Small molecules for fat combustion: targeting obesity

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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

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

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

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1. Introduction

Overweight and obesity have reached epidemic proportions worldwide, for both children and adults. 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, atherosclerosis and several forms of cancer. 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. 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. 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). Non-shivering thermogenesis in brown and beige adipocytes has been recognized to play a crucial role in energy balance in rodents and humans. Thermogenic activity of brown and beige adipocytes is positively correlated with energy expenditure, and dysregulation of thermogenesis is linked to obesity in humans. Studies have disclosed that the “brown” fat in human adults is composed primarily of beige adipocytes. 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. 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. 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. 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. A clinic study showed treatment of CL316243 on healthy men enhanced fat oxidation and insulin-stimulated glucose disposal. 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. CDP-12177A is a β3-AR agonist, which enhanced uncoupling content in BAT and inguinal white adipose tissue (WAT) of NMRI mice. Arotinolon, a weak β3-AR agonist, stimulated oxygen consumption in brown adipocytes from hamsters or rats, but did not change thermogenesis in intact animals. 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.

Surprisingly, several other β3-AR agonists, including ZD7114, ZD2079 and TAK-677, didn’t change energy expenditure in obese humans. 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 agents, especially those obese patients, lacked brown and beige adipocytes, which led to
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

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**Table 1** Thermogenic regulators in clinical trials.

| Name                        | Identifier          | Condition                                      | Phase |
|-----------------------------|---------------------|-------------------------------------------------|-------|
| Propranolol                 | NCT03379181         | Hyperthyroidism                                 | 4     |
|                             | NCT01791114         | Insulin sensitivity, obesity                    | –     |
| Prednisone                  | NCT03269747         | BAT activity                                    | 4     |
| Fluavastatin                | 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.
the stimulatory receptors $A_{2A}$ and $A_{2B}$\textsuperscript{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 \textsuperscript{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 adipocytes\textsuperscript{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\textsuperscript{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\textsuperscript{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), $Pgc-1\alpha$ (peroxisome proliferator-activated receptor $\gamma$ coactivator-1$\alpha$), and $UCP1$, in both BAT and abdominal and subcutaneous WAT\textsuperscript{29}. Similarly, chenodeoxycholic acid was found to increase $UCP1$ expression and activate thermogenesis in human BAT\textsuperscript{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 1. 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 11. 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 12. 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, dihydrocapsiati, and nordihyrocapsiati, 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 13. In addition, administration of 4 mg/kg capsinoids for 1 month showed enhanced energy expenditure and decreased body weight 14. Another trial on healthy humans also showed that 8-week capsinoids treatment (9 mg/kg per day) increased BAT capacity using 18F-fluorodeoxyglucose positron emission tomography combined with computed tomography (PET-CT) 15. 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 therogenesis 12. Capsaicin, one principle constituent of hot pepper, was reported to enhance energy expenditure and fatty acid β-oxidation via stimulating TRPV1-SNS axis 16. Treatment with high dose of capsaicin (135 mg/day) for 3 months significantly increased fat oxidation without obvious adverse effect 17. 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 18. In 2011, monoacylglycerol was identified as a TRPV1 agonist, which increased UCP1 expression in BAT and prevented visceral fat accumulation in C57BL/6Cr mice 19. 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 20. A 12-week intervention with nonivamide, a TRPV1 agonist, prevented a dietary-induced body fat gain and increased peripheral serotonin in moderately overweight subjects 21. 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 22. 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 23. These results suggested that the role of TRPV2 in the treatment of obesity is still remaining elusive.

TRPV4 is highly expressed in adipocytes 24. 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 25. 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 26. In addition, it is notable intracerebroventricular treatment with RN-1747, a chemical selective agonist of TRPV4, did not cause hyperthermia. It indicated that the observed response was indeed due to activation of TRPV4 channels in the periphery 27.

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 28-30. Menthol or 1,8-cineole activates TRPM8 to trigger UCP1-induced non-shivering thermogenesis and locomotor activity 31,32. Allyl isothiocyanate and cinnamaldehyde were reported to enhance thermogenesis and inhibit heat diffusion in mice, through activating TRPA1 33.

