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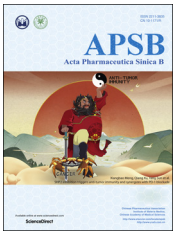

REVIEW

Small molecules for fat combustion: targeting obesity

Jingxin Liu, Yitao Wang, Ligen Lin*

State Key Laboratory of Quality Research in Chinese Medicine, Institute of Chinese Medical Sciences, University of Macau, Taipa 999078, Macau, China

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KEY WORDS

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Brown adipose tissue;
Beige cells;
Obesity;
Uncoupling protein 1

Abstract Obesity is increasing in an alarming rate worldwide, which causes higher risks of some diseases, such as type 2 diabetes, cardiovascular diseases, and cancer. Current therapeutic approaches, either pancreatic lipase inhibitors or appetite suppressors, are generally of limited effectiveness. Brown adipose tissue (BAT) and beige cells dissipate fatty acids as heat to maintain body temperature, termed non-shivering thermogenesis; the activity and mass of BAT and beige cells are negatively correlated with overweight and obesity. The existence of BAT and beige cells in human adults provides an effective weight reduction therapy, a process likely to be amenable to pharmacological intervention. Herein, we combed through the physiology of thermogenesis and the role of BAT and beige cells in combating with obesity. We summarized the thermogenic regulators identified in the past decades, targeting G protein-coupled receptors, transient receptor potential channels, nuclear receptors and miscellaneous pathways. Advances in clinical trials were also presented. The main purpose of this review is to provide a comprehensive and up-to-date knowledge from the biological importance of thermogenesis in energy homeostasis to the representative thermogenic regulators for treating obesity. Thermogenic regulators

Abbreviations: AKT, protein kinase B; ALDH9, aldehyde dehydrogenase 9; AMPK, AMP-activated protein kinase; ATP, adenosine triphosphate; β3-AR, β3-adrenergic receptor; BA, bile acids; BAT, brown adipose tissue; BMP8b, bone morphogenetic protein 8b; cAMP, cyclic adenosine monophosphate; C/EBPα, CCAAT/enhancer binding protein α; cGMP, cyclic guanosine monophosphate; Cidea, cell death-inducing DNA fragmentation factor α-like effector A; CLA, *cis*-12 conjugated linoleic acid; CRABP-II, cellular RA binding protein type II; CRE, cAMP response element; Dio2, iodothyronine deiodinase type 2; ERs, estrogen receptors; ERE, estrogen response element; FAS, fatty acid synthase; FGF21, fibroblast growth factor 21; GPCRs, G protein-coupled receptors; HFD, high fat diet; LXR, liver X receptors; MAPK, mitogen-activated protein kinase; OXPHOS, oxidative phosphorylation; PDEs, phosphodiesterases; PET-CT, positron emission tomography combined with computed tomography; PGC-1α, peroxisome proliferator-activated receptor γ coactivator 1-α; PKA, protein kinase A; PPARs, peroxisome proliferator-activated receptors; PPREs, peroxisome proliferator response elements; PRDM16, PR domain containing 16; PTP1B, protein-tyrosine phosphatase 1B; PXR, pregnane X receptor; RA, retinoic acid; RAR, RA receptor; RARE, RA response element; RMR, resting metabolic rate; RXR, retinoid X receptor; SIRT1, silent mating type information regulation 2 homolog 1; SNS, sympathetic nervous system; TFAM, mitochondrial transcription factor A; TMEM26, transmembrane protein 26; TRPs, transient receptor potential cation channels; UCP1, uncoupling protein 1; VDR, vitamin D receptor; VDRE, VDR response elements; WAT, white adipose tissue

*Corresponding author.
E-mail address: ligenl@umac.mo (Ligen Lin).

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might have a large potential for further investigations to be developed as lead compounds in fighting obesity.

1. Introduction

Overweight and obesity have reached epidemic proportions worldwide, for both children and adults[1]. The updated World Health Organization data showed more than 1.9 billion adults aged over eighteen were overweight and over 650 million were obese in 2016, which were almost double those in 1980. Obesity always increases the risk of some complications, such as type 2 diabetes[2], atherosclerosis[3] and several forms of cancer[4–6]. The mainstay anti-obesity approach remains in having low calorie diet and increasing physical activity. While, the anti-obesity therapeutic agents are the only choice for obese patients who have other comorbid conditions to restrict physical activity, such like hypertension, type 2 diabetes and arthritis. Currently, only five U. S. Food and Drug Administration-approved small molecule drugs for obesity treatment were sold on market. These anti-obesity drugs could be classified into two types, pancreatic lipase inhibitors to reduce intestinal fat absorption, and anorectics to suppress appetite. Most of them have unhappy adverse effects[7]. Thus, there is still a desperate demand for effective and safe candidates to get the obesity under control.

Obesity is characterized by fat mass expansion, occurred *via* adipocytes hyperplasia (increased number of adipocytes) and hypertrophy (increased size of adipocytes), and dysfunction of adipose tissues. Under positive energy conditions, pre-adipocytes proliferate and differentiate into mature adipocytes (hyperplasia), and excessive lipid stores within adipocytes (hypertrophy). There are 3 types of adipocytes: white adipocytes store excess calories in the form of triglycerides; brown adipocytes contain large amounts of mitochondria and disperse lipids to generate heat by uncoupling protein 1 (UCP1); beige adipocytes express low UCP1 at basal status, which resemble white adipocytes, and have a highly inducible thermogenic capacity upon stimulation[8,9]. Upon cold-stimulus, the sympathetic nervous system (SNS) is activated to release noradrenaline, which binds to β3-adrenergic receptor (β3-AR) on brown and beige adipocytes (Fig. 1). Subsequently, UCP1 is highly expressed and activated in mitochondria, promoting lipid β-oxidation and heat production (Fig. 1)[8]. Non-shivering thermogenesis in brown and beige adipocytes has been recognized to play a crucial role in energy balance in rodents and humans[10]. Thermogenic activity of brown and beige adipocytes is positively correlated with energy expenditure, and dysregulation of thermogenesis is linked to obesity in humans[11]. Studies have disclosed that the “brown” fat in human adults is composed primarily of beige adipocytes[9]. Therefore, interventions to increase “brown” fat mass and/or activity are attractive strategies for prevention/treatment of obesity.

Increasing evidences have revealed that thermogenic regulators have therapeutic effects towards obesity. With growing demands for treatment of obesity safely and effectively, more and more clinical studies were carried out recent years. Through searching on the data base of clinical registration in USA (https//clinicaltrails.gov), 10 preparations have been involved into clinical trials (Table 1). One (NCT02937298), three (NCT03171415, NCT01783470 and NCT00302276), two (NCT02048215 and NCT00302276) and four (NCT03379181, NCT03269747, NCT03189511 and NCT00781586) products have been involved in phases 1, 2, 3 and 4 clinical trials, respectively. These agents target on treatment of obesity, insulin resistance and hyperthyroidism. Till now, there is still no drug in clinic targeting thermogenesis for treatment of obesity. This review summarized recent research progresses in thermogenic regulators, and speculated their potential as anti-obesity agents.

