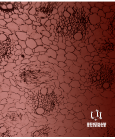**REVIEW ARTICLE**

Classification of Therapeutic and Experimental Drugs for Brown Adipose Tissue Activation: Potential Treatment Strategies for Diabetes and Obesity

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Abstract: **Objective**: Increasing efforts are being made towards pharmacologic activation of brown adipose tissue (BAT) in animals and humans for potential use in the treatment of obesity and diabetes. We and others have reported a number of animal studies using either experimental or therapeutic drugs. There are now efforts to translate these findings to human studies. The goal of this review is to evaluate the various drugs currently being used that have the potential for BAT activation.

Methods: Drugs were classified into 4 classes based on their mechanism of action. Class 1 drugs include the use of β3 adrenoceptor agonists for BAT activation. Class 2 drugs include drugs that affect norepinephrine levels and activate BAT with the potential of reducing obesity. Class 3 includes activators of peroxisome proliferator-activated receptor-γ in pursuit of lowering blood sugar, weight loss and diabetes and finally Class 4 includes natural products and other emerging drugs with limited information on BAT activation and their effects on diabetes and weight loss.

Results: Class 1 drugs are high BAT activators followed by Class 2 and 3. Some of these drugs have now been extended to diabetes and obesity animal models and human BAT studies. Drugs in Class 3 are used clinically for Type 2 diabetes, but the extent of BAT involvement is unclear.

Conclusion: Further studies on the efficacy of these drugs in diabetes and measuring their effects on BAT activation using noninvasive imaging will help in establishing a clinical role of BAT.

1. INTRODUCTION

Brown adipose tissue (BAT) in mammals helps to maintain body temperature during prolonged exposure to cold temperature by generating heat using energy in the body. This extraordinary metabolic capacity has the potential of regulating body fat stores and holds promise in combating obesity and diabetes [1-3]. Mitochondria in the brown adipocytes express uncoupling protein-1 (UCP1) which uses lipids and carbohydrates to generate heat by uncoupling electron transport from oxidative phosphorylation [4]. Activation of brown adipocytes results in unrestrained oxidation by drawing lipids and carbohydrates from outside the cell [5]. The role of BAT in understanding the mechanism of insulin sensitivity [6], lowering adiposity and improving type-2 diabetes [7], are being pursued and therefore make it a valuable target to study pathogenicity of obesity and diabetes.

Norepinephrine contained in neuronal fibers in BAT interact with β3 adrenoceptors (β3AR) present in the adipocyte cell surface [8]. This results in an increase in cyclic AMP (cAMP) which subsequently results in overexpression of UCP1 resulting in the enhancement of glycolysis [9]. Thus, agonist-mediated activation of β3AR on brown adipocytes has been evaluated as a strategy for studying BAT biology, and as a potential therapeutic approach for diabetes and obesity. Studies on presynaptic proteins which can elevate norepinephrine levels (*e.g.* norepinephrine transporter, NET) or at the level of secondary messenger changes (*e.g.* adenylyl cyclase) and peroxisome proliferator-activated receptor-γ (PPAR-γ) are limited and less understood. Other potential modulating factors of UCP1 levels have been recently reviewed [10].

Due to the growing incidence of obesity and diabetes globally, studies on BAT across different species are being pursued with great urgency. Several recent reviews have evaluated the potential role of BAT in energy use. These reviews have summarized the various approaches of imaging BAT and their shortcomings [11]. Other reviews have pointed to the value of diet-induced thermogenesis [12]. More recently, pharmacological strategies for BAT recruitment have been reported as a target of obesity and insulin sensitivity [13, 14].

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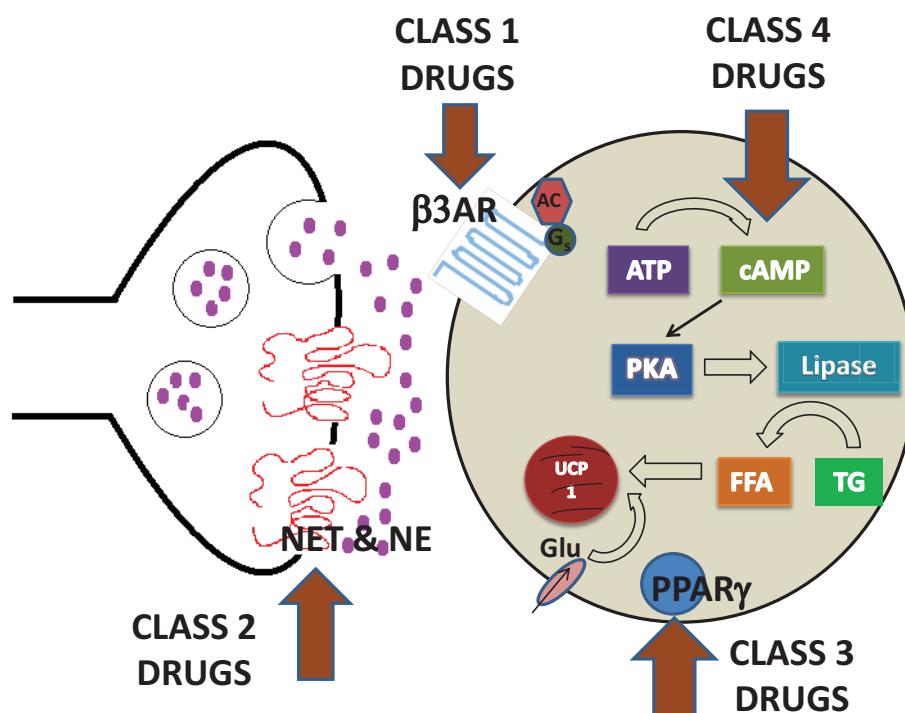
Fig. (1). Schematic of Sites of Drug Action: Class 1 drugs act on the adipocyte cell membrane bound β3 adrenergic receptor (β3AR) triggering a cascade of events *via* cAMP. Class 2 drugs act on the norepinephrine transporter (NET) on the sympatheic nerve terminal and increase norepinephrine (NE) levels which then stimulates β3AR. Class 3 drugs activate peroxisome proliferator-activated receptor gamma (PPARγ). Class 4 drugs act on various pathways within the adipocyte. Abbreviations: AC: adenylate cyclase; Gs: stimulatory G-protein; ATP: adenosine triphosphate; cAMP: cyclic adenosine monophosphate; PKA: protein kinase A; TG: triglycerides; FFA: free fatty acids; UCP-1: uncoupling protein-1(found in mitochondria); Glu: glucose. ● Norepinephrine.

We have previously reported several studies on drug-induced BAT activation [15]. This review summarizes our findings on BAT activation by various drugs used in the experimental and therapeutic approaches along with other published findings. It is by no means exhaustive, and at the time of writing this review, there were more than 9000 citations on "brown adipose tissue" in Pubmed and over 2200 occurred in the last 5 years.

2. COLD-INDUCED BAT ACTIVATION

Thermogenesis has been known for several decades and various studies have been reported on increased metabolic activity of BAT. Assessing the potential of BAT received an impetus from resolving the uptake of 2-deoxy-2-^{18}F-fluoro-D-glucose (^{18}F-FDG) in human BAT positron emission tomography/computed tomography (PET/CT) studies [16-18]. Around this time, BAT was visualized in rats using ^{123}I-MIBG, an analog of norepinephrine [19], and more recently, norepinephrine transporters were visualized in BAT using ^{11}C-MRB and ^{11}C-TAZA [20, 21]. Additional studies have also included the use of ^{11}C-acetate, ^{11}C-palmitate and radio-labeled fatty acids as metabolic substrates [22, 23]. Measuring metabolic activity of BAT and assessing factors that influence BAT activity are important for the development of novel strategies in the regulation of body weight. BAT is active when its thermogenic function is stimulated [24], and accumulation of metabolic substrates such as ^{18}F-FDG, ^{11}C-acetate and ^{11}C-palmitate is a consequence of UCP1 activity [9]. Activated BAT may thus have therapeutic potential to combat both diabetes and obesity with its ability to reduce plasma triglyceride levels [25]. The well-established literature of BAT biology in humans and animal models is now supported by quantitative analysis of ^{18}F-FDG PET/CT imaging data [15, 27-29].

Cold temperatures increase ^{18}F-FDG uptake in activated rodent BAT [30], and studies have been performed in both humans (~16 °C) [31] and rodents (~4 °C) [28], with some degree of success in demonstrating BAT activation [26]. Long-time exposure to cold temperature prior to PET was the only method until recently to study BAT in humans--a function mediated by the β-adrenergic system [31]. The BAT prevalence from these studies ranged from 30% to 95%, which is higher than those of the retrospective studies [26, 31, 32].

