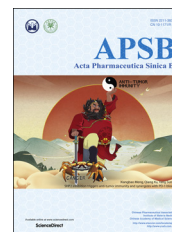




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REVIEW

Small molecules for fat combustion: targeting obesity

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KEY WORDS

Thermogenesis;
Brown adipose tissue;
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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; β 3-AR, β 3-adrenergic receptor; BA, bile acids; BAT, brown adipose tissue; BMP8b, bone morphogenetic protein 8b; cAMP, cyclic adenosine monophosphate; C/EBP α , 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-1 α , 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

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

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1. Introduction

Overweight and obesity have reached epidemic proportions worldwide, for both children and adults¹. 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 diabetes², atherosclerosis³ 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 effects⁷. 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 β 3-adrenergic receptor (β 3-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 humans¹⁰. Thermogenic activity of brown and beige adipocytes is positively correlated with energy expenditure, and dysregulation of thermogenesis is linked to obesity in humans¹¹. Studies have disclosed that the “brown” fat in human adults is composed primarily of beige adipocytes⁹. 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://clinicaltrials.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. β 3-AR

β 3-AR, one isoform of adrenergic receptors, is pivotal in thermogenesis because it's selectively expressed in brown and beige adipocytes in both rodents and humans¹². Many studies have been focusing on the potential of β 3-AR agonists as anti-obesity agents (Table 2). Two β 3-AR agonists, BRL-37344 and CL316243, were reported to induce lipolysis and thermogenesis in brown adipocytes from rats¹³. 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 thermoneutrality¹⁴. A clinic study showed treatment of CL316243 on healthy men enhanced fat oxidation and insulin-stimulated glucose disposal¹⁵. Acute administration of another β 3-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 β 3-AR agonist, which enhanced uncoupling content in BAT and inguinal white adipose tissue (WAT) of NMRI mice^{18,19}. Arotinolol, a weak β 3-AR agonist, stimulated oxygen consumption in brown adipocytes from hamsters or rats, but did not change thermogenesis in intact animals²⁰. Mirabegron, with a high specific affinity to human β 3-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 men¹².

Surprisingly, several other β 3-AR agonists, including ZD7114, ZD2079 and TAK-677, didn't change energy expenditure in obese humans^{21,22}. The failure of β 3-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

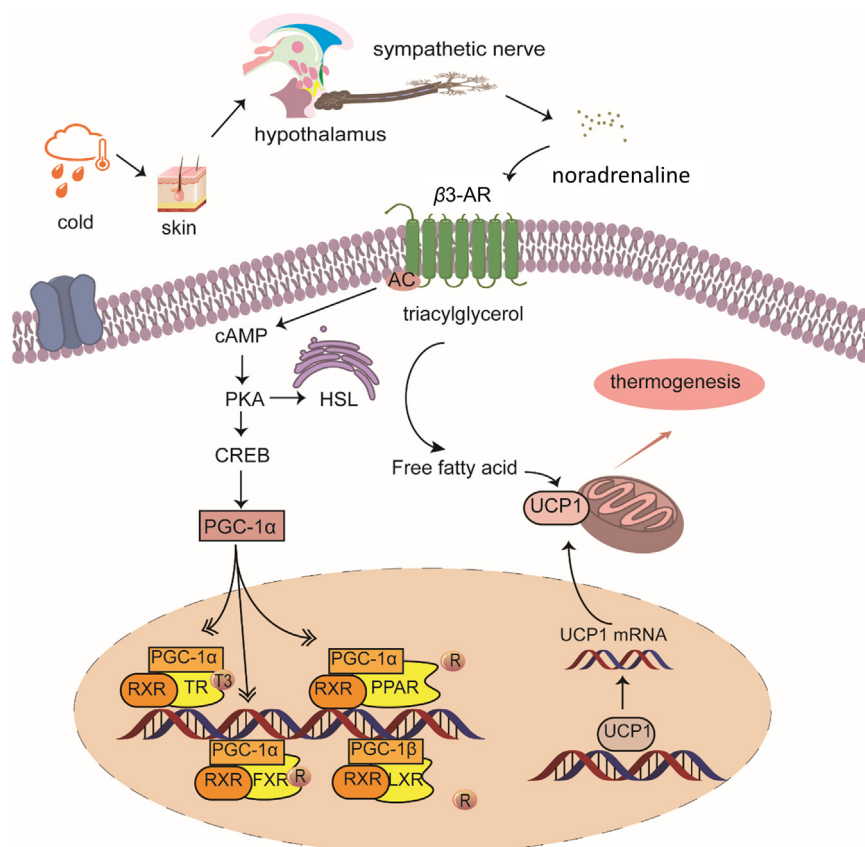


Figure 1 Schematic diagram of cold-activated thermogenesis. Sympathetic nerve is activated in response to cold exposure to release noradrenaline. Noradrenaline binds to the β_3 -AR on brown and beige cells to initiate signaling cascades for triglycerides hydrolysis and protein kinase A (PKA) activation. In response to PKA activation, CREB (cAMP response element-binding protein) recruits PGC-1 α 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
β_3 -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.

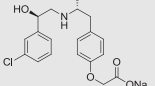
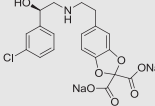
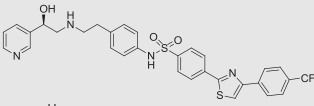
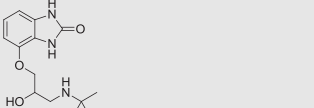
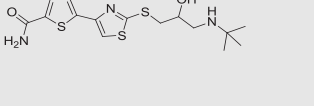
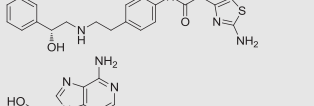
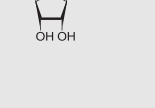
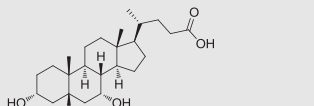
attenuation of the effect of β_3 -AR agonists on energy expenditure; 2) the objects were treated with β_3 -AR agonists for a short period of time in the most of trials, ranging from few hours to a few days; however, the activation of BAT might be observed in a long period of time; 3) the β_3 -AR expression and function are different in rodents and humans. Most β_3 -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 β_3 -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 β_3 -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 <i>UCP1</i> 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 <i>Dio2</i> , <i>PGC-1α</i> , and <i>UCP1</i>	29
			BAT from human	Increase BAT activity	30
Chenodeoxycholic acid					

the stimulatory receptors A_{2A} and A_{2B} ²³. The distribution of the adenosine 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 due to higher expression of A_{2A} receptor and higher A_{2A}/A_1 ratio in brown adipocytes²⁵. 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 activation²⁸. 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-1 α* (peroxisome proliferator-activated receptor γ coactivator-1 α), and *UCP1*, in both BAT and abdominal and subcutaneous WAT²⁹. Similarly, chenodeoxycholic acid was found to increase *UCP1* expression and activate thermogenesis in human BAT³⁰. 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^{+} ions³¹. 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 channel³¹. 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 thermogenesis³². 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 loss³³. In addition, administration of 4 mg/kg capsinoids for 1 month showed enhanced energy expenditure and decreased body weight³⁴. 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 axis³⁷. Treatment with high dose of capsaicin (135 mg/day) for 3 months significantly increased fat oxidation without obvious adverse effect³⁸. 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 remodeling³⁹. In 2011, monoacylglycerol was identified as a TRPV1 agonist, which increased *UCP1* expression in BAT and prevented visceral fat accumulation in C57BL/6Cr mice⁴⁰. 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 TRPV1⁴¹. A 12-week intervention with nonivamide, a TRPV1 agonist, prevented a dietary-induced body fat gain and increased peripheral serotonin in moderately overweight subjects⁴². 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 sensor 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 activation⁴³. 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 adipocytes⁴⁴. These results suggested that the role of TRPV2 in the treatment of obesity is still remaining elusive.

