

Non-shivering thermogenesis and its current advances in clinical trials targeting obesity

Zhiyu Wu *

McMaster University, 123 Royal Ave, Hamilton ON

* Corresponding author. Email: Wuz140@mcmaster.ca

Abstract. Obesity is a major risk factor for adverse cardiometabolic events such as diabetes and cardiovascular diseases. Cardiometabolic diseases are the number one cause of death globally. Despite being the leading cause of death, many therapeutics targeted at its risk factors such as obesity have limited effectiveness. This limited effectiveness warrants research into novel strategies to combat obesity. Past literature established an inverse relationship between obesity and thermogenic activity. Research in thermogenesis has made unprecedented progress in the past decade. Based on this progress, thermogenesis has been proposed as a novel target for treating obesity. Thermogenesis is targeted due to its ability to expend excess energy such as fat in the form of heat. This conversion from fat to heat is mostly done by brown and brite adipocytes in brown adipose tissue (BAT). This review presents current advances in clinical trials related to the therapeutic application of non-shivering thermogenesis. Each clinical trial topic is highlighted and summarized. This paper summarized sympathetic nervous system activation (cold-induced, pharmacologically activated, and thyroid hormones), and transient receptor potential (TRP) channels on non-shivering thermogenesis. Advanced knowledge in non-shivering thermogenesis allows researchers to harness its vast therapeutic potential to combat obesity.

Keywords: Thermogenesis, obesity, cardiometabolic.

1. Introduction

Obesity is the top risk factor for many complex and fatal diseases such as stroke, diabetes, and heart attack. Stroke, diabetes, and heart attack are conditions that categorize under cardiometabolic diseases (CMD) [1]. Obesity directly contributes to CMD pathogenesis and is primarily caused by an unhealthy lifestyle, which includes risk factors like smoking, alcohol consumption, physical inactivity, and an unhealthy diet [2]. Treatment options for obesity such as lipase inhibitors and appetite suppressors are often ineffective [3]. Finding a treatment target that can effectively prevent and treat obesity is much needed. Recent studies have begun exploring thermogenesis as a potential therapeutic target for obesity.

Thermogenesis is defined as the production of heat, and it is commonly observed in endotherms [4]. In humans, thermogenesis plays an important role in metabolic homeostasis by dissipating excess energy in the form of heat. This process occurs in specialized tissue such as the brown adipose tissue and skeletal muscles [5]. There are two main forms of thermogenesis: shivering thermogenesis (ST) and non-shivering thermogenesis (NST). ST is primarily activated upon cold exposure. Cold temperatures can lead to a drop in body temperature, stressing vital organs. To protect vital organs, cold exposure leads to involuntary skeletal muscle contraction, generating heat through exothermic biochemical reactions to raise the body's core temperature to counteract the cold [6]. On the other hand, non-shivering thermogenesis (NST) is regulated by the sympathetic nervous system (SNS) and mainly occurs in brown adipose tissue (BAT). NST can be meal or cold-induced, also known as meal-induced thermogenesis (MIT) and cold-induced thermogenesis (CIT) respectively. MIT is activated upon feeding; this activates changes to the SNS and BAT generating heat. CIT is activated upon cold exposure, stressing the SNS and increasing norepinephrine (NE) activity, activating BAT, and producing heat [7].

In the past decade, scientists have made remarkable progress in understanding NST in both mice and humans. Understanding how NST behaves in humans is critical for researching its therapeutic application as ST is uncomfortable and inefficient. This review presents a summary of clinical trials

in the past decade exploring the relationship between obesity and NST. This review hopes to identify potential NST pathways that can be targeted for developing future therapies for obesity and metabolic syndrome.

