**, 167–174.

Porter C (2021). Adipocyte browning in response to trauma: some important methodological considerations. *Shock* **56**, 870–871.

Porter C, Herndon DN, Chondronikola M, Chao T, Annamalai P, Bhattarai N, Saraf MK, Capek KD, Reidy PT, Daquinag AC, Kolonin MG, Rasmussen BB, Borsheim E, Toliver-Kinsky T & Sidossis LS (2016*a*). Human and mouse brown adipose tissue mitochondria have comparable UCP1 function. *Cell Metab* **24**, 246–255.

Porter C, Tompkins RG, Finnerty CC, Sidossis LS, Suman OE & Herndon DN (2016*b*). The metabolic stress response to burn trauma: current understanding and therapies. *Lancet North Am Ed* **388**, 1417–1426.

Rasmussen SG, Choi HJ, Rosenbaum DM, Kobilka TS, Thian FS, Edwards PC, Burghammer M, Ratnala VR, Sanishvili R, Fischetti RF, Schertler GF, Weis WI & Kobilka BK (2007). Crystal structure of the human beta2 adrenergic G-protein-coupled receptor. *Nature* **450**, 383–387.

Redman LM, de Jonge L, Fang X, Gamlin B, Recker D, Greenway FL, Smith SR & Ravussin E (2007). Lack of an effect of a novel beta3-adrenoceptor agonist, TAK-677, on energy metabolism in obese individuals: a double-blind, placebo-controlled randomized study. *J Clin Endocrinol Metab* **92**, 527–531.

Reeds PJ, Hay SM, Dorward PM & Palmer RM (1988). The effect of beta-agonists and antagonists on muscle growth and body composition of young rats (Rattus sp.). *Comp Biochem Physiol C* **89**, 337–341.

Reynisdottir S, Wahrenberg H, Carlstrom K, Rossner S & Arner P (1994). Catecholamine resistance in fat cells of women with upper-body obesity due to decreased expression of beta 2-adrenoceptors. *Diabetologia* **37**, 428–435.

Ricks CA, Dalrymple RH, Baker PK & Ingle DL (1984). Use of a β-agonist to alter fat and muscle deposition in steers. *J Anim Sci* **59**, 1247–1255.

Rosenbaum DM, Rasmussen SG & Kobilka BK (2009). The structure and function of G-protein-coupled receptors. *Nature* **459**, 356–363.

Ryall JG, Church JE & Lynch GS (2010). Novel role for ss-adrenergic signalling in skeletal muscle growth, development and regeneration. *Clin Exp Pharmacol Physiol* **37**, 397–401.

Salpeter SR, Ormiston TM & Salpeter EE (2004). Cardiovascular effects of beta-agonists in patients with asthma and COPD: a meta-analysis. *Chest* **125**, 2309–2321.

Schiffelers SLH, Saris WHM, Boomsma F & van Baak MA (2001). β1- andβ 2-adrenoceptor-mediated thermogenesis and lipid utilization in obese and lean men1. *The Journal of Clinical Endocrinology & Metabolism* **86**, 2191–2199.

Schwartz MW, Seeley RJ, Zeltser LM, Drewnowski A, Ravussin E, Redman LM & Leibel RL (2017). Obesity pathogenesis: an endocrine society scientific statement. *Endocr Rev* **38**, 267–296.

Seale P, Bjork B, Yang W, Kajimura S, Chin S, Kuang S, Scimè A, Devarakonda S, Conroe HM, Erdjument-Bromage H, Tempst P, Rudnicki MA, Beier DR & Spiegelman BM (2008). PRDM16 controls a brown fat/skeletal muscle switch. *Nature* **454**, 961–967.

Shaw WN (1993). Long-term treatment of obese Zucker rats with LY255582 and other appetite suppressants. *Pharmacol Biochem Behav* **46**, 653–659.

Shenoy SK, Drake MT, Nelson CD, Houtz DA, Xiao K, Madabushi S, Reiter E, Premont RT, Lichtarge O & Lefkowitz RJ (2006). β-Arrestin-dependent, G Protein-independent ERK1/2 activation by the β2 adrenergic receptor∗. *J Biol Chem* **281**, 1261–1273.

Sidossis LS, Porter C, Saraf MK, Borsheim E, Radhakrishnan RS, Chao T, Ali A, Chondronikola M, Mlcak R, Finnerty CC, Hawkins HK, Toliver-Kinsky T & Herndon DN (2015). Browning of subcutaneous white adipose tissue in humans after severe adrenergic stress. *Cell Metab* **22**, 219–227.

Simonsen L, Bulow J, Madsen J & Christensen NJ (1992). Thermogenic response to epinephrine in the forearm and abdominal subcutaneous adipose tissue. *Am J Physiol* **263**, E850–E855.

Simonsen L, Stallknecht B & Bulow J (1993). Contribution of skeletal muscle and adipose tissue to adrenaline-induced thermogenesis in man. *Int J Obes Relat Metab Disord* **17**(Suppl 3), S47–S51; discussion S68.

Simonsen S & Kjekshus JK (1978). The effect of free fatty acids on myocardial oxygen consumption during atrial pacing and catecholamine infusion in man. *Circulation* **58**, 484–491.