2. Thermogenic regulators targeting G protein-coupled receptors (GPCRs)

GPCRs, a protein family comprised of more than 600 members, are associated with many physiological and pathological conditions. Thermogenic regulators targeting GPCRs have been widely investigated (Table 2).

2.1. β3-AR

β3-AR, one isoform of adrenergic receptors, is pivotal in thermogenesis because it's selectively expressed in brown and beige adipocytes in both rodents and humans[12]. Many studies have been focusing on the potential of β3-AR agonists as anti-obesity agents (Table 2). Two β3-AR agonists, BRL-37344 and CL316243, were reported to induce lipolysis and thermogenesis in brown adipocytes from rats[13]. CL316243 treatment increased brown adipose tissue (BAT) activity and energy expenditure of C57BL/6J mice in a thermoneutral state, but did not reduced adiposity in mice housed below thermoneutrality[14]. A clinic study showed treatment of CL316243 on healthy men enhanced fat oxidation and insulin-stimulated glucose disposal[15]. Acute administration of another β3-AR agonist, L-796568, in overweight men significantly increased energy expenditure after 4 h, while chronic administration of this compound for 4 weeks failed to increase energy expenditure[16,17]. CGP-12177A is a β3-AR agonist, which enhanced uncoupling content in BAT and inguinal white adipose tissue (WAT) of NMRI mice[18,19]. Arotinolol, a weak β3-AR agonist, stimulated oxygen consumption in brown adipocytes from hamsters or rats, but did not change thermogenesis in intact animals[20]. Mirabegron, with a high specific affinity to human β3-AR, is being applied to treat overactive bladder in clinic. High dose of mirabegron was reported to increase resting metabolic rate (RMR) and BAT thermogenesis in healthy young men[12].

Surprisingly, several other β3-AR agonists, including ZD7114, ZD2079 and TAK-677, didn't change energy expenditure in obese humans[21,22]. The failure of β3-AR agonists to reduce body weight or increase energy expenditure in the clinical trials might be due to the following reasons: 1) the objects, especially those obese patients, lacked brown and beige adipocytes, which led to

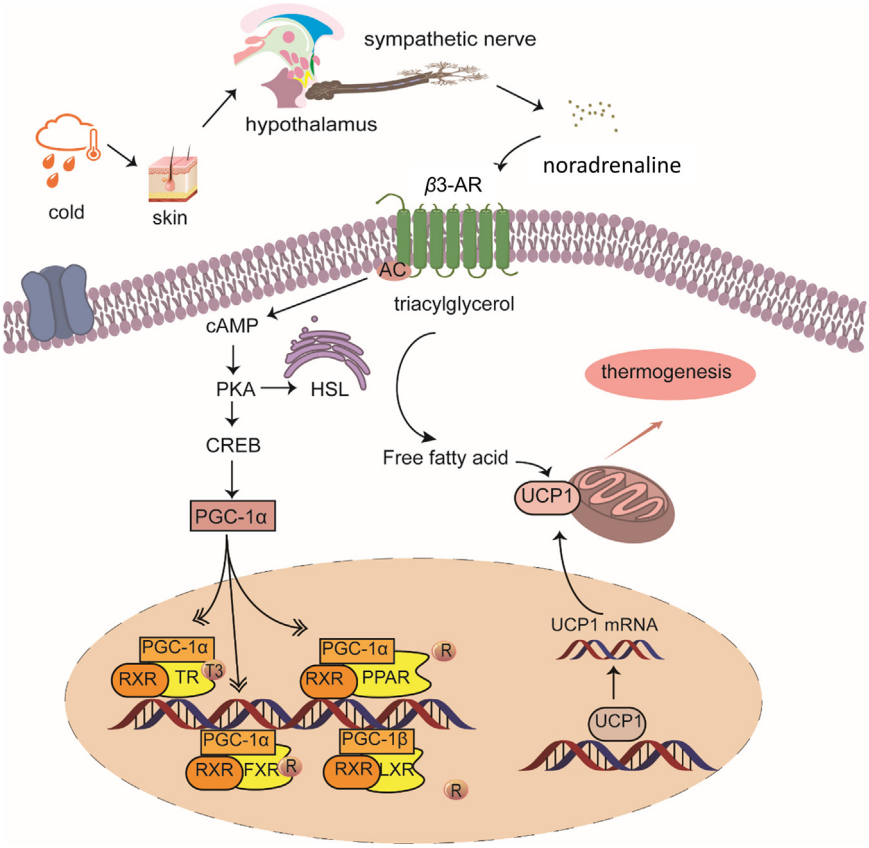


Figure 1 Schematic diagram of cold-activated thermogenesis. Sympathetic nerve is activated in response to cold exposure to release noradrenaline. Noradrenaline binds to the β3-AR on brown and beige cells to initiate signaling cascades for triglycerides hydrolysis and protein kinase A (PKA) activation. In response to PKA activation, CREB (cAMP response element-binding protein) recruits PGC-1α to facilitate RXR heterodimerization, which then interacts with nuclear receptors, like PPARs and LXR, to enhance *UCP1* gene transcription. Then, UCP1 dispersed the released fatty acids to generate heat.

Table 1 Thermogenic regulators in clinical trials.

Name	Identifier	Condition	Phase
Propranolol	NCT03379181	Hyperthyroidism	4
	NCT01791114	Insulin sensitivity, obesity	–
Prednisone	NCT03269747	BAT activity	4
Fluvastatin	NCT03189511	Brown fat activity, insulin resistance	4
RZL-012	NCT03171415	Obesity	2
Caffeine, ephedrine	NCT02048215	Obesity	3
β3-AR agonist	NCT01783470	Obesity	2
Caffeine	NCT00781586	Energy expenditure	4
Zantrex-3	NCT02937298	Diet-induced thermogenesis, obesity	–
Metobes-compound	NCT00302276	Obesity	2 and 3
Tyrosine, green tea, caffeine	NCT02937298	Diet-induced thermogenesis, obesity	1

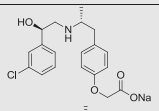
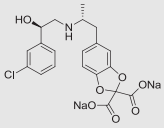
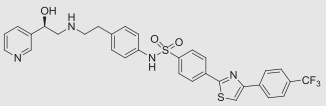
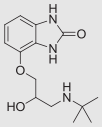
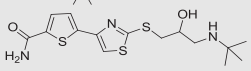
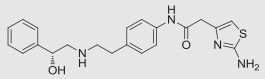
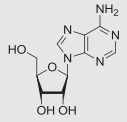
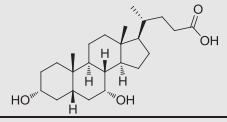
–Not applicable.

attenuation of the effect of β3-AR agonists on energy expenditure; 2) the objects were treated with β3-AR agonists for a short period of time in the most of trials, ranging from few hours to a few days; however, the activation of BAT might be observed in a long period of time; 3) the β3-AR expression and function are different in rodents and humans. Most β3-AR agonists were authenticated to be efficient on rodents, but failed in clinical trials. The human setting from *in vitro* to *in vivo* need to be addressed. Some β3-AR agonists also showed adverse effects due to insufficient selectivity. The structure and function mechanism of different ARs need to be further investigated to discover and develop more specific β3-AR agonists as a mean of activating brown and beige adipocytes.