3. DRUG-INDUCED BAT ACTIVATION

In order to activate BAT at ambient temperatures, several pharmacological agents have been reported [27, 33-35] and some of these findings have been reviewed recently [13, 14, 36]. In this review, the various experimental and therapeutic drugs used for BAT related studies have been divided into 4 major classes. The classification is primarily based on the most probable site of action of the drugs. Fig. (1) depicts classification of the drugs based on their site of action. Class 1 drugs are the β3AR agonists which act on the β3AR located on the adipocyte cell surface. These drugs have been used in animal and human studies. Class 2 consists of drugs that have an effect on altering the norepinephrine levels or
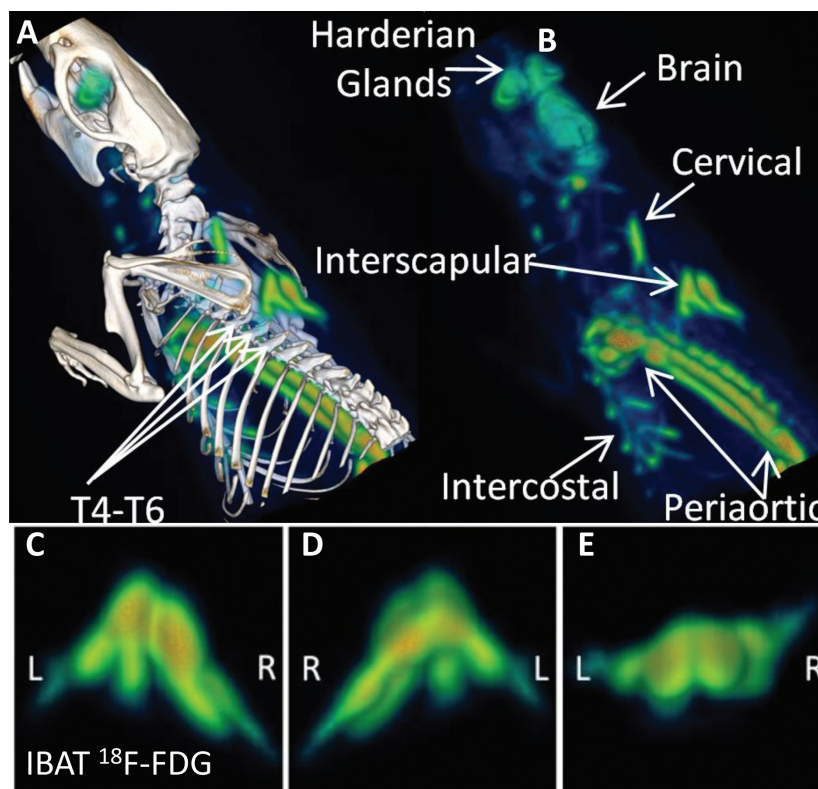
Fig. (2). PET Images from Class 1 Drug Effects on Rat BAT: CL316,243 induced activation of BAT is seen in the PET image (B) and regional localization confirmed by PET/CT (A). Interscapular, periaortic, cervical and intercostal BAT regions are evident. The bilateral structure of activated interscapular BAT (IBAT) is evident in the ventral (C), dorsal (D) and caudal (E) views of IBAT (Figure adapted from Mirbolooki **et al.**, 2011) [29].

directly mimicking norepinephrine effect or by blocking the norepinephrine transporter (NET) located on the sympathetic nerve terminal. Class 3 drugs are activators of peroxisome proliferator-activated receptor-γ (PPAR-γ) and act within the adipocyte. Class 4 are other drugs including natural products on which information is limited or is now emerging.

3.1. Class 1 Drugs: β3 Adrenoceptor Agonists

Agonists for β3AR are currently used clinically for over-active bladder (OAB) [37]. The β3AR are G-protein coupled receptors (GPCR) and are found in significant levels on brown adipocytes [38-41]. BAT is innervated by sympathetic nerves containing norepinephrine which activate β3AR. A significant effort has been made to evaluate β3AR selective agonists as possible therapeutic agents for the treatment of obesity [42].

Table **1** shows a list of β3AR selective agonists which are derivatives of the "2-hydroxyethylamino" backbone mimicking norepinephrine. BRL 37344, an active metabolite of BRL 35135 is known to be selective for adipocyte lipolytic response [43]. Furthermore, 2-deoxy-[^{3}H]-glucose has been used to investigate glucose utilization index (GUI) of BRL-35135. It has been shown that chronic treatment with BRL 37344 causes a 34 fold increase in basal GUI of BAT with no effect on GUI of other tissues [44]. BRL 35135 was also effective in improving glucose tolerance in genetically obese (ob/ob) mice and obese Zucker (fa/fa) rats at doses that had no significant anti-obesity activity [45].
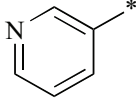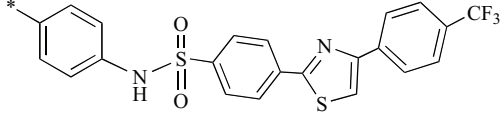
CL316,243, (*R,R*)-5-[2-[2,3-(3-chlorphenyl)-2-hydroxy-ethyl-amino]propyl]-1,3-benzodioxole-2,2-dicarboxylate, di-sodium salt is a β3AR selective agonist [34,46]. CL316,243 activated interscapular BAT (IBAT), cervical, periaortic and intercostal BAT, which were clearly visualized by PET (Fig. **2**) [29]. Because of the selective nature of CL316,243, it may be inferred that the increase in ^{18}F-FDG uptake occurred due to stimulation of the β3AR. This is consistent with the reported effects of CL316,243 on overall energy expenditure in BAT [33]. CL316,243 promotes BAT mitochondrial proliferation and energy expenditure in brown fat is capable of ranging over many orders of magnitude, controlled primarily by sympathetic stimulation mediated by rapid changes in UCP1 intrinsic activity [47]. In initial human studies with CL316,243 energy expenditure after 8 weeks in young lean males did not differ from baseline [48]. Human studies using CL316,243 were discontinued due to poor bioavailability of the drug.

Three closely related derivatives, rafabegron, mirabegron and solabegron are being pursued for clinical use in OAB and irritable bowel syndrome (IBS) [37]. Rafabegron exhibited some increase (~50 kcal/ day) in 24-h energy expenditure (EE) at highest dose in obese men and women [49]. Solabegron which is being pursued for IBS has not been studied for effects on EE. Mirabegron, a β3AR selective agonist [50] is approved for use in OAB [51]. Mirabegron was shown to activate rat [52] IBAT and human [53] BAT metabolic activity as measured by ^{18}F-FDG PET/CT. Thus, mirabegron-induced increased glucose metabolism in BAT

Table 1. Class 1 Drugs: β3 Adrenoceptor Agonists.
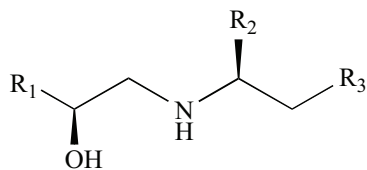
Drug	R_1	R_2	R_3	Status
BRL37344		CH_3		Increases glucose utilization index [44]. No human studies reported.
CL316,243		CH_3		Animal studies continue. Human studies discontinued due to bioavailability [29, 33, 46, 48].
Rafabegron		CH_3		Potential to treat overactive bladder [49].
Mirabegron		H		Clinically used for overactive bladder [37, 52, 53]. BAT activated in rats and humans.
Solabegron		H		Clinically used for overactive bladder [37].
Amibegron		H		Initial clinical trial for depression discontinued [61].
ICID7114		H		BAT activation in dogs. No effect on humans [57, 58].
ZD7114		H		No effect on energy expenditure. No further studies reported [54-56].
Talibegron (ZD2079)		H		Little effect on energy expenditure. No further studies reported [56].

Drug	R$_1$	R$_2$	R$_3$	Status
L-796568		H		Little effect on energy expenditure. No further studies reported [59].

across species is of potential interest for obesity and diabetes.

ZD2079 (talibegron) and ZD7114 are selective β3AR drugs which increase EE *via* non-shivering and reduced weight gain and activated thermogenesis [54]. ZD7114 also has been reported to have antagonist properties at β3AR in isolated rat ileum [55]. ZD7114 had no effect on 24 h EE in obese women and men, while ZD2079 had a very small stimulatory effect on EE [56]. Their value is for weight loss or diabetes is therefore questionable. The structurally similar ICID7114 has been reported to stimulate BAT and oxygen consumption in canine studies [57, 58]. However, no further reports on its effects on weight loss or diabetes have appeared. In the case of the somewhat larger molecule, L-796568, after a 28-day treatment with L-796568 in nondiabetic men no major effect was observed on lipolytic or thermogenic measures [59].

Other clinically used β3AR agonists, amibegron (SR 58611A) [60, 61] have been pursued as antidepressants in clinical trials, but have now been discontinued. β3 adrenoceptors are mostly found in BAT, white adipose tissue, myocardium, skeletal muscle, and liver [40, 62]. Expression of β3 adrenoceptor mRNA in the brain is lower than in BAT [63]. It is unclear if the low brain concentration of β3AR affected the poor outcome with amibegron.

3.2. Class 2 Drugs: Norepinephrine Altering Drugs

Norepinephrine activates β3AR and cold temperatures may promote metabolism indirectly by elevating norepinephrine levels [64, 65]. Uptake of 2-[^{3}H]-DG (glucose metabolic index) in BAT was elevated with increasing doses of norepinephrine [66]. Thus, the capacity of BAT thermogenesis is increased with norepinephrine [67]. In UCP1 ablated mice, addition of norepinephrine in brown adipocytes resulted in no increase in oxygen consumption rate. It has been shown that BAT activity increases with ephedrine (structurally related to norepinephrine, Table **2**) in lean but not in obese participants. The change in BAT activity after ephedrine compared with placebo was negatively correlated with various indices of body fatness [68]. Chronic ephedrine treatment reduced body fat content, but this was not associated with an increase in BAT activity; chronic ephedrine suppressed BAT glucose disposal, suggesting that treatment decreased, rather than increased, BAT activity [69].

Atomoxetine is a potent and highly selective blocker of presynaptic NET that is used for treatment of attention deficit hyperactivity disorder (ADHD) [70]. Atomoxetine leads to increased synapse concentrations of norepinephrine and therefore an increase in adrenergic neurotransmission [71]. Uptake of a highly selective NET ligand, ^{11}C-MRB,

Uptake of a highly selective NET ligand, ^{11}C-MRB, suggests the existence of these transporters in BAT [20]. Uptake of ^{11}C-TAZA *via* the NET in the IBAT as well as other BAT regions was also very evident as can be seen in the Supplementary (Fig. **1**) using PET [21]. Atomoxetine effects on BAT metabolism in rats were quantified by ^{18}F-FDG PET and have recently been reported [72]. This increase is substantially higher than that of ephedrine [27]. Propranolol inhibited atomoxetine-induced BAT activation to control levels and confirmed the likelihood of action of atomoxetine *via* the β3AR. There are few reports introducing atomoxetine as a weight loss agent. A preliminary study to evaluate short-term anti-obesity efficacy demonstrated modest short-term weight loss in obese women [73]. In a trial on outpatients with binge-eating disorder, atomoxetine was found to be efficacious [74]. However, it was not effective for weight loss in those who have gained weight on either clozapine or olanzapine [75].

Nisoxetine, another potent and selective inhibitor of NET uptake was shown to bind IBAT [76]. Increased IBAT binding density from angiotensin II infusion led to promising results of body weight reduction due to increased sympathetic neurotransmission [77]. Sibutramine another NET reuptake inhibitor exhibited thermogenic effects but had cardiovascular side effects [78]. Fibromyalgia patients on another NET reuptake inhibitor, milnacipram showed an approximately 5% weight loss in 3-6 months [79].

