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Molecular pathways linking non-shivering thermogenesis and obesity: focusing on brown adipose tissue development

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ABSTRACT

An increase in energy intake and/or a decrease in energy expenditure lead to fat storage, causing overweight and obesity phenotypes. The objective of this review was to analyse, for the first time using a systematic approach, all published evidence from the past 8 years regarding the molecular pathways linking non-shivering thermogenesis and obesity in mammals, focusing on mechanisms involved in brown adipose tissue development. Two major databases were scanned from 2006 to 2013 using 'brown adipose tissue' AND 'uncoupling protein-1' AND 'mammalian thermoregulation' AND 'obesity' as key words. A total of 61 articles were retrieved using the search criteria. The available research used knockout methodologies, various substances, molecules and agonist treatments, or different temperature and diet conditions, to assess the molecular pathways linking non-shivering thermogenesis and obesity. By integrating the results of the evaluated animal and human studies, our analysis identified specific molecules that enhance non-shivering thermogenesis and metabolism by: (*i*) stimulating 'brite' (brown-like) cell development in white adipose tissue; (*ii*) increasing uncoupling protein-1 expression in brite adipocytes; and (*iii*) augmenting brown and/or brite adipose tissue mass. The latter can be also increased through low temperature, hibernation and/or molecules involved in brown adipocyte differentiation. Cold stimuli and/or certain molecules activate uncoupling protein-1 in the existing brown adipocytes, thus increasing total energy expenditure by a magnitude proportional to the number of available brown adipocytes. Future research should address the interplay between body mass, brown adipose tissue mass, as well as the main molecules involved in brite cell development.

Key words: brite cells, metabolism, energy expenditure, white adipose tissue, retinaldehyde dehydrogenase 1, tri-iodothyronine, uncoupling protein-1.

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I. INTRODUCTION

Since 1980, worldwide mean body mass index has been increasing by 0.45 kg/m^2 per decade (Finucane *et al.*, 2011). By the end of 2010, around 43 million children under the age of five were overweight, while rates of overweightness and obesity are projected to increase in almost all countries, leading to 1.5 billion overweight individuals by 2015 (World Health Organization, 2010). The countermeasure often proposed against overweightness and obesity is a reduction of energy intake (Golubovic *et al.*, 2013) and/or an increase in physical activity (Flouris *et al.*, 2008; Bischoff *et al.*, 2012; Carrillo *et al.*, 2013). However, increasing basal metabolic rate (BMR) through frequent stimulation of non-shivering thermogenesis (NST) – *via* changes in the thyroid hormones – is also a viable alternative that can be beneficial (Koutedakis *et al.*, 2005; Feldmann *et al.*, 2009; Shabalina *et al.*, 2010; Carrillo & Flouris, 2011). This is because human data summarized in a recent review showed that up to 20% of average daily metabolic rate may consist of cold-induced NST (van Marken Lichtenbelt & Schrauwen, 2011). In rodents, NST takes place exclusively in brown adipose tissue (BAT) by uncoupling protein-1 (UCP1) (Mattson, 2010), a mitochondrial inner membrane protein, while in humans NST can also occur in skeletal muscle (Wijers *et al.*, 2008; Mitchell *et al.*, 2010; van Marken Lichtenbelt & Schrauwen, 2011). The prevailing hypothesis to date is that the function of BAT in mammals is to maintain body temperature during exposure to cold which, as a consequence, leads to excess energy expenditure (EE) (Kozak, 2010). Indeed, frequent stimulation of NST irrevocably increases BMR, resulting in decreased body mass and stored fat (Feldmann *et al.*, 2009; Shabalina *et al.*, 2010).

Adipose tissue biology pertaining to UCP1-mediated thermogenesis is one of the most rapidly advancing areas in obesity research, with an ever-increasing number of molecular, physiological, pharmacological and dietary factors being implicated in the transdifferentiation of white adipocytes to 'brite' (i.e. brown-like) adipocytes and potential targets for obesity management. In rodents, brite cells in white adipose tissue express UCP1 and may play an important role in NST and metabolism when UCP1 is activated through pathways similar to those of brown adipocytes. Nevertheless, brite cells possess a different gene expression pattern (e.g. transcription factors) than classic brown adipocytes (Petrovic *et al.*, 2010; Walden *et al.*, 2012).

BMR is reduced with ageing in humans (Carrillo & Flouris, 2011), while an ageing effect on NST has been also observed in rodents (Feldmann *et al.*, 2009; Shabalina *et al.*, 2010), leading to overweightness or obesity. Moreover, a recent human study reported that obese individuals demonstrate low BAT activity and EE in response to cold compared with lean controls, suggesting that BAT is either reduced or absent in obese individuals (Vijgen *et al.*, 2011). Therefore, it is crucial to intensify research efforts aimed at the prevention of BAT loss and/or BAT regeneration to treat human adipose excess.

The importance of systematic analyses is based on reducing the likelihood of bias on the effects of certain phenomena across a wide range of settings and empirical methods (Wieseler & McGauran, 2010). However, recent research findings concerning BAT biology and its role in obesity have not yet been reviewed using a systematic methodology. Thus, the purpose of this review is to summarise critically– for the first time using a systematic approach – all the recent evidence on the molecular pathways linking NST and obesity in mammals, focusing on the mechanisms involved in BAT development.

II. METHODS

This systematic review was performed according to published guidelines (Wieseler & McGauran, 2010). The databases PubMed and Scopus were searched for articles published from the beginning of 2006 to the end of October 2013, using the key words 'brown adipose tissue' AND 'uncoupling protein-1' AND 'mammalian thermoregulation' AND 'obesity'. We used 2006 as a start year for this systematic search because, in that year, UCP1 was established as the only mediator of NST in mammalian BAT (Barger, Barnes & Boyer, 2006). The limitation 'research articles' was applied to the search parameters in order to exclude reviews and conference proceedings. Only English-language literature was considered eligible. Titles and abstracts were screened independently by two reviewers (A.V. and A.D.F.) to identify relevant articles.

Due to the methodology outlined above, our searching and selection procedure eliminated bias since the inclusion (or not) of a study was based on content (i.e. investigating molecular pathways linking NST and obesity with a focus on the mechanisms involved in mammalian BAT development), and not on quality, journal, or other factors. A total of 56 articles were retrieved during the search and all were identified as relevant (Fig. 1). Five additional articles were

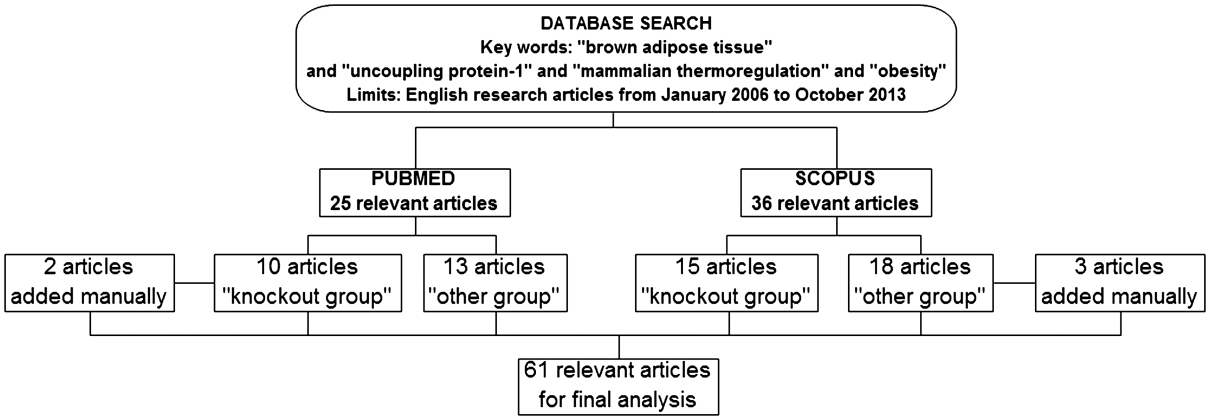

Fig. 1. Systematic selection process used to identify relevant studies within the adopted criteria.

included manually. These five articles met all the set criteria but were excluded erroneously from the 56 articles when the search limits were applied. Therefore, 61 articles meeting all the established criteria were considered for subsequent analysis. To enable a systematic synthesis of the presented evidence, the articles were separated into two categories based on research methodology: 'knockout studies' in which gene-function analysis was performed using knockout (KO) animals lacking the gene under study (Table 1), and 'other studies' in which the analysis was carried out using various substances, molecules/agonist treatments, as well as different temperature and diet conditions (Table 2).

III. RESULTS

(1) Knockout studies

The analysed studies adopting KO gene analysis targeted: (*a*) UCP1, (*b*) molecules of the nervous system, or (*c*) receptors, enzymes, and transcription factors to analyse their impact on NST heat production and obesity. Thus, the 'knockout studies' were separated into 'UCP1-KO', 'Brain', and 'Receptors, enzymes, and transcription factors' groups, respectively (Table 1).

(*a*) *UCP1-KO group*

Fatty acid-binding protein 3 is essential for accelerating fatty acid flux into the tissue from the bloodstream and for stimulating UCP1 activity for NST in rat BAT (Yamashita *et al.*, 2008). UCP1 activity prevents the development of obesity and is the only protein that can mediate diet-induced thermogenesis in mouse BAT (Feldmann *et al.*, 2009). Specifically, β3-adrenergic stimulation induces BAT NST which is fully UCP1-dependent (Inokuma *et al.*, 2006). UCP2 and UCP3 are not involved in this process but they play roles in the regulation of cellular energy levels and/or oxidation of fatty acids (Inokuma *et al.*, 2006). Therefore, given that UCP1 is the only player in NST heat production, its ablation becomes obesogenic in mice fed with both high-fat and normal diets due to an inability to stimulate diet-induced thermogenesis (Feldmann *et al.*, 2009). The UCP1-KO mice also demonstrate slow arousal from torpor because re-warming is less effective and requires additional energy (Oelkrug, Heldmaier & Meyer, 2011). Despite their dysfunctional NST, UCP1-KO mice are cold tolerant probably due to: (*i*) persistent muscle shivering (Shabalina *et al.*, 2010); (*ii*) up-regulation of ATP-Mg^{2+}/Pi inner mitochondrial membrane solute transporter gene in skeletal muscle [which could be a candidate for shivering thermogenesis (Anunciado-Koza *et al.*, 2011)]; and/or (*iii*) reduction of heat loss through adrenergic vasoconstriction (Meyer *et al.*, 2010). On the other hand, ectopic UCP1 expression in skeletal muscles results in reduced adiposity and augmented EE in mice (Adjeitey *et al.*, 2013). Moreover, the activity of UCP1 in muscle mitigates the production of reactive oxygen species in the mitochondria but not in BAT (Adjeitey *et al.*, 2013).

(*b*) *Brain group*

It is well known that the hypothalamus links the nervous system to endocrine secretion *via* the pituitary and that both the hypothalamus and the pituitary express factors involved in NST and the regulation of temperature homeostasis in mammals. Specifically, cytosolic brain-type creatine kinase deficiency in mice inhibits neuronal communication between different hypothalamic circuits involved in NST (Streijger *et al.*, 2009). Moreover, dorsomedial hypothalamic neuropeptide Y knockdown in rats increases EE and the thermogenic response to cold, and stimulates brown adipocyte formation in inguinal white adipose tissue (WAT) (Chao *et al.*, 2011). Other central circuits, including the pro-opiomelanocortin neurons that are expressed in the pituitary, also regulate energy balance (De Jonghe *et al.*, 2011). Down-regulation of pro-opiomelanocortin-protein tyrosine phosphatase 1B, a negative regulator of leptin signalling, reduces food intake and body mass in mice and increases their core temperature. Furthermore, pro-opiomelanocortin-protein tyrosine phosphatase 1B KO mice show an increase in BAT mass, UCP1 mRNA in BAT,

Table 1. The 'Knockout studies' articles identified by our search criteria and their main results

Group	Article	Main results
UCP1-KO	Yamashita *et al.* (2008)	FABP3 affects free fatty acid flux and ↑ UCP1 thermogenesis.
	Feldmann *et al.* (2009)	UCP1 activity affects obesity development in mice and humans.
	Inokuma *et al.* (2006)	Anti-obesity effect of β3-adrenergic stimulation is largely attributable to UCP1 in BAT.
	Oelkrug *et al.* (2011)	Functional BAT is essential for rapid arousal from torpor.
	Shabalina *et al.* (2010)	↑ shivering muscle and ↓ heat loss induces cold adaptations in UCP1-KO mice.
	Anunciado-Koza *et al.* (2011)	SLC25A25 induces alternative thermogenesis.
	Meyer *et al.* (2010)	↓ heat loss by ↑ vasoconstriction and ↓ thermal conductance in UCP1-KO mice.
	Adjeitey *et al.* (2013)	UCP1 expression in muscles ↑ EE, ↓ body mass, and ↓ reactive oxygen species production.
Brain	Streijger *et al.* (2009)	Inefficient neuronal transmission induces dysfunction in NST.
	Chao *et al.* (2011)	Deficiency of NPY induces ↑ both WAT lipogenesis and BAT thermogenesis.
	De Jonghe *et al.* (2011)	Deficiency of PTP1B in POMC induces ↑ cold-induced thermogenesis through thyroid axis.
Receptors, enzymes, and transcription factors	Korach-Andréa *et al.* (2011)	Deficiency of LXRα and LXRβ induces ↑ EE, ↑ UCP1 expression and ↓ body mass loss.
	Vila-Bedmar *et al.* (2012)	GRK2 hemizygous (+/−) induces ↑ NST, ↑ EE and ↓ body mass.
	Pelletier *et al.* (2008)	Chronic muscle shivering in mice with dysfunctional NST.
	Hudson-Davies *et al.* (2009)	Deficiency of RIP140 ↓ metabolism and ↓ thermoregulatory adaptations.
	Gray *et al.* (2006)	PPAR-γ is required for full activation and recruitment of brown adipocytes.
	Giralt *et al.* (2011)	Sirt3 is essential for the differentiation of fully thermogenic-competent brown adipocytes.
	Bordicchia *et al.* (2012)	Cardiac NPs induce ↑ BAT thermogenesis, ↑ PGC-1α and ↑ UCP1 and ↓ WAT mass *via* p38 MAPK.
	Tseng *et al.* (2008)	BMP-7 induces brown adipocyte differentiation and NST.
	Jimenez-Preitner *et al.* (2011)	Plac8 is required for BAT differentiation and thermogenic capacity.
	Czyzyk *et al.* (2012)	δ-opioid receptor deficiency induces ↑ UCP1 and ↓ adiposity.
	Satyanarayana *et al.* (2012)	Id1 deficiency induces ↑ UCP1, ↑ EE, and ↑ fatty acid oxidation.
	Lin *et al.* (2011)	GHS-R ablation induces ↑ insulin sensitivity, ↑ EE, ↑ resting metabolic rate and ↑ UCP1 during ageing.
	Ma *et al.* (2011)	GHS-R regulates BAT NST, adiposity, metabolism and energy homeostasis during ageing.
	Mano-Otagiri *et al.* (2010)	GHS-R/ghrelin induces ↓ EE and ↑ EI by suppressing the SNS innervating BAT.
	Kiefer *et al.* (2012)	Rald induces ↑ transcription of brown fat markers in WAT.
	Bauwens *et al.* (2011)	α1-AMPK does not play a key role in NST and body mass.

↑, increase; ↓, decrease; α1-AMPK, α1-AMP-activated protein kinase; BAT, brown adipose tissue; BMP-7, bone morphogenetic protein 7; EE, energy expenditure; EI, energy intake; FABP3, fatty acid-binding protein 3; GHS-R, growth hormone secretagogue receptor; GRK2, G-protein-coupled receptor kinase 2; Id1, inhibitor of DNA binding 1; KO, knockout; LXR, liver-X receptor; NPs, natriuretic peptides; NPY, hypothalamic neuropeptide Y; NST, non-shivering thermogenesis; p38 MAPK, p38 mitogen-activated protein kinase; PGC-1α, peroxisome proliferator-activated receptor gamma co-activator 1-α; Plac8, placenta-specific 8; POMC, proopiomelanocortin; PPAR-γ, peroxisome proliferator-activated receptor γ; PTP1B, protein tyrosine phosphatase 1B; Rald, retinaldehyde dehydrogenase 1; RIP140, receptor interacting protein 140; Sirt3, silent mating type information regulation 2, homolog 3; SLC25A25, solute carrier family 25 (mitochondrial carrier; phosphate carrier), member 25; SNS, sympathetic nervous system; UCP1, uncoupling protein-1; WAT, white adipose tissue.

and serum tri-iodothyronine (T3) and type 2 iodothyronine deiodinase in response to cold exposure despite stable core temperature, food intake, and body mass (De Jonghe *et al.*, 2011). These results highlight the negative effect of the expression of dorsomedial hypothalamic neuropeptide Y and pro-opiomelanocortin-protein tyrosine phosphatase 1B on body mass regulation *via* food intake, adiposity, NST, and EE (Chao *et al.*, 2011; De Jonghe *et al.*, 2011).

(*c*) *Receptors, enzymes, and transcription factors group*

The brain plays an important role in metabolism and thermoregulation, yet the function of the heart in these pathways also appears to be important. This is due to the release of natriuretic peptides from myocardiocytes which can regulate UCP1 expression promoting the binding of its transcription factors (Bordicchia *et al.*, 2012). Indeed, nuclear receptors (described in detail below) play an important role in

Table 2. The 'other studies' identified by our search criteria and their main results

Group	Article	Main results
Molecule treatment	Lehr *et al.* (2009)	T3 in brown adipocyte medium induces differentiation of WAT stroma vascular cells in brown adipocytes-like cells.
	Bartolomucci *et al.* (2006)	TLQP-21 ↑ EE and blocks the early phase of high-fat-diet-induced obesity.
	Kim *et al.* (2006)	PE extract ↑ BAT thermogenesis and WAT fatty acid oxidation.
	Matsen *et al.* (2013)	Thyroid hormone β-selective agonist GC-1 induces ↑ UCP1, ↑EE, and ↓ adiposity.
	Ma *et al.* (2012)	L-menthol induces ↑ Tc, ↑ UCP1, and ↓ body mass.
	Romero del Mar *et al.* (2009)	OE maintains thermogenic potential.
	Wargent *et al.* (2013)	ZAG induces no effect on UCP1 expression and NST activity.
	Moriya *et al.* (2006)	QRFP43 ↑ feeding, ↓ EE, and induces obesity.
Sex and polymorphisms	Rodriguez-Cuenca *et al.* (2007*a*)	Variation of sex steroid receptor expression in BAT of male, female, pregnant and lactating females.
	Rodriguez-Cuenca *et al.* (2007*b*)	Sex hormones affect mitochondrial transcription factors and mitochondriogenesis.
	Shore *et al.* (2012)	CpG methylation in the UCP1 promoter has no role in UCP1 expression.
	Rose *et al.* (2011)	UCP1 transcription is ↑ by progesterone and retinoic acid but ↓ by 17-β estradiol.
	Nikolic *et al.* (2011)	Sex-difference effect of PKG-I activation on BAT thermogenesis and WAT lipolysis.
Temperature	Wells *et al.* (2012)	Opa3 gene mutation indices ↓ NST and ↑ lipid storage in BAT.
	Oelkrug *et al.* (2013)	Original function of BAT in defending mammal high body temperature for migration to cold.
	Cypess *et al.* (2013)	Higher UCP1 expression in longus colli and carotid sheath than superficial BAT depots.
	van der Lans *et al.* (2013)	Cold acclimation induces ↑ BAT activity and ↑ NST but no effect on brite development.
	Yoneshiro *et al.* (2013)	Cold exposure induces ↑EE, ↑ BAT activity, and ↓ body fat mass.
	Li *et al.* (2013)	Cold exposure induces ↑ Bmal1 which is involved in lipid catabolism.
	Chappuis *et al.* (2013)	Cold exposure induces ↑ Per2 which is a co-activator of UCP1 transcription factors.
	Barger *et al.* (2006)	UCP1 is the only mediator of NST in BAT.
	Liu *et al.* (2009)	Low temperature ↑NST, ↑RMR and ↑ total respiratory capacity of BAT.
	Zhang *et al.* (2009)	Cold exposure ↑EE, ↑ thermogenic capacity, and ↑EI.
	Zhang *et al.* (2012)	Cold exposure induces ↑ UCP1, ↑ body mass, and ↓ leptin.
	Kitao & Hashimoto (2012)	Hibernation induces ↑ BAT thermogenesis at low temperature.
	Chen *et al.* (2012)	Cold exposure and short photoperiod induce ↑ T3, ↑ UCP1, ↑NST, ↓ leptin and ↓ body mass.
Diet and reproductive state	Xiao *et al.* (2007)	Early postnatal over-nutrition induces adverse BAT impact reducing NST in adulthood.
	Zhao & Wang (2009)	Changes in nutritional fibre content impact BAT activity, basal metabolic rate, and NST.
	Primeaux *et al.* (2007)	Spinal cord injury induces ↓ body mass, ↓ WAT mass, ↓ UCP1 mRNA and ↑ caloric intake.
	Zhang *et al.* (2008)	Lactating voles ↑ caloric intake, ↑resting metabolic rate, ↓ UCP1 and ↓ body fat mass.
	Leitner & Bartness (2009)	MSG ↓BAT NST and induces obesity.
	Cheng *et al.* (2010)	Leucine deprivation induces ↑ EE, ↑ UCP1, ↓ EI, ↓ body mass.
	Du *et al.* (2012)	Isoleucine or valine deprivation induces ↑ EE, ↑ UCP1, ↓ EI, ↓ body mass.
	Noatsch *et al.* (2011)	↑ proteins or leucine in diet have no effect on energy homeostasis and UCP1 expression.

↑, increase; ↓, decrease; BAT, brown adipose tissue; Bmal1, brain and muscle arnt-like 1; CpG, cytosine–phosphate–guanine; EE, energy expenditure; EI, energy intake; GC-1 = 2-[4-[(4-hydroxy-3-propan-2-ylphenyl)methyl]-3,5-dimethylphenoxy]acetic acid; MSG, monosodium glutamate; NST, non-shivering thermogenesis; OE, oleoyl-oestrone; Opa3, optic atrophy 3; PE, *Pinellia ternata*; Per2, period2; PKG-I, cyclic guanosine monophosphate-dependent protein kinase I; QRFP43, pyroglutamylated arginine-phenylalanine-amide peptide; RMR, resting metabolic rate; T3, tri-iodothyronine; Tc, core temperature; TLQP-21, VGF-derived peptide; UCP1, uncoupling protein 1; WAT, white adipose tissue; ZAG, zinc-α2-glycoprotein.

the control of metabolism and thermoregulation, regulating the expression of specific genes involved in both mechanisms. The activity of these nuclear receptors is mediated by a variety of cofactors and enzymes (also described in detail below) whose activity, in turn, depends on specific G-protein-coupled receptors. To evaluate effectively the gene function of these receptors, enzymes and transcription factors on NST and metabolism, the relevant studies were separated into: positive-effect genes (the activation of which promotes NST and EE) and negative or null-effect genes (the activation of which decreases or does not affect BAT activity and metabolism, respectively).

(*i*) *Positive-effect genes*. Liver-X receptors α and β play key roles in both NST and metabolism in mice, controlling EE and body mass loss through UCP1 expression in BAT (Korach-Andréa *et al.*, 2011). Other receptors directly involved in the control of EE and body mass balance include the G-protein-coupled receptor kinase 2 (Vila-Bedmar *et al.*, 2012), the thyroid hormone receptor (Pelletier *et al.*, 2008), and the receptor interacting protein 140 (Hudson-Davies *et al.*, 2009). G-protein-coupled receptor kinase 2 hemizygous (+/−) adult mice exhibit increased EE, BAT activity, white and brown fat lipid catabolism as well as core temperature after a cold exposure compared with wild-type animals (Vila-Bedmar *et al.*, 2012). On the other hand, studies using KO mice for thyroid hormone receptor or receptor interacting protein 140 report a significant reduction in BAT mass associated with poor NST, as well as disturbed BAT metabolic gene expression (Hudson-Davies *et al.*, 2009). These results indicate that the activation of these receptors results in a positive effect on NST and metabolism.

Transcription factors such as peroxisome proliferator-activated receptor γ (PPAR-γ) (Gray *et al.*, 2006) as well as molecules including silent mating type information regulation 2, homolog 3 (Sirt3; (Giralt *et al.*, 2011), cardiac natriuretic peptides (Bordicchia *et al.*, 2012), bone morphogenetic protein 7 (Tseng *et al.*, 2008) and placenta-specific gene 8 protein (Jimenez-Preitner *et al.*, 2011) are involved in mouse NST playing key roles in both UCP1 activation and brown adipocyte differentiation. Specifically, the transcription factor peroxisome proliferator-activated receptor γ co-factor 1α (PGC-1α) is unable to activate the brown adipocyte differentiation and the full programme of thermogenic genes, such as UCP1 and type 2 iodothyronine deiodinase expression, in mice lacking Sirt3 (Giralt *et al.*, 2011). Moreover, cardiac natriuretic peptides promote brite cell development (and, thus, brite mass) and UCP1 expression in both natriuretic peptide receptor C-null mice and *in vitro* in human white adipocytes through the activation of natriuretic peptide receptor A (Bordicchia *et al.*, 2012).

(*ii*) *Negative- or null-effect genes*. Despite the aforementioned effect of specific receptors and transcription factors on increasing metabolism and NST, the expression of a group of G-protein-coupled receptors, δ-opioid receptors (Czyzyk *et al.*, 2012), as well as inhibitor of DNA-binding protein 1 (Satyanarayana *et al.*, 2012), attenuates the activity of UCP1 in mice. Indeed, δ-opioid receptor (Czyzyk *et al.*, 2012) or inhibitor of DNA-binding protein 1 (Satyanarayana *et al.*, 2012) deficient mice demonstrate increased NST and EE as well as reduced body mass. Similarly, the ablation