Molecular and Cellular Endocrinology 418 (2015) 184–190

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

journal homepage: www.elsevier.com/locate/mce

Review

Non-pharmacological and pharmacological strategies of brown adipose tissue recruitment in humans

Paul Lee [a, b, *], Jerry R. Greenfield [a, b, c]

[a] Diabetes and Metabolism Division, Garvan Institute of Medical Research, 384 Victoria Street, Darlinghurst, Sydney, NSW 2010, Australia
[b] Department of Endocrinology, St Vincent's Hospital, New South Wales, Australia
[c] Diabetes Centre, St Vincent's Hospital, New South Wales, Australia

ARTICLE INFO

Article history:
Received 11 December 2014
Received in revised form
31 March 2015
Accepted 4 May 2015
Available online 27 May 2015

Keywords:
Beige adipose tissue
Thermogenesis
Energy balance
Metabolism
FGF21
Irisin

ABSTRACT

Humans maintain core temperature through a complex neuroendocrine circuitry, coupling environmental thermal and nutritional cues to heat-producing and dissipating mechanisms. Up to 40% of resting energy expenditure contributes to thermal homeostasis maintenance. Recent re-discovery of thermogenic brown adipose tissue (BAT) has brought the relation between ambient temperature, thermogenesis and systemic energy and substrate metabolism to the forefront. In addition to well-known pituitary −thyroid−adrenal axis, new endocrine signals, such as FGF21 and irisin, orchestrate crosstalk between white adipose tissue (WAT), BAT and muscle, tuning non-shivering and shivering thermogenesis responses. Cold exposure modulates the endocrine milieu, and cold-induced hormones cause bioenergetics transformation sufficient to impact whole body metabolism. This review will appraise the nature of human BAT and the basis of BAT-centred therapeutics, highlighting how the interaction between hormones and adipose tissue impacts metabolic responses. Non-pharmacological and pharmacological strategies of BAT recruitment and/or fat browning for metabolic benefits will be discussed.

Contents

* Corresponding author. Diabetes and Metabolism Division, Garvan Institute of Medical Research, 384 Victoria Street, Darlinghurst, Sydney, NSW 2010, Australia.
Tel.: +61 2 9295 8416; fax: +61 2 9295 8481.
E-mail address: p.lee@garvan.org.au (P. Lee).

http://dx.doi.org/10.1016/j.mce.2015.05.025
0303-7207/

1. Introduction

Despite global research effort, current strategies targeting obesity and related metabolic disorders have been ineffective in halting their expansion. Identification of significant depots of brown adipose tissue (BAT) in adult humans and its relation to leanness represent exciting developments with potential therapeutic implications. In this review, we offer a critical appraisal on the premise that human BAT is an obesity/diabetes treatment target, as well as challenges and pitfalls of BAT-centred therapeutics.

2. Why is BAT an attractive target of obesity treatment?

Adipose tissue can be broadly classified into two main types: white and brown adipose tissues. White adipose tissue (WAT) is primarily a site of energy storage while BAT dissipates heat to maintain core temperature in defence against cold exposure (Cannon and Nedergaard, 2004). It harbours the unique protein, uncoupling protein 1 (UCP1) in the inner mitochondrial membrane. UCP1 is able to induce proton leak in the respiratory chain and releasing energy as heat. This process utilises fatty acid and glucose as substrates. In animal models, triglyceride and glucose clearance in cold-activated BAT amount to as much as two-thirds of total body substrate clearance (Bartelt et al., 2011), thus attesting its remarkable energy utilising capacity, and its potential to be harnessed for metabolic benefits.

In rodents and infants, the most prominent BAT depot is situated in the interscapular region (known as "classic BAT"). Recently, the spectrum of BAT has been extended, with studies revealing the presence of BAT-like cells within WAT, known as beige or brite (brown-in-white) adipose tissue (BeAT). While both classic BAT and BeAT expresses UCP1, they are distinguished by their unique gene signatures that point to their different developmental origins. However, they share similar bioenergetics profiles. In the basal state, BeAT resembles WAT; but upon cold exposure, BeAT within WAT expresses UCP1 and acquires similar energetic capacity as classic BAT (Wu et al., 2012a, 2012b). Pharmacological, genetic and transplantational models of high BAT and/or BeAT states are associated with resistance against high fat-induced weight gain, glycaemic improvement, hyperlipidaemia reversal and hepatic steatosis resolution (Wu et al., 2013), independent of diet and physical activity. In other words, recruitment of classic BAT or induction of BeAT may both lead to metabolic benefits, and represents a mechanism beyond traditional emphasis on diet and exercise that is highly relevant in an over-nutritious and sedentary contemporary society.

3. Do humans have BAT or BeAT or both?

^{18}F-fluorodeoxyglucose (^{18}FDG)-positron emission tomography (PET)/CT imaging, which was introduced in the early 2000s, revealed adipose tissue of high metabolic activity around the supraclavicular and cervical areas in some patients (Hany et al., 2002). Subsequent PET/CT studies and PET-guided biopsies in healthy adults confirmed the presence of BAT in majority of adults (Cypess et al., 2009; Saito et al., 2009; van Marken Lichtenbelt et al., 2009; Virtanen et al., 2009). More recent studies reveal expression of BeAT-specific genes in FDG-avid supraclavicular fat and classic BAT genes in deeper neck fat in adult humans (Cypess et al., 2013; Jespersen et al., 2013; Sharp et al., 2012; Wu et al., 2012a). This is in contrast to infants whose interscapular fat resembles that in animals and retains classic BAT gene signature (Lidell et al., 2013). These results suggest the presence of mixed classic brown and beige adipocytes in adult human "BAT depots". However, as beige adipocytes are interspersed within WAT depots, whole body BeAT abundance may be greater than what PET/CT is able to capture. These findings set the stage for investigations into BAT/BeAT recruitment strategies in humans.