3.3. Class 3 Drugs. PPAR-γ Activators

Activation of PPARγ by the glitazone class of drugs (also referred as thiazolidinediones) affects carbohydrate and lipid metabolism by several mechanisms and have been pusued for type 2 diabetes [80]. Given the role of brown adipocytes in the enhancement of energy expenditure, promotion of brown fat adipogenesis by thiazolidinediones could contribute to the beneficial effects of these drugs on insulin sensitivity in humans. Table **3** shows the structural similarities of the thiazolidinediones.

Rosiglitazone (BRL-49653), has been shown to promote differentiation of the brown pre-adipocyte cell line and to increase rat IBAT mass. Rosiglitazone treatment of human pre-adipocytes prepared from all depots resulted in increased levels of UCP1 mRNA [81]. Previous studies have shown that rodents treated with high doses of troglitazone, another type of thiazolidinedione, increased IBAT [82].

Ciglitazone decreased blood glucose, triglycerides, and food intake without affecting body weight in obese hyperglycemic mice. It did show a decrease in human blood sugar but is not currently used in any medication form [83]. Trogli-
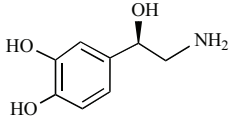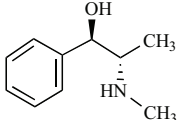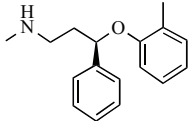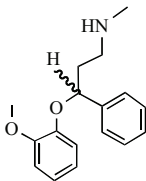
Table 2. Class 2 Drugs: Norepinephrine Elevators.
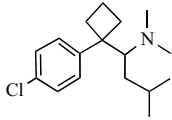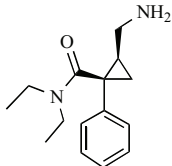
Drug Name	Specific Target(s)	Structure	Current Status
Norepinephrine	Increases NE		Treatment of critically low blood pressure. BAT activation reported in rats [64-67].
Ephedrine	Increases NE-like activity (from natural product ephedra)		BAT activation reported in rats. BAT activated in lean humans [68].
Atomoxetine	NET blocker increases NE levels		Rodent BAT activation [72]. Small change in weight in obese women [73].
Nisoxetine	NET blocker increases NE levels		BAT activation reported in rats [76,77]. No human studies on BAT activation.
Sibutramine	Serotonin-norepinephrine up-take inhibitor		Used to reduces apetitte and promote weight loss. Cardiovascular effect concern [78, 128].
Milnacipram	Serotonin-norepinephrine up-take inhibitor		Reduces body weight in fibromyalgia patients [79].

Table 3. Class 3 Drugs: PPAR-γ Activators.
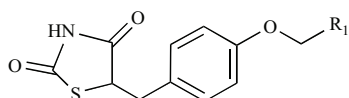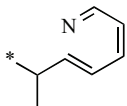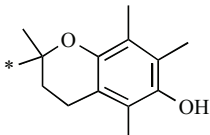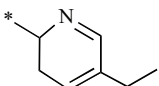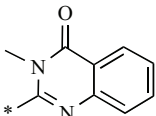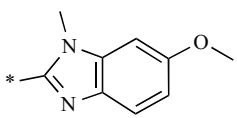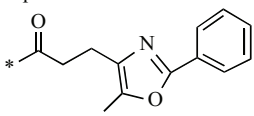
Drug	R₁	Status
Rosiglitazone		Enhanced BAT lipogenesis. Human studies have been reported. Still available in US, but with serious side-effects [81].
Ciglitazone		Decreased blood glucose, triglycerides, and food intake affecting body weight in obese hyperglycemic mice [83]. Not currently used in any medication form.
Troglitazone		Was clinically used as an anti-diabetic drug, now discontinued. Increases insulin sensitivity in non-insulin-dependent diabetes mellitus but with serious liver side effects [84].
Pioglitazone		Currently used to treat diabetes mellitus 2, with bladder side-effects in some cases [85, 86].

Drug	R$_1$	Status
Balaglitazone	(structure)	Lowered glucose levels. Effects on glucose levels and HbA(1c) in type 2 diabetes patients [85, 86].
Rivoglitazone	(structure)	Lowers glucose levels by improving insulin resistance in diabetic animal models [87]. Undergoing trials in treatment of type 2 diabetes mellitus [88].
Darglitazone	replaces the ether side chain (structure)	BAT size increased with altered morphology in rats. No human studies reported. Serious side-effects [89].

tazone improves GLUT4 expression in obese type 2 diabetic rat model and increases insulin sensitivity in non-insulin-dependent diabetes mellitus but with serious liver side-effects [84]. It was used as an anti-diabetic, but has now been discontinued. Pioglitazone is currently used to treat diabetes mellitus and has urinary bladder side-effects in some cases [85,86]. It has been shown to play a role in remodeling of adipocytes in the rat model [87]. Balaglitazone lowered glucose levels and did not affect fluid retention or bone formation in obese rats. It had effects on blood glucose levels and HbA1c in type 2 diabetes patients [85, 86]. Rivaglitazone also lowers glucose levels by improving insulin sensitivity in diabetic animal models. Improved glycemic control in type 2 diabetic patients short time. Rivoglitazone is undergoing trials in treatment of type 2 diabetes mellitus to asses potential health risks with this drug [87, 88]. Darglitazone exhibited an increase in BAT with altered morphology in rats [89]. Clinical development of darglitazone has been discontinued.

Thus, pioglitazone is currently the most promising agent in this class of drugs. Although blood sugar has been lowered by pioglitazone, its ability to induce browning of adipocytes and assist in weight loss has yet to be demonstrated, No PET imaging studies to study BAT activation (either animal or human) using pioglitazone have been reported. It may be useful to evaluate if BAT is activated *in vivo* using pioglitazone and compare these findings with those of mirabegron from class 1 drugs.

3.4. Class 4 Drugs. Other Products/Natural Products

Intraperitoneal injection of nicotine causes the release of catecholamines, including norepinephrine, which stimulates thermogenesis in BAT for energy expenditure [90]. Nicotine causes increases in ^{18}F-FDG uptake in BAT, and the effect is further enhanced when nicotine is combined with ephedrine [27]. These results suggest that nicotine stimulates norepinephrine turnover and BAT thermogenesis while also promoting resting metabolic rate, all of which contribute to the mitigation of obesity [91].

Forskolin is known as an inducer of thermogenic response in BAT [92]. It activates the adenylyl cyclase enzyme directly and increases the intracellular levels of camp [93].

Thus, forskolin is capable of enhancing BAT metabolism as measured by ^{18}F-FDG PET/CT [15].

Caffeine significantly elevated BAT temperature with less effect on core temperature, and oxygen consumption in BAT mitochondria suggesting caffeine activates BAT thermogenesis [94]. Adenosine receptors, A2A have been suggested to play a role in BAT activation [95]. It remains to be demonstrated if interaction of caffeine with adenosine receptors plays a role on its effects on BAT.
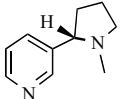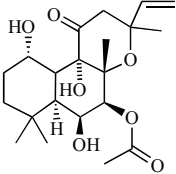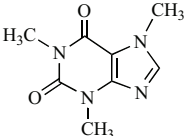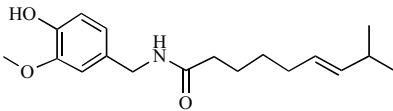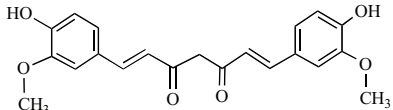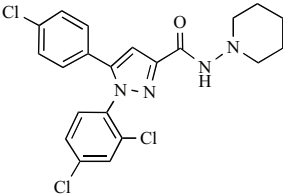
Previous studies have shown a significant reduction in adiposity after prolonged ingestion of capsinoids (capsacin) in humans. BAT is involved in the capsinoid-induced increase in energy expenditure, as presented in small rodents. Increased UCP1 expression was also shown in rats treated with capsinoids for 2 weeks [96]. Capsinoid ingestion increases energy expenditure through the activation of brown adipose tissue in humans [97].

Curcumin is a yellow pigment found in turmeric and has been investigated as a treatment for obesity-related diseases. It interacts directly with adipocytes, pancreatic cells, hepatic stellate cells, macrophages, and muscle cells. Curcumin has been used to reverse insulin sensitivity, hyperglycemia, hyperlipidemia, and other symptoms linked to obesity. It also has the capability of binding to PPAR-γ in order to stimulate differentiation of human adipocytes [98]. It has been further demonstrated to improve cold tolerance in mice and to promote β3 adrenoceptor gene expression in inguinal WAT. Elevation of plasma norepinephrine levels were enhanced with curcumin treatment [99].

Rimonabant, a cannabinoid CB1 receptor drug caused weight loss which was thought to due to elevated BAT temperature mediated by the peripheral endocannbinoid system which was confirmed by the peripheral CB1 receptor antagonist AM6545 [100, 101]. However, rimonabant has been withdrawn from the market due to side effects [102]. Use of peripherally acting CB1 receptor drugs, such as AM6545 in PET imaging may be useful for further evaluation of the role of this target receptor.

ShK-186, a selective Kv1.3 peptide inhibitor, exhibits robust therapeutic effects in a mouse model of diet-induced

Table 4. Class 4 Drugs: Natural and Other Products.