2.2. *Adenosine receptor*

The innate ligand to adenosine receptor is adenosine, which binds to four P1 GPCR subtypes, the inhibitory receptors A1 and A3 and

Table 2 Thermogenic regulators targeting GPCRs.

Name	Molecule	Receptor	Object	Mechanism	Ref.
BRL-37344		β_3-AR	Brown adipocytes from Sprague–Dawley rats	Enhance respiration	13
CL316,243			Brown adipocytes from Sprague–Dawley rats	Enhance respiration	13
			C57BL/6J mice	Increase BAT activity and energy expenditure	14
			Humans	Enhance fat oxidation, lower 24h respiratory quotient	15
L-796568			Overweight humans	Increase lipolysis and energy expenditure	16, 17
CGP-12177A			NMRI mice	Enhance expression of *UCP1* in WAT and BAT	18, 19
Arotinolol			Brown adipocytes from Syrian hamster and Sprague–Dawley rats	Stimulate oxygen consumption	20
Mirabegron			Healthy humans	Increase RMR and BAT thermogenesis	12
Adenosine		Adenosine receptor	White adipocytes from female Sprague–Dawley rats	Enhance oxygen consumption	24
			Brown adipocytes from hamster	Enhance oxygen consumption and lipolysis	23
			Human and murine BAT and WAT	Enhance the thermogenic program	25
Bile acids	–	TGR5	BAT in male C57BL/6J and KK-Ay mice	Increase energy expenditure	28
			BAT and WAT from male Wistar Hannover GALAS rats	Induce gene expression of *Dio2, PGC-1α,* and *UCP1*	29
Chenodeoxycholic acid			BAT from human	Increase BAT activity	30

the stimulatory receptors A_{2A} and A_{2B}[23]. The distribution of the adenine receptor subtypes varies greatly by tissues and species, resulting in distinct response in different tissue contexts. Adenosine and its analogues (including 2-chloroadenosine, 2′-deoxyadenosine, 3′-deoxyadenosine and 2′-deoxyadenosine monophosphate) were found to inhibit isoproterenol-induced lipolysis, adenylate cyclase activation and 3′,5′-cyclic monophosphate generation in adipocytes from rodents[23,24]. While, another study suggested adenosine enhanced the thermogenic program in brown and white adipocytes at nanomolar concentrations, either from human or murine; and the effect of adenosine was stronger in brown adipocytes than white adipocytes duo to higher expression of A_{2A} receptor and higher A_{2A}/A1 ratio in brown adipocytes[25]. These findings indicated the role of adenosine signaling in thermogenesis is still controversial, and more studies are needed.

2.3. *G protein-coupled bile acid receptor (TGR5)*

G protein-coupled bile acid receptor, named TGR5, is involved in energy homeostasis[26,27]. Administration of bile acids (BA) increased energy expenditure in BAT of mice, through inducing the cyclic adenosine monophosphate (cAMP)-dependent thyroid hormone activation[28]. Interestingly, the increase of plasma BA concentration in rats was associated with the induction of genes involved in energy metabolism, including *Dio2* (iodothyronine deiodinase type 2), *Pgc-1α* (peroxisome proliferator-activated receptor γ coactivator-1α), and *UCP1*, in both BAT and abdominal and subcutaneous WAT[29]. Similarly, chenodeoxycholic acid was found to increase *UCP1* expression and activate thermogenesis in human BAT[30]. BA also has other hormonal actions through the farnesoid X receptor, which makes it not applicable for treatment of obesity.

3. Thermogenic regulators targeting transient receptor potential (TRP) channels

TRP channels are a group of transmembrane cation channels that are relatively non-selective for Ca^{2+}, Mg^{2+}, and Na^{+} ions[31]. Unlike the K^{+} selective ion channels, the TRP channels are constitutively open and are gated by a wide spectrum of physical and chemical stimuli, such as voltage, adenosine triphosphate (ATP), pH, redox agents, and multiple sensory stimuli. Upon stimulation, TRP channels initiate SNS activity, which, in turn, cascade a set of physiological processes, leading to defending responses to environmental changes. All TRP channels family members display six transmembrane α-helical protein domains which are assembled as tetramers to produce the overall functional channel[31]. Based on sequence and topological differences, the TRP channels family are classified into seven subfamily members, the five group 1 TRPs (TRPC, TRPV, TRPM, TRPN, and TRPA) and two group 2 TRPs (TRPP and TRPML). Among them, TRPV, TRPM and TRPA belong to thermally activated members. TRPV1, TRPV2, TRPV3 and TRPV4 are for warm sensation, and TRPM8 and TRPA1 are for cold sensation.

Upon stimulation from pain, heat, cold, capsaicin, and even mechanical motion, TRP channels receptors are sufficient to activate SNS-noradrenaline-BAT axis to enhance thermogenesis[32]. However, it's still debating whether activation or inhibition of TRP channels has benefit for thermogenesis, central or peripheral expressed TRP channels are the most critical. TRP channels regulators have received considerable attention in the field of obesity and diabetes (Table 3).

Capsinoids, including capsiate, dihydrocapsiate, and nordihydrocapsiate, are chemical constituents naturally present in chili peppers. In 2009, a clinical trial on healthy humans showed oral treatment with 6 mg capsinoids each day for 12 weeks caused obvious abdominal fat loss[33]. In addition, administration of 4 mg/kg capsinoids for 1 month showed enhanced energy expenditure and decreased body weight[34]. Another trial on healthy humans also showed that 8-week capsinoids treatment (9 mg/kg per day) increased BAT capacity using ^{18}F-fluorodeoxyglucose positron emission tomography combined with computed tomography (PET-CT)[35]. Through activating TRPV1 receptor, capsinoids not only enhances BAT thermogenesis, which always occurs in minutes, but also stimulates browning of WAT, which is adaptive process through increasing capacity of thermogenesis[32,36]. Capsaicin, one principle constituent of hot pepper, was reported to enhance energy expenditure and fatty acid β-oxidation *via* stimulating TRPV1-SNS axis[37]. Treatment with high dose of capsaicin (135 mg/day) for 3 months significantly increased fat oxidation without obvious adverse effect[38]. Importantly, dietary capsaicin activated TRPV1-evoked Ca^{2+} influx in the process of adipocyte-to-adipocyte communication, which, in turn, promoted lipolysis both *in vitro* and *in vivo*, improving visceral fat remodeling[39]. In 2011, monoacylglycerol was identified as a TRPV1 agonist, which increased *UCP1* expression in BAT and prevented visceral fat accumulation in C57BL/6Cr mice[40]. 10-Oxo-12(*Z*)-octadecenoic acid, a linoleic acid metabolite produced by gut lactic acid bacteria, was reported to enhance energy metabolism by activation of TRPV1[41]. A 12-week intervention with nonivamide, a TRPV1 agonist, prevented a dietary-induced body fat gain and increased peripheral serotonin in moderately overweight subjects[42]. It is interested to note that most TRPV1 receptor agonists are constituents from edibles, such as Guinea pepper seeds, with high content, which indicate they are safe for long term application. Activation of TRPV1 may mimic chronic cold exposure to increase thermogenesis in BAT that a process for body to adapt the change of environment.