2. Sympathetic Nervous System (SNS) Stimulation Activates Thermogenesis

2.1. Cold stimulation

Upon cold stimulation, the cold sensitive thermoreceptors send an afferent signal to the hypothalamus and brain stem, activating the SNS. After receiving the signal, post ganglionic sympathetic nerves release NE to activate beta 3 adrenergic receptors (B3AR). B3ARs are highly expressed on brown adipocytes. B3AR is coupled to g-protein receptors, thereby activating adenylyl cyclase (AC). AC activation promotes an increase in cyclic AMP (cAMP) and activates the protein kinase A (PKA). PKA activates the MAPK pathway and increases the activities of several enzymes like adipose triglyceride lipase (ATGL), hormone-sensitive lipase (HSL), monoglyceride lipase (MGL). MGL hydrolyze triglycerides and release free fatty acids (FFAs) into the circulation. Lipolysis is activated in white adipose tissue (WAT) and thermogenesis is activated in brown and beige adipocytes [8]. In brown and beige adipocytes, FFAs are transported into the mitochondria through the carnitine palmitoyl transferase 1A (CPT1A), where it is used to fuel thermogenesis [9]. NST is facilitated by uncoupling protein 1 (UCP1) (Figure 1), also known as thermogenin. An increase in FFA concentration leads to an increase in proton conductance through UCP1 [10]. More proton flux across the inner mitochondrial membrane results in less ATP synthesis and more dissipation of energy in the form of heat [9].

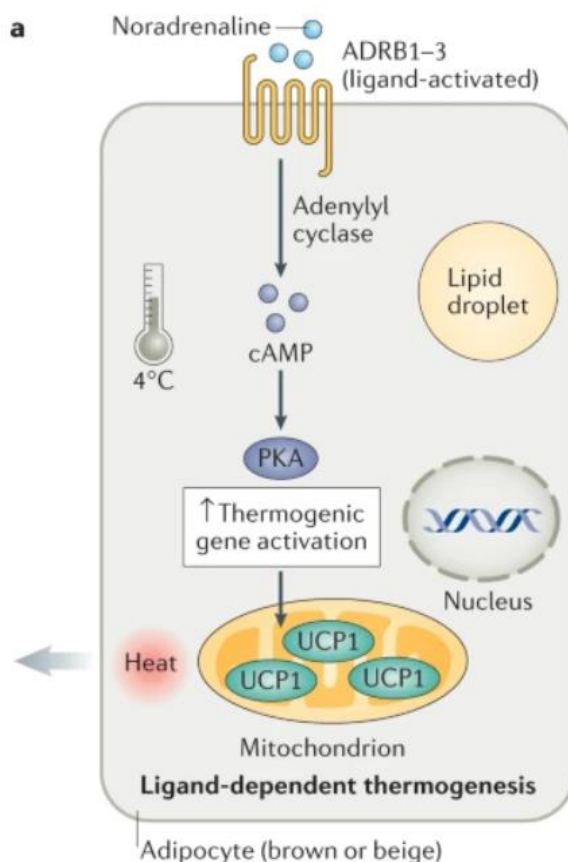


Figure 1. Simplified figure of cold-induced thermogenesis [11].

To confirm the effects of cold exposure on BAT activation, a study examined participants at room temperature and with acute cold exposure. Radiotracers were given to each participant to track BAT oxygen consumption, blood flow and fatty acid uptake with positron emission tomography-computed

tomography (PET-CT) scan. Indirect calorimetry was used to measure energy expenditure in both temperature conditions. Results show that BAT is a minor contributor while deep muscles are a major contributor to cold-induced NST [12]. To use cold exposure to induce NST, one current clinical trial is attempting to study the effect of BAT activation on insulin sensitivity. The researchers are using PET-CT to measure BAT activity, and the result of this study is not yet available [13]. However, it does show current progress in research where scientists are exploring the use of NST as new therapeutic targets to prevent obesity and metabolic syndrome.

2.2. Pharmacologically induced

As previously discussed, the activation of B3AR ultimately leads to thermogenesis activation. (Figure 1) Many studies have attempted to use B3AR agonist drugs to pharmacologically induce thermogenesis to combat obesity. Despite numerous attempts, there is yet to be an approved drug targeting obesity and metabolic syndrome. This failure is largely attributed to factors including poor oral bioavailability, and cross reactivity with beta 1 AR resulting in undesirable cardiovascular events [14].

Several studies (NCT03012113, NCT02354807) are exploring the effect of a B3AR drug – Mirabegron- and its therapeutic potential for obesity. A study (NCT02354807) investigated the use of Mirabegron, a drug approved to treat overactive bladder, to stimulate BAT activity. The groups were divided into 200mg dose of Mirabegron and placebo. Results were measured using 18F-fluorodeoxyglucose (18F-FDG) PET-CT. Results indicate that B3AR agonist Mirabegron increased basal metabolic rate, stimulating human thermogenesis [15,16]. Following this, further research is encouraged to explore the use of B3AR agonists to develop pharmacological trials to treat obesity and metabolic syndrome. NCT03012113 also explores the therapeutic potential of B3AR to improve metabolic phenotype in South Asians [17].