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 34. 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) | ![Molecule](image) | TRPV1 | Humans | Abdominal fat loss<br>Enhance energy expenditure and decrease body weight<br>Increase BAT capacity<br>Enhance BAT thermogenesis and stimulate browning of WAT | 33, 34, 35, 36 |
| Capsaicin | ![Molecule](image) | | Humans | Enhance energy expenditure and fatty acid β-oxidation<br>Increase fat oxidation | 37, 38 |
| | | | | 3T3-L1, visceral adipose tissues from humans and wild-type and TRPV1-deficient mice | 39 |
| Monoacylglycerol | ![Molecule](image) | C57BL/6Cr mice | Increase UCP1 in BAT and prevent visceral fat accumulation | 40 |
| 10-Oxo-12(Z)-octadecenoic acid | ![Molecule](image) | Male C57BL/6 mice and KK-Ay mice | Enhance energy metabolism | 41 |
| Nonivamide | ![Molecule](image) | Overweight humans | Prevent a dietarily induced body fat gain and increase peripheral serotonin | 42 |
| 2-Aminoethoxydiphenyl borate | ![Molecule](image) | TRPV2 | Mouse brown adipocytes | Suppress differentiation | 44 |
| Lysophosphatidylcholine | ![Molecule](image) | | | | |
| GSK205 | ![Molecule](image) | TRPV4 | HFD treated C57BL/6J mice | Induce WAT browning | 46 |
| GSK1016790A | ![Molecule](image) | | | Repress thermogenic genes expression | 46 |
| HIC-067047 | ![Molecule](image) | Wistar rats | Increase core body temperature and oxygen consumption | 47 |
| RN-1734 | ![Molecule](image) | | | | |
| RN-1747 | ![Molecule](image) | | No effect | | |
| Menthol | ![Molecule](image) | TRPM8 | C57BL/6 mice | Trigger UCP1-induced non-shivering thermogenesis and locomotor activity | 51, 52 |
| 1,8-Cineole | ![Molecule](image) | | | | |
| Allyl isothiocyanate | ![Molecule](image) | TRPA1 | C57BL/6 mice | Enhance thermogenesis and inhibit heat diffusion | 51 |
| Cinamaldehyde | ![Molecule](image) | | | | |
4.1. Peroxisome proliferator-activated receptors (PPAR)

PPARs belong to nuclear receptor super family, and so far three PPAR isoforms have been identified. PPARδ and PPARγ are directly linked to thermogenesis. PPARγ has capacity to increase fat acid oxidation. 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.

Rosiglitazone (Table 4), a PPARγ agonist, was found to promote mitochondrial biogenesis in 3T3-L1 adipocytes, accompanied with increased thermogenesis capacity and browning character. In addition, chronic treatment of rosiglitazone to human multipotent adipose-derived stem cells showed lowered expression of the mitochondrial (Cox4i1, Cox4i2), thermogenic (FGF21, Prdm16) and fatty-acid β-oxidation related genes. It suggested PPARγ receptor agonists promote adipocyte remodeling in epidydimal 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 PPARα, participating in cell survival. RA activated PPARδ in preadipocytes and adipocytes to increase UCP1 and Aldh9 (aldehyde dehydrogenase 9), a key enzyme in fatty acid oxidation. It suggested activation of PPARδ shifts substrate oxidation towards combustion of lipids. In addition, administration of PPARδ selective agonist GW0742 (Table 4) effectively suppressed adiogenesis and enhanced lipolysis through AKT (protein kinase B) signaling pathway.

4.2. Liver X receptors (LXR)

LXRs play a vital role in bile acid synthesis, lipid and glucose homeostasis. LXRs present in two isofoms, LXRα and LXRβ. Both isoforms are expressed in mature murine and human adipocytes. 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). 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. It suggested that LXR suppression has a positive correlation with thermogenesis. Consistently, a report showed T0901317 (Table 4), a potent and selective LXRα agonist, has significant effect on suppression of Dio2 expression in primary brown adipocytes. Moreover, administration of LXRs agonist GW3965 repressed UCP1 in BAT and browning of subcutaneous WAT. 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. 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. Rhein is a multitarget molecule, which still need further investigation for pharmaceutical application as an anti-obesity agent.