TRPV4 is highly expressed in adipocytes⁴⁵. 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 expression⁴⁶. 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 °C⁴⁷. 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 periphery⁴⁷.

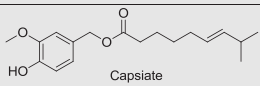
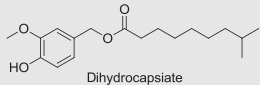
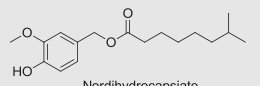
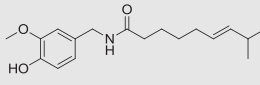
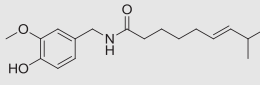
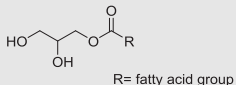
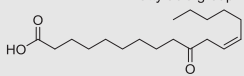
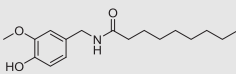
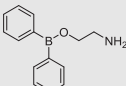
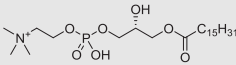
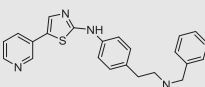
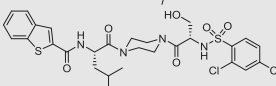
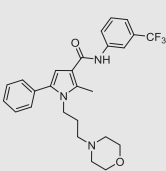
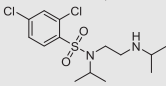
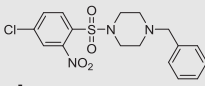
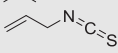
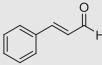
At lower experimental temperature like 20 °C, TRPM8 or TRPA1 is more likely to respond to cold stimulation. Previous studies have validated that TRPM8 plays a vital role in the detection of environmental temperature in mammals and is responsible for cold and chemical stimulation like menthol^{48–50}. Menthol or 1,8-cineole activates TRPM8 to trigger *UCP1*-induced non-shivering thermogenesis and locomotor activity^{51,52}. Allyl isothiocyanate and cinamaldehyde were reported to enhance thermogenesis and inhibit heat diffusion in mice, through activating TRPA1⁵¹.

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 metabolism⁵³. 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)		TRPV1	Humans	Abdominal fat loss	33
				Enhance energy expenditure and decrease body weight	34
				Increase BAT capacity	35
				Enhance BAT thermogenesis and stimulate browning of WAT	32, 36
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			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 dietary-induced 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 identified⁵⁴. PPAR α and PPAR γ are directly linked to thermogenesis. PPAR δ has capacity to increase fat acid oxidation⁵⁵. 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 function⁵⁶.

Rosiglitazone (Table 4), a PPAR γ agonist, was found to promote mitochondrial biogenesis in 3T3-L1 adipocytes, accompanied with increased thermogenesis capacity and browning character⁵⁷. 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 expressions⁵⁸. Another study showed chronic treatment of rosiglitazone promoted browning in epididymal WAT⁵⁹. Rosiglitazone induced BAT recruitment and lipolytic mRNA levels independently of tissue innervation status⁶⁰. However, activation of PPAR γ by rosiglitazone strongly exacerbated cold-induced upregulation of thyroid status and *PGC-1 α* and *Dio2* in BAT⁶¹. Treatment of pioglitazone (Table 4), another PPAR γ agonist, not only increased mitochondrial copy number but also enhanced *PGC-1 α* and *TFAM* (mitochondrial transcription factor A) expressions on human subcutaneous WAT⁶². PPAR pathway represented an alternative, potent, and fully competent mechanism for BAT recruitment. It is noteworthy that PPAR γ induced *UCP1* expression involves cross-talk between PKA (protein kinase A) and PPAR γ 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 promoters⁶³. It suggested that increasing energy expenditure via BAT thermogenesis maybe more potential to stimulate both PKA and PPAR γ signaling pathways. However, it should not be neglected that PPAR γ 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-1 α* ⁶⁵. Collectively, PPAR γ activation goes prior to functioning on browning in WAT.

PPAR α , also known as NR1C1, is a master regulator of fatty acid oxidation, which is highly expressed in tissues consuming fatty acids at a rapid rate⁶⁶. In term of thermogenesis, PPAR α plays an important role in the expression of *UCP1*⁶⁷. In primary human fat cells, activation of PPAR α 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. PPAR α agonist may stimulate combustion of lipid instead of glucose⁶⁸. Surprisingly, thiazolidinedione treatment did not show this effect⁶⁹. In addition, PPAR α agonists, GW7647 or WY14643 (Table 4), directly activated *PGC-1 α* and *Prdm16* (PR domain containing 16) expression, resulting in induction of thermogenic genes, mitochondrial genes, and lipid oxidation genes in brown fat⁷⁰. Similarly, oleoylethanolamide (Table 4), a PPAR α agonist, enhanced β 3-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 genes⁷¹. It suggested PPAR α receptor agonists promote adipocyte remodeling in epididymal WAT, and therefore have a potential clinical utility in the treatment of obesity.

PPAR δ 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 PPAR δ in WAT plays a role in regulating lipid mobilization and energy storage. To be frustrated, there are only few effective PPAR δ 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 PPAR δ , participating in cell survival⁷². RA activated PPAR δ in preadipocytes and adipocytes to increase *UCP1* and *Aldh9* (aldehyde dehydrogenase 9), a key enzyme in fatty acid oxidation⁷³. It suggested activation of PPAR δ shifts substrate oxidation towards combustion of lipids. In addition, administration of PPAR δ selective agonist GW0742 (Table 4) effectively suppressed adipogenesis and enhanced lipolysis through AKT (protein kinase B) signaling pathway⁷³.

4.2. Liver X receptors (LXR)

LXRs play a vital role in bile acid synthesis, lipid and glucose homeostasis. LXRs present in two isoforms, LXR α and LXR β . Both isoforms are expressed in mature murine and human adipocytes⁷⁴. The role of LXR in adipose tissue and obesity is still controversial, due to the complicated interaction among LXR, PPAR γ and C/EBP α (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 LXR α and LXR β dual antagonist, which reduced body weight gain and white adipocytes size in high fat diet (HFD)-treated mice⁷⁸. It suggested that LXR suppression has a positive correlation with thermogenesis. Consistently, a report showed TO901317 (Table 4), a potent and selective LXR α agonist, has significant effect on suppression of *Dio2* expression in primary brown adipocytes⁷⁹. Moreover, administration of LXRs agonist GW3965 repressed *UCP1* in BAT and browning of subcutaneous WAT⁸⁰. 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 BAT⁸³. Rhein is a multi-target molecule, which still need further investigation for pharmaceutical application as an anti-obesity agent⁸⁴.

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 adipocytes⁸⁵. Twenty years ago, RA was found to activate primary brown preadipocytes, which stimulated

Table 4 Thermogenic regulators targeting nuclear receptors.