Drug Name	Specific Target(s)	Structure	Current Status
Nicotine	Agonist at nicotinic receptors		BAT activated in rats [90, 91].
Forskolin	Adenylate cyclase activator to produce cAMP		BAT activated in mice [15]. Extracts of forskolin used for obesity [139].
Caffeine	Potential mechanism *via* adenosine receptors		Activates BAT thermogenesis [94].
Capsacin (Capsinoids)	Increases UCP1 expression		Increases EE by activation of BAT [97].
Curcumin	Various mechanisms including PPARγ		Stimulates human adipocyte differentiation [98].
Rimonabant (SR141716)	Cannabinoid CB-1 receptor inverse agonist		May promote weight loss [100,101]: problems with CNS side effects. Drug discontinued [102].
SHK186	Kv1.3 potassium ion-channel blocker; may act via PPARγ	35-amino acid peptide derivative	BAT activation reported in DIO mice [103]. Human trials ongoing for MS [146].
Fibroblast Growth Factor 21 (FGF21)	Endocrine factor present in liver, pancreas and adipose tissue	Secreted protein, 210 amino acid (mouse), 209 amino acids (humans)	Energy expenditure may be associated with BAT [135, 149]. Analogs pursued as antidiabetic agents [150].

obesity and insulin sensitivity [103]. ShK-186 activated BAT as evidenced by increased glucose uptake, enhanced β-oxidation, and elevated transcription of the UCP1 gene involved in BAT thermogenesis. In mice fed an obesity-inducing diet, ShK-186 reduced weight gain despite voracious calorie consumption. These beneficial changes may be associated with elevated membrane remodeling and a simultaneous increase in PPARγ expression and the metabolites that activate PPARγ. Since PPARγ agonists improve insulin sensitivity and diabetes control [104], enhanced PPARγ signaling in ShK-186-treated mice may contribute to the peptide's therapeutic effects.

4. THERAPEUTIC POTENTIAL

4.1. Class 1 Drugs

The presence of β3AR in human BAT allows for a targeted therapeutic strategy [62]. However, concerns such as selectivity and bioavailability of the drugs as well as measureable effects on weight loss have yet to be fully understood for class I drugs. CL 316,243 has only a 10-fold selectivity for human β3 over β2 adrenocoptor and β3AR mRNA is also expressed in the human heart [105], which increases the concerns regarding its cardiovascular side effects. However, CL 316,243 has not been reported to affect heart rate, systolic and/or diastolic blood pressures, ECG intervals or to cause development of tremors [48]. Newer drugs such as mirabegron, targeting this receptor have now been approved for clinical use in OAB but their potential for the treatment of type 2 diabetes has yet to be established [39].

Chronic CL316,243 administration has been shown to have an anti-obesity effect in mice and rats [33,106,107]. Quantitative analysis of ^{18}F-FDG uptake in rats treated with CL316,243 has provided evidence on the ability of acute β3AR stimulation by CL316,243 to increase BAT metabolism *in vivo* using PET. In the early stages of exposure to
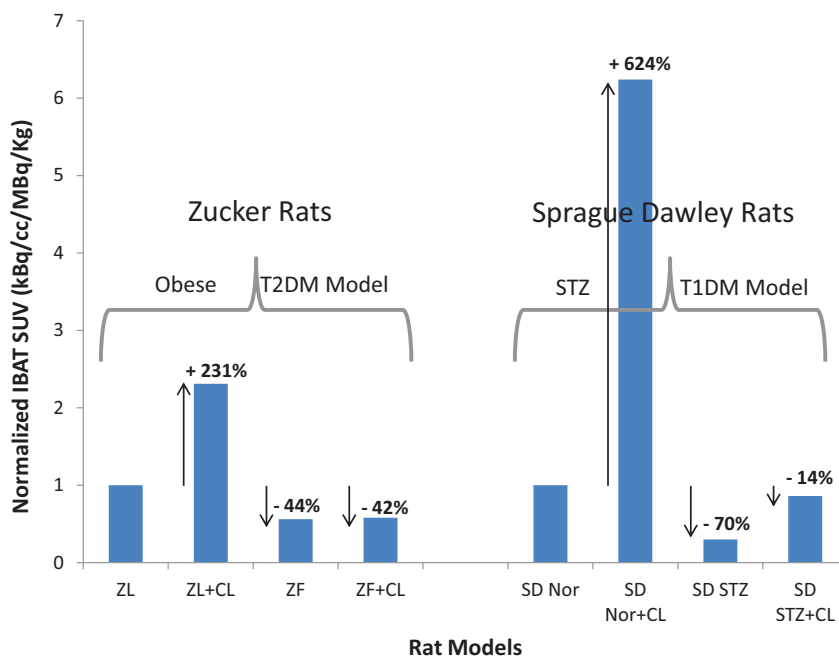
Fig. (3). Class 1 Drug Effects on Obesity and Type1 Diabetes Model: Graph showing effects of CL316,243 (CL) in the two rodent models. In Zucker rat obese models (and T2DM), uptake of ^{18}F-FDG in Zucker lean (ZL) increased by +231% with CL316,243 while Zucker fat (ZF) were reduced by -44% with little effect of CL316,243. In T1DM streptozotocin (STZ) model, uptake of ^{18}F-FDG in Sprague-Dawley normal (SD Nor) increased by +624% with CL316,243, whereas SD STZ was reduced by -70%. An increased uptake of ^{18}F-FDG was seen SD STZ upon CL316,243 treatment, suggesting some recovery of BAT function.

cold temperatures, mobilization of fatty acids from WAT is also known to be a primary source for activation of BAT rather than the breakdown of fat depot stored in BAT [108,109]. Our histology studies showed that the number of lipid vacuoles in BAT was substantially decreased after stimulation by CL316,243, while there was no significant change in WAT lipid content between the two conditions [29]. Therefore, in acute administration of CL316,243, glucose metabolism and lipolysis of stored lipids in BAT are primary sources for activation of the tissue rather than the mobilization of fatty acids from WAT. Although its *in vitro* binding to the human β3AR is similar to that of the rodent receptor, it is only a partial (60%) agonist at the human β3AR —in contrast to the rodent receptor, where CL 316,243 is a full agonist— and its bioavailability is poor, with ~10% of an oral dose being absorbed [48].

β3 adrenoceptor agonist mediated BAT activation using ^{18}F-FDG PET/CT has been investigated in Zucker lean (ZL) and obese (ZF) rats. Brain ^{18}F-FDG PET studies in the ZF model have been reported to study the central effects of leptin-receptor deficiency [110,111]. CL316,243 activated BAT in ZL up by 4-fold and in ZF up by two-fold compared to saline [112]. The decreased activation was consistent with lower β3 adrenoceptor levels in ZF rats [113]. Despite the lower β3 adrenoceptor levels and reduced G-protein coupling in the ZF rat model, the agonist CL316,243 showed some measureable effects on BAT. The CT scans showed a significantly low opacity in ZF compared to ZL, suggesting low abundance of brown adipocytes in the IBAT region. There is renewed focus on the development of therapeutics to restore leptin receptor function in order to address human obesity [114]. Thus, the leptin-receptor deficient *fa/fa* rat model demonstrates that the residual β3AR conserved in this

rat model are functional with respect to enhancing metabolic activity. In addition, the coupling of the β3AR with the G-protein is reportedly reduced in white adipocytes [115]. Abnormalities in central metabolism regulation and neuroendocrine metabolism may also contribute to BAT thermogenesis impairment [113]. Chronic β3AR drug treatment studies of this rat model may be of value to study restoration of brown adipocytes.

In an early study done on type 1 diabetes mellitus (TIDM) streptozotocin-treated rat model, results show that the metabolic capacity of IBAT in streptozotocin-diabetic rats is decreased [116]. Our recent findings confirmed the loss of metabolic activity in streptozotocin-diabetic rats [117]. Comparing the two diabetic models, it appears that the reduction in IBAT activity in the Zucker fat rat may be driven by impaired β3AR signaling, whereas for the reduction in the streptozotocin-treated rats, the impairment may be driven by mitochondrial dysfunction. Our results also suggest that IBAT is activated by stimulation of β3AR in this T1DM rat model and is able to enhance metabolic activity. However, attempts to alter norepinephrine levels using atomoxetine had little effect, possibly due to impaired norepinephrine turnover. Blockage of the insulin receptors in BAT transplant streptozotocin-treated mice lead to impaired glucose tolerance, similar to what is seen in nondiabetic animals, indicating that insulin receptor activity plays a role in reversing diabetes [118].

Since mirabegron is a selective β3AR agonist in clinical use for OAB, studies in diabetes rodent models as described above may be worthwhile. Compared to CL316,243, mirabegron has better agonist potency for human β3AR [29, 51]. Amibegron is another selective β3AR agonist that crosses the BBB and has anti-depressant like properties such as its

Table 5. Therapeutic Potential of BAT Activators.

Drug Class	Observed Physiological Effects	Effect on Caloric Consumption; Weight Loss or Gain	Current Therapeutic Status
Class 1 Selective β3 Adreno-ceptor Agonists	Increase in BAT activation in animal and human studies.	Burns calories by consuming glucose. Weight loss in animals—no human data. Significant loss of β3AR activation in obese models. Chronic treatment studies needed to demonstrate regeneration of BAT.	*Mirabegron* used clinically in OAB. Use in IBS of related drugs being pursued. Use for weight loss unclear.
Class 2 Norepinephrine Elevators	Lower blood glucose; Increase BAT activation and thermogenesis.	Weight reduction shown in obese women.	*Atomoxetine* used clinically for ADHD. Potential for small weight loss.
Class 3 PPAR-γ Activators	Increased energy expenditure and improved cold tolerance.	Does not affect caloric intake. Pioglitazone shown to aid in weight loss.	*Pioglitazone* used clinically for T2DM. Other analogs have side effects and not used.
Class 4 Natural and Other Products	Increased energy expenditure and UCP1 gene expression.	Increases fat metabolism and reduces weight gain. ShK-186 reduced weight gain in DIO mice.	No clinically approved product for obesity or diabetes. *ShK-186* undergoing trials for MS.

ability to increase serotonin synthesis [61]. Thus, further studies are warranted on the various disease models using the newer, human translatable β3AR drugs.

4.2. Class 2 Drugs

Atomoxetine is a selective norepinephrine reuptake inhibitor and has low abuse potential [70]. Atomoxetine, structurally related to the antidepressant fluoxetine acts by elevating synaptic norepinephrine levels with few side effects [119, 120]. Cardiovascular side effects in adult placebo-controlled trials showed increased heart rate (3.0%) and increased blood pressure [121, 122]. It has been used in psychiatry for the treatment of both adult and pediatric ADHD, with relatively benign side effects [123, 124]. Under fasting conditions, atomoxetine initiated extensive ^{18}F-FDG increase in BAT compared to control rats [72].