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. 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       | ![Rosiglitazone](image) | PPARy    | 3T3-L1 adipocytes | Promote mitochondrial biogenesis, increase thermogenesis capacity and browning | 57   |
|                     |                    |          | Human multipotent adipose-derived stem cells | Increase UCP1 and CIDEB 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        | ![Pioglitazone](image) | Human subcutaneous WAT | Induce mitochondrial biogenesis and enhance PGC-1α and TFAM expression | 62   |
| Berberine           | ![Berberine](image) | db/db mice | Up-regulate fatty acid oxidation and heat production | 65   |
| GW7647              | ![GW7647](image)   | PPARα    | Primary human fat cells | Up-regulate β-oxidation genes and enhance palmitate oxidation | 68   |
| WY14,643            | ![WY14,643](image) | Male Sprague–Dawley rats | Induce thermogenic genes, mitochondrial genes, and lipid oxidation genes in brown fat | 70   |
| Oleoylethanolamide  | ![Oleoylethanolamide](image) | Male Sprague–Dawley rats | Enhance β3-adrenergic-mediated thermogenesis and browning in epididymal WAT | 71   |
| All-trans RA        | ![All-trans RA](image) | PPARδ    | C57BL/6Ntac mice | Increase Ucp1 and Aldh9 expression | 73   |
| GW0742              | ![GW0742](image)   | 3T3-L1 cells and C57BL/6Ntac mice | Suppress adipogenesis and enhance lipolysis | 73   |
| Morin               | ![Morin](image)    | 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   |
| Name                        | Receptor(s) | cells/tissue/strain | action/phenotype                                                                 |
|-----------------------------|-------------|---------------------|----------------------------------------------------------------------------------|
| TO901317                    | LXRα        | Primary brown adipocytes | Suppress Dio2 expression                                                          |
| GW3965                      | LXRα        | Female high-carbohydrate diet C57BL/6J mice | Decrease UCP1 expression                                                          |
| Rhein                       |             | Female C57BL/6J mice | Upregulate UCP1 and increase energy expenditure                                  |
| All-trans-RA or 9-cis-RA    | RAR and RXR | HIB1B brown adipocytes and male NMRI mice | Increase UCP1 content in BAT                                                       |
| Vitamin A                   | RXRα, RXRα  | Male F-344 X BN rats | Increase mitochondrial biogenesis and Ucp1 expression                              |
| p-[(E)-2-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthal enylyl-1-propenyl)benzoic acid | RAR | Primary brown adipocytes from Swiss mice | Increase Ucp1 expression                                                          |
| Methoprene                  |             |                     |                                                                                  |
| Femretinide                 | RAR         | 3T3-L1 cells and HFD-fed mice | Suppress differentiation and prevent obesity and insulin resistance |
| 1,25-Dihydroxyvitamin D3    | VDR         | Brown adipocytes | Suppress Ucp1 expression                                                          |
| 17β-Estradiol               | ERα         | Primary brown adipocytes | Promote mitochondrial biogenesis and thermogenesis                                 |
| Diethylstilbestrol          | ERα, ERβ    | C57BL/6J mice       | Increase Bmp8β and Fgf family genes in BAT                                         |
UCP1 gene expression through a RA-responsive region but independent of adrenergic pathway. Puigserver et al. 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. 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. In addition, adipocytes from humans with hereditary vitamin D resistant rickets showed increased UCP1 expression and a browning phenotype. 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.

4.4. 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. The hormonal form of vitamin D, 1,25-dihydroxy vitamin D3 (Table 4), suppressed the expression of UCP1 and vitamin D or VDR deficiency decreased adiposity and increased UCP1 in rodents. In addition, adipocytes from humans with hereditary vitamin D resistant rickets showed increased UCP1 expression and a browning phenotype. 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. Pregnen 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. PXR activation is associated with thermogenesis. Pregnenolone 16α-carbonitrile (Table 4) enhanced thermogenesis by induction the mRNA expression of Dio2, PGC-1α, Pgc-1β and Cidea in BAT of mice. 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. A decade ago, 17β-estradiol was found to promote mitochondrial biogenesis and thermogenic function in primary brown adipocytes. It is notable that 17β-estradiol negatively modulated the ATP synthase activity through direct binding to the oligomycin sensitive-conferring protein, which may result in decreasing of mitochondrial ATP generation. ERs are highly expressed in the hypothalamus and estradiol upregulated BAT thermogenesis via hypothalamic AMPK. The 3-day-treatment with a selective ERβ agonist, LY3201 (Table 4), induced browning of subcutaneous abdominal fat pad in obese female mice. Consistently, acute 17β-estradiol or estradiol benzoate (Table 4) treatment...
led to thermogenesis in female sheep but not chronic estrogen treatment\textsuperscript{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.