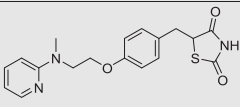
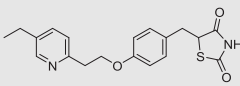
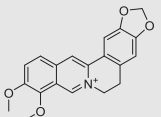
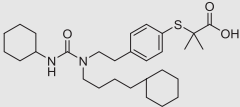
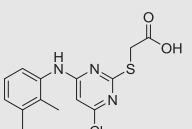
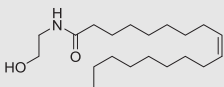
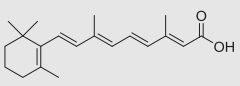
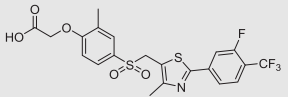
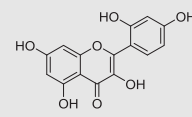
Names	Molecules	Receptor	Object	Mechanism	Ref.
Rosiglitazone		PPAR γ	3T3-L1 adipocytes	Promote mitochondrial biogenesis, increase thermogenesis capacity and browning	57
			Human multipotent adipose-derived stem cells	Increase <i>UCP1</i> and <i>CIDEA</i> mRNA expression	58
			NMRI mice	Promote browning 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 <i>Pgc-1α</i> and <i>Dio2</i> in BAT	61
Pioglitazone			Human subcutaneous WAT	Induce mitochondrial biogenesis and enhance <i>PGC-1α</i> and <i>TFAM</i> expression	62
Berberine			<i>db/db</i> mice	Up-regulate fatty acid oxidation and heat production	65
GW7647		PPAR α	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
Oleylethanolamide			Male Sprague–Dawley rats	Enhance β_3 -adrenergic-mediated thermogenesis and browning in epididymal WAT	71
All-trans RA		PPAR δ	C57BL/6Ntac mice	Increase <i>Ucp1</i> and <i>Aldh9</i> expression	73
GW0742			3T3-L1 cells and C57BL/6Ntac mice	Suppress adipogenesis and enhance lipolysis	73
Morin		LXR α and LXR β	Female HFD C57BL/6J mice	Reduce body weight gains and the size of white adipocytes and increase <i>Ucp1</i> and <i>Pgc-1α</i> expressions in WAT	78

Table 4. (continued)

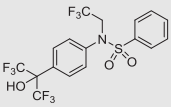
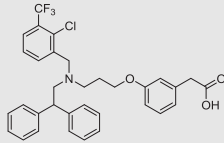
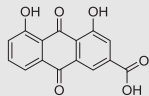
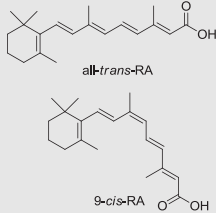
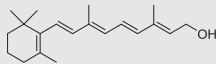
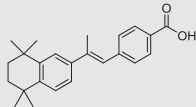
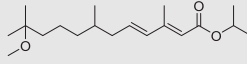
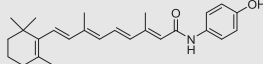
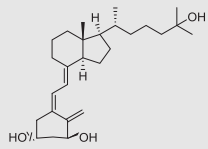
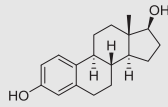
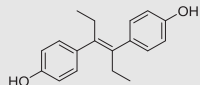
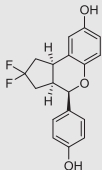
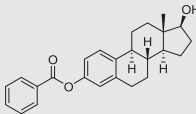
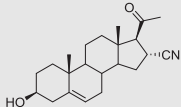
TO901317		LXR α	Primary brown adipocytes	Suppress <i>Dio2</i> expression	79
GW3965		LXR α s	Female high-carbohydrate diet C57BL/6J mice	Decrease UCP1 expression	80
Rhein			Female C57BL/6J mice	Upregulate UCP1 and increase energy expenditure	83
All- <i>trans</i> -RA or 9- <i>cis</i> -RA		RAR and RXR	HIB1B brown adipocytes and male NMRI mice	Increase UCP1 content in BAT	87
Vitamin A		RXR α , RAR α	Male F-344 X BN rats	Increase mitochondrial biogenesis and <i>Ucp1</i> expression	88
<i>p</i> -[(<i>E</i>)-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 <i>Ucp1</i> 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 <i>Ucp1</i> expression	97
17 β -Estradiol		ER α	Primary brown adipocytes	Promote mitochondrial biogenesis and thermogenesis	107
Diethylstilbestrol		ERs	C57BL/6J mice	Increase <i>Bmp8b</i> and <i>Fgf</i> family genes in BAT	111

Table 4. (continued)

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

UCP1 gene expression through a RA-responsive region but independent of adrenergic pathway⁸⁶. 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 mice⁸⁸. Feeding a vitamin A-deficient diet triggered opposite effects to those of all-*trans*-RA treatment, including increased body weight and reduced BAT thermogenic potential⁸⁹. 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* promoter⁹⁰. 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 process⁹¹. RA suppressed adipogenesis *in vivo* by activating the cellular PPAR δ associated binding proteins cellular RA binding protein type II (CRABP-II)/RAR γ path in preadipocytes⁹². 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 differentiation⁹³. 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 adipocytes⁹⁴. 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 RXR α , 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 phenotype¹⁰². 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.

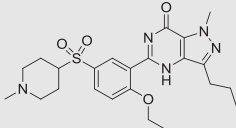
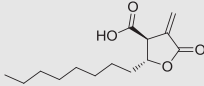
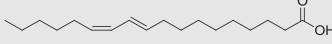
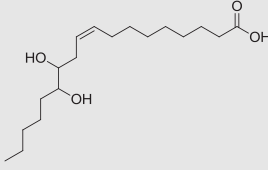
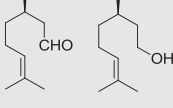
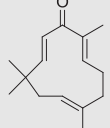
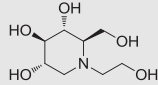
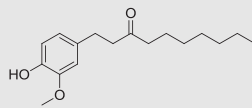
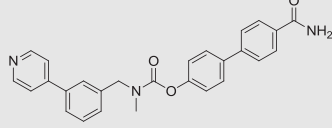
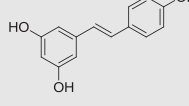
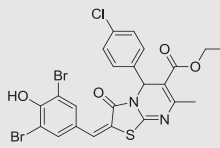
4.5. Pregnane X receptor

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 body¹⁰³. PXR activation is associated with thermogenesis¹⁰⁴. Pregnenolone 16 α -carbonitrile (Table 4) enhanced thermogenesis by induction the mRNA expression of *Dio2*, *PGC-1 α* , *PGC-1 β* and *Cidea* in BAT of mice¹⁰⁵. 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, ER α and ER β ¹⁰⁶. A decade ago, 17 β -estradiol was found to promote mitochondrial biogenesis and thermogenic function in primary brown adipocytes¹⁰⁷. It is notable that 17 β -estradiol negatively modulated the ATP synthase activity through direct binding to the oligomycin sensitive-conferring protein¹⁰⁸, which may result in decreasing of mitochondrial ATP generation. ERs are highly expressed in the hypothalamus¹⁰⁹ and estradiol upregulated BAT thermogenesis *via* hypothalamic AMPK¹¹⁰. In the brown fat, diethylstilbestrol increased the expression of *Bmp8b* (bone morphogenetic protein 8b) and *FGF* family genes involved in BAT activity¹¹¹. A 3-day-treatment with a selective ER β agonist, LY3201 (Table 4), induced browning of subcutaneous abdominal fat pad in obese female mice¹¹². Consistently, acute 17 β -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 <i>UCP1</i> and <i>PGC-1α</i> expressions in WAT	115
C75		FAS	Male Wistar rats	Activate sympathetic outflow and thermogenesis in BAT	116
<i>trans</i> -10, <i>cis</i> -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
<i>R</i> -(+)-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	125
		SIRT1 independent pathway	Male C57BL/6J mice	and expressions of thermogenic markers	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 treatment¹¹³. 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 expenditure¹¹⁴. Surprisingly, the *UCP1* level was significantly lower in BAT from sildenafil-treated mice¹¹⁴. While, short-term sildenafil treatment showed no change on *UCP1* or *PGC-1 α* levels in BAT; however, it caused an increase of *UCP1* and *PGC-1 α* expressions in WAT and browning features like appearance of multilocular adipocytes within WAT¹¹⁵. 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 thermogenesis¹¹⁶.