4. Does BAT abundance matter to metabolism in humans?

Although BAT constitutes only a small fraction of body cell mass (~0.1%) in adults based on PET-CT estimation, its remarkable energy utilisation suggests it could contribute to whole body energy expenditure. Studies have estimated energy equivalent attributable to cold-simulated BAT. Based on nearly 10-fold increase in ^{18}FDG uptake in BAT on cold exposure (Orava et al., 2011; Ouellet et al., 2012; Virtanen et al., 2009), and the assumption that glucose represents 10% of BAT fuel (Ma and Foster, 1986), the extrapolated contribution of BAT metabolism to basal EE could be as high as 20%. If such BAT activation were "continuous", one may further hypothesise that the excess energy expenditure equates to an energy consumption of ~100 kcal/day, representing ~5 kg of fat loss in 1 year (Virtanen et al., 2009). As the contribution of physical activity to total energy expenditure dwindles in modern sedentary societies (Healy et al., 2008), the thermogenic contribution of activated BAT may be important since even minor changes in basal energy expenditure can translate into changes in body weight over the long-term (Astrup et al., 1999; Cunningham, 1982; Esparza et al., 2000; Ravussin and Swinburn, 1993). This is supported by associative studies showing negative correlations between BAT activity with fat mass, BMI and fasting glycaemia (Cypess et al., 2009; Lee et al., 2010; Ouellet et al., 2011; Saito et al., 2009; Yoneshiro et al., 2011). Our group showed that body weight was nearly 4 kg lower in 145 individuals when BAT was active (Lee et al., 2010). We also observed greater *UCP1* mRNA abundance in supraclavicular fat among leaner individuals, with UCP1 accounting for nearly 50% of BMI variance (Lee et al., 2011).

5. Can humans recruit BAT?

5.1. Genes vs. environment

The observation that some adults possess cold-activated BAT, and that these individuals are leaner can be interpreted in two ways. Since BAT is present in infancy, is it possible that certain genetic traits are associated with BAT retention through "to adulthood" and that BAT presence on PET/CT scanning merely represents a genetically determined phenotype. Conversely, human BAT may be "plastic" to some extent, with its abundance and activity determined by physiologic and/or environmental cues. The latter is a fundamental question as it forms the basis of the quest for BAT-harnessing therapeutics.

5.2. Do common genetic variations in humans influence BAT?

Given the importance of UCP1 to BAT function, polymorphism of the *UCP1* gene was the first candidate to be examined. A common

A/G substitution at position −3826 upstream of the *UCP1* transcriptional start site influences *UCP1* mRNA expression in human peritoneal adipose tissues (Esterbauer et al., 1998). This polymorphism interacts with a common amino acid substitution (Trp64Arg) in the gene encoding the β3-adrenoceptor, *ADRB3*, a chief regulator of BAT differentiation, and individuals possessing both variants manifest lower basal metabolic rate (Valve et al., 1998), lower sympathetic nervous system activity (Shihara et al., 2001), and lesser weight loss on a very low calorie diet (Fogelholm et al., 1998). The mechanisms were elusive as these studies were performed before the re-discovery of BAT in humans, and a link to BAT was not made. Very recently, Yoneshiro and colleagues examined the relationship between BAT positivity and *UCP1*/*ADRB3* genotype (Yoneshiro et al., 2012). In older subjects (>40 years old), BAT prevalence was significantly lower in those possessing 2 or more variant alleles at *UCP1* −3826 A/G or *ADRB3* Trp64Arg, relative to subjects with <2 variant alleles (0% *vs.* 24%).

Taken together, common genetic variation in metabolic pathways may indirectly modulate BAT activity and contribute to obesity by influencing metabolic rate and the response to weight loss. Whether there is any interaction with environmental cues, such as ambient temperature, physical activity or caloric exposure has not been studied.

5.3. Does temperature affect BAT recruitment?

PET/CT studies display the remarkable responsiveness of BAT to cold stimulation. Acute (1–2 hours) mild cold exposure (18–19 °C) is sufficient to activate BAT (*i.e.* "switching on" pre-existing BAT). Whether BAT can be recruited (increase in abundance) has only been investigated recently in cold acclimation studies.

Duration of cold exposure ranged from 2 to 6 hour per day at 10–17 °C for a period between 10 days and 6 weeks was tested in three studies (Blondin et al., 2014; van der Lans et al., 2013; Yoneshiro et al., 2013). The results were similar, with significant increase in BAT quantity and activity (chiefly the cervical–supraclavicular depots), up to 45%, following cold acclimation, even among individuals with low or negligible BAT at baseline. Our group investigated the plasticity of BAT systematically in a long-term temperature acclimation study, and rotated volunteers through monthly mild cold (19 °C), mild warm (27 °C) and thermoneutral (24 °C) temperature exposure for a total duration of 4 months (Lee et al., 2014a). PET/CT studies at the end of each temperature month showed inverse relation between exposed temperature and BAT, with BAT abundance/activity peaking in the mild cold month and completely abolished in the warm month, thus demonstrating not only cold recruitability of human BAT, but also its suppressibility in the warmth.

Although FDG-avid fat biopsies were not performed, based on previous identification of a beige gene signature in these depots [Section III], it is tempting to speculate that fat-browning/beiging is the responsible mechanism. This is corroborated by our demonstration of functional thermogenic beige adipogenesis in pre-adipocytes isolated from human neck fat and the capacity of human adipocytes to acquire functional beige fat features *in vitro* (Lee et al., 2014b). Such "fat browning" occurs in precursors isolated from individuals devoid of BAT on PET/CT too (Lee et al., 2011). Recently, an adipose tissue comparative analysis in humans between seasons showed a 4- to 10-fold higher *UCP1* and beige gene expression in subcutaneous adipose tissue in winter compared to summer (Kern et al., 2014).

Collectively, these results provide proof of concept evidence demonstrating recruitability and plasticity of human BAT/BeAT, both *in vivo* and *in vitro*.

6. Does BAT or BeAT recruitment benefit metabolism in humans?

6.1. Body composition

Yoneshiro and colleagues examined changes in body composition in their cold acclimation study. Total body weight and lean mass did not change significantly following 6 weeks of mild cold exposure. However, body fat mass decreased by 5% following BAT recruitment (Yoneshiro et al., 2013). Based on the known energy-utilising capacity of BAT, this study provides first evidence of possible metabolic benefits of BAT recruitment as significant adiposity loss.

6.2. Glucose metabolism

In our long-term temperature acclimation study, we probed how BAT acclimation relates to energy balance (Lee et al., 2014a). Sequential monthly acclimation to four temperatures (24 °C → 19 °C → 24 °C → 27 °C) modulated BAT reversibly, boosting and suppressing its abundance and activity in mild cold and warm conditions, respectively. Cold-acclimated BAT recruitment was accompanied by 30% enhancement of diet-induced thermogenesis following a mixed meal. Postprandial insulin sensitivity increases after cold acclimation, the month when BAT abundance was the highest. These results echo known glucose-utilising capacity of BAT and suggest BAT glucose clearance may potentially translate into glycaemic benefits in humans.