BAT in patients with pheochromocytoma (excess release of epinephrine and norepinephrine from adrenal gland) has been reported to exhibit very intense ^{18}F-FDG uptake [125, 126]. Due to the adrenergic interaction with β1 and β2 adrenoceptors serious cardiovascular side effects were noted in in these patients [127]. Thus, any potential adrenergic agonist for BAT activation should be highly specific for β3AR.

Sibutramine is a combined norepinephrine and serotonin reuptake inhibitor. It is used as an anti-obesity agent to reduce appetite and promote weight loss in combination with diet and exercise. It improves insulin sensitivity and glucose metabolism; however it is believed that most of these effects result from weight loss rather than from an intrinsic effect of the drug [128]. Milnacipran is another serotonin-norepinephrine reuptake inhibitor anti-depressant. It has been used in co-morbid depression which is common in patients with diabetes mellitus. It improves blood glucose and HbA1c levels in type 2 diabetics. It is suggested that the effective treatment of depression results in higher sense of self-care which leads to improvement in the metabolic parameters [129], and BAT activation is protective against hyperglycemia [130].

4.3. Class 3 Drugs

Of the many thiazolididiones investigated as agents affecting adipogenesis [131, 132] serious side effects have hampered studies in humans in order to investigate BAT activation [13, 80]. Pioglitazone is currently the one PPARγ activator used for type 2 diabetes [133]. A recent study includes pioglitazone in a India-specific algorithm for management of type 2 diabetes [134]. The role of BAT in the glucose lowering effect of pioglitazone remains to be demonstrated, since UCP1 in human epicardial adipose tissue remained unaltered after pioglitazone treatment [135]. Thus, thermogenic effect of thiazolidinones *via* PPARγ remains to be demonstrated [136]. Measurements of the effect of pioglitazone on animal or human BAT using ^{18}F-FDG imaging methodology would be useful to confirm increased metabolic activity.

4.4. Class 4 Drugs

Nicotine has been shown to activate BAT [137]. However, the effect on weight loss/gain associated with smoking has been attributed to the effect of nicotine in brain regions such as the hypothalamus [138]. Forskolin directly activates adenylyl cyclase and raises cAMP levels in a wide variety of cell types [139]. Forskolin increased BAT ^{18}F-FDG SUV 1.6-fold compared to control mice [15]. On the other hand, forskolin increases heart myocardium ^{18}F-FDG, with side effects including headaches, decreased blood pressure, and a rapid heart rate. It has inotropic and vasodilatory properties both *in vitro* and *in vivo*, and changes in contractility parallel an increase in cAMP concentration as well as calcium transport into the myocardium [140]. Evidence for a role of forskolin in weight loss in humans is limited [141]. Caffeine appears to have some small effects on increasing fat metabolism which is enhanced when used in combination with ephedra [141]. Anti-obesity effects of capsaicin may occur through activation of brown and beige adipocytes [142, 143]. Curcumin has been shown to promote browning of white adipose tissue [144]. A bioavailable form of curcumin was

recently shown to increase weight loss in overweight people with metabolic syndrome [145]. Interesting findings on the role of the cannabinoid receptor system in weight loss have been reported [146, 147]. Although rimonabant has CNS side effects, other agents targeting the peripheral receptor may have promise. ShK-186, a selective Kv1.3 peptide inhibitor, is undergoing clinical trials as a therapeutic for autoimmune diseases [148]. It exhibited robust therapeutic effects in a mouse model of diet-induced obesity and insulin sensitivity [103]. Fibroblast growth factor 21 (FGF21) has been the focus of recent studies for obesity and may have the ability, at least in part to activate BAT [149]. Recent reviews have focused on therapeutic potential of engineered FGF21 analogs [150].

4.5. BAT Transplantation

Transplantation of BAT in obese subjects will be advantageous over pharmacological drug effects due to the significantly lower levels of BAT in the obese subjects. Several reports have been published and recent reviews have summarized their findings. Efforts have focused on BAT transplantation as a potential therapeutic tool for obesity by improving control over body composition and metabolism and were recently reviewed [151]. In order to overcome issues related to transplanting harvested BAT, tissue-engineering pathways, including stem cells to develop adipose tissue implants is currently underway in order to provide BAT for human therapeutic purposes [152, 153]. These pathways offer alternatives to pharmacological approaches or may be used in conjunction with pharmacological approaches in order to tackle obesity and diabetes.

5. SUMMARY

Currently, the prevalence of BAT in the adult population is reportedly low [154-157], which dampens its potential significance for altering adult human metabolism. BAT is only active when its thermogenic function is required or pharmacologically stimulated [24, 27], and ^{18}F-FDG uptake is a direct consequence of tissue activity [9]. Thus, inactive BAT would not be visible on PET scans. Due to the potential role of BAT in obesity [158, 159] efforts towards pharmacological activation have increased [160, 161]. Pharmacologically induced brown adipocyte biogenesis along with engineered tissue transplantation is now possible thus raising the possibility for drug development in combating diabetes and obesity.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is available on the publishers Web site along with the published article.

REFERENCES

[1] Jacene HA, Cohade CC, Zhang Z, Wahl RL. The relationship between patients' serum glucose levels and metabolically active brown adipose tissue detected by PET/CT. Mol Imag Biol 2011; 13: 1278-83.

[2] Enerbäck S: Brown adipose tissue in humans. Int J Obes 2010; 34: 43-46.

[3] Nedergaard J, Bengtsson T, Cannon B. New powers of brown fat: fighting the metabolic syndrome. Cell Metab 2011; 13: 238-40.

[4] Nicholls DG, Locke RM: Thermogenic mechanisms in brown fat. Physol Rev 1984; 64: 1–64.

[5] Watanabe M, Yamamoto T, Mori C, *et al.* Cold-induced changes in gene expression in brown adipose tissue: implications for the activation of thermogenesis. Biol Pharm Bull 2008; 31: 775-84.

[6] Valverde AM, Benito M: The brown adipose cell: a unique model for understanding the molecular mechanism of insulin resistance. Mini Rev Med Chem 2005; 5: 269-78.

[7] Kato H, Ohue M, Kato K, *et al.* Mechanism of amelioration of insulin resistance by beta3-adrenoceptor agonist AJ-9677 in the KK-Ay/Ta diabetic obese mouse model. Diabetes 2001; 50: 113-22.

[8] De Matteis R, Ricquier D, Cinti S. 1998. TH-, NPY-, SP-, and CGRP-immunoreactive nerves in interscapular brown adipose tissue of adult rats acclimated at different temperatures: an immunohistochemical study. J Neurocytol 1998; 27: 877-86.

[9] Inokuma K, Ogura-Okamatsu Y, Toda C, Kimura K, Yamashita H, Saito M: Uncoupling protein 1 is necessary for norepinephrine-induced glucose utilization in brown adipose tissue. Diabetes 2005; 54: 1385-91.

[10] Poher A-L, Altirriba J, Veyrat-Durebex C, Rohner-Jeanrenaud F. Brown adipose tissue activity as a target for the treatment of obesity/insulin resistance. Front Physiol 2015; 6: 4. doi: 10.3389/fphys.2015.00004

[11] Cypress AM, Haft CR, Laughlin MR, Hu HH. Brown fat in humans: consensus points and experimental guidelines. Cell Metab 2014; 20: 408-15.

[12] Halpern B, Mancini MC, Halpern A. Brown adipose tissue: what have we learnt since its recent idenitification in human adults. Arq Bras Endocrinol Metabol 2014; 58: 889-99.

[13] Lee P, Greenfiled JR. Non-pharmacological and pharmacological strategies of brown adipose tissue recruitment in humans. Mol Cell Endocrinol 2015; 418: 184-90.

[14] Peng X-R, Gennemark P, O'Mahoney G, Bartesaghi S. Unlock the thermogenic potential of adipose tissue: Pharmacological modulation and implications for treatment of diabetes and obesity. Front. Endocrinol (Lausanne) 2015; 6: 174. doi: 10.3389/fendo.2015.00174.

[15] Mirbolooki MR, Upadhyay SK, Constantinescu C, Pan ML, Mukherjee J. Adrenergic pathway activation enhances brown adipose tissue metabolism:A ^{18}F-FDG PET/CT study in mice. Nucl Med Biol 2014; 41: 10-16.

[16] Hany TF, Gharehpapagh E, Kamel EM, Buck A, Himms-Hagen J, von Schulthess GK. Brown adipose tissue: a factor to consider in symmetrical tracer uptake in the neck and upper chest region. Eur J Nucl Med Mol Imag 2002; 29: 1393-98.

[17] Cohade C, Osman M, Pannu HK, Wahl RL. Uptake in supraclavicular area fat ("USA-Fat"): description on 18F-FDG PET/CT. J Nucl Med 2003; 44: 170-76.

[18] Truong MT, Erasmus JJ, Munden RF, *et al.* Focal FDG uptake in mediastinal brown fat mimicking malignancy: A potential pitfall resolved on PET/CT. Amer J Radiol 2004; 183: 1127-32.

[19] Okuyama C, Sakane N, Yoshida T, *et al.* 123I- or 125I-Metaiodobenzylguanidine visualization of brown adipose tissue. J Nucl Med 2002; 43: 1234-40.

[20] Lin SF, Fan X, Yeckel CW, *et al.* Ex Vivo and *In Vivo* Evaluation of the Norepinephrine Transporter Ligand [(11)C]MRB for Brown Adipose Tissue Imaging. Nucl Med Biol 2012; 39: 1081-86.

[21] Pan ML, Mukherjee MT, Patel HH, *et al.* Evaluation of [^{11}C]TAZA for amyloid Aβ plaque imaging in postmortem Alzheimer's disease

brain region and whole body distribution in rodent PET/CT. Synapse 2016; 70: 163-76.

[22]   Nemanich S, Rani S, Shoghi K. *In vivo* multi-tissue efficacy of peroxisome proliferator-activated receptor-g therapy on glucose and fatty acid metabolism in obese type 2 diabetic rats. Obesity 2013; 21: 2522-29.

[23]   Labbe SM, Caron A, Bakan I, *et al. In vivo* measurement of energy substrate contribution to cold-induced brown adipose tissue thermogenesis. The FASEB J 2015; 29: 2046-58.