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-9Z-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 CD36¹¹⁹. 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 BAT¹²⁰. In another study, the major component of *Zingiber zerumbet*, zerumbone, was found to enhance sympathetic nerve activity and temperature in BAT¹²¹. 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 signaling¹²². 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 level¹²³. 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 heartbeat¹²⁴. 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 pathway¹²⁶. 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 obesity¹²⁷. A novel chemical uncoupler, CZ5, was found to elevate energy expenditure without change *UCP1* level¹²⁸.

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 expenditure¹²⁴. 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 cycle¹³² and calcium cycle¹³³. *UCP1* knockdown animals was found to be acclimated to cold temperature¹³⁴ and WAT contributes to *UCP1*-independent thermogenesis¹³⁵. 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 FGF21⁹. Using a brown adipocyte culture system, PPAR activation was found to represent a nonadrenergic, potent, and fully competent mechanism for BAT recruitment⁵⁹. 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 profiles⁹. 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 brain¹³⁶. 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 cancer¹³⁸. 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 metabolism¹⁴¹. 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 phosphatases¹⁴⁴. 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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References

1. Ng M, Fleming T, Robinson M, Thomson B, Graetz N, Margono C, et al. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* 2014;**384**:766–81.
2. Gallagher EJ, LeRoith D. Obesity and diabetes: the increased risk of cancer and cancer-related mortality. *Physiol Rev* 2015;**95**:727–48.
3. Lovren F, Teoh H, Verma S. Obesity and atherosclerosis: mechanistic insights. *Can J Cardiol* 2015;**31**:177–83.
4. Deng T, Lyon CJ, Bergin S, Caligiuri MA, Hsueh WA. Obesity, inflammation, and cancer. *Annu Rev Pathol* 2016;**11**:421–49.
5. Himbert C, Delphan M, Scherer D, Bowers LW, Hursting S, Ulrich CM. Signals from the adipose microenvironment and the obesity–cancer link—a systematic review. *Cancer Prev Res* 2017;**10**:494–506.
6. Park J, Morley TS, Kim M, Clegg DJ, Scherer PE. Obesity and cancer—mechanisms underlying tumour progression and recurrence. *Nat Rev Endocrinol* 2014;**10**:455–65.
7. Daneschvar HL, Aronson MD, Smetana GW. FDA-approved anti-obesity drugs in the United States. *Am J Med* 2016;**129**:879. e1–6.
8. Cannon B, Nedergaard J. Brown adipose tissue: function and physiological significance. *Physiol Rev* 2004;**84**:277–359.
9. Wu J, Bostrom P, Sparks LM, Ye L, Choi JH, Giang AH, et al. Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. *Cell* 2012;**150**:366–76.
10. Cyppess AM, Lehman S, Williams G, Tal I, Rodman D, Goldfine AB, et al. Identification and importance of brown adipose tissue in adult humans. *N Engl J Med* 2009;**360**:1509–17.
11. van Marken Lichtenbelt WD, Vanhommerig JW, Smulders NM, Drossaerts JM, Kemerink GJ, Bouvy ND, et al. Cold-activated brown adipose tissue in healthy men. *N Engl J Med* 2009;**360**:1500–8.
12. Cyppess AM, Weiner LS, Roberts-Toler C, Franquet Elia E, Kessler SH, Kahn PA, et al. Activation of human brown adipose tissue by a β 3-adrenergic receptor agonist. *Cell Metab* 2015;**21**:33–8.
13. Atgie C, D'Allaire F, Bukowiecki LJ. Role of β 1- and β 3-adrenoceptors in the regulation of lipolysis and thermogenesis in rat brown adipocytes. *Am J Physiol* 1997;**273**:C1136–42.
14. Xiao C, Goldgof M, Gavrilova O, Reitman ML. Anti-obesity and metabolic efficacy of the β 3-adrenergic agonist, CL316243, in mice at thermoneutrality compared to 22 degrees C. *Obesity* 2015;**23**:1450–9.
15. Weyer C, Tataranni PA, Snitker S, Danforth Jr. E, Ravussin E. Increase in insulin action and fat oxidation after treatment with CL 316,243, a highly selective β 3-adrenoceptor agonist in humans. *Diabetes* 1998;**47**:1555–61.
16. Larsen TM, Toubro S, van Baak MA, Gottesdiener KM, Larson P, Saris WH, et al. Effect of a 28-d treatment with L-796568, a novel β 3-adrenergic receptor agonist, on energy expenditure and body composition in obese men. *Am J Clin Nutr* 2002;**76**:780–8.