6.3. Cold exposure and weight loss

BAT expansion upon cold exposure, and its accompanying metabolic benefits, raises the question of whether cold exposure may be a simple strategy to tackle obesity and diabetes. Indeed, in rodents, BAT activation has been shown to decrease plasma triglyceride/cholesterol and accelerate hepatic clearance of cholesterol-enriched remnants through the enhancement of fatty acid uptake from triglyceride-rich lipoproteins (Berbee et al., 2015). Animal studies therefore suggest BAT activation to be a protective mechanism against atherosclerosis. There are 3 caveats to consider in this approach.

First, cold exposure increases energy expenditure. Although there was no increase in total caloric intake during cold acclimation month in our study (Lee et al., 2014a), volunteers reported subjectively "feeling more hungry" on visual analogue scale questionnaires. One cannot exclude the possibility of appetite increase during long-term cold acclimation negating weight loss benefits of BAT recruitment outside of a controlled research setting. Second, clothing is standardised in all cold acclimation studies to ensure uniform and sufficient cold exposure. The natural tendency to seek thermal comfort means humans are likely to increase thermal insulation (*e.g.* through clothing) in a sub-thermal environment. This may explain why some (Cohade et al., 2003; Cypess et al., 2009; Ouellet et al., 2011; Saito et al., 2009), but not all (Kim et al., 2008; Lee et al., 2010) cross-sectional PET/CT studies observed higher BAT prevalence rates in winter than in summer, likely a result of differing clothing habits across seasons. Third, cold exposure has been associated with increased cardiovascular risk (Bhaskaran et al., 2009). We hypothesise this is because metabolic response to cold may vary according to thermogenic "fitness", not dissimilar to exercise tolerance. For example, 60 minutes of jogging may represent good aerobic training for some individuals, it may be hazardous for sedentary people with poor aerobic reserve. Accordingly, acute cold exposure can result in a stress response in individuals, characterised by an increase in insulin resistance and

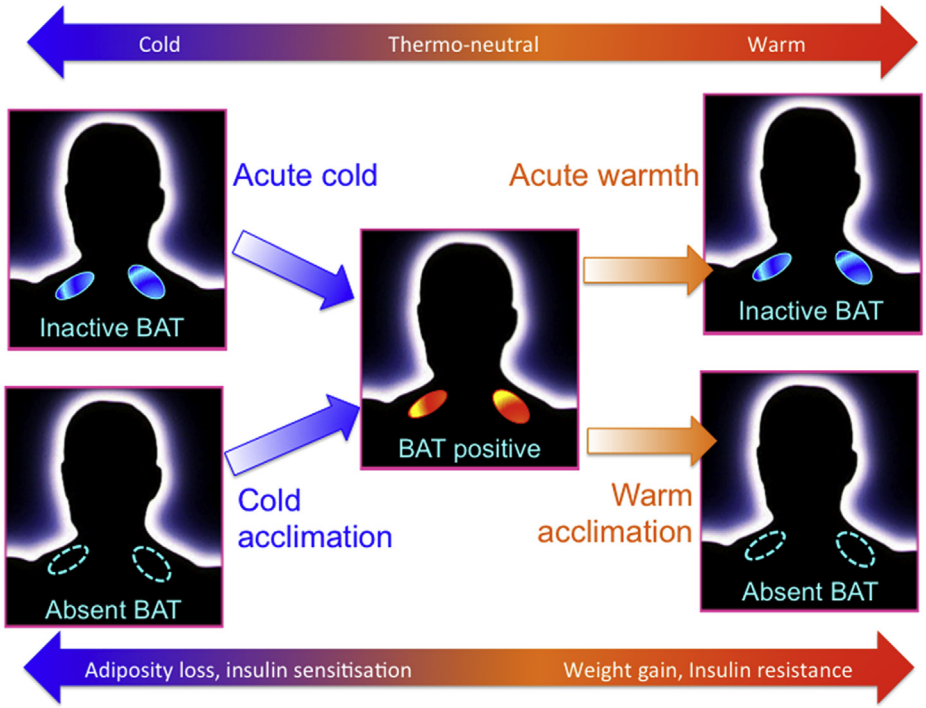

Fig. 1. Schematic diagram of relation between temperature, BAT and metabolism. Acute cold exposure (*e.g.* 2 hours of 19 °C) activates pre-existing BAT as depicted on PET/CT and cold acclimation (*e.g.* 4 weeks of overnight 19 °C) recruits BAT, even among BAT negative individuals. BAT recruitment is associated with adiposity loss and improvement in insulin sensitivity. Conversely, acute warming (*e.g.* 2 hours of 24–27 °C) mutes BAT activity and warm acclimation (*e.g.* 4 weeks of 27 °C) diminishes BAT quantity. Warm-acclimated suppression of BAT is associated with increase in insulin resistance in previously BAT positive individuals.

urinary cortisol excretion (Celi et al., 2010). One must also be cautious of blood pressure and pulse rate elevation as a consequence of cold-induced heightened sympathetic drive, both are well-known cardiovascular risk factors. In certain strains of mice, cold triggered lipolysis and increased plasma levels of small low-density lipoprotein (LDL) remnants, leading to accelerated development of atherosclerotic lesions (Dong et al., 2013).

Taken together, while cold acclimation studies have provided "proof of concept" evidence to BAT recruitability and potential metabolic benefits, there are inherent logistic and practical barriers, as well as safety concerns in translating these findings to real-life obesity/diabetes management. Other strategies, such as BAT-activating pharmacological approaches, are highly desirable. However, on a public health level, muting of BAT by warm acclimation may be a hidden contributor to obesity/diabetes in current thermoneutral society [Fig. 1]. Future studies should evaluate whether allowance of indoor climate control to track safely outdoor seasonal changes may modulate body weight on a population level.

7. What are current potential BAT/BeAT pharmacological recruitment strategies?

7.1. BAT activation/recruitment vs. fat browning

Harnessing BAT for therapeutic benefits is the ultimate goal of human BAT research. Cold exposure studies have established the foundation for developing BAT-based therapeutics. Although the developmental origin of human BAT and BeAT remains to be clarified, the metabolic phenotype of therapeutic interest is the high energy utilisation state of UCP1-expressing brown adipocytes, regardless of whether it is classic BAT or "BAT-like" BeAT. Whether the enhanced brown fat function arises from boosted activity of pre-existing BAT, from newly recruited classic brown adipocytes, or from the induction of brown fat-like cells (*i.e.* fat browning) within WAT becomes an academic matter from a clinical standpoint.