| Name                     | Molecule | Receptor | Object               | Mechanism                                                                 | Ref. |
|--------------------------|----------|----------|----------------------|---------------------------------------------------------------------------|------|
| Sildenafil               | ![Image] | PDEs     | C57BL/6J mice        | Increase energy expenditure and UCP1 level                                | 114  |
| C75                      | ![Image] | FAS      | Male Wistar rats     | Increase UCP1 and PGC-1α expressions in WAT                                | 115  |
| trans-10, cis-12 CLA     | ![Image] |          | Sv129 mice           | Activate sympathetic outflow and thermogenesis in BAT                      | 116  |
| 12,13-DiHOME             | ![Image] | 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 | ![Image] |          | Male SD rats | Increase BAT temperature                                                    | 120  |
| Zerumbone                | ![Image] |          | Male SD rats | Increase BAT temperature                                                    | 121  |
| Miglitol                 | ![Image] |          | Male C57BL/6J mice   | Increase energy expenditure by upregulating UCP1 in BAT                    | 122  |
| Paradol analogues        | ![Image] | SNS      | C57BL/6J mice        | Increase energy expenditure                                                | 123  |
| WWL113U                  | ![Image] |          | Male C57BL/6J mice   | Increase the content of UCP1 in BAT                                       | 124  |
| Resveratrol              | ![Image] | SIRT1    | Male SD rats         | Increase energy expenditure and expressions of thermogenic markers         | 125  |
|                          |          |          | Male C57BL/6J mice   |                                                                           | 126  |
|                          |          |          | independent pathway  |                                                                           |      |
| CZ5                      | ![Image] | Mitochondrial uncoupler | L6 myotubes, 3T3-L1 adipocytes and rat primary hepatocytes | Elevate energy expenditure                                                | 128  |

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Not known
5. Miscellaneous

Some other small molecules have also been reported to induce thermogenesis (Table 5). Prostaglandins (PG) and adenosine monophosphate (cAMP). Chronic treatment with sildenafil, a PDE-5 inhibitor, resulted in increased energy expenditure. Surprisingly, the UCP1 level was significantly lower in BAT from sildenafil-treated mice. 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. 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.

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. 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 CD36. 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.

In another study, the major component of Zingiber zerumbet, zerumbone, was found to enhance sympathetic nerve activity and temperature in BAT. 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 β3-adrenergic signaling. However, it needs further investigation to fully elucidate the thermogenic effect of miglitol. Another study showed paradox analogues increased energy metabolism in the BAT via the activation of sympathetic nerve activity; and the length of the acyl chain of the paradox analogues had a significant impact on the extent of UCP1 expression level. 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. Resveratrol increased energy expenditure and the expression of thermogenic markers through activating SIRT1 (silent mating type information regulation 2 homolog 1). However, another group reported resveratrol induced thermogenesis through SIRT1 independent pathway. 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. A novel chemical uncoupler, CZS, was found to elevate energy expenditure without change UCP1 level.

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. 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. 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 and calcium cycle. UCP1 knockdown animals was found to be acclimated to cold temperature and WAT contributes to UCP1-independent thermogenesis. Evidences have showed that beige cells have higher respiratory capacity than brown adipocytes, which are supposed to occupy high level of UCP1. In addition, beige cells express beige-selective marker, TMEM26 (transmembrane protein 26), CD137, and other thermogenic markers including mitochondrial genes Cox7a1 and Cox8b, transcriptional corregulatory PRDM16 and PGC-1α, and the thermogenic hormone FGF21. Using a brown adipocyte culture system, PPAR activation was found to represent a nonadrenergic, potent, and fully competent mechanism for BAT recruitment. 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. 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. 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. 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. 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. 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. Either a whole-body or whole-brain deletion of PTP1B causes lean, leptin-hypersensitive and resistant to HFD-induced obesity in mice. 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. 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. 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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