[24]   Cannon B, Nedergaard J. Brown adipose tissue: function and physiological significance. Physiol Rev 2004; 84: 277-359.

[25]   Bartelt A, Heeren J. Adipose tissue browning and metabolic health. Nat Rev Endocrinol 2014; 10: 24-36.

[26]   Saito M,Okamatsu-Ogura Y, Matsushita M, *et al.* High incidence of metabolically active brown adipose tissue in healthy adult humans: effects of cold exposure and adiposity. Diabetes 2009; 58: 1526-31.

[27]   Baba S, Tatsumi M, Ishimori T, Lilien DL, Engles JM, Wahl RL. Effect of nicotine and ephedrine on the accumulation of 18F-FDG in brown adipose tissue. J Nucl Med 2007; 48: 981-86.

[28]   Baba S, Jacene HA, Engles JM, Honda H, Wahl RL. CT Hounsfield units of brown adipose tissue increase with activation: preclinical and clinical studies. J Nucl Med 2010; 51: 246-50.

[29]   Mirbolooki MR, Constantinescu CC, Pan ML, Mukherjee J. Quantitative assessment of brown adipose tissue metabolic activity and volume using ^{18}F-FDG PET/CT and β3-adrenergic receptor activation. EJNMMI Res 2011; 1: 30.

[30]   Tatsumi M, Engles JM, Ishimori T, Nicely O, Cohade C, Wahl RL. Intense (18)F-FDG uptake in brown fat can be reduced pharmacologically. J Nucl Med 2004; 45: 1189-93.

[31]   van Marken Lichtenbelt WD, Vanhommerig JW, Smulders NM, *et al.* Cold-activated brown adipose tissue in healthy men. N Engl J Med 2009; 360: 1500-08.

[32]   Pfannenberg C, Werner MK, Ripkens S, *et al.* Impact of age on the relationships of brown adipose tissue with sex and adiposity in humans. Diabetes 2010; 59: 1789-93.

[33]   Himms-Hagen J, Cui J, Danforth E Jr, *et al.* Effect of CL-316,243, a thermogenic beta 3-agonist, on energy balance and brown and white adipose tissues in rats. Am J Physiol 1994; 266: R1371-82.

[34]   Yoshida T, Sakane N, Wakabayashi Y, Umekawa T, Kondo M: Anti-obesity effect of CL316,243, a highly specific ß 3-adrenergic receptor agonist, in yellow KK mice. Life Sci 1994; 54: 491–98.

[35]   Wu C, Cheng W, Xing H, Dang Y, Li F, Zhu Z: Brown adipose tissue can be activated or inhibited within an hour before 18F-FDG injection: a preliminary study with microPET. J Biomed Biotechnol 2011; 2011: 159834. doi: 10.1155/2011/159834

[36]   Whittle A, Relat-Pardo J, Vidal-Puig A. Pharmacological strategies for targeting BAT thermogenesis. Trends Pharm Sci 2013; 34: 347-55.

[37]   Michel MC, Korstanje C. β3-Adrenoceptor agonists for overactive badder syndrome: Role of translational pharmacology in a repositioning clinical drug development project. Pharm. Therapeutics 2016; dx.doi.org/10.1016/j.pharmthera.2016.01.007

[38]   Arch JR, Ainsworth AT, Cawthorne MA, *et al.* Atypical ß-adrenoceptor on brown adipocytes as target for anti-obesity drugs. Nature 1984; 309: 163–65.

[39]   Arch JR. β3-Adrenoceptor agonists: potential, pitfalls and progress. Eur J Pharmacol 2002; 440: 99-107.

[40]   Ursino MG, Vasina V, Raschi E, Crema F, DePonti F. The β3-adrenoceptor as a therapeutic target: Current perspectives. Pharm Res 2009; 59: 221-34.

[41]   Michel MC, Ochodnicky P, Summers RJ. Tissue functions mediated by b3-adrenoceptors- findings and challenges. Naunyn Sch Arch Pharmacol 2010; 382: 103-8.

[42]   Sen A, Nichani N. Exploring beta3 adrenoceptors for potential clinical applications. Int J Pharm Sci Rev Res 2010; 5: 55-8.

[43]   Wilson C, Wilson S, Piercy V, Sennitt MV, Arch JR. The rat lipolytic beta-adrenoceptor: studies using novel beta-adrenoceptor agonists. Eur J Pharm 1984; 100: 309-19.

[44]   Liu YL, Stock MJ. Acute effects of the beta 3-adrenoceptor agonist, BRL 35135, on tissue glucose utilisation. Br J Pharm 1995; 114: 888-94.

[45]   Cawthorne MA, Sennitt MV, Arch JR, Smith SA. BRL 35135, a potent and selective atypical beta-adrenoceptor agonist. Am J Clin Nutr. 1992; 55(1 Suppl): 252S-7S.

[46]   Bloom JD, Dutia MD, Johnson BD, *et al.* Disodium(R,R)-5-[2-[[2-(3-chlorophenyl)-2-hydroxyethyl]-amino]propyl]-1,3-benzodioxole-2,2-dicarboxylate (CL 316,243). A potent beta-adrenergic agonist virtually specific for beta 3 receptors. A promising antidiabetic and antiobesity agent. J Med Chem 1992; 35: 3081-84.

[47]   Lowell BB, Flier JS: Brown adipose tissue, beta 3-adrenergic receptors, and obesity. Annu Rev Med 1997; 48: 307-16.

[48]   Weyer C, Tataranni PA, Snitker S, Danforth E, Jr., Ravussin E. Increase in insulin action and fat oxidation after treatment with CL 316,243, a highly selective beta3-adrenoceptor agonist in humans. Diabetes 1998; 7: 1555-1561.

[49]   Redman LM, de Jonge I, Fang X, *et al.* Lack of an effect of novel beta3-adrenoceptor agonist, TAK-677, on energy metabolism in obese individuals: a double-blind, placebo-controlled randomized study. J Clin Endocrinol Metab 2007; 92: 527-531.

[50]   Takasu T, Ukai M, Sato S, *et al.* Effect of (R)-2-(2-aminothiazol-4-yl)-4'-{2-[((2-hydroxy-2-phenylethyl)-amino]ethyl}acetanilide (YM178), a novel selective b3-adrenoceptor agonist, on bladder function. J Pharm Exp Ther 2007; 321: 642-647.

[51]   Australian public assessment report for Mirabegron, Australian Government, Department of Health. AusPAR 2014;PM-2012-01928-3-3.

[52]   Mirbolooki MR, Schade KN, Constantinescu CC, Pan M-L, Mukherjee J. Enhancement of ^{18}F-fluorodeoxyglucose metabolism in rat brain frontal cortex using a β3 adrenoceptor agonist. Synapse 2015; 69: 96-8.

[53]   Cypess AM, Weiner LS, Roberts-Toler C, *et al.* Activation of human brown adipose tissue by a β3-adrenergic receptor agonist. Cell Metab 2015; 21: 33-8.

[54]   Savontaus E, Pesonen U, Rouru J, Huupponen R, Koulu M. Effects of ZD7114, a selective beta3-adrenoceptor agonist, on neuroendocrine mechanisms controlling energy balance. Eur J Pharm. 1998; 347: 265-74.

[55]   Growcott JW, Holloway B, Green M, Wilson C. Zeneca ZD7114 acts as an antagonist at b3-adrenoreceptors in rat isolated ileum. Br J Pharm 1993; 110: 1375-80.

[56]   Buemann B, Toubro S, Astrup A. Effects of the two beta3-agonists, ZD7114 and ZD2079 on 24 hour energy expenditure and respiratory quotient in obese subjects. Int J Obes Relat Metab Disord 2000; 24: 1553-60.

[57]   Holloway BR, Howe R, Rao BS, *et al.* ICI D7114 a novel selective beta-adrenoceptor agonist selectively stimulates brown fat and increases whole-body oxygen consumption. Br J Pharm 1991; 104: 97-104.

[58]   Champigny O, Ricquier D, Blondel O, Mayers RM, Briscoe MG, Holloway BR. β3-Adrenergic receptor stimulation restores message and expression of brown-fat mitochondrial uncoupling protein in adult dogs. Proc Natl Acad Sci 1991; 88: 10774-7.

[59]   Larsen TM, Toubro S, van Baak MA, *et al.* Effect of a 28-day treatment with L-796568, a novel beta3-adrenergic receptor agonist, on energy expenditure and body composition of obese men. Am J Clin Nutr 2002; 76: 780-8.

[60]   Bianchetti A, Manara L. *In vitro* inhibition of intestinal motility by phenylethanolaminotetralines: evidence of atypical beta-adrenoceptors in rat colon. Br J Pharm 1990; 100: 831–9.

[61]   Stemmelin J, Cohen C, Yalcin I, Keane P, Griebel G. Implication of β3-adrenoceptors in the antidepressant-like effects of amibegron using Adrb3 knockout mice in the chronic mild stress. Behav Brain Res 2009; 206: 310–2.

[62]   Coman O, Paunescu H, Ghita I, Coman L, Badararu A, Fulga I. Beta 3 adrenergic receptors: molecular, histological, functional and pharmalogical approaches. RJME 2009; 50: 169–79.

[63]   Summers RJ, Papaioannou M, Harris S, Evans BA. Expression of β3-adrenoceptor mRNA in rat brain. Br J Pharm 1995; 116: 2547-48.

[64]   Chernogubova E, Cannon B, Bengtsson T. Norepinephrine increases glucose transport in brown adipocytes *via* beta3-adrenoceptors through a cAMP, PKA, and PI3-kinase-dependent pathway stimulating conventional and novel PKCs. Endocrinology 2004; 145: 269-80.

[65]   Vijgen GH, Bouvy ND, Teule GJ, Brans B, Schrauwen P, van MarkenLichtenbelt WD. Brown adipose tissue in morbidly obese subjects. PLoS One 2011, 6:e17247.

[66] Shimizu Y, Nikami H, Saito M. Sympathetic activation of glucose utilization in brown adipose tissue in rats. J Biochem 1991; 110: 688-92.

[67] Matthias A, Kerstin BE, Ohlson J, *et al.* Thermogenic Responses in Brown Fat Cells Are Fully UCP1-dependent UCP2 or UCP3 cannot substitute for UCP1 in adrenergically or fatty acid-induced thermogenesis. J Biol Chem 2000; 275: 25073-081.