TRPV1 is a temperature sensor which gets activated at 42 °C or over. It suggests that TRPV1 is the transmitter or amplifier to thermogenesis. When initial senor gets the signal of cold and cascade a series of action to initiate thermogenesis, TRPV1 can amplify the effect of thermogenesis. TRPV2 gets activated with an activation temperature threshold of higher than 52 °C. It is notable that loss of TRPV2 in mice showed increased WAT and larger brown adipocytes, and less BAT temperature increase in response to sympathetic activation[43]. However, it has been reported that activation of TRPV2 with non-selective TRPV2 agonists, 2-aminoethoxydiphenyl borate or lysophosphatidylcholine, inhibited the differentiation of mouse brown adipocytes[44]. These results suggested that the role of TRPV2 in the treatment of obesity is still remaining elusive.

TRPV4 is highly expressed in adipocytes[45]. Interestingly, TRPV4 expression is higher in WAT than BAT; and inactivation TRPV4 with its antagonist GSK205 led to WAT browning, while activation of TRPV4 with its agonist, GSK1016790A, repressed thermogenic genes expression[46]. It inferred that inactivation of TRPV4 might stimulate the formation of beige cells in WAT. Consistently, intravenous blockade of TRPV4 channel with chemical selective antagonists, HC-067047 or RN-1734, caused an increase in core body temperature and oxygen consumption at ambient temperature of 26 °C[47]. In addition, it is notable intracerebroventricular treatment with RN-1747, a chemical selective agonist of TRPV4, did not cause hypothermia. It indicated that the observed response was indeed due to activation of TRPV4 channels in the periphery[47].

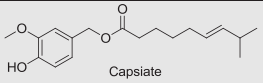
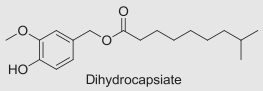
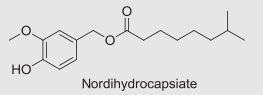
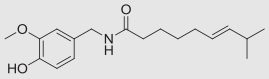
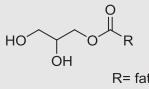
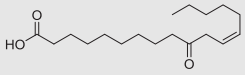
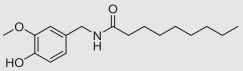
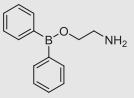
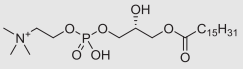
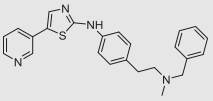
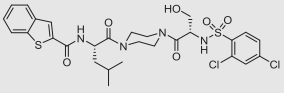
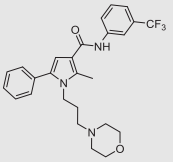
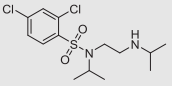
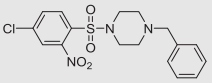
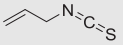
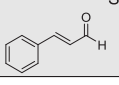
At lower experimental temperature like 20 °C, TRPM8 or TRPA1 is more likely to respond to cold stimulation. Previous studies have validated that TRPM8 plays a vital role in the detection of environmental temperature in mammals and is responsible for cold and chemical stimulation like menthol[48–50]. Menthol or 1,8-cineole activates TRPM8 to trigger UCP1-induced non-shivering thermogenesis and locomotor activity[51,52]. Allyl isothiocyannate and cinamaldehyde were reported to enhance thermogenesis and inhibit heat diffusion in mice, through activating TRPA1[51].

There are controversial results from dietary supplementation of TRP ligands (*e.g.*, capsaicin), either showing beneficial effects on body weight, metabolism, and hormone levels, or no effects. Selectivity of activators or inhibitors should be taken into consideration. Large clinical trials are needed to confirm the role of TRP ligands in the treatment of obesity. TRP channels are expressed in many tissues and organs important for the maintenance of whole body metabolism. Manipulation of TRP with small molecules is a potential strategy for induction of thermogenesis and treatment of obesity.

4. Thermogenic regulators targeting nuclear receptors in adipocyte

Nuclear receptors are a class of proteins directly binding to DNA to regulate expression of specific genes, which are highly related with energy homeostasis and metabolism[53]. Thermogenic regulators targeting nuclear receptors were listed in Table 4.

Table 3 Thermogenic regulators targeting TRPs.

Name	Molecule	Receptor	Objects	Mechanisms	Ref.
Capsinoids (capsiate, dihydrocapsiate and nordihydrocapsiate)	 Capsiate	TRPV1	Humans	Abdominal fat loss	33
				Enhance energy expenditure and decrease body weight	34
				Increase BAT capacity	35
	 Dihydrocapsiate				
				Enhance BAT thermogenesis and stimulate browning of WAT	32, 36
	 Nordihydrocapsiate				
Capsaicin			Humans	Enhance energy expenditure and fatty acid β-oxidation	37
				Increase fat oxidation	38
			3T3-L1, visceral adipose tissues from humans and wild-type and TRPV1-deficient mice	Promote lipolysis and improve visceral fat remodeling	39
Monoacylglycerol	 R= fatty acid group		C57BL/6Cr mice	Increase UCP1 in BAT and prevent visceral fat accumulation	40
10-Oxo-12(*Z*)-octadecenoic acid			Male C57BL/6 mice and KK-Ay mice	Enhance energy metabolism	41
Nonivamide			Overweight humans	Prevent a dietaryinduced body fat gain and increase peripheral serotonin	42
2-Aminoethoxydiphenyl borate		TRPV2	Mouse brown adipocytes	Suppress differentiation	44
Lysophosphatidylcholine					
GSK205		TRPV4	HFD treated C57BL/6J mice	Induce WAT browning	46
GSK1016790A				Repress thermogenic genes expression	46
HC-067047			Wistar rats	Increase core body temperature and oxygen consumption	47
RN-1734					
RN-1747				No effect	
Menthol		TRPM8	C57BL/6 mice	Trigger UCP1-induced non-shivering thermogenesis and locomotor activity	51, 52
1,8-Cineole					
Allyl isothiocyanate		TRPA1	C57BL/6 mice	Enhance thermogenesis and inhibit heat diffusion	51
Cinamaldehyde					

4.1. Peroxisome proliferator-activated receptors (PPAR)

PPARs belong to nuclear receptor super family, and so far three PPAR isoforms have been identified[54]. PPARα and PPARγ are directly linked to thermogenesis. PPARδ has capacity to increase fat acid oxidation[55]. When activated by specific ligands, PPARs bind to RXR (retinoid X receptor) to form heterodimers, which translocate to nucleus and bind peroxisome proliferator response elements (PPREs) to exert its function[56].