2.3. Thyroid hormone

Thyroid hormone, adrenergic stimulation and UCP1 expression are all essential components of thermogenesis in BAT. Mice models with hypothyroidism have been shown to have lower body temperature and those with hyperthyroidism with higher body temperature [18]. During cold exposure, activation of BAT can induce deiodinase 2 (Dio2). Dio2 converts thyroxine (T4) to active T3. An increase in intracellular T3 acts to stimulate SNS and increase UCP 1, which leads to mitochondrial respiration and fatty acid oxidation, and thermogenesis. Without T3, thermogenic capacity is greatly reduced, and this was observed in patients with hypothyroidism. In mouse models, hypothyroidism is also associated with less BAT, FFA oxidation and glucose uptake [19]. Hypothyroid rats can continue to perform diet induced thermogenesis but experience impaired NST, indicating the codependent relationship between thyroid hormones and SNS [20]. Thyroid hormones can also accelerate the rate of ATP turnover, increasing thermogenesis [21].

A study explored the impact of cold exposure on thyroid hormones and NST. Participant groups were divided into Hunters who live in remote settlements in East Greenland with no modern housing (more cold exposure) and men who live in West Greenland of a major town (less cold exposure). The research team measured TSH, free thyroxine, free T3 (fT3), thyroglobulin antibody and TG levels. Results show that those who were most exposed to cold (hunters and settlement dwellers) had the highest serum TG and lowest fT3 compared to the other group. This indicates a change in long-term cold exposure on thyroid hormones and TG. There is a high thyroid hormone consumption and activation of BAT from chronic cold exposure [22]. This finding shows the potential for thyroid hormones as a target for preventing obesity.

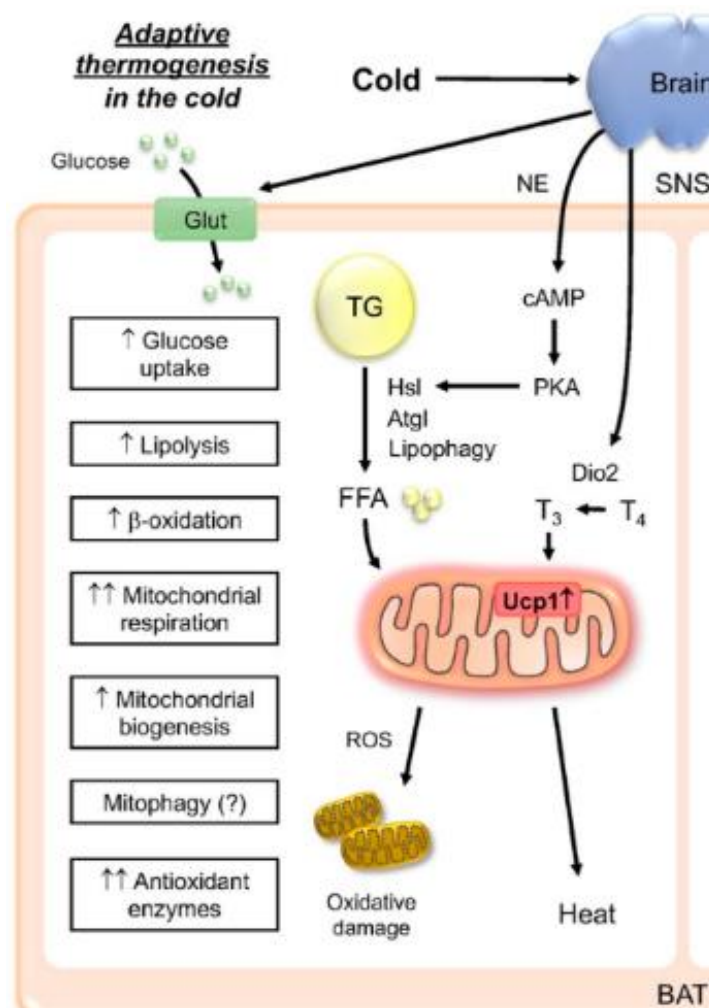


Figure 2. Cold exposure stimulating the SNS leading to to NE and Dio2 production [18].