[68] Carey AL, Formosa MF, Van Every B, *et al.* Ephedrine activates brown adipose tissue in lean but not obese humans. Diabetologia 2012; 56: 147-55.

[69] Carey AL, Pajtak R, Formosa MF, *et al.* Chronic ephedrine administration decreases brown adipose tissue activity in a randomised controlled human trial: implications for obesity. Diabetologia 2015; 58: 1045-54.

[70] Garnock-Jones KP, Keating GM. Atomoxetine: a review of its use in attention-deficit hyperactivity disorder in children and adolescents. Paediatr Drugs 2009; 11: 203-26.

[71] Bymaster FP, Katner JS, Nelson DL, *et al.* Atomoxetine increases extracellular levels of norepinephrine and dopamine in prefrontal cortex of rat: a potential mechanism for efficacy in attention deficit/hyperactivity disorder. Neuropsychopharmacology 2002; 27: 699-711.

[72] Mirbolooki MR, Constantinescu C, Pan ML, Mukherjee J. Targeting presynaptic norepinephrine transporter in brown adipose tissue: A novel imaging approach and potential treatment for diabetes and obesity. Synapse 2013; 67: 79-93.

[73] Gadde KM, Yonish GM, Wagner HR, 2nd, Foust MS, Allison DB. Atomoxetine for weight reduction in obese women: a preliminary randomised controlled trial. Int J Obes 2006; 30: 1138-42.

[74] McElroy SL, Guerdjikova A, Kotwal R, *et al.* Atomoxetine in the treatment of binge-eating disorder: a randomized placebo-controlled trial. J Clin Psych 2007; 68: 390-98.

[75] Ball MP, Warren KR, Feldman S, McMahon RP, Kelly DL, Buchanan RW. Placebo-controlled trial of atomoxetine for weight reduction in people with schizophrenia treated with clozapine or olanzapine. Clin Schizophr Relat Psychoses 2011; 5: 17-25.

[76] King VL, Dwoskin LP, Cassis LA. Cold exposure regulates the norepinephrine uptake transporter in rat brown adipose tissue. Am J Physiol 1999; 276: R143-51.

[77] King VL, English VL, Bharadwaj K, Cassis LA. Angiotensin II Stimulates Sympathetic Neurotransmission to Adipose Tissue. Physiol Rep 2013; 1:e00014.

[78] James WP, Caterson ID, Coutinho W, *et al.* Effect of sibutramine on cardiovascular outcomes in overweight and obese subjects. N. Engl. J. Med. 2010; 363: 905-917.

[79] Arnold LM, Palmer RH, Hufford MR, Chen W. Effect of milnacipram on body weight in patients with fibromyalgia. Int J Gen Med 2012; 5: 879-887.

[80] Salomone S. Pleiotropic effects of glitazones: double edge sword? Front Pharm 2011; 2: 1-6.

[81] Digby JE, Mantague CT, Sewter CP, *et al.* Thiazolidinedione exposure increases the expression of uncoupling protein 1 in cultured human preadipocytes. Diabetes 1998; 47: 138-41.

[82] Breider MA, Gough AW, Haskins JR, Sobocinski G, De La Iglesia FA. Troglitazone-Induced Heart and Adipose Tissue Cell Proliferation in Mice. Toxicol Pathol 1999; 27 :545-52.

[83] Chang AY, Wyse BM, Gilchrist BJ, Peterson T, Diani AR. Ciglitazone, a new hypoglycemic agent. I. Studies in ob/ob and db/db mice, diabetic Chinese hamsters, and normal and streptozotocin-diabetic rats. Diabetes 1983; 32: 830-38.

[84] Yamamoto Y, Nakajima M, Yamazaki H, Yokoi T. Cytotoxicity and apoptosis produced by troglitazone in human hepatoma cells. Life Sci 2000; 70: 471-82.

[85] Henriksen K, Byrjalsen I, Nielsen RH, *et al.* A comparison of glycemic control, water retention, and musculoskeletal effects of balaglitazone and pioglitazone in diet-induced obese rats. Eur J Pharm. 2009; 616: 340-45.

[86] Henriksen K, Byrjalsen I, Qvist P, *et al.* BALLET Trial Investigators. Efficacy and safety of the PPARγ partial agonist balaglitazone compared with pioglitazone and placebo: a phase III, randomized, parallel-group study in patients with type 2 diabetes on stable insulin therapy. Diabetes Metab Res Rev 2011; 27: 392-401.

[87] Kanda S, Nakashima R, Takahashi K *et al.* Potent antidiabetic effects of rivoglitazone, a novel peroxisome proliferator-activated

[88] receptor-gamma agonist, in obese diabetic rodent models. J Pharm Sci 2009; 111: 155-66.

[88] Koffarnus RL, Wargo KA, Phillippe HM. Rivoglitazone: A New Thiazolidinedione for the Treatment of Type 2 Diabetes Mellitus. Ann Pharmacother 2013; 47: 877-85.

[89] Aleo MD, Lundeen GR, Blackwell DK, *et al.* Mechanism and implications of brown adipose tissue proliferation in rats and monkeys treated with the thiazolidinone darglitazone, a potent peroxisome-activated receptor-gamma agonist. J Pharm Exp Ther 2003; 305: 1173-82.

[90] Mano-Otagiri A, Iwasaki-Sekino A, Ohata H, Arai K, Shibasaki T. Nicotine suppresses energy storage through activation of sympathetic outflow to brown adipose tissue *via* corticotropin-releasing factor type 1 receptor. Neurosci Lett 2009; 455: 26-29.

[91] Yoshida T, Yoshioka K, Hiraoka N, Kondo M. Effect of nicotine on norepinephrine turnover and thermogenesis in brown adipose tissue and metabolic rate in MSG obese mice. J Nutr Sci Vitaminol (Tokyo) 1990; 36: 123-30.

[92] Scarpace PJ, Matheny M. Thermogenesis in brown adipose tissue with age: post-receptor activation by forskolin. Eur J Physiol 1996; 431: 388-94.

[93] De Jesus LA, Carvalho SD, Ribeiro MO, *et al.* The type 2 iodothyronine deiodinase is essential for adaptive thermogenesis in brown adipose tissue. J Clin Invest 2001; 108: 1379-85.

[94] Yoshida T, Yoshioka K, Kamanaru K, Hiraoka N, Kondo M. Caffeine activates brown adipose tissue thermogenesis and metabolic rate in mice. J Nutr Sci Vitaminol (Tokyo) 1990; 36: 173-8.

[95] Gnad T, Scheiber S, Kugelgen IV, *et al.* Adenosine activates brown adipose tissue and recruits beige adipocytes *via* A2A receptors. Nature 2014; 516: 395-99.

[96] Yoneshiro T, Aita S, Kawai Y, Iwanaga T, Saito M. Nonpungent capsaicin analogs (capsinoids) increase energy expenditure thorugh the activation of brown adipose tissue in humans. Am J Clin Nutri 2012; 95: 845-50.

[97] Leung FW. Capsaicin as an anti-obesity drug. Prog Drug Res. 2014; 68:171-9.

[98] Aggarwal, BB. Targeting Inflammation-Induced Obesity and Metabolic Diseases by Curcumin and Other Nutraceuticals, Annu Rev Nutr 2010; 30: 173-99.

[99] Wang S, Wang X, Zichen Y, *et al.* Curcumin promotes browning of white adipose tissue in a norepinephrine dependent way. Biochem Biophys Res Commun 2015; 466: 247-53.

[100] Verty ANA, Allen AM, Oldfield BJ. The effects of rimonabant on brown adipose tissue in rat: implications for energy expenditure. Obesity 2009; 17: 254-61.

[101] Boon MR, Kooijman S, van Dam AD, *et al.* Perpheral cannabinoid receptor blockade activates brown adipose tissue and diminishes dyslipidemia and obesity. FASEB J 2014; 28: 5361-95.

[102] Moreira FA, Crippa JA. The psychiatric side-effects of rimonabant. Rev Bras Psiquiatr 2009; 31: 145-53.

[103] Upadhyay SK, Eckel-Mahan KL, Mirbolooki MR, *et al.* Selective Kv1.3 channel blocker as therapeutic for obesity and insulin resistance. Proc Natl Acad Sci USA 2013; 110: E2239-48.

[104] Wilding JP. PPAR agonists for the treatment of cardiovascular disease in patients with diabetes. Diabetes Obes Metab 2012; 14: 973-982.

[105] Michel MC, Harding SE, Bond RA. Are there functional beta(3)-adrenoceptors in the human heart? Br J Pharmacol 2011; 162: 817-22.

[106] De Souza CJ, Hirshman MF, Horton ES: CL-316,243, a beta3-specific adrenoceptor agonist, enhances insulin-stimulated glucose disposal in non-obese rats. Diabetes 1997; 46: 1257-63.

[107] Park JW, Jung K-H, Lee JH, *et al.* ^{18}F-FDG PET/CT monitoring of b3 agonist-stimulated brown adipocyte recruitment in white adipose tissue. J. Nucl. Med 2015; 56: 153-8.

[108] Baba S, Engles JM, Huso DL, Ishimori T, Wahl RL. Comparison of uptake of multiple clinical radiotracers into brown adipose tissue under cold-stimulated and nonstimulated conditions. J Nucl Med 2007; 48: 1715-23.

[109] Deiuliis JA, Liu LF, Belury MA, Rim JS, Shin S, Lee K: Beta(3)-adrenergic signaling acutely down regulates adipose triglyceride lipase in brown adipocytes. Lipids 2010; 45: 479-89.

[110] Virtanen KA, Haaparanta M, Gronroos T, *et al.* 2-[18F]fluoro-2-deoxy-D-glucose combined with microdialysis can be used for the

comparison of tissue glucose metabolism in obese and lean rats. Diabetes, Obesity Metab 2002; 4: 60-8.

[111]　Liistro T, Guiducci L, Burchielli S, *et al.* Brain glucose overexposure and lack of acute metabolic flexibility in obesity and type 2 diabetes: a PET-[18F]FDG study in Zucker and ZDF rats. J Cereb Blood Flow Metab. 2010; 30: 895-9.