Rosiglitazone (Table 4), a PPARγ agonist, was found to promote mitochondrial biogenesis in 3T3-L1 adipocytes, accompanied with increased thermogenesis capacity and browning character[57]. In addition, chronic treatment of rosiglitazone to human multipotent adipose-derived stem cells showed browning phenotypes by increased *UCP1* and *CIDEA* (cell death-inducing DNA fragmentation factor α-like effector A) mRNA expressions[58]. Another study showed chronic treatment of rosiglitazone promoted browning in epididymal WAT[59]. Rosiglitazone induced BAT recruitment and lipolytic mRNA levels independently of tissue innervation status[60]. However, activation of PPARγ by rosiglitazone strongly exacerbated cold-induced upregulation of thyroid status and *PGC-1α* and *Dio2* in BAT[61]. Treatment of pioglitazone (Table 4), another PPARγ agonist, not only increased mitochondrial copy number but also enhanced *PGC-1α* and *TFAM* (mitochondrial transcription factor A) expressions on human subcutaneous WAT[62]. PPAR pathway represented an alternative, potent, and fully competent mechanism for BAT recruitment. It is noteworthy that PPARγ induced *UCP1* expression involves cross-talk between PKA (protein kinase A) and PPARγ signaling systems. A study showed that rosiglitazone and forskolin synergistically activated the *UCP1* promoter involving cross-talk between the signaling systems regulating the cAMP response element (CRE) and PPRE on the promoters[63]. It suggested that increasing energy expenditure *via* BAT thermogenesis maybe more potential to stimulate both PKA and PPARγ signaling pathways. However, it should not be neglected that PPARγ is modulated by other factors such as FGF21 (fibroblast growth factor 21)[64]. Berberine (Table 4) was found to increase energy expenditure and cold tolerance, and enhance BAT activity in *db/db* mice. In addition, administration of berberine led to increased expressions of *UCP1* and thermogenic genes in WAT and primary brown adipocytes *via* a mechanism involving AMPK (AMP-activated protein kinase) and PGC-1α[65]. Collectively, PPARγ activation goes prior to functioning on browning in WAT.

PPARα, also known as NR1C1, is a master regulator of fatty acid oxidation, which is highly expressed in tissues consuming fatty acids at a rapid rate[66]. In term of thermogenesis, PPARα plays an important role in the expression of *UCP1*[67]. In primary human fat cells, activation of PPARα with its agonist GW7647 (Table 4) resulted in up-regulation of β-oxidation genes and enhanced palmitate oxidation. Particularly, in this process glucose oxidation was decreased. PPARα agonist may stimulate combustion of lipid instead of glucose[68]. Surprisingly, thiazolidinedione treatment did not show this effect[69]. In addition, PPARα agonists, GW7647 or WY14643 (Table 4), directly activated *PGC-1α* and *Prdm16* (PR domain containing 16) expression, resulting in induction of thermogenic genes, mitochondrial genes, and lipid oxidation genes in brown fat[70]. Similarly, oleoylethanolamide (Table 4), a PPARα agonist, enhanced β3-adrenergic-mediated thermogenesis and browning in epididymal WAT in rat, with increased expressions of the mitochondrial (*Cox4i1*, *Cox4i2*), thermogenic (*FGF21*, *Prdm16*) and fatty-acid β-oxidation related genes[71]. It suggested PPARα receptor agonists promote adipocyte remodeling in epididymal WAT, and therefore have a potential clinical utility in the treatment of obesity.

PPARδ is a nuclear receptor that governs a variety of biological processes, which ubiquitously distributes in brain, skin, liver, skeletal muscle and adipose tissue. It has been validated PPARδ in WAT plays a role in regulating lipid mobilization and energy storage. To be frustrated, there are only few effective PPARδ agonists and the mechanism remains elusive in terms of thermogenic regulation. Interestingly, previous study showed retinoic acid (RA, Table 4), a vitamin A metabolite, acted as a physiological ligand of PPARδ, participating in cell survival[72]. RA activated PPARδ in preadipocytes and adipocytes to increase *UCP1* and *Aldh9* (aldehyde dehydrogenase 9), a key enzyme in fatty acid oxidation[73]. It suggested activation of PPARδ shifts substrate oxidation towards combustion of lipids. In addition, administration of PPARδ selective agonist GW0742 (Table 4) effectively suppressed adipogenesis and enhanced lipolysis through AKT (protein kinase B) signaling pathway[73].

4.2. Liver X receptors (LXR)

LXRs play a vital role in bile acid synthesis, lipid and glucose homeostasis. LXRs present in two isoforms, LXRα and LXRβ. Both isoforms are expressed in mature murine and human adipocytes[74]. The role of LXR in adipose tissue and obesity is still controversial, due to the complicated interaction among LXR, PPARγ and C/EBPα (CCAAT/enhancer binding protein α)[75–77]. Inhibition of LXR in BAT induced thermogenesis contributing to weight loss. Morin (Table 4), a naturally occurring flavonoid, was found as an LXRα and LXRβ dual antagonist, which reduced body weight gain and white adipocytes size in high fat diet (HFD)-treated mice[78]. It suggested that LXR suppression has a positive correlation with thermogenesis. Consistently, a report showed TO901317 (Table 4), a potent and selective LXRα agonist, has significant effect on suppression of *Dio2* expression in primary brown adipocytes[79]. Moreover, administration of LXRs agonist GW3965 repressed UCP1 in BAT and browning of subcutaneous WAT[80]. Although there are still confused findings that activation of LXRs in white adipocytes have positive correlation with fatty acid oxidation, it should be noted that all these results were obtained in white adipocytes[81,82]. Interestingly, rhein (Table 4), a lipophilic anthraquinone derived from a traditional Chinese herbal medicine *Rheum palmatum* L., was found to maintain energy balance by targeting LXRs and protect against obesity through LXRs-mediated *UCP1* upregulation in BAT[83]. Rhein is a multi-target molecule, which still need further investigation for pharmaceutical application as an anti-obesity agent[84].

4.3. Retinoid X receptor (RXR) and RA receptor (RAR)

RAR and RXR are members of the steroid/thyroid hormone receptor superfamily. RAR is activated by binding either all-*trans* RA or 9-*cis* RA (Table 4); while RXR is activated only by 9-*cis* RA but not all-*trans* RA. Cellular responses to RA are mediated by RAR and RXR, which are activated to form dimeric transcriptional factors that bind to specific RA response element (RARE) to regulate thermogenesis in adipocytes[85]. Twenty years ago, RA was found to activate primary brown preadipocytes, which stimulated

Table 4 Thermogenic regulators targeting nuclear receptors.

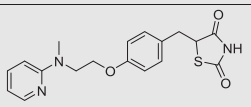
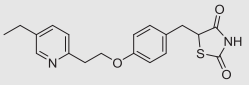
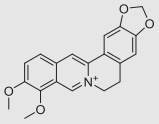
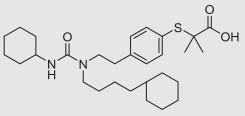
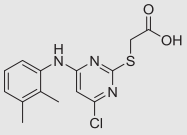
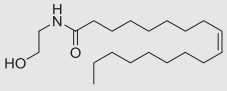
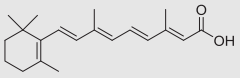
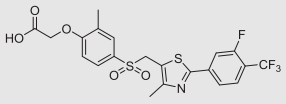
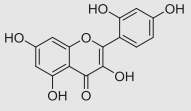
Names	Molecules	Receptor	Object	Mechanism	Ref.
Rosiglitazone		PPARγ	3T3-L1 adipocytes	Promote mitochondrial biogenesis, increase thermogenesis capacity and browning	57
			Human multipotent adipose-derived stem cells	Increase *UCP1* and *CIDEA* mRNA expression	58
			NMRI mice	Promote browning in epididymal WAT	59
			Sprague–Dawley rats	Induce BAT recruitment and lipolytic mRNA levels	60
			Male Sprague–Dawley rats	Exacerbate cold-induced upregulation of thyroid status and *Pgc-1α* and *Dio2* in BAT	61
Pioglitazone			Human subcutaneous WAT	Induce mitochondrial biogenesis and enhance *PGC-1α* and *TFAM* expression	62
Berberine			*db/db* mice	Up-regulate fatty acid oxidation and heat production	65
GW7647		PPARα	Primary human fat cells	Up-regulate β-oxidation genes and enhance palmitate oxidation	68
WY14,643			Male Sprague–Dawley rats	Induce thermogenic genes, mitochondrial genes, and lipid oxidation genes in brown fat	70
Oleoylethanolamide			Male Sprague–Dawley rats	Enhance β_3-adrenergic-mediated thermogenesis and browning in epididymal WAT	71
All-*trans* RA		PPARδ	C57BL/6Ntac mice	Increase *Ucp1* and *Aldh9* expression	73
GW0742			3T3-L1 cells and C57BL/6Ntac mice	Suppress adipogenesis and enhance lipolysis	73
Morin		LXRα and LXRβ	Female HFD C57BL/6J mice	Reduce body weight gains and the size of white adipocytes and increase *Ucp1* and *Pgc-1α* expressions in WAT	78