3. Thermogenesis Activation Through Transient Receptor Potential (TRP) Channels

3.1. Capsinoids Activating Thermogenesis Through Vanilloid (TRPV1) Channel

The TRP channels are a group of transmembrane cation channels. Most TRP channels are nonselective cation channels. Once activated, it causes a depolarization of the cell membrane which activates the voltage gated ion channels, causing an influx of Ca^{2+} . An influx of Ca^{2+} increases intracellular Ca^{2+} concentration which can initiate an SNS signal, activating several physiological processes including thermogenesis. TRP family is divided into seven subfamilies, among which vanilloid (TRPV1) is called the capsaicin receptor [24]. Past literature highlighted the strong association between capsasinoids and NST through the TRPV1 channel. Capsasinoids are a group of compounds in chili peppers with bioactive properties. Capsasinoids are the main compound that makes a food 'spicy'. Due to many people's intolerance to spicy taste, scientists explored the use of capsinoids. Capsinoids are derived from sweet pepper and have similar effects to capsasinoids but without a pungent taste [25]. Capsasinoids activate TRPV1 channels. Studies have shown the activation of TRPV1 leads to prevention of adipogenesis, obesity and non-alcoholic fatty liver disease [26,27]. In addition, the activation of TRPV1 by capsaicin has led to the promotion of lipolysis, which promotes NST. Studies showed that TRPV1 antagonists decreased those effects. TRPV1 is expressed in mesenteric adipose tissue. In the mesenteric adipose tissue, ingestion of capsaicin was found to increase levels of PPAR gamma and HSL in wild type mice and obese humans. PPAR gamma and HSL are both crucial to NST activation [28].

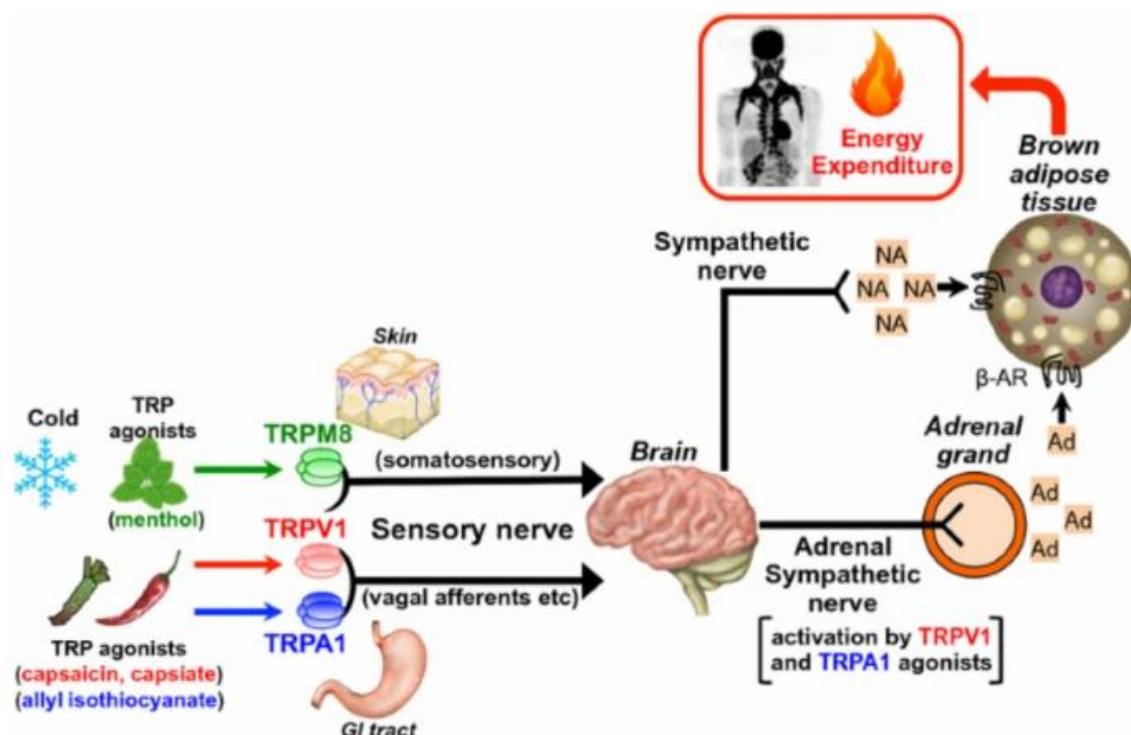


Figure 3. TRP agonists activating thermogenesis [23].