The premise of fat browning as an alternative strategy to boosting of BAT mass is based on the expression of beige fat genes in human BAT depots, and the observation that human white adipocytes are endowed with the potential for functional thermogenic fat beig-ing (Lee et al., 2014b). Animal models exhibiting high BeAT states (as a result of fat browning) (Cinti, 2009; Wu et al., 2012b) display profound metabolic benefits. Importantly, the level of UCP1 reached in beige adipocytes was generally 4–6% of the UCP1 amount in murine BAT and ectopic expression of UCP1 in transgenic mice at 1% of BAT UCP1 content is sufficient to impede the development of obesity (Kopecky et al., 1995).

In the last few years, a plethora of transcription factors, small molecules and cytokines with BAT/BeAT enhancing potential have been identified in animal models. Here we will discuss those that have been tested in humans, either *in vivo* or *in vitro* in human adipocytes.

7.2. Sympathomimetic

Noradrenaline is the chief mediator of cold exposure. As acute cold activates and chronic cold recruits BAT [see Section V], pharmacological activation of β-adrenergic pathway is conceivably a feasible way of stimulating BAT and recruiting BAT/BeAT by mimicking the effects of cold exposure.

Non-selective β-agonists, such as ephedrine and isoproterenol (Astrup et al., 1995), and β_1-selective agonists, such as dobutamine (Schiffelers et al., 2001), and β_2-selective agonists, such as formoterol (Lee et al., 2015a), all increase energy expenditure in humans. Whether BAT can be recruited by β-adrenergic activation was evaluated in 4 studies. Treatment with therapeutic dosages of ephedrine (Cypess et al., 2012) or isoproterenol (Vosselman et al., 2012) increased energy expenditure in healthy volunteers to comparable extent to cold exposure; however, only cold exposure,

but not β-agonist administration enhanced BAT activity in these volunteers (Cypess et al., 2012; Vosselman et al., 2012). The third study (Carey et al., 2012) utilised a dose of ephedrine 2.5 times higher than that in the previous study (Cypess et al., 2012), and BAT activity was only modestly increased, compared to that achieved by cold stimulation; however blood pressure rose twice as high, indicating β-adrenergic BAT activation occurs in concert with inadvertent cardiovascular cross-stimulation. This led to a recent study investigating the effect of a β_3-selective agonist, mirabegron, on BAT activation (Cypess et al., 2015). This is based on knowledge that BAT is enriched with β_3-adrenoceptors. A single dose of mirabegron was capable of activating BAT in young lean men as detected by PET/CT, to similar extent of cold exposure. The study thus provides proof-of-concept evidence that β_3-selective agonists could increase BAT activity. However, blood pressure and heart rate also rose significantly in treated-individuals. Issue of cardiovascular safety awaits investigation in future studies.

In addition to BAT activation, noradrenaline is capable of inducing brown adipogenesis. Abdominal subcutaneous fat from patients with pheochromocytoma, a state of noradrenaline excess, revealed evidence of fat browning, with high abundance of brown fat-like cells, which were otherwise absent in control patients (Lean et al., 1986; Melicow, 1977; Ricquier et al., 1982). Since PET/CT became available, investigators have observed positive correlation between BAT activity and plasma catecholamine levels in patients with phaeochromocytoma (Wang et al., 2011), and FDG avidity in cervical–supraclavicular BAT disappeared after resection of phaeochromocytoma (English et al., 1973; Fukuchi et al., 2004).

These results therefore suggest while possibly an effective BAT activating/fat browning agent, therapeutic dosage of traditional β-agonist is unlikely to achieve sufficient efficacy without incurring undesirable cardiovascular side effects. Given the differential effects of cold and β-agonist on BAT, it is possible that pathways beyond the sympathetic nervous system are involved in cold activation of BAT in humans.

7.3. Non-adrenergic cold mimics

To explore potential non-adrenergic factors that could stimulate BAT, our group exposed volunteers to graded cold exposure, eliciting non-shivering and shivering thermogenesis, and obtained hormonal profile during each phase of cold exposure (Lee et al., 2014c). In addition to the expected pituitary–thyroid–adrenal activation, two hormones were augmented by cold exposure: Fibroblast growth factor 21 (FGF21) during non-shivering thermogenesis and irisin during shivering. Even mild cold exposure (19 °C) is sufficient to increase FGF21 secretion, with its output correlating positively with lipolysis and cold induced thermogenesis (Lee et al., 2013). Since BAT abundance measured by PET/CT determined FGF21 secretory magnitude in these volunteers, and shivering intensity correlated with irisin rise, these findings suggest that FGF21 is a brown adipokine and irisin a myokine in humans. Along this line, treatment of human white adipocytes with FGF21 and/or irisin resulted in robust upregulation of brown fat thermogenic programme, and augmentation of oxygen consumption, fatty acid oxidation and heat production (Lee et al., 2014c).

Taken together, these results highlight the presence of cold-activated muscle–WAT–BAT crosstalk, and support evidence in favour of FGF21 and irisin being cold-induced endocrine BAT activators that may be exploited for fat browning benefits, without directly stimulating sympathetic nervous system.

7.4. Thiazolididiones

Aside sympathetic stimulation, PPAR-γ agonism is the most widely recognised strategy in promoting brown adipogenesis in animals and *in vitro*. In contrast to sympathomimetic inducers, PPAR-γ agonists such as rosiglitazone, induce brown adipogenesis and can transform murine (Walden et al., 2012) and human (Lee et al., 2011) white adipocytes into a brite/beige phenotype; they do not activate the newly acquired thermogenic function *per se*, which necessitates sympathetic activation (*e.g.* by noradrenaline). The *in vivo* effect of thiazolidinedione on BAT and/or fat browning has not been investigated in humans. However, given the known glucose-lowering and insulin-sensitising effects of thiazolidinedione in patients with diabetes, it leads one to wonder if some of these benefits arise from fat browning. The utility of thiazolidinedione as a BAT-based therapeutic is somewhat hampered by concerns regarding their cardiovascular safety (Schernthaner and Chilton, 2010), possible risks of bladder cancer (Turner et al., 2014) and long-term bone loss/fractures (Zhu et al., 2014). However, a recent review of the benefits and risks of pioglitazone suggests it remains an effective and feasible option in people with type 2 diabetes (Schernthaner et al., 2013), and that its fat browning potential should be explored in future studies. In addition, as new PPARγ agonists emerge (Fukunaga et al., 2014), with perhaps more bone-sparing effects, thiazolidinediones hold promise as browning agents, that may serve a useful role in combination with BAT specific β-agonists.