Table 4. (*continued*)

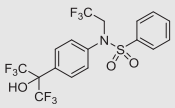
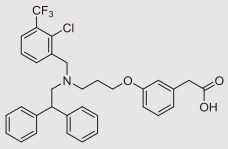
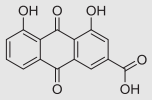
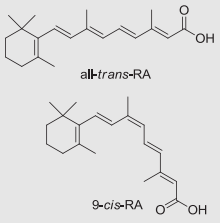
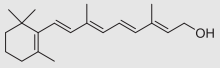
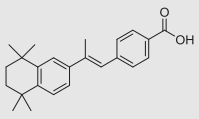
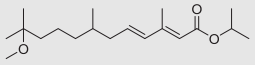
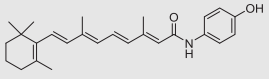
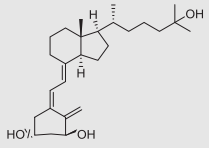
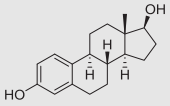
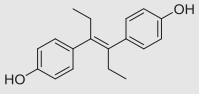
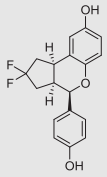
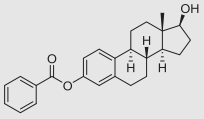
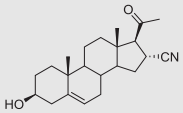
TO901317		LXRα	Primary brown adipocytes	Suppress *Dio2* expression	79
GW3965		LXRs	Female high-carbohydrate diet C57BL/6J mice	Decrease UCP1 expression	80
Rhein			Female C57BL/6J mice	Upregulate UCP1 and increase energy expenditure	83
All-*trans*-RA or 9-*cis*-RA		RAR and RXR	HIB1B brown adipocytes and male NMRI mice	Increase UCP1 content in BAT	87
Vitamin A		RXRα, RARα	Male F-344 X BN rats	Increase mitochondrial biogenesis and *Ucp1* expression	88
p-[(*E*)-2-(5,6,7,8,-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-propenyl]benzoic acid		RAR	Primary brown adipocytes from Swiss mice	Increase *Ucp1* expression	90
Methoprene		RXR			
Fenretinide		RAR	3T3-L1 cells and HFD-fed mice	Suppress differentiation and prevent obesity and insulin resistance	93
1,25-Dihydroxyvitamin D3		VDR	Brown adipocytes	Suppress *Ucp1* expression	97
17β-Estradiol		ERα	Primary brown adipocytes	Promote mitochondrial biogenesis and thermogenesis	107
Diethylstilbestrol		ERs	C57BL/6J mice	Increase *Bmp8b* and *Fgf* family genes in BAT	111

Table 4. (*continued*)

LY3201		ERβ	C57BL/6J mice	Induce browning of subcutaneous abdominal fat	112
Estradiol benzoate		ERs	Female sheep	Induce thermogenesis	113
Pregnenolone 16*a*-carbonitrile		PXR	AKR/J mice	Induce of *Dio2*, *Pgc-1α*, *Pgc-1β* and *Cidea* expression in BAT	105

UCP1 gene expression through a RA-responsive region but independent of adrenergic pathway[86]. Puigserver et al.[87] firstly reported that administration of all-*trans*-RA or 9-*cis*-RA led to an increase in the BAT specific UCP1 content in mice, as well as in HIB1B brown adipocytes. Dietary vitamin A (Table 4) supplementation increased *UCP1* expression in BAT of mice[88]. Feeding a vitamin A-deficient diet triggered opposite effects to those of all-*trans*-RA treatment, including increased body weight and reduced BAT thermogenic potential[89]. In addition, either synthetic RAR-specific agonist, *p*-[(*E*)-2-(5,6,7,8,-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-propenyl]benzoic acid (Table 4), or RXR-specific agonist, methoprene (Table 4), increased the expression of *UCP1* mRNA and the activity of chloramphenicol acetyltransferase expression vectors driven by the *UCP1* promoter[90]. It indicated both RAR- and RXR-dependent signaling pathways mediate the induction of *UCP1* in BAT by retinoids. It is noteworthy that RA's effects on induction of UCP1 might associate with p38 mitogen-activated protein kinase (p38MAPK) activation. Inhibition of p38MAPK activity with PD169316 hindered retinoid on induction of UCP1, suggesting p38MAPK participating in this process[91]. RA suppressed adipogenesis *in vivo* by activating the cellular PPARδ associated binding proteins cellular RA binding protein type II (CRABP-II)/RARγ path in preadipocytes[92]. These findings indicated RA as common dietary supplements help to counteract diet-induced obesity. Fenretinide (Table 4), a synthetic retinoid, was found to prevent obesity and insulin resistance in HFD-fed mice and completely suppress 3T3-L1 preadipocyte differentiation[93]. It is promising to apply RAR agonist to counteract diet-induced obesity. It has been reported that all-*trans* RA induced UCP1 expression in mouse white and brown adipocytes, but not in human adipocyte cell lines or primary human white adipocytes[94]. More studies on human adipocytes are needed to verify RAs as thermogenesis inducers.

4.4. Vitamin D receptor

The vitamin D receptor (VDR), a member of the steroid/thyroid/retinoid nuclear receptor superfamily, dimerizes with RXRα, and binds to VDR response elements (VDREs). It was reported that VDR is expressed in adipose tissue and dynamically up-regulated during adipocytes differentiation[95,96]. The hormonal form of vitamin D, 1,25-dihydroxy vitamin D3 (Table 4), suppressed the expression of *UCP1*[97], and vitamin D or VDR deficiency decreased adiposity and increased UCP1 in rodents[98–101]. In addition, adipocytes from humans with hereditary vitamin D resistant rickets showed increased UCP1 expression and a browning phenotype[102]. To be frustrated, there is no data reported about VDR antagonist on thermogenesis. On the other hand, vitamin D's positive effect on adipogenesis might be suitable for some special populations like cachexia patients.