Several studies (NCT02964442, NCT03110809, UMIN000006073) examined the effect of capsinoids on NST and brown fat activation through TRPV1 receptor in the gastrointestinal tract. A randomized controlled trial (RCT) (UMIN000006073) examined the effects of capsinoids on human BAT activation and energy expenditure [29]. Using a cross over design, participants were tested for whole body energy expenditure and skin temperature after capsinoid ingestion under warm 27 degrees Celsius (C) conditions. Results show an increase in energy expenditure through activating BAT after ingestion of capsinoids. Another study (NCT02964442) compared capsinoids and cold exposure on BAT activation [30]. Similar to Yoneshiro et al., Sun et al. used a cross over design to compare the effects of ingesting 12mg capsinoids and effect of 1–2-hour cold exposure at 14.5 C. To assess BAT activation, scientists used 18F-FDG PET and whole-body calorimetry. NCT03110809 aims to explore the effect of capsinoids on brown fat activation and its impact on obesity. Their results have yet to be posted [31]. Results of these studies demonstrate that capsinoids significantly increased energy expenditure in BAT positive individuals compared to BAT negative group. Both results indicate a slight increase in energy expenditure after capsinoid ingestion, showing the potential for capsinoids to increase BAT activity and have anti-obesity effects. Due to small effects, additional studies are encouraged to continue exploring this idea.

3.2. Menthol Activating Thermogenesis Through Transient Receptor Potential Cation Channel Subfamily Melastatin Member 8 (TRPM8)

One of the seven TRP channels include the TRPM8 that is expressed in BAT. Upon cold stimulation, or activation by testosterone, ilicin, or cooling agents like menthol and eucalyptol, TRPM8 activates PKA and upregulate UCP1 expression [32, 33, 34]. Upregulation of UCP1 leads to increase thermogenesis. Aside from brown adipose tissue, TRPM 8 was found to activate UCP1, mitochondria, and produce heat in a white adipocyte cell line. TRPM8 is a thermal sensor and is predominantly located on sensory neuron membrane, but it is recently discovered that they also exist on brown and white adipocytes [35].

A study explored the use of L-Menthol to induce NST. L-Menthol stimulates TRPM8, which display similar effects to B3AR activation. Once L-Menthol enters the body, it increases intracellular Ca^{2+} concentration, activating PKA, stimulating UCP1 activation. Participants were placed into oral and skin administration of menthol. Results show that skin administration of menthol increases

metabolic rate and NST [36]. Findings present L-Menthol as a promising therapeutic candidate for treating obesity and metabolic diseases.

4. Limitations

Studies exploring cold induced thermogenesis pathways may present bias due to insufficient sample size and limited to the complications involving PET imaging and radiotracers. These biases may result in an underestimation of the effect of BAT activation [12,13]. The pilot study exploring pharmacological activation of the SNS through Mirabegron is limited in their sample size calculation due to no prior experimental data to support the required sample size. This study is also limited to a short duration; thus, it is unfit for estimating long term effects of Mirabegron on metabolism. In addition, the population is limited to young, lean, and healthy males, and thus is not applicable to women and other population. The high dose of 200mg Mirabegron is selected due to it being able to selectively target B3AR activation. The approved daily dose of Mirabegron for overactive bladder is 50mg. Therefore, this pilot study has limited applicability [17]. Studies exploring thyroid hormone activation from cold stimulation also has limited applicability. Its limited applicability largely stems from only selecting elderly participants. Although the authors provided clear explanations for the reason for selection, however; the applicability of this study remains for elderly populations [22]. Studies examining capsinoids are limited due to their small sample sizes. PET imaging conducted to assess BAT activity was also not standardized. Further, this study receives funding which may introduce bias to the reported results. Lastly, studies exploring L-menthol and thermogenesis is limited to its short duration [29, 30, 31]. Long-term exploration of the effects of L-menthol on thermogenesis is recommended [36]. Overall, these biases introduce limitations to the applicability of these studies. These limitations may overestimate or underestimate the effects of these therapeutic applications on obesity and metabolic syndrome. However, due to this field being relatively new and slowly emerging in recent years, more data is becoming available, and these explanatory studies can assist in informing future directions.

5. Conclusion

Non-shivering thermogenesis can be activated by a multitude of biological pathways, presenting potential treatment candidates for obesity and metabolic syndrome. Cold stimulation and B3AR agonists were shown to stimulate the SNS, increasing NST. Current clinical trials, although are limited in number, still demonstrate the rising interest among the scientific community to target NST as a treatment for obesity and metabolic syndrome. Future studies are encouraged to explore the use of NST pathways to treat obesity and metabolic syndrome.