[112]　Schade KN, Baranwal A, Liang C, Mirbolooki MR, Mukherjee J. Preliminary evaluation of 3-adrenoceptor agonist-induced ^{18}F-FDG metabolic activity in brown adipose tissue of obese Zucker rat. Nucl Med Biol 2015; 42: 691-94.

[113]　Levin BE, Finnegan MB, Marquet E, Sullivan AC. Defective brown adipose oxygen consumption in obese Zucker rats. Am J Physiol 1984; 47: E94-E100.

[114]　Roujeau C, Jockers R, Dam J. New Pharmacological Perspectives for the Leptin Receptor in the Treatment of Obesity. Front Endocrinol (Lausanne) 2014; 5: 167. doi: 10.3389/fendo.2014.00167

[115]　Mory G, Wiel M, Adli H, Diot-Dupuy F, Ferré P, Bazin R, Ferré P, Impaired beta-adrenergic signaling pathway in white adipocytes of suckling fa/fa Zucker rats: a defect in receptor coupling. Int J Obes Relat Metab Disord 2001; 25: 1592-8.

[116]　Seydoux J, Chinet A, Schneider-Picard G, *et al.* Brown adipose tissue metabolism in streptozotocin-diabetic rats. J Endocrin 1983; 113: 604-10.

[117]　Baranwal A, Mirbolooki MR, Mukherjee J. Initial assessment of brown adipose tissue activity in streptozotocin-induced type 1 diabetes rodent model using 18F-FDG PET/CT. Mol Imag 2015; 14: 22-33.

[118]　Gunawardana SC, Piston DW. Reversal of type 1 diabetes in mice by brown adipose tissue transplant. Diabetes 2012; 61: 674–82.

[119]　Scherer D, Hassel D, Bloehs R, *et al.* Selective noradrenaline reuptake inhibitor atomoxetine directly blocks hERG currents. Br J Pharm 2009; 156: 226-36.

[120]　Ledbetter M. Atomoxetine: a novel treatment for child and adult ADHD. Neuropsychiatr Dis Treat 2006; 2: 455-66.

[121]　Habel LA, Cooper WO, Sox CM, *et al.* ADHD medications and risk of serious cardiovascular events in young and middle-aged adults. JAMA 2011; 306: 2673-83.

[122]　Adler LA, Spencer TJ, Milton DR, Moore RJ, Michelson D. Long-term, open-label study of the safety and efficacy of atomoxetine in adults with attention-deficit/hyperactivity disorder: an interim analysis. J Clin Psych 2005; 66: 294-99.

[123]　Michelson D, Adler L, Spencer T, *et al.* Atomoxetine in adults with ADHD: two randomized, placebo-controlled studies. Biol Psychiatry 2003; 53: 112-20.

[124]　Rosler M, Casas M, Konofal E, Buitelaar J. Attention deficit hyperactivity disorder in adults. World J Biol Psychiatry 2010; 11: 684-8.

[125]　Iyer RB, Guo CC, Perrier N. Adrenal pheochromocytoma with surrounding brown fat stimulation. AJR 2009; 192: 300-1.

[126]　Yamaga LY, Thom AF, Wagner J, Baroni RH, Hidal JT, Funari MG. The effect of catecholamines on the glucose uptake in brown adipose tissue demonstrated by (18)F-FDG PET/CT in a patient with adrenal pheochromocytoma. Eur J Nuc Med Mol 2008; 35: 446-7.

[127]　Zelinka T, Petrak O, Turkova H, *et al.* High incidence of cardiovascular complications in pheochromocytoma. Horm Metab Res 2012; 44: 379-84.

[128]　Scheen AJ. Cardiovascular risk-benefit profile of sibutramine. Am J Cardiovasc Drugs 2010; 10: 321-4.

[129]　Abrahamian H, Hofmann P, Prager R, Toplak H. Diabetes mellitus and co-morbid depression: treatment with milnacipran results in significant improvement of both diseases (results from the Austrian MDDM study group). Neuropsych Dis Treat 2009; 5: 261-6.

[130]　Bartelt A, Bruns OT, Reimer R, *et al.* Brown adipose tissue activity controls triglyceride clearance. Nat Med 2011; 17: 200-5.

[131]　Lowell BB. PPARg: an essential regulator of adipogenesis and modulator of fat cell function. Cell 1999; 99: 239-42.

[132]　De Souza CJ, Eckhardt M, Gagen K, *et al.* Effects of pioglitazone on adipose tissue remodeling within the setting of obesity and insulin resistance. Diabetes 2001; 50: 1863-71.

[133]　Schernthaner G, Currie CJ, Schernthaner GH. Do we still need pioglitazone for the treatment of type2 diabetes? A risk-benefit critique in 2013. Diabetes Care 2013; 36(suppl2): S155-61.

[134]　India Diabetes Management Algorithm Proposal Group. A proposed India-specific algorithm for management of type 2 diabetes. Diabetes Tech Ther 2016; 18: doi:10.1089/dia.2015.0308.

[135]　Sacks HS, Fain JN, Holman B, *et al.* Uncoupling protein-1 and related messenger ribonucleic acids in human epicardial and other adipose tissues: Epicardial fat functioning as brown fat. J Clin Endocrinol Metab 2009; 94: 3611-15.

[136]　Rosen ED, Spiegelman BM. What we talk about when we talk about fat. Cell 2014; 156: 20-44.

[137]　Wellman PJ, Marmon MM, Reich S, Ruddle J. Effects of nicotine on body weight, food intake and brown adipose tissue thermogenesis. Pharm Biochem Behav 1986; 24: 1605-9.

[138]　Martinez de Morentin PB, Whittle AJ, Ferno J, *et al.* Nicotine induces negative energy balance through hypothalamic AMP-activated protein kinase. Diabetes 2012; 61: 807-17.

[139]　Insel PA, OstromRS. Forskolin as a tool for examining adenylyl cyclase expression, regulation, and G protein signaling. Cell Mol Neurobiol 2003; 23: 305-14.

[140]　Storstein L. Non-receptor-mediated inotropic drugs. Eur Heart J 1988; 9 Suppl H: 91-3.

[141]　Jeukendrup AE, Randell R. Fat burners: nutrition supplements that increase fat metabolism. Obes Rev 2011; 12: 841-51.

[142]　Ohyama K, Nogusa Y, Shinoda K, Bannai M, Kajimura S. A synergistic anti-obesity effect by a combination of capsinoids and cold temperature through promoting beige adipocyte biogenesis. Diabetes 2016; pii: db150662.

[143]　Kida R, Yoshida H, Murakami M, *et al.* Direct action of capsaicin in brown adipogenesis and activation of brown adipocytes. Cell Biochem Funct 2016; 34: 34-41.

[144]　Wang S, Wang X, Ye Z, *et al.* Curcumin promotes browning of white adipose tissue in a norepinephrine-dependent way. Biochem Biophys Res Commun 2015; 466: 247-53.

[145]　DiPierro F, Bressan A, Ranaldi D, Rapacioli G, Giacomelli L, Bertuccioli A. Potential role of bioavailable curcumin in weight loss and omental adipose tissue decrease: preliminary data of a randomized, controlled trial in overweight people with metabolic syndrome. Preliminary study. Eur Rev Med Pharm Sci 2015; 19: 4195-202.

[146]　Addy C, Wright H, Van Laere K, *et al.* The acyclic CB1R inverse agonist taranabant mediates weight loss by increasing energy expenditure and decreasing caloric intake. Cell Metab 2008; 7: 68-78.

[147]　Gadde KM, Allison DB. Cannabinoid-1 Receptor Antagonist, Rimonabant, for Management of Obesity and Related Risks. Circulation 2006; 114: 974-84.

[148]　Chi Y, Pennington MW, Norton RS, *et al.* Development of a sea anemone toxin as an immunomodulatory for therapy of autoimmune disease. Toxicon 2012; 59: 529-46.

[149]　Straub L, Wolfrum. FGF21, energy expenditure and weight loss-how much brown fat do you need. Mol Med 2015; 4:605-9.

[150]　So WY, Leung PS. Fibroblast growth factor 21 as an emerging therapeutic target for Type2 diabetes Mellitus. Med Res Rev 2016; DOI: 10.1002/med.21390

[151]　Stanford KI, Middelbeek RJW, Townsend KL *et al.* Brown adipose tissue regulates glucose homeostasis and insulin sensitivity. J Clin Invest 2013; 123: 215-23.

[152]　Tharp KM, Stahl A. Bioengineering beige adipose tissue therapeutics. Front Endocrinol 2015; 6:164.

[153]　Roman S, Agil A, Peran M *et al.* Brown adipose tissue and novel therapeutic approaches to treat metabolic disorders. Transl Res 2015; 65: 464-79.

[154]　Nedergaard J, Bengtsson T, Cannon B. Unexpected evidence for active brown adipose tissue in adult humans. Am J Physiol 2007; 293: E444-52.

[155]　Au-Yong IT, Thorn N, Ganatra R, Perkins AC, Symonds ME. Brown adipose tissue and seasonal variation in humans. Diabetes 2009; 58: 2583-87.

[156]   Cypess AM, Lehman S, Williams G, *et al.* Identification and importance of brown adipose tissue in adult humans. N Engl J Med 2009; 360: 1509-17.

[157]   Stefan N, Pfannenberg C, Haring HU. The importance of brown adipose tissue. New Eng J Med 2009; 361: 416-17.

[158]   Carey A and Kingwell B. Brown Adipose Tissue in Humans: Therapeutic Potential to Combat Obesity. Pharmacol Ther 2013; 140: 26-33.

[159]   Cohen P, Spiegelman BM. Brown and Beige Fat: Molecular parts of a thermogenic machine. Diabetes 2015; 64: 2346-51.

[160]   Cemeka H, Sand C, Michel MC. The odd sibling: Features of β3-adrenoceptor pharmacology. Mol Pharm 2014; 86: 479-84.

[161]   Arch JR, Trayhurn P. Detection of thermogenesis in rodents in response to anti-obesity drugs and genetic modification. Front Physiol 2013; dx.doi.org/10.3389/fphys.2013.00064.