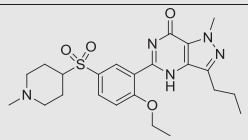
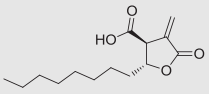
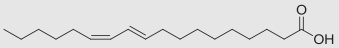
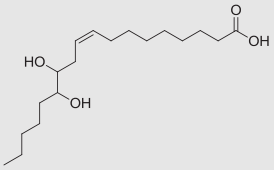
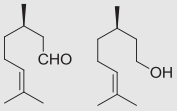
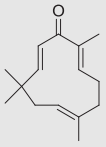
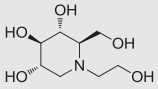
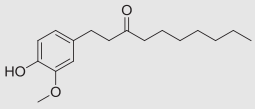
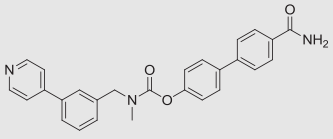
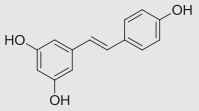
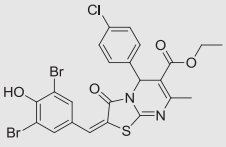
4.5. Pregnane X receptor

The primary function of pregnane X receptor (PXR) is to sense the presence of xenobiotic substances and respond to detoxification and clearance of these substances from the body[103]. PXR activation is associated with thermogenesis[104]. Pregnenolone 16α-carbonitrile (Table 4) enhanced thermogenesis by induction the mRNA expression of *Dio2*, *PGC-1α*, *PGC-1β* and Cidea in BAT of mice[105]. However, there was no significant increase in *UCP1* mRNA in adipocytes. The mechanism of PXR on thermogenesis is still unclear.

4.6. Estrogen receptors (ERs)

The rat, mouse and human ERs exist as two subtypes, ERα and ERβ[106]. A decade ago, 17β-estradiol was found to promote mitochondrial biogenesis and thermogenic function in primary brown adipocytes[107]. It is notable that 17β-estradiol negatively modulated the ATP synthase activity through direct binding to the oligomycin sensitive-conferring protein[108], which may result in decreasing of mitochondrial ATP generation. ERs are highly expressed in the hypothalamus[109] and estradiol upregulated BAT thermogenesis *via* hypothalamic AMPK[110]. In the brown fat, diethylstilbestrol increased the expression of *Bmp8b* (bone morphogenetic protein 8b) and *FGF* family genes involved in BAT activity[111]. A 3-day-treatment with a selective ERβ agonist, LY3201 (Table 4), induced browning of subcutaneous abdominal fat pad in obese female mice[112]. Consistently, acute 17β-estradiol or estradiol benzoate (Table 4) treatment

Table 5 Miscellaneous small molecules inducing thermogenesis.

Name	Molecule	Receptor	Object	Mechanism	Ref.
Sildenafil		PDEs	C57BL/6J mice	Increase energy expenditure and UCP1 level	114
			C57BL/6J mice	Increase *UCP1* and *PGC-1α* expressions in WAT	115
C75		FAS	Male Wistar rats	Activate sympathetic outflow and thermogenesis in BAT	116
trans-10, *cis*-12 CLA		–	Sv129 mice	Up-regulate fatty acid oxidation and heat production	117, 118
12,13-DiHOME		Fatty acid transport protein 1 and CD36	C57BL/6J mice	Activated BAT fuel uptake, enhance cold tolerance, and decrease serum triglycerides	119
R-(+)-Citronellal and *β*-citronellol		–	Male SD rats	Increase BAT temperature	120
Zerumbone		–	Male SD rats	Increase BAT temperature	121
Miglitol		–	Male C57BL/6J mice	Increase energy expenditure by upregulating UCP1 in BAT	122
Paradol analogues		SNS	Male C57BL/6J mice	Increase energy expenditure	123
WWL113U		–	Male C57BL/6J mice	Increase the content of UCP1 in BAT	124
Resveratrol		SIRT1	Male SD rats	Increase energy expenditure and expressions of thermogenic markers	125
		SIRT1 independent pathway	Male C57BL/6J mice		126
CZ5		Mitochondrial chemical uncoupler	L6 myotubes, 3T3-L1 adipocytes and rat primary hepatocytes	Elevate energy expenditure	128

– Not known

led to thermogenesis in female sheep but not chronic estrogen treatment[113]. One possible explanation was that the distribution of ER subtypes varies by tissues and species, resulting in distinct response in different tissue contexts. Taken together, a stable estrogen level or ER agonist is essential to keep thermogenesis in BAT and energy homeostasis in female.

5. Miscellaneous

Some other small molecules have also been reported to induce thermogenesis (Table 5).

Phosphodiesterases (PDEs) hydrolyze cGMP (cyclic guanosine monophosphate) and cAMP. Chronic treatment with sildenafil, a PDE-5 inhibitor, resulted in increased energy expenditure[114]. Surprisingly, the *UCP1* level was significantly lower in BAT from sildenafil-treated mice[114]. While, short-term sildenafil treatment showed no change on *UCP1* or *PGC-1α* levels in BAT; however, it caused an increase of *UCP1* and *PGC-1α* expressions in WAT and browning features like appearance of multilocular adipocytes within WAT[115]. It suggested that beige cells are responsible for sildenafil induced thermogenesis.

FAS (fatty acid synthase) is a multi-enzyme protein that catalyzes fatty acid synthesis, especially the synthesis of palmitate. Inhibition of FAS by its inhibitor C75 activated sympathetic outflow and thermogenesis in BAT, indicating FAS might serve as a potential target of thermogenesis[116].

Low dosage of *trans*-10, *cis*-12 conjugated linoleic acid (CLA) increased browning in overweight SV129 mice. CLA led to reduction in percentage of body fat, and increased *UCP1* level and fatty acid oxidation[117,118]. 12,13-Dihydroxy-9*Z*-octadecenoic acid (12,13-diHOME), a lipid to stimulate BAT activity, is negatively correlated with body-mass index. A study showed the injection of 12,13-diHOME activated BAT fuel uptake, enhanced cold tolerance and decreased levels of serum triglycerides through promoting the membrane translocation of the fatty acid transporters fatty acid transport protein 1 and CD36[119]. The identification of BAT-specific lipid utilization may spark potential way to unlock the maximum therapeutic potential of brown fat in humans. *R*-(+)-citronellal and *β*-citronellol from citronella oil, one of the most famous Indonesian essential oils, have ability to increase temperature and sympathetic nerve activity in BAT[120]. In another study, the major component of *Zingiber zerumbet*, zerumbone, was found to enhance sympathetic nerve activity and temperature in BAT[121]. Some oral anti-diabetic drugs showed thermogenic effect. Miglitol, *α*-glucosidase inhibitor, was able to increase energy expenditure by upregulating UCP1 in BAT. Miglitol has capability in enhancement of *β*3-adrenergic signaling[122]. However, it needs further investigation to fully elucidate the thermogenic effect of miglitol. Another study showed paradol analogues increased energy metabolism in the BAT *via* the activation of sympathetic nerve activity; and the length of the acyl chain of the paradol analogues had a significant impact on the extent of UCP1 expression level[123]. Using a transgenic animal model expressing luciferase to mimics endogenous UCP1 expression, a potential modulator WWL113 was discovered with capacity to increase UCP1 expression and thermogenic response without significant change in locomotor activity, food intake, or heartbeat[124]. Resveratrol increased energy expenditure and the expression of thermogenic markers through activating SIRT1 (silent mating type information regulation 2 homolog 1)[125]. However, another group reported resveratrol induced thermogenesis through SIRT1 independent pathway[126]. The role of SIRT1 in resveratrol induced thermogenesis remains elusive. Experiments using transgenic mice overexpressing UCP1 in metabolic tissues showed that locally uncoupling oxidative phosphorylation (OXPHOS) could combat obesity[127]. A novel chemical uncoupler, CZ5, was found to elevate energy expenditure without change *UCP1* level[128].