17. van Baak MA, Hul GB, Toubro S, Astrup A, Gottesdiener KM, DeSmet M, et al. Acute effect of L-796568, a novel β_3 -adrenergic receptor agonist, on energy expenditure in obese men. *Clin Pharmacol Ther* 2002;**71**:272–9.
18. Oliver P, Pico C, Martinez N, Bonet ML, Palou A. *In vivo* effects of CGP-12177 on the expression of leptin and uncoupling protein genes in mouse brown and white adipose tissues. *Int J Obes Relat Metab Disord* 2000;**24**:423–8.
19. Pico C, Bonet ML, Palou A. Stimulation of uncoupling protein synthesis in white adipose tissue of mice treated with the β_3 -adrenergic agonist CGP-12177. *Cell Mol Life Sci* 1998;**54**:191–5.
20. Zhao J, Golozoubova V, Cannon B, Nedergaard J. Arctinolol is a weak partial agonist on β_3 -adrenergic receptors in brown adipocytes. *Can J Physiol Pharmacol* 2001;**79**:585–93.
21. Buemann B, Toubro S, Astrup A. Effects of the two β_3 -agonists, ZD7114 and ZD2079 on 24 h energy expenditure and respiratory quotient in obese subjects. *Int J Obes Relat Metab Disord* 2000;**24**:1553–60.
22. Redman LM, de Jonge L, Fang X, Gamlin B, Recker D, Greenway FL, et al. Lack of an effect of a novel β_3 -adrenoceptor agonist, TAK-677, on energy metabolism in obese individuals: a double-blind, placebo-controlled randomized study. *J Clin Endocrinol Metab* 2007;**92**:527–31.
23. Schimmel RJ, McCarthy L. Role of adenosine as an endogenous regulator of respiration in hamster brown adipocytes. *Am J Physiol* 1984;**246**:C301–7.
24. Fain JN, Pointer RH, Ward WF. Effects of adenosine nucleosides on adenylate cyclase, phosphodiesterase, cyclic adenosine monophosphate accumulation, and lipolysis in fat cells. *J Biol Chem* 1972;**247**:6866–72.
25. Gnad T, Scheibler S, von Kugelgen I, Scheele C, Kilic A, Glode A, et al. Adenosine activates brown adipose tissue and recruits beige adipocytes via A2A receptors. *Nature* 2014;**516**:395–9.
26. Ding L, Yang L, Wang Z, Huang W. Bile acid nuclear receptor FXR and digestive system diseases. *Acta Pharm Sin B* 2015;**5**:135–44.
27. Zhu Y, Liu H, Zhang M, Guo GL. Fatty liver diseases, bile acids, and FXR. *Acta Pharm Sin B* 2016;**6**:409–12.
28. Watanabe M, Houten SM, Matakai C, Christoffolete MA, Kim BW, Sato H, et al. Bile acids induce energy expenditure by promoting intracellular thyroid hormone activation. *Nature* 2006;**439**:484–9.
29. Liaset B, Hao Q, Jorgensen H, Hallenborg P, Du ZY, Ma T, et al. Nutritional regulation of bile acid metabolism is associated with improved pathological characteristics of the metabolic syndrome. *J Biol Chem* 2011;**286**:28382–95.
30. Broeders EP, Nascimento EB, Havelkes B, Brans B, Roumans KH, Tailleux A, et al. The bile acid chenodeoxycholic acid increases human brown adipose tissue activity. *Cell Metab* 2015;**22**:418–26.
31. Venkatachalam K, Montell C. TRP channels. *Annu Rev Biochem* 2007;**76**:387–417.
32. Yoneshiro T, Saito M. Transient receptor potential activated brown fat thermogenesis as a target of food ingredients for obesity management. *Curr Opin Clin Nutr Metab Care* 2013;**16**:625–31.
33. Snitker S, Fujishima Y, Shen H, Ott S, Pi-Sunyer X, Furuhashi Y, et al. Effects of novel capsinoid treatment on fatness and energy metabolism in humans: possible pharmacogenetic implications. *Am J Clin Nutr* 2009;**89**:45–50.
34. Inoue N, Matsunaga Y, Satoh H, Takahashi M. Enhanced energy expenditure and fat oxidation in humans with high BMI scores by the ingestion of novel and non-pungent capsaicin analogues (capsinoids). *Biosci Biotechnol Biochem* 2007;**71**:380–9.
35. Nirengi S, Homma T, Inoue N, Sato H, Yoneshiro T, Matsushita M, et al. Assessment of human brown adipose tissue density during daily ingestion of thermogenic capsinoids using near-infrared time-resolved spectroscopy. *J Biomed Opt* 2016;**21**:091305.
36. Yoneshiro T, Aita S, Kawai Y, Iwanaga T, Saito M. Nonpungent capsaicin analogs (capsinoids) increase energy expenditure through the activation of brown adipose tissue in humans. *Am J Clin Nutr* 2012;**95**:845–50.
37. Whiting S, Derbyshire E, Tiwari BK. Capsaicinoids and capsinoids. a potential role for weight management? A systematic review of the evidence. *Appetite* 2012;**59**:341–8.
38. Lejeune MP, Kovacs EM, Westerterp-Plantenga MS. Effect of capsaicin on substrate oxidation and weight maintenance after modest body-weight loss in human subjects. *Br J Nutr* 2003;**90**:651–9.
39. Chen J, Li L, Li Y, Liang X, Sun Q, Yu H, et al. Activation of TRPV1 channel by dietary capsaicin improves visceral fat remodeling through connexin43-mediated Ca^{2+} influx. *Cardiovasc Diabetol* 2015;**14**:22.
40. Iwasaki Y, Tamura Y, Inayoshi K, Narukawa M, Kobata K, Chiba H, et al. TRPV1 agonist monoacylglycerol increases UCP1 content in brown adipose tissue and suppresses accumulation of visceral fat in mice fed a high-fat and high-sucrose diet. *Biosci Biotechnol Biochem* 2011;**75**:904–9.
41. Kim M, Furuzono T, Yamakuni K, Li Y, Kim YI, Takahashi H, et al. 10-Oxo-12(Z)-octadecenoic acid, a linoleic acid metabolite produced by gut lactic acid bacteria, enhances energy metabolism by activation of TRPV1. *FASEB J* 2017;**31**:5036–48.
42. Hochkogler CM, Lieder B, Rust P, Berry D, Meier SM, Pignitter M, et al. A 12-week intervention with nonivamide, a TRPV1 agonist, prevents a dietary-induced body fat gain and increases peripheral serotonin in moderately overweight subjects. *Mol Nutr Food Res* 2017;**61**:1600731.
43. Sun W, Uchida K, Suzuki Y, Zhou Y, Kim M, Takayama Y, et al. Lack of TRPV2 impairs thermogenesis in mouse brown adipose tissue. *EMBO Rep* 2016;**17**:383–99.
44. Sun WP, Uchida K, Takahashi N, Iwata Y, Wakabayashi S, Goto T, et al. Activation of TRPV2 negatively regulates the differentiation of mouse brown adipocytes. *Pflugers Arch* 2016;**468**:1527–40.
45. Liedtke W, Choe Y, Marti-Renom MA, Bell AM, Denis CS, Sali A, et al. Vanilloid receptor-related osmotically activated channel (VR-OAC), a candidate vertebrate osmoreceptor. *Cell* 2000;**103**:525–35.
46. Ye L, Kleiner S, Wu J, Sah R, Gupta RK, Banks AS, et al. TRPV4 is a regulator of adipose oxidative metabolism, inflammation, and energy homeostasis. *Cell* 2012;**151**:96–110.
47. Vizin RC, Scarpellini Cda S, Ishikawa DT, Correa GM, de Souza CO, Gargaglioni LH, et al. TRPV4 activates autonomic and behavioural warmth-defence responses in Wistar rats. *Acta Physiol* 2015;**214**:275–89.
48. Voets T, Owsianik G, Janssens A, Talavera K, Nilius B. TRPM8 voltage sensor mutants reveal a mechanism for integrating thermal and chemical stimuli. *Nat Chem Biol* 2007;**3**:174–82.
49. Colburn RW, Lubin ML, Stone Jr. DJ, Wang Y, Lawrence D, D'Andrea MR, et al. Attenuated cold sensitivity in TRPM8 null mice. *Neuron* 2007;**54**:379–86.
50. Bautista DM, Siemens J, Glazer JM, Tsuruda PR, Basbaum AI, Stucky CL, et al. The menthol receptor TRPM8 is the principal detector of environmental cold. *Nature* 2007;**448**:204–8.
51. Masamoto Y, Kawabata F, Fushiki T. Intragastric administration of TRPV1, TRPV3, TRPM8, and TRPA1 agonists modulates autonomic thermoregulation in different manners in mice. *Biosci Biotechnol Biochem* 2009;**73**:1021–7.
52. Ma S, Yu H, Zhao Z, Luo Z, Chen J, Ni Y, et al. Activation of the cold-sensing TRPM8 channel triggers UCP1-dependent thermogenesis and prevents obesity. *J Mol Cell Biol* 2012;**4**:88–96.
53. Evans RM, Mangelsdorf DJ. Nuclear receptors, RXR, and the big bang. *Cell* 2014;**157**:255–66.
54. van Bilsen M, van der Vusse GJ, Gilde AJ, Lindhout M, van der Lee KA. Peroxisome proliferator-activated receptors: lipid binding proteins controlling gene expression. *Mol Cell Biochem* 2002;**239**:131–8.
55. Roberts LD, Murray AJ, Menassa D, Ashmore T, Nicholls AW, Griffin JL. The contrasting roles of PPAR δ and PPAR γ in regulating