References

- [1] De Waard A-KM, Hollander M, Korevaar JC, Nielen MMJ, Carlsson AC, Lionis C, et al. Selective prevention of cardiometabolic diseases: activities and attitudes of general practitioners across Europe. *European Journal of Public Health*. 2019, pp. 88 – 93.
- [2] Chatterjee A, Harris SB, Leiter LA, Fitchett DH, Teoh H, Bhattacharyya OK. Managing cardiometabolic risk in primary care. *Can Fam Physician*. 2012, pp. 389 – 93.
- [3] Liu J, Wang Y, Lin L. Small molecules for fat combustion: targeting obesity. *Acta Pharm Sin B*. 2019, pp. 220 – 36.
- [4] Thermogenesis. In: *The Free Dictionary*. [cited 2022 Mar 30]. Available from: <https://medical-dictionary.thefreedictionary.com/thermogenesis>.
- [5] Thermogenesis Encyclopedia.com. [cited 2022]. <https://www.encyclopedia.com/medicine/anatomy-and-physiology/anatomy-and-physiology/thermogenesis>.
- [6] Haman F, Blondin DP. Shivering thermogenesis in humans: Origin, contribution and metabolic requirement. *Temperature*. 2017, pp. 217 – 26.

- [7] Nonshivering thermogenesis - an overview | sciencedirect topics. [cited 2022 Apr 14]. Available from: <https://www.sciencedirect.com/topics/medicine-and-dentistry/nonshivering-thermogenesis>.
- [8] Contreras C, Nogueiras R, Diéguez C, Rahmouni K, López M. Traveling from the hypothalamus to the adipose tissue: The thermogenic pathway. *Redox Biol.* 2017, pp. 854 – 63.
- [9] Contreras C, Nogueiras R, Diéguez C, Medina-Gómez G, López M. Hypothalamus and thermogenesis: heating the bat, browning the wat. *Molecular and Cellular Endocrinology.* 2016, 107 – 15.
- [10] Chouchani ET, Kazak L, Spiegelman BM. New advances in adaptive thermogenesis: ucp1 and beyond. *Cell Metabolism.* 2019, pp. 27 – 37.
- [11] Cioffi F, Gentile A, Silvestri E, Goglia F, Lombardi A. Effect of iodothyronines on thermogenesis: focus on brown adipose tissue. *Frontiers in Endocrinology.* 2018, p. 9.
- [12] u Din M, Raiko J, Saari T, Kudomi N, Tolvanen T, Oikonen V, et al. Human brown adipose tissue [15O] O₂ PET imaging in the presence and absence of cold stimulus. *Eur J Nucl Med Mol Imaging.* 2016, 1878 – 86.
- [13] The effect of brown adipose tissue activation on insulin sensitivity in humans - full text view - clinicaltrials. Gov. [cited 2022 Apr 21]. Available from: <https://clinicaltrials.gov/ct2/show/NCT01791114>.
- [14] Arch JRS. Challenges in β 3 -adrenoceptor agonist drug development. *Therapeutic Advances in Endocrinology.* 2022, pp. 59–64. Available from: <http://journals.sagepub.com/doi/10.1177/2042018811398517>.
- [15] Muzik O. Efficacy of pharmacological stimulation of brown and white fat in lean and obese young adults [Internet]. *clinicaltrials.gov*; 2017 May [cited 2022 Apr 19]. Report No.: results/NCT02354807.
- [16] Cypess AM, Weiner LS, Roberts-Toler C, Elía EF, Kessler SH, Kahn PA, et al. Activation of human brown adipose tissue by a β 3-adrenergic receptor agonist. *Cell Metab.* 2015, pp. 33 – 8.
- [17] Jazet I. The effect of mirabegron on brown adipose tissue in healthy young white caucasian and south asian men [Internet]. *clinicaltrials.gov*; 2017 Jan [cited 2022 Apr 19]. Report No.: NCT03012113.
- [18] Yau WW, Yen PM. Thermogenesis in adipose tissue activated by thyroid hormone. *Int J Mol Sci* [Internet]. 2020, p.3020.
- [19] Yau WW, Singh BK, Lesmana R, Zhou J, Sinha RA, Wong KA, et al. Thyroid hormone (T3) stimulates brown adipose tissue activation via mitochondrial biogenesis and MTOR-mediated mitophagy. *Autophagy.* 2018, pp. 131–50. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6287687/>.
- [20] Curcio C, Lopes AM, Ribeiro MO, Francoso OA, Carvalho SD, Lima FB, et al. Development of compensatory thermogenesis in response to overfeeding in hypothyroid rats. *Endocrinology.* 1999, pp. 3438 – 43.
- [21] Silva JE. Thyroid hormone control of thermogenesis and energy balance. *Thyroid.* 1995, pp. 481–92.
- [22] Andersen S, Kleinschmidt K, Hvingel B, Laurberg P. Thyroid hyperactivity with high thyroglobulin in serum despite sufficient iodine intake in chronic cold adaptation in an Arctic Inuit hunter population. *European Journal of Endocrinology.* 2012, pp. 433 – 40.
- [23] Uchida K, Dezaki K, Yoneshiro T, Watanabe T, Yamazaki J, Saito M, et al. Involvement of thermosensitive TRP channels in energy metabolism. *J Physiol Sci* [Internet]. 2017, pp. 549 – 60.
- [24] Nilius B, Owsianik G. The transient receptor potential family of ion channels. *Genome Biol.* 2011, p. 218.
- [25] Whiting S, Derbyshire E, Tiwari BK. Capsaicinoids and capsinoids. A potential role for weight management? A systematic review of the evidence. *Appetite.* 2012, pp. 341 – 8.
- [26] Zhang LL, Yan Liu D, Ma LQ, Luo ZD, Cao TB, Zhong J, et al. Activation of transient receptor potential vanilloid type-1 channel prevents adipogenesis and obesity. *Circ Res.* 2007, pp.1063 – 70.
- [27] Li Q, Li L, Wang F, Chen J, Zhao Y, Wang P, et al. Dietary capsaicin prevents nonalcoholic fatty liver disease through transient receptor potential vanilloid 1-mediated peroxisome proliferator-activated receptor δ activation. *Pflugers Arch.* 2013, pp. 1303 – 16.
- [28] 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 Ca²⁺ Influx. *Cardiovasc Diabetol.* 2015, p. 22.