7.5. Capsinoids and capsinoid-like compounds

The thermogenic action of capsinoid and related compounds on BAT has recently been investigated in humans. Saito and colleagues explored the effects of two compounds on BAT function: capsinoids and grains of paradise (Aframomum melegueta) extracts. Capsinoids are non-pungent capsaicin analogues, while grains of paradise are rich in non-volatile pungent compounds such as 6-paradol, 6-gingerol and 6-shogaol. In randomised, placebo-controlled designs, acute administration of capsinoids or grains of paradise in healthy adults augmented energy expenditure by 5–6% in BAT positive individuals (Sugita et al., 2013; Yoneshiro et al., 2013). In contrast, energy expenditure did not change among BAT negative subjects.

These data shed novel insight on the critical dependence of "thermic-food" action on BAT status. Capsinoid compounds activate TRPV1 and TRPA1 receptors present on brown adipocytes (Shintaku et al., 2012). It is therefore conceivable why such food items increase energy expenditure only among BAT positive individuals, as it would not have an effect if the effector tissue is absent (*i.e.* BAT negative individuals). These findings suggest BAT activating effect of capsinoid compounds holds promise as obesity therapeutics. Indeed, long-term administration of capsinoids (6 weeks) and grains of paradise (4 weeks) decreased total body fat and visceral fat by 2% and 7%, respectively (Sugita et al., 2014; Yoneshiro et al., 2013). While these results require confirmation in larger trials, they are exciting as they provide first evidence that thermic compounds may impart anti-obesity effects through BAT activation, without the need for cold exposure. The question begging to be answered is whether longer-term administration of capsinoid compounds could *induce* BAT expansion, thereby benefiting BAT negative individuals. In other words, are capsinoid compounds fat-browning/BAT-recruiting as well as BAT-activating agents? Fig. 2 is a schematic diagram illustrating how various factors achieve fat browning through cold exposure and the potential of FGF21, irisin and capsinoids in mimicking these fat browning effects for metabolic benefits.

7.6. New directions

In addition to pituitary–thyroid–adrenal and sympathetic

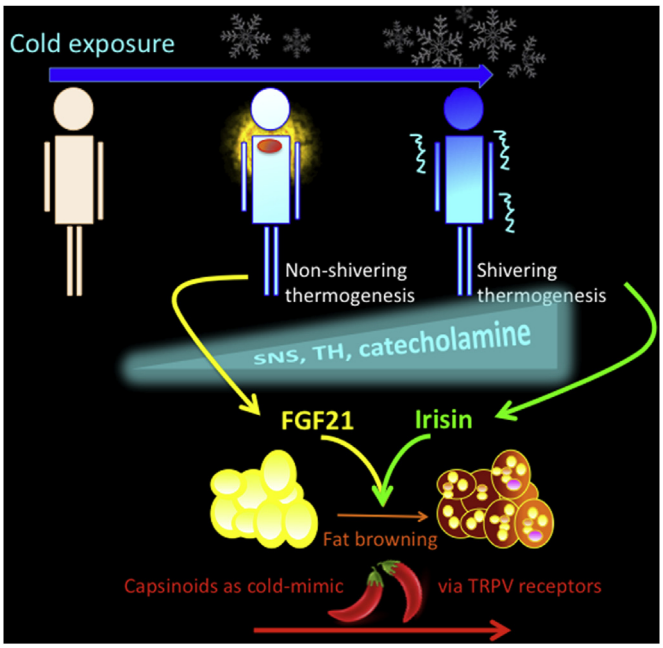

Fig. 2. Muscle–WAT–BAT endocrine axis in fat browning. Cold exposure is the best-recognised mechanism leading to BAT activation and WAT browning. Pituitary–thyroid (TH)–adrenal (PTA) hormone secretion and sympathetic nervous system (SNS) activity progressively increase with extent of cold exposure, resulting in BAT recruitment. Recent evidence points to induction of brown adipokine (*e.g.* FGF21) and myokine (*e.g.* irisin) release during cold-induced thermogenesis, capable of fat browning, beyond SNS and PTA axes. Capsinoid compounds, by stimulating TPRV receptors, could induce UCP1 on adipocytes *in vitro*. Whether fat browning occurs *in vivo* in humans await future studies.

nervous system pathways, recent animal studies have revealed crosstalk between immune system and BeAT. Upon cold stimulation, alternatively-activated macrophages within WAT are capable of releasing Il-4 to induce fat browning (Nguyen et al., 2011). The mechanisms are beginning to be elucidated in rodents, which involve eosinophil recruitment and release of type 2 cytokines (Brestoff et al., 2015; Lee et al., 2015), as well as a newly discovered circulating factor known as meteorin-like (Metrnl) (Rao et al., 2014). These studies reveal new interactions between host adaptive response to cold, energy homeostasis and adipose inflammation. How such adipose–immune crosstalk applies to humans has not been investigated. It is tempting to speculate their involvement underlying previously demonstrated correlation between higher eosinophil count and decreased risk of diabetes in adults (Zhu et al., 2013). Improved understanding of the relationship between type 2 innate immunity and adaptive thermogenesis may open new directions to obesity immunotherapy.

8. Conclusion

The re-discovery of BAT has marked a renaissance in metabolic research. Human BAT studies in the last 6 years have shown that (i) metabolically active BAT is present in most adults, (ii) BAT is cold-activated and its abundance is associated with a healthy metabolic phenotype, (iii) BAT-like adipose tissue can be recruited in humans, both *in vivo* and *in vitro* and (iv) recruited BAT and/or BeAT is associated with fat loss and insulin sensitising benefits. These findings have created a new paradigm relating thermogenesis to metabolism in humans, opening novel therapeutic directions in obesity and diabetes treatment through BAT-activating or fat browning strategies (Lee et al., 2015b). The challenges ahead are to identify pharmacological agents that achieve these goals in a BAT-specific manner, without co-stimulating the cardiovascular system or triggering metabolic compensation (*e.g.* increase in appetite). Capsinoids and related compounds are showing promise and a plethora of pre-clinical brown-ing agents await testing and translation in human studies.

Acknowledgements

PL is supported by an Early Career Fellowship (1035997) of the NHMRC (APP1088123) and a Diabetes Australia Research Trust Grant (Y14G-LEEP). JGR is the recipient of the Don Chisholm Fellowship (funds from Garvan Research Foundation, including support from GlaxoSmithKline, Australia, Diabetes Australia Research Trust, the Commonwealth Department of Health and Ageing).