- the metabolic switch between oxidation and storage of fats in white adipose tissue. *Genome Biol* 2011;**12**:R75.
56. Castelein H, Gulick T, Declercq PE, Mannaerts GP, Moore DD, Baes MI. The peroxisome proliferator activated receptor regulates malic enzyme gene expression. *J Biol Chem* 1994;**269**:26754–8.
 57. Wilson-Fritch L, Burkart A, Bell G, Mendelson K, Leszyk J, Nicoloro S, et al. Mitochondrial biogenesis and remodeling during adipogenesis and in response to the insulin sensitizer rosiglitazone. *Mol Cell Biol* 2003;**23**:1085–94.
 58. Elabd C, Chiellini C, Carmona M, Galitzky J, Cochet O, Petersen R, et al. Human multipotent adipose-derived stem cells differentiate into functional brown adipocytes. *Stem Cells* 2009;**27**:2753–60.
 59. Petrovic N, Shabalina IG, Timmons JA, Cannon B, Nedergaard J. Thermogenically competent nonadrenergic recruitment in brown preadipocytes by a PPAR γ agonist. *Am J Physiol Endocrinol Metab* 2008;**295**:E287–96.
 60. Festuccia WT, Blanchard PG, Richard D, Deshaies Y. Basal adrenergic tone is required for maximal stimulation of rat brown adipose tissue UCP1 expression by chronic PPAR- γ activation. *Am J Physiol Regul Integr Comp Physiol* 2010;**299**:R159–67.
 61. Festuccia WT, Blanchard PG, Oliveira TB, Magdalon J, Paschoal VA, Richard D, et al. PPAR γ activation attenuates cold-induced upregulation of thyroid status and brown adipose tissue PGC-1 α and D2. *Am J Physiol Regul Integr Comp Physiol* 2012;**303**:R1277–85.
 62. Bogacka I, Xie H, Bray GA, Smith SR. Pioglitazone induces mitochondrial biogenesis in human subcutaneous adipose tissue *in vivo*. *Diabetes* 2005;**54**:1392–9.
 63. Chen HY, Liu Q, Salter AM, Lomax MA. Synergism between cAMP and PPAR γ signalling in the initiation of UCP1 gene expression in HIB1B brown adipocytes. *PPAR Res* 2013;**2013**:476049.
 64. Dutchak PA, Katafuchi T, Bookout AL, Choi JH, Yu RT, Mangelsdorf DJ, et al. Fibroblast growth factor-21 regulates PPAR γ activity and the antidiabetic actions of thiazolidinediones. *Cell* 2012;**148**:556–67.
 65. Zhang Z, Zhang H, Li B, Meng X, Wang J, Zhang Y, et al. Berberine activates thermogenesis in white and brown adipose tissue. *Nat Commun* 2014;**5**:5493.
 66. Rigano D, Sirignano C, Tagliatela-Scafati O. The potential of natural products for targeting PPAR α . *Acta Pharm Sin B* 2017;**7**:427–38.
 67. Barbera MJ, Schluter A, Pedraza N, Iglesias R, Villarroya F, Giralt M. Peroxisome proliferator-activated receptor α activates transcription of the brown fat uncoupling protein-1 gene. A link between regulation of the thermogenic and lipid oxidation pathways in the brown fat cell. *J Biol Chem* 2001;**276**:1486–93.
 68. Lee JY, Hashizaki H, Goto T, Sakamoto T, Takahashi N, Kawada T. Activation of peroxisome proliferator-activated receptor- α enhances fatty acid oxidation in human adipocytes. *Biochem Biophys Res Commun* 2011;**407**:818–22.
 69. Ribet C, Montastier E, Valle C, Bezaire V, Mazzucotelli A, Mairal A, et al. Peroxisome proliferator-activated receptor- α control of lipid and glucose metabolism in human white adipocytes. *Endocrinology* 2010;**151**:123–33.
 70. Hondares E, Rosell M, Diaz-Delfin J, Olmos Y, Monsalve M, Iglesias R, et al. Peroxisome proliferator-activated receptor α (PPAR α) induces PPAR γ coactivator 1 α (PGC-1 α) gene expression and contributes to thermogenic activation of brown fat: involvement of PRDM16. *J Biol Chem* 2011;**286**:43112–22.
 71. Suarez J, Rivera P, Arrabal S, Crespillo A, Serrano A, Baixeras E, et al. Oleoylethanolamide enhances β -adrenergic-mediated thermogenesis and white-to-brown adipocyte phenotype in epididymal white adipose tissue in rat. *Dis Model Mec* 2014;**7**:129–41.
 72. Schug TT, Berry DC, Shaw NS, Travis SN, Noy N. Opposing effects of retinoic acid on cell growth result from alternate activation of two different nuclear receptors. *Cell* 2007;**129**:723–33.
 73. Berry DC, Noy N. All-*trans*-retinoic acid represses obesity and insulin resistance by activating both peroxisome proliferation-activated receptor β/δ and retinoic acid receptor. *Mol Cell Biol* 2009;**29**:3286–96.
 74. Juvet LK, Andresen SM, Schuster GU, Dalen KT, Tobin KA, Hollung K, et al. On the role of liver X receptors in lipid accumulation in adipocytes. *Mol Endocrinol* 2003;**17**:172–82.
 75. Seo JB, Moon HM, Kim WS, Lee YS, Jeong HW, Yoo EJ, et al. Activated liver X receptors stimulate adipocyte differentiation through induction of peroxisome proliferator-activated receptor γ expression. *Mol Cell Biol* 2004;**24**:3430–44.
 76. Hummasti S, Laffitte BA, Watson MA, Galardi C, Chao LC, Ramamurthy L, et al. Liver X receptors are regulators of adipocyte gene expression but not differentiation: identification of apoD as a direct target. *J Lipid Res* 2004;**45**:616–25.
 77. Zheng F, Zhang S, Lu W, Wu F, Yin X, Yu D, et al. Regulation of insulin resistance and adiponectin signaling in adipose tissue by liver X receptor activation highlights a cross-talk with PPAR γ . *PLoS One* 2014;**9**:e101269.
 78. Gu M, Zhang Y, Liu C, Wang D, Feng L, Fan S, et al. Morin, a novel liver X receptor α/β dual antagonist, has potent therapeutic efficacy for nonalcoholic fatty liver diseases. *Br J Pharmacol* 2017;**174**:3032–44.
 79. Shu L, Hoo RL, Wu X, Pan Y, Lee IP, Cheong LY, et al. A-FABP mediates adaptive thermogenesis by promoting intracellular activation of thyroid hormones in brown adipocytes. *Nat Commun* 2017;**8**:14147.
 80. Korach-Andre M, Archer A, Barros RP, Parini P, Gustafsson JA. Both liver-X receptor (LXR) isoforms control energy expenditure by regulating brown adipose tissue activity. *Proc Natl Acad Sci U S A* 2011;**108**:403–8.
 81. Stenson BM, Ryden M, Steffensen KR, Wahlen K, Pettersson AT, Jocken JW, et al. Activation of liver X receptor regulates substrate oxidation in white adipocytes. *Endocrinology* 2009;**150**:4104–13.
 82. Dib L, Bugge A, Collins S. LXR α fuels fatty acid-stimulated oxygen consumption in white adipocytes. *J Lipid Res* 2014;**55**:247–57.
 83. Sheng X, Zhu X, Zhang Y, Cui G, Peng L, Lu X, et al. Rhein protects against obesity and related metabolic disorders through liver X receptor-mediated uncoupling protein 1 upregulation in brown adipose tissue. *Int J Biol Sci* 2012;**8**:1375–84.
 84. Sun H, Luo G, Chen D, Xiang Z. A comprehensive and system review for the pharmacological mechanism of action of rhein, an active anthraquinone ingredient. *Front Pharmacol* 2016;**7**:247.
 85. Rabelo R, Reyes C, Schiffman A, Silva JE. A complex retinoic acid response element in the uncoupling protein gene defines a novel role for retinoids in thermogenesis. *Endocrinology* 1996;**137**:3488–96.
 86. Alvarez R, de Andres J, Yubero P, Vinas O, Mampel T, Iglesias R, et al. A novel regulatory pathway of brown fat thermogenesis. retinoic acid is a transcriptional activator of the mitochondrial uncoupling protein gene. *J Biol Chem* 1995;**270**:5666–73.
 87. Puigserver P, Vazquez F, Bonet ML, Pico C, Palou A. *In vitro* and *in vivo* induction of brown adipocyte uncoupling protein (thermogenin) by retinoic acid. *Biochem J* 1996;**317**:827–33.
 88. Kumar MV, Sunvold GD, Scarpace PJ. Dietary vitamin A supplementation in rats: suppression of leptin and induction of UCP1 mRNA. *J Lipid Res* 1999;**40**:824–9.
 89. Bonet ML, Oliver J, Pico C, Felipe F, Ribot J, Cinti S, et al. Opposite effects of feeding a vitamin A-deficient diet and retinoic acid treatment on brown adipose tissue uncoupling protein 1 (UCP1), UCP2 and leptin expression. *J Endocrinol* 2000;**166**:511–7.
 90. Alvarez R, Checa M, Brun S, Vinas O, Mampel T, Iglesias R, et al. Both retinoic-acid-receptor- and retinoid-X-receptor-dependent signalling pathways mediate the induction of the brown-adipose-tissue-uncoupling-protein-1 gene by retinoids. *Biochem J* 2000;**345**:91–7.
 91. Teruel T, Hernandez R, Benito M, Lorenzo M. Rosiglitazone and retinoic acid induce uncoupling protein-1 (UCP-1) in a p38 mitogen-activated protein kinase-dependent manner in fetal primary brown adipocytes. *J Biol Chem* 2003;**278**:263–9.
 92. Berry DC, DeSantis D, Soltanian H, Croniger CM, Noy N. Retinoic acid upregulates preadipocyte genes to block adipogenesis and suppress diet-induced obesity. *Diabetes* 2012;**61**:1112–21.