6. Conclusions

Direct ways to evaluate thermogenic capacity are mainly comprised of determination of expressions of thermogenic genes, oxygen consumption rate and mitochondrial function in brown adipocytes, as well as measurement of core temperature, locomotor activity, energy expenditure and sympathetic nerve activity in animals. Each the above method has limitation; and results from one or few assays might cause misleading. A systemic evaluation including *in vitro* and *in vivo* models should be carried out to authenticate thermogenic regulators.

UCP proteins are able to mediate directly adaptive non-shivering thermogenesis and metabolic inefficiency[129–131]. Among them, UCP1 protein is the most important marker to predict thermogenesis capacity. At the mitochondrial inner membrane, the energy of nutrients such as glucose and lipids is converted into a proton gradient, but instead of storing the potential energy in the generation of ATP, UCP1 catalyzes an inducible proton leak to release the energy of the proton gradient directly as heat. It should be noted that UCP1 does not primarily evolve as an anti-obesity protein but as a means of quickly generating heat. Over-activated UCP1 posed a threat on thermogenic response when confronted with acute cold stimulation. Activation of UCP1 is not an automatic process and requires extra stimulation such as hormones, chemical agents, nutritional or even environmental factors. Therefore, additional variables including housing temperature, mouse strain and diet should be accurately controlled. No exact evidence showed there is a definite link between the expression of UCP1 and basal brown adipocyte metabolic rate. On the contrary, a previous work showed enhancement in UCP1 expression was accompanied with no difference in basal energy expenditure[124]. To analyze thermogenic capacity, measurements of UCP1 at both mRNA and protein levels with functional and metabolic assessments are necessary.

There are some indications of alternative uncoupling mechanisms besides UCP1, such as the creatine kinase cycle[132] and calcium cycle[133]. UCP1 knockdown animals was found to be acclimated to cold temperature[134] and WAT contributes to UCP1-independent thermogenesis[135]. Evidences have showed that beige cells have higher respiratory capacity than brown adipocytes, which are supposed to occupy high level of UCP1[9]. In addition, beige cells express beige-selective marker, TMEM26 (transmembrane protein 26), CD137, and other thermogenic markers including mitochondrial genes *Cox7a1* and *Cox8b*, transcriptional coregulatory PRDM16 and PGC-1*β*, and the thermogenic hormone FGF21[9]. Using a brown adipocyte culture system, PPAR activation was found to represent a nonadrenergic, potent, and fully competent mechanism for BAT recruitment[59]. The complementary ways to increase energy expenditure in BAT remain to be unexplored.

The major brown fat deposits in adult humans are composed of beige adipocytes, which express distinct gene profiles[9]. It's also notable that classic brown fat exists in adult human, mainly distributes in the cervical, supraclavicular, axillary, and paravertebral regions; it may be involved in protecting the brain by warming up the blood supplied to the brain[136]. While, greater proportion of brown adipocytes and less proportion of beige cells exist in adult rodents. Most of the thermogenic regulators in previous studies were investigated on either *in vitro* brown adipocytes or *in vivo* murine models. It might explain why some thermogenic inducers did not show activity in humans. Human WAT derived beige cells should be recruited to screen

thermoregulatory molecules and investigate underlying mechanisms. The content and function of brown adipocytes and the beige cells are declined with age, contributing to an obesity-prone character in aged objects[10,137]. The design of clinical trials in future need to include a broader age range, both genders, and diverse genetic or ethnic backgrounds to reveal important information for stratified therapeutic approaches.

Uncontrolled thermogenic treatments can produce excessive heat, promote cachexia, and muscle waste, similar to victims of severe burns and cancer[138]. Thus, developing pharmacological brakes for thermogenesis also has an important therapeutic value.

There are a constantly expanding numbers of regulatory nodes and pathways that integrate BAT function with physiological changes. Some endogenous molecules have been identified to control thermogenesis. The increasing levels of key endogenous molecules, such as irisin and FGF21, are associated with metabolically beneficial in obese states, which might be potential targets of some of the molecules described in this review. The metabolic changes in certain disease states are disproportionately inhibiting thermogenesis; thus, identifying the molecular pathways other than thermogenesis is likely to supply new therapeutic opportunities.

The allosteric regulation triggering the protein's functional activity *via* conformational changes is an intrinsic function of protein under many physiological and pathological conditions, including metabolism[139,140]. Protein-tyrosine phosphatase 1B (PTP1B) is the prototype for the superfamily of PTPs involving in regulation of insulin, leptin and adiponectin to govern food intake and energy metabolism[141]. Either a whole-body or whole-brain deletion of PTP1B causes lean, leptin-hypersensitive and resistant to HFD-induced obesity in mice[142,143]. PTP1B allosteric inhibitors prevent formation of the active form of PTP1B by blocking mobility of the catalytic loop, thereby exploiting a general mechanism used by tyrosine phosphatases[144]. However, it remains elusive how PTP1B allosteric inhibition regulates energy metabolism. Modern allosteric drug discovery faces considerable challenges; in particular, there is the vast majority of allosteric sites in proteins which are undiscovered[145,146]. Thus, the allosteric regulation might be a potential pathway for discovery of thermogenic regulators.

The key for the medicinal utilization of small molecules targeting thermogenesis is specificity and efficacy. The unique qualities of brown adipocytes with unique regulatory systems will help address the issue of specificity. It's more difficult to elucidate the complex central regulatory mechanisms that sense heat production and modulate sympathetic nervous stimulation of thermogenesis. There should be key nuclei that integrate information on temperature and energy availability, which might be specific targets to control BAT activation. The approaches of thermogenic regulation at multiple levels are likely to be the most effective.

Given the growing world-wide prevalence and increasing healthcare burden of obesity and associated diseases and the current lack of effective treatment strategies, new anti-obesity therapies are urgently needed. Emerging evidences have indicated BAT, mostly beige adipocytes, is present in human adults, and activation of BAT is inversely associated with obesity and metabolic disease. In either rodent models or clinical trials, several pharmacological approaches increasing thermogenic capacity have been proven to effectively prevent obesity, facilitate weight reduction, and ameliorate insulin resistance. Although, there are still many issues to be solved for the therapeutic agents targeting activation or expansion of BAT, including: 1) the effectiveness of a thermogenic enhancer in treating obesity and insulin resistance is still unstable; 2) compensating mechanisms, such as increased appetite, could reduce the benefits of this approach; 3) the risks of drugs in central nervous system and sympathetic nerve activation should also be considered. The pharmacological approaches targeting stimulation of BAT activity and increase of energy expenditure would provide exciting new options in obesity therapy.

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