References

Astrup, A., Breum, L., Toubro, S., 1995. Pharmacological and clinical studies of ephedrine and other thermogenic agonists. Obes. Res 3 (Suppl. 4), 537S–540S.
Astrup, A., Gotzsche, P.C., van de Werken, K., Ranneries, C., Toubro, S., Raben, A., et al., 1999. Meta-analysis of resting metabolic rate in formerly obese subjects. Am. J. Clin. Nutr 69, 1117–1122.
Bartelt, A., Bruns, O.T., Reimer, R., Hohenberg, H., Ittrich, H., Peldschus, K., et al., 2011. Brown adipose tissue activity controls triglyceride clearance. Nat. Med 17, 200–205.
Berbee, J.F., Boon, M.R., Khedoe, P.P., Bartelt, A., Schlein, C., Worthmann, A., et al., 2015. Brown fat activation reduces hypercholesterolaemia and protects from atherosclerosis development. Nat. Commun 6, 6356.
Bhaskaran, K., Hajat, S., Haines, A., Herrett, E., Wilkinson, P., Smeeth, L., 2009. Effects of ambient temperature on the incidence of myocardial infarction. Heart 95, 1760–1769.
Blondin, D.P., Labbe, S.M., Tingelstad, H.C., Noll, C., Kunach, M., Phoenix, S., et al., 2014. Increased brown adipose tissue oxidative capacity in cold-acclimated humans. J. Clin. Endocrinol. Metab 99, E438–E446.
Brestoff, J.R., Kim, B.S., Saenz, S.A., Stine, R.R., Monticelli, L.A., Sonnenberg, G.F., et al., 2015. Group 2 innate lymphoid cells promote beiging of white adipose tissue and limit obesity. Nature 519, 242–246.
Cannon, B., Nedergaard, J., 2004. Brown adipose tissue: function and physiological significance. Physiol. Rev 84, 277–359.
Carey, A.L., Formosa, M.F., Van Every, B., Bertovic, D., Eikelis, N., Lambert, G.W., et al., 2012. Ephedrine activates brown adipose tissue in lean but not obese humans. Diabetologia 56, 147–155.
Celi, F.S., Brychta, R.J., Linderman, J.D., Butler, P.W., Alberobello, A.T., Smith, S., et al., 2010. Minimal changes in environmental temperature result in a significant increase in energy expenditure and changes in the hormonal homeostasis in healthy adults. Eur. J. Endocrinol 163, 863–872.
Cinti, S., 2009. Transdifferentiation properties of adipocytes in the Adipose Organ. Am. J. Physiol. Endocrinol. Metab 297, E977–E986.
Cohade, C., Mourtzikos, K.A., Wahl, R.L., 2003. "USA-Fat": prevalence is related to ambient outdoor temperature-evaluation with 18F-FDG PET/CT. J. Nucl. Med 44, 1267–1270.
Cunningham, J.J., 1982. Body composition and resting metabolic rate: the myth of feminine metabolism. Am. J. Clin. Nutr 36, 721–726.
Cypess, A.M., Lehman, S., Williams, G., Tal, I., Rodman, D., Goldfine, A.B., et al., 2009. Identification and importance of brown adipose tissue in adult humans. N. Engl. J. Med 360, 1509–1517.
Cypess, A.M., Chen, Y.C., Sze, C., Wang, K., English, J., Chan, O., et al., 2012. Cold but not sympathomimetics activates human brown adipose tissue in vivo. Proc. Natl. Acad. Sci. U.S.A. 109, 10001–10005.
Cypess, A.M., White, A.P., Vernochet, C., Schulz, T.J., Xue, R., Sass, C.A., et al., 2013. Anatomical localization, gene expression profiling and functional characterization of adult human neck brown fat. Nat. Med 19, 635–639.
Cypess, A.M., Weiner, L.S., Roberts-Toler, C., Elia, E.F., Kessler, S.H., Kahn, P.A., et al., 2015. Activation of human brown adipose tissue by a beta3-adrenergic receptor agonist. Cell Metab 21, 33–38.
Dong, M., Yang, X., Lim, S., Cao, Z., Honek, J., Lu, H., et al., 2013. Cold exposure promotes atherosclerotic plaque growth and instability via UCP1-dependent lipolysis. Cell Metab 18, 118–129.
English, J.T., Patel, S.K., Flanagan, M.J., 1973. Association of pheochromocytomas with brown fat tumors. Radiology 107, 279–281.
Esparza, J., Fox, C., Harper, I.T., Bennett, P.H., Schulz, L.O., Valencia, M.E., et al., 2000. Daily energy expenditure in Mexican and USA Pima Indians: low physical activity as a possible cause of obesity. Int. J. Obes. Relat. Metab. Disord 24, 55–59.
Esterbauer, H., Oberkofler, H., Liu, Y.M., Breban, D., Hell, E., Krempler, F., et al., 1998. Uncoupling protein-1 mRNA expression in obese human subjects: the role of sequence variations at the uncoupling protein-1 gene locus. J. Lipid Res 39, 834–844.