93. McIlroy GD, Tammireddy SR, Maskrey BH, Grant L, Doherty MK, Watson DG, et al. Fenretinide mediated retinoic acid receptor signalling and inhibition of ceramide biosynthesis regulates adipogenesis, lipid accumulation, mitochondrial function and nutrient stress signalling in adipocytes and adipose tissue. *Biochem Pharmacol* 2016;**100**:86–97.
94. Murholm M, Isidor MS, Basse AL, Winther S, Sorensen C, Skovgaard-Petersen J, et al. Retinoic acid has different effects on UCP1 expression in mouse and human adipocytes. *BMC Cell Biol* 2013;**14**:41.
95. Kamei Y, Kawada T, Kazuki R, Ono T, Kato S, Sugimoto E. Vitamin D receptor gene expression is up-regulated by 1,25-dihydroxyvitamin D3 in 3T3-L1 preadipocytes. *Biochem Biophys Res Commun* 1993;**193**:948–55.
96. Fu M, Sun T, Bookout AL, Downes M, Yu RT, Evans RM, et al. A nuclear receptor atlas: 3T3-L1 adipogenesis. *Mol Endocrinol* 2005;**19**:2437–50.
97. Ricciardi CJ, Bae J, Esposito D, Komarnytsky S, Hu P, Chen J, et al. 1,25-Dihydroxyvitamin D3/vitamin D receptor suppresses brown adipocyte differentiation and mitochondrial respiration. *Eur J Nutr* 2015;**54**:1001–12.
98. Narvaez CJ, Matthews D, Broun E, Chan M, Welsh J. Lean phenotype and resistance to diet-induced obesity in vitamin D receptor knockout mice correlates with induction of uncoupling protein-1 in white adipose tissue. *Endocrinology* 2009;**150**:651–61.
99. Wong KE, Szeto FL, Zhang W, Ye H, Kong J, Zhang Z, et al. Involvement of the vitamin D receptor in energy metabolism: regulation of uncoupling proteins. *Am J Physiol Endocrinol Metab* 2009;**296**:E820–8.
100. Wong KE, Kong J, Zhang W, Szeto FL, Ye H, Deb DK, et al. Targeted expression of human vitamin D receptor in adipocytes decreases energy expenditure and induces obesity in mice. *J Biol Chem* 2011;**286**:33804–10.
101. Bhat M, Noolu B, Qadri SS, Ismail A. Vitamin D deficiency decreases adiposity in rats and causes altered expression of uncoupling proteins and steroid receptor coactivator3. *J Steroid Biochem Mol Biol* 2014;**144**:304–12.
102. Malloy PJ, Feldman BJ. Cell-autonomous regulation of brown fat identity gene UCP1 by unliganded vitamin D receptor. *Mol Endocrinol* 2013;**27**:1632–42.
103. Brewer CT, Chen T. PXR variants: the impact on drug metabolism and therapeutic responses. *Acta Pharm Sin B* 2016;**6**:441–9.
104. He J, Gao J, Xu M, Ren S, Stefanovic-Racic M, O'Doherty RM, et al. PXR ablation alleviates diet-induced and genetic obesity and insulin resistance in mice. *Diabetes* 2013;**62**:1876–87.
105. Ma Y, Liu D. Activation of pregnane X receptor by pregnenolone 16 alpha-carbonitrile prevents high-fat diet-induced obesity in AKR/J mice. *PLoS One* 2012;**7**:e38734.
106. Kuiper GG, Lemmen JG, Carlsson B, Corton JC, Safe SH, van der Saag PT, et al. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor β . *Endocrinology* 1998;**139**:4252–63.
107. Rodriguez-Cuenca S, Monjo M, Gianotti M, Proenza AM, Roca P. Expression of mitochondrial biogenesis-signaling factors in brown adipocytes is influenced specifically by 17 β -estradiol, testosterone, and progesterone. *Am J Physiol Endocrinol Metab* 2007;**292**:E340–6.
108. Moreno AJ, Moreira PI, Custodio JB, Santos MS. Mechanism of inhibition of mitochondrial ATP synthase by 17 β -estradiol. *J Bioenerg Biomembr* 2013;**45**:261–70.
109. Shughrue PJ, Lane MV, Merchenthaler I. Comparative distribution of estrogen receptor- α and - β mRNA in the rat central nervous system. *J Comp Neurol* 1997;**388**:507–25.
110. Martinez de Morentin PB, Gonzalez-Garcia I, Martins L, Lage R, Fernandez-Mallo D, Martinez-Sanchez N, et al. Estradiol regulates brown adipose tissue thermogenesis via hypothalamic AMPK. *Cell Metab* 2014;**20**:41–53.
111. Grefhorst A, van den Beukel JC, van Houten EL, Steenbergen J, Visser JA, Themmen AP. Estrogens increase expression of bone morphogenetic protein 8b in brown adipose tissue of mice. *Biol Sex Differ* 2015;**6**:7.
112. Miao YF, Su W, Dai YB, Wu WF, Huang B, Barros RP, et al. An ER β agonist induces browning of subcutaneous abdominal fat pad in obese female mice. *Sci Rep* 2016;**6**:38579.
113. Clarke SD, Clarke IJ, Rao A, Evans RG, Henry BA. Differential effects of acute and chronic estrogen treatment on thermogenic and metabolic pathways in ovariectomized sheep. *Endocrinology* 2013;**154**:184–92.
114. Ayala JE, Bracy DP, Julien BM, Rottman JN, Fueger PT, Wasserman DH. Chronic treatment with sildenafil improves energy balance and insulin action in high fat-fed conscious mice. *Diabetes* 2007;**56**:1025–33.
115. Mitschke MM, Hoffmann LS, Gnad T, Scholz D, Kruithoff K, Mayer P, et al. Increased cGMP promotes healthy expansion and browning of white adipose tissue. *FASEB J* 2013;**27**:1621–30.
116. Cassola P, Uchoa ET, Mansur Machado FS, Guimaraes JB, Rissato Garofalo MA, de Almeida Brito N, et al. The central administration of C75, a fatty acid synthase inhibitor, activates sympathetic outflow and thermogenesis in interscapular brown adipose tissue. *Pflugers Arch* 2013;**465**:1687–99.
117. Shen W, Chuang CC, Martinez K, Reid T, Brown JM, Xi L, et al. Conjugated linoleic acid reduces adiposity and increases markers of browning and inflammation in white adipose tissue of mice. *J Lipid Res* 2013;**54**:909–22.
118. Shen W, Baldwin J, Collins B, Hixson L, Lee KT, Herberg T, et al. Low level of trans-10, cis-12 conjugated linoleic acid decreases adiposity and increases browning independent of inflammatory signaling in overweight Sv129 mice. *J Nutr Biochem* 2015;**26**:616–25.
119. Lynes MD, Leiria LO, Lundh M, Bartelt A, Shamsi F, Huang TL, et al. The cold-induced lipokine 12,13-diHOME promotes fatty acid transport into brown adipose tissue. *Nat Med* 2017;**23**:631–7.
120. Batubara I, Suparto IH, Sa'diah S, Matsuoka R, Mitsunaga T. Effects of inhaled citronella oil and related compounds on rat body weight and brown adipose tissue sympathetic nerve. *Nutrients* 2015;**7**:1859–70.
121. Batubara I, Suparto IH, Sa'diah S, Matsuoka R, Mitsunaga T. Effect of *Zingiber zerumbet* essential oils and zerumbone inhalation on body weight of Sprague Dawley rat. *Pak J Biol Sci* 2013;**16**:1028–33.
122. Sugimoto S, Nakajima H, Kodo K, Mori J, Matsuo K, Kosaka K, et al. Miglitol increases energy expenditure by upregulating uncoupling protein 1 of brown adipose tissue and reduces obesity in dietary-induced obese mice. *Nutr Metab* 2014;**11**:14.
123. Haratake A, Watase D, Setoguchi S, Terada K, Matsunaga K, Takata J. Relationship between the acyl chain length of paradol analogues and their antiobesity activity following oral ingestion. *J Agric Food Chem* 2014;**62**:6166–74.
124. Galmozzi A, Sonne SB, Altshuler-Keylin S, Hasegawa Y, Shinoda K, Luijten IH, et al. ThermoMouse: an *in vivo* model to identify modulators of UCP1 expression in brown adipose tissue. *Cell Rep* 2014;**9**:1584–93.
125. Andrade JM, Frade AC, Guimaraes JB, Freitas KM, Lopes MT, Guimaraes AL, et al. Resveratrol increases brown adipose tissue thermogenesis markers by increasing SIRT1 and energy expenditure and decreasing fat accumulation in adipose tissue of mice fed a standard diet. *Eur J Nutr* 2014;**53**:1503–10.
126. Alberdi G, Rodriguez VM, Miranda J, Macarulla MT, Churrua I, Portillo MP. Thermogenesis is involved in the body-fat lowering effects of resveratrol in rats. *Food Chem* 2013;**141**:1530–5.
127. Kopecky J, Clarke G, Enerback S, Spiegelman B, Kozak LP. Expression of the mitochondrial uncoupling protein gene from the α P2 gene promoter prevents genetic obesity. *J Clin Invest* 1995;**96**:2914–23.
128. Fu YY, Zhang M, Turner N, Zhang LN, Dong TC, Gu M, et al. A novel chemical uncoupler ameliorates obesity and related phenotypes in mice with diet-induced obesity by modulating energy expenditure and food intake. *Diabetologia* 2013;**56**:2297–307.
129. Affourtit C, Crichton PG, Parker N, Brand MD. Novel uncoupling proteins. *Novartis Found Symp* 2007;**287**:70–91.