Slack RJ, Barrett VJ, Morrison VS, Sturton RG, Emmons AJ, Ford AJ & Knowles RG (2013). In vitro pharmacological characterization of vilanterol, a novel long-acting beta2-adrenoceptor agonist with 24-hour duration of action. *J Pharmacol Exp Ther* **344**, 218–230.

Spiller HA, James KJ, Scholzen S & Borys DJ (2013). A descriptive study of adverse events from clenbuterol misuse and abuse for weight loss and bodybuilding. *Substance Abuse* **34**, 306–312.

Stainsby WN & Lambert CR (1979). Determination of oxygen uptake in skeletal muscle. *Exerc Sport Sci Rev* **7**, 125–151.

Stratton JR, Pfeifer MA, Ritchie JL & Halter JB (1985). Hemodynamic effects of epinephrine: concentration-effect study in humans. *J Appl Physiol (1985)* **58**, 1199–1206.

Sun Y, Huang J, Xiang Y, Bastepe M, Juppner H, Kobilka BK, Zhang JJ & Huang XY (2007). Dosage-dependent switch from G protein-coupled to G protein-independent signaling by a GPCR. *EMBO J* **26**, 53–64.

Takenaka A, Nakamura S, Mitsunaga F, Inoue-Murayama M, Udono T & Suryobroto B (2012). Human-specific SNP in obesity genes, adrenergic receptor beta2 (ADRB2), Beta3 (ADRB3), and PPAR gamma2 (PPARG), during primate evolution. *PLoS One* **7**, e43461.

Vergnes L, Davies GR, Lin JY, Yeh MW, Livhits MJ, Harari A, Symonds ME, Sacks HS & Reue K (2016). Adipocyte browning and higher mitochondrial function in periadrenal but not SC fat in pheochromocytoma. *J Clin Endocrinol Metab* **101**, 4440–4448.

Vosselman MJ, van der Lans AA, Brans B, Wierts R, van Baak MA, Schrauwen P & van Marken Lichtenbelt WD (2012). Systemic beta-adrenergic stimulation of thermogenesis is not accompanied by brown adipose tissue activity in humans. *Diabetes* **61**, 3106–3113.

Ward JK, Dow J, Dallow N, Eynott P, Milleri S & Ventresca GP (2000). Enantiomeric disposition of inhaled, intravenous and oral racemic-salbutamol in man–no evidence of enantioselective lung metabolism. *Br J Clin Pharmacol* **49**, 15–22.

Watt MJ, Holmes AG, Pinnamaneni SK, Garnham AP, Steinberg GR, Kemp BE & Febbraio MA (2006). Regulation of HSL serine phosphorylation in skeletal muscle and adipose tissue. *Am J Physiol Endocrinol Metab* **290**, E500–E508.

Weyer C, Gautier JF & Danforth E (1999). Development of beta 3-adrenoceptor agonists for the treatment of obesity and diabetes–an update. *Diabetes Metab* **25**, 11–21.

Whyte KF, Addis GJ, Whitesmith R & Reid JL (1987). The mechanism of salbutamol-induced hypokalaemia. *Br J Clin Pharmacol* **23**, 65–71.

Wijers SL, Saris WH & van Marken Lichtenbelt WD (2009). Recent advances in adaptive thermogenesis: potential implications for the treatment of obesity. *Obes Rev* **10**, 218–226.

Williams RS, Caron MG & Daniel K (1984). Skeletal muscle beta-adrenergic receptors: variations due to fiber type and training. *Am J Physiol* **246**, E160–E167.

Wilson SR, Amoroso P, Moxham J & Ponte J (1993). Modification of the thermogenic effect of acutely inhaled salbutamol by chronic inhalation in normal subjects. *Thorax* **48**, 886–889.

Wolfe RR, Herndon DN, Jahoor F, Miyoshi H & Wolfe M (1987). Effect of severe burn injury on substrate cycling by glucose and fatty acids. *N Engl J Med* **317**, 403–408.

Wolfe RR, Klein S, Carraro F & Weber J-M (1990). Role of triglyceride-fatty acid cycle in controlling fat metabolism in humans during and after exercise. *American Journal of Physiology-Endocrinology And Metabolism* **258**, E382–E389.

Zhang H, Wu J & Yu L (2014). Association of Gln27Glu and Arg16Gly polymorphisms in Beta2-adrenergic receptor gene with obesity susceptibility: a meta-analysis. *PLoS One* **9**, e100489.

Zhang J, He Q, Liu QY, Guo W, Deng XM, Zhang WW, Hu XX & Li N (2007). Differential gene expression profile in pig adipose tissue treated with/without clenbuterol. *BMC Genomics* **8**, 433.

Zurlo F, Larson K, Bogardus C & Ravussin E (1990). Skeletal muscle metabolism is a major determinant of resting energy expenditure. *J Clin Invest* **86**, 1423–1427.

Additional information

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None to declare.

Author contributions

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Supporting information

Additional supporting information can be found online in the Supporting Information section at the end of the HTML view of the article. Supporting information files available:

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