Fogelholm, M., Valve, R., Kukkonen-Harjula, K., Nenonen, A., Hakkarainen, V., Laakso, M., et al., 1998. Additive effects of the mutations in the beta3-adrenergic receptor and uncoupling protein-1 genes on weight loss and weight maintenance in Finnish women. J. Clin. Endocrinol. Metab 83, 4246–4250.
Fukuchi, K., Tatsumi, M., Ishida, Y., Oku, N., Hatazawa, J., Wahl, R.L., 2004. Radionuclide imaging metabolic activity of brown adipose tissue in a patient with pheochromocytoma. Exp. Clin. Endocrinol. Diabetes 112, 601–603.
Fukunaga, T., Zou, W., Rohatgi, N., Colca, J.R., Teitelbaum, S.L., 2014. An insulin-sensitizing thiazolidinedione, which minimally activates PPAR?, does not cause bone loss. J. Bone Miner. Res 30, 481–488.
Hany, T.F., Gharehpapagh, E., Kamel, E.M., Buck, A., Himms-Hagen, J., von Schulthess, G.K., 2002. Brown adipose tissue: a factor to consider in symmetrical tracer uptake in the neck and upper chest region. Eur. J. Nucl. Med. Mol. Imaging 29, 1393–1398.
Healy, G.N., Wijndaele, K., Dunstan, D.W., Shaw, J.E., Salmon, J., Zimmet, P.Z., et al., 2008. Objectively measured sedentary time, physical activity, and metabolic risk: the Australian Diabetes, Obesity and Lifestyle Study (AusDiab). Diabetes Care 31, 369–371.
Jespersen, N.Z., Larsen, T.J., Peijs, L., Daugaard, S., Homoe, P., Loft, A., et al., 2013. A classical brown adipose tissue mRNA signature partly overlaps with brite in the supraclavicular region of adult humans. Cell Metab 17, 798–805.
Kern, P.A., Finlin, B.S., Zhu, B., Rasouli, N., McGehee Jr., R.E., Westgate, P.M., et al., 2014. The effects of temperature and seasons on subcutaneous white adipose tissue in humans: evidence for thermogenic gene induction. J. Clin. Endocrinol. Metab 99, E2772–E2779 jc20142440.
Kim, S., Krynyckyi, B.R., Machac, J., Kim, C.K., 2008. Temporal relation between temperature change and FDG uptake in brown adipose tissue. Eur. J. Nucl. Med. Mol. Imaging 35, 984–989.
Kopecky, J., Clarke, G., Enerback, S., Spiegelman, B., Kozak, L.P., 1995. Expression of the mitochondrial uncoupling protein gene from the aP2 gene promoter prevents genetic obesity. J. Clin. Invest 96, 2914–2923.
Lean, M.E., James, W.P., Jennings, G., Trayhurn, P., 1986. Brown adipose tissue in patients with phaeochromocytoma. Int. J. Obes 10, 219–227.
Lee, M.W., Odegaard, J.I., Mukundan, L., Qiu, Y., Molofsky, A.B., Nussbaum, J.C., et al., 2015. Activated type 2 innate lymphoid cells regulate beige fat biogenesis. Cell 160, 74–87.
Lee, P., Greenfield, J.R., Ho, K.K., Fulham, M.J., 2010. A critical appraisal of the prevalence and metabolic significance of brown adipose tissue in adult humans. Am. J. Physiol. Endocrinol. Metab 299, E601–E606.
Lee, P., Swarbrick, M.M., Zhao, J.T., Ho, K.K., 2011. Inducible brown adipogenesis of supraclavicular fat in adult humans. Endocrinology 152, 3597–3602.
Lee, P., Zhao, J.T., Swarbrick, M.M., Gracie, G., Bova, R., Greenfield, J.R., et al., 2011. High prevalence of brown adipose tissue in adult humans. J. Clin. Endocrinol. Metab 96, 2450–2455.
Lee, P., Brychta, R., Linderman, J., Smith, S., Chen, K.Y., Celi, F.S., 2013. Mild cold exposure modulates fibroblast growth factor 21 (FGF21) diurnal rhythm in humans: relationship between FGF21 levels, lipolysis and cold-induced thermogenesis. J. Clin. Endocrinol. Metab 98, E98–E102.
Lee, P., Smith, S., Linderman, J., Courville, A.B., Brychta, R.J., Dieckmann, W., et al., 2014. Temperature-acclimated brown adipose tissue modulates insulin sensitivity in humans. Diabetes 63, 3686–3698.
Lee, P., Werner, C.D., Kebebew, E., Celi, F.S., 2014. Functional thermogenic beige adipogenesis is inducible in human neck fat. Int. J. Obes. (Lond) 38, 170–176.
Lee, P., Linderman, J.D., Smith, S., Brychta, R.J., Wang, J., Idelson, C., et al., 2014. Irisin and FGF21 are cold-induced endocrine activators of brown fat function in humans. Cell Metab 19, 302–309.
Lee, P., Birzniece, V., Umpleby, A.M., Poljak, A., Ho, K.K., 2015. Formoterol, a highly beta2-selective agonist, induces gender-dimorphic whole body leucine metabolism in humans. Metabolism 64, 506–512.
Lee, P., Swarbrick, M.M., Greenfield, J.R., 2015. The sum of all browning in FGF21 therapeutics. Cell Metab 21, 795–796.
Lidell, M.E., Betz, M.J., Dahlqvist Leinhard, O., Heglind, M., Elander, L., Slawik, M., et al., 2013. Evidence for two types of brown adipose tissue in humans. Nat. Med 19, 631–634.
Ma, S.W., Foster, D.O., 1986. Uptake of glucose and release of fatty acids and glycerol by rat brown adipose tissue in vivo. Can. J. Physiol. Pharmacol 64, 609–614.
Melicow, M.M., 1977. One hundred cases of pheochromocytoma (107 tumors) at the Columbia-Presbyterian Medical Center, 1926–1976: a clinicopathological analysis. Cancer 40, 1987–2004.
Nguyen, K.D., Qiu, Y., Cui, X., Goh, Y.P., Mwangi, J., David, T., et al., 2011. Alternatively activated macrophages produce catecholamines to sustain adaptive thermogenesis. Nature 480, 104–108.
Orava, J., Nuutila, P., Lidell, M.E., Oikonen, V., Noponen, T., Viljanen, T., et al., 2011. Different metabolic responses of human brown adipose tissue to activation by cold and insulin. Cell Metab 14, 272–279.
Ouellet, V., Routhier-Labadie, A., Bellemare, W., Lakhal-Chaieb, L., Turcotte, E., Carpentier, A.C., et al., 2011. Outdoor temperature, age, sex, body mass index, and diabetic status determine the prevalence, mass, and glucose-uptake activity of 18F-FDG-detected BAT in humans. J. Clin. Endocrinol. Metab 96, 192–199.
Ouellet, V., Labbe, S.M., Blondin, D.P., Phoenix, S., Guerin, B., Haman, F., et al., 2012. Brown adipose tissue oxidative metabolism contributes to energy expenditure during acute cold exposure in humans. J. Clin. Invest 122, 545–552.