130. Echtay KS. Mitochondrial uncoupling proteins—what is their physiological role?. *Free Radic Biol Med* 2007;**43**:1351–71.
131. Diano S, Horvath TL. Mitochondrial uncoupling protein 2 (UCP2) in glucose and lipid metabolism. *Trends Mol Med* 2012;**18**:52–8.
132. Kazak L, Chouchani ET, Jedrychowski MP, Erickson BK, Shinoda K, Cohen P, et al. A creatine-driven substrate cycle enhances energy expenditure and thermogenesis in beige fat. *Cell* 2015;**163**:643–55.
133. Ikeda K, Kang Q, Yoneshiro T, Camporez JP, Maki H, Homma M, et al. UCP1-independent signaling involving SERCA2b-mediated calcium cycling regulates beige fat thermogenesis and systemic glucose homeostasis. *Nat Med* 2017;**23**:1454–65.
134. Ukropec J, Anunciado RP, Ravussin Y, Hulver MW, Kozak LP. UCP1-independent thermogenesis in white adipose tissue of cold-acclimated *Ucp1*^{−/−} mice. *J Biol Chem* 2006;**281**:31894–908.
135. Granneman JG, Burnazi M, Zhu Z, Schwamb LA. White adipose tissue contributes to UCP1-independent thermogenesis. *Am J Physiol Endocrinol Metab* 2003;**285**:E1230–6.
136. Sidossis L, Kajimura S. Brown and beige fat in humans: thermogenic adipocytes that control energy and glucose homeostasis. *J Clin Invest* 2015;**125**:478–86.
137. Saito M, Okamatsu-Ogura Y, Matsushita M, Watanabe K, Yoneshiro T, Nio-Kobayashi J, 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.
138. Abdullahi A, Jeschke MG. White adipose tissue browning: a double-edged sword. *Trends Endocrinol Metab* 2016;**27**:542–52.
139. Lu S, Jang H, Gu S, Zhang J, Nussinov R. Drugging Ras GTPase: a comprehensive mechanistic and signaling structural view. *Chem Soc Rev* 2016;**45**:4929–52.
140. Shen Q, Cheng F, Song H, Lu W, Zhao J, An X, et al. Proteome-scale investigation of protein allosteric regulation perturbed by somatic mutations in 7000 cancer genomes. *Am J Hum Genet* 2017;**100**:5–20.
141. Tsou RC, Zimmer DJ, De Jonghe BC, Bence KK. Deficiency of PTP1B in leptin receptor-expressing neurons leads to decreased body weight and adiposity in mice. *Endocrinology* 2012;**153**:4227–37.
142. Zabolotny JM, Bence-Hanulec KK, Stricker-Krongrad A, Haj F, Wang Y, Minokoshi Y, et al. PTP1B regulates leptin signal transduction *in vivo*. *Dev Cell* 2002;**2**:489–95.
143. Bence KK, Delibegovic M, Xue B, Gorgun CZ, Hotamisligil GS, Neel BG, et al. Neuronal PTP1B regulates body weight, adiposity and leptin action. *Nat Med* 2006;**12**:917–24.
144. Li S, Zhang J, Lu S, Huang W, Geng L, Shen Q, et al. The mechanism of allosteric inhibition of protein tyrosine phosphatase 1B. *PLoS One* 2014;**9**:e97668.
145. Huang W, Wang G, Shen Q, Liu X, Lu S, Geng L, et al. ASBench: benchmarking sets for allosteric discovery. *Bioinformatics* 2015;**31**:2598–600.
146. Yang T, Xiao T, Sun Q, Wang K. The current agonists and positive allosteric modulators of $\alpha 7$ nAChR for CNS indications in clinical trials. *Acta Pharm Sin B* 2017;**7**:611–22.