- [29] 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, pp. 845 – 50.
- [30] Sun L, Camps SG, Goh HJ, Govindharajulu P, Schaefferkoetter JD, Townsend DW, et al. Capsinoids activate brown adipose tissue (Bat) with increased energy expenditure associated with subthreshold 18-fluorine fluorodeoxyglucose uptake in BAT-positive humans confirmed by positron emission tomography scan. *The American Journal of Clinical Nutrition.* 2018, pp. 62 – 70. Available from: <https://academic.oup.com/ajcn/article/107/1/62/4825215>.
- [31] Neff L. The effects of capsinoids on brown adipose tissue recruitment and activation in obesity [Internet]. clinicaltrials.gov; 2021 Dec [cited 2022 Apr 19]. Report No.: study/NCT03110809. Available from: <https://clinicaltrials.gov/ct2/show/study/NCT03110809>.
- [32] Wei ET, Seid DA. AG-3-5: a chemical producing sensations of cold. *J Pharm Pharmacol.* 1983, pp.110 – 2.
- [33] Asuthkar S, Demirkhanyan L, Sun X, Elustondo PA, Krishnan V, Baskaran P, et al. The trpm8 protein is a testosterone receptor. *J Biol Chem.* 2015, pp. 2670 – 88.
- [34] Sakellariou P, Valente A, Carrillo AE, Metsios GS, Nadolnik L, Jamurtas AZ, et al. Chronic l-menthol-induced browning of white adipose tissue hypothesis: A putative therapeutic regime for combating obesity and improving metabolic health. *Med Hypotheses.* 2016, pp. 21 – 6.
- [35] Rossato M, Granzotto M, Macchi V, Porzionato A, Petrelli L, Calcagno A, et al. Human white adipocytes express the cold receptor TRPM8 which activation induces UCP1 expression, mitochondrial activation and heat production. *Molecular and Cellular Endocrinology.* 2014, pp.137 – 46.
- [36] Valente A, Carrillo AE, Tzatzarakis MN, Vakonaki E, Tsatsakis AM, Kenny GP, et al. The absorption and metabolism of a single L-menthol oral versus skin administration: Effects on thermogenesis and metabolic rate. *Food and Chemical Toxicology.* 2015, pp. 262 – 73.