Rao, R.R., Long, J.Z., White, J.P., Svensson, K.J., Lou, J., Lokurkar, I., et al., 2014. Meteorin-like is a hormone that regulates immune-adipose interactions to increase beige fat thermogenesis. Cell 157, 1279–1291.
Ravussin, E., Swinburn, B.A., 1993. Metabolic predictors of obesity: cross-sectional versus longitudinal data. Int. J. Obes. Relat. Metab. Disord 17 (Suppl. 3), S28–S31 discussion S41-2.
Ricquier, D., Nechad, M., Mory, G., 1982. Ultrastructural and biochemical characterization of human brown adipose tissue in pheochromocytoma. J. Clin. Endocrinol. Metab 54, 803–807.
Saito, M., Okamatsu-Ogura, Y., Matsushita, M., Watanabe, K., Yoneshiro, T., Nio-Kobayashi, J., et al., 2009. High incidence of metabolically active brown adipose tissue in healthy adult humans: effects of cold exposure and adiposity. Diabetes 58, 1526–1531.
Schernthaner, G., Chilton, R.J., 2010. Cardiovascular risk and thiazolidinediones—-what do meta-analyses really tell us? Diabetes Obes. Metab 12, 1023–1035.
Schernthaner, G., Currie, C.J., Schernthaner, G.H., 2013. Do we still need pioglitazone for the treatment of type 2 diabetes? A risk-benefit critique in 2013. Diabetes Care 36 (Suppl. 2), S155–S161.
Schiffelers, S.L., Saris, W.H., Boomsma, F., van Baak, M.A., 2001. beta(1)- and beta(2)-Adrenoceptor-mediated thermogenesis and lipid utilization in obese and lean men. J. Clin. Endocrinol. Metab 86, 2191–2199.
Sharp, L.Z., Shinoda, K., Ohno, H., Scheel, D.W., Tomoda, E., Ruiz, L., et al., 2012. Human BAT possesses molecular signatures that resemble beige/brite cells. PLoS ONE 7, e49452.
Shihara, N., Yasuda, K., Moritani, T., Ue, H., Uno, M., Adachi, T., et al., 2001. Synergistic effect of polymorphisms of uncoupling protein 1 and beta3-adrenergic receptor genes on autonomic nervous system activity. Int. J. Obes. Relat. Metab. Disord 25, 761–766.
Shintaku, K., Uchida, K., Suzuki, Y., Zhou, Y., Fushiki, T., Watanabe, T., et al., 2012. Activation of transient receptor potential A1 by a non-pungent capsaicin-like compound, capsiate. Br. J. Pharmacol 165, 1476–1486.
Sugita, J., Yoneshiro, T., Hatano, T., Aita, S., Ikemoto, T., Uchiwa, H., et al., 2013. Grains of paradise (Aframomum melegueta) extract activates brown adipose tissue and increases whole-body energy expenditure in men. Br. J. Nutr 110, 733–738.
Sugita, J., Yoneshiro, T., Sugishima, Y., Ikemoto, T., Uchiwa, H., Suzuki, I., et al., 2014. Daily ingestion of grains of paradise (Aframomum melegueta) extract increases whole-body energy expenditure and decreases visceral fat in humans. J. Nutr. Sci. Vitaminol. (Tokyo) 60, 22–27.
Turner, R.M., Kwok, C.S., Chen-Turner, C., Maduakor, C.A., Singh, S., Loke, Y.K., 2014. Thiazolidinediones and associated risk of bladder cancer: a systematic review and meta-analysis. Br. J. Clin. Pharmacol 78, 258–273.
van der Lans, A.A., Hoeks, J., Brans, B., Vijgen, G.H., Visser, M.G., Vosselman, M.J., et al., 2013. Cold acclimation recruits human brown fat and increases nonshivering thermogenesis. J. Clin. Invest 123, 3395–3403.
van Marken Lichtenbelt, W.D., Vanhommerig, J.W., Smulders, N.M., Drossaerts, J.M., Kemerink, G.J., Bouvy, N.D., et al., 2009. Cold-activated brown adipose tissue in healthy men. N. Engl. J. Med 360, 1500–1508.
Valve, R., Heikkinen, S., Rissanen, A., Laakso, M., Uusitupa, M., 1998. Synergistic effect of polymorphisms in uncoupling protein 1 and beta3-adrenergic receptor genes on basal metabolic rate in obese Finns. Diabetologia 41, 357–361.
Virtanen, K.A., Lidell, M.E., Orava, J., Heglind, M., Westergren, R., Niemi, T., et al., 2009. Functional brown adipose tissue in healthy adults. N. Engl. J. Med 360, 1518–1525.
Vosselman, M.J., van der Lans, A.A., Brans, B., Wierts, R., van Baak, M.A., Schrauwen, P., et al., 2012. Systemic beta-adrenergic stimulation of thermogenesis is not accompanied by brown adipose tissue activity in humans. Diabetes 61, 3106–3113.
Walden, T.B., Hansen, I.R., Timmons, J.A., Cannon, B., Nedergaard, J., 2012. Recruited vs. nonrecruited molecular signatures of brown, “brite,” and white adipose tissues. Am. J. Physiol. Endocrinol. Metab 302, E19–E31.
Wang, Q., Zhang, M., Ning, G., Gu, W., Su, T., Xu, M., et al., 2011. Brown adipose tissue in humans is activated by elevated plasma catecholamines levels and is inversely related to central obesity. PLoS ONE 6, e21006.
Wu, J., Bostrom, P., Sparks, L.M., Ye, L., Choi, J.H., Giang, A.H., et al., 2012. Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. Cell 150, 366–376.
Wu, J., Bostrom, P., Sparks, L.M., Ye, L., Choi, J.H., Giang, A.H., et al., 2012. Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. Cell 150, 366–376.
Wu, J., Cohen, P., Spiegelman, B.M., 2013. Adaptive thermogenesis in adipocytes: is beige the new brown? Genes Dev 27, 234–250.
Yoneshiro, T., Aita, S., Matsushita, M., Okamatsu-Ogura, Y., Kameya, T., Kawai, Y., et al., 2011. Age-related decrease in cold-activated brown adipose tissue and accumulation of body fat in healthy humans. Obesity (Silver Spring) 19, 1755–1760.
Yoneshiro, T., Ogawa, T., Okamoto, N., Matsushita, M., Aita, S., Kameya, T., et al., 2012. Impact of UCP1 and beta3AR gene polymorphisms on age-related changes in brown adipose tissue and adiposity in humans. Int. J. Obes. (Lond) 37, 993–998.
Yoneshiro, T., Aita, S., Matsushita, M., Kayahara, T., Kameya, T., Kawai, Y., et al., 2013. Recruited brown adipose tissue as an antiobesity agent in humans. J. Clin. Invest 123, 3404–3408.
Zhu, L., Su, T., Xu, M., Xu, Y., Li, M., Wang, T., et al., 2013. Eosinophil inversely associates with type 2 diabetes and insulin resistance in Chinese adults. PLoS ONE 8, e67613.
Zhu, Z.N., Jiang, Y.F., Ding, T., 2014. Risk of fracture with thiazolidinediones: an updated meta-analysis of randomized clinical trials. Bone 68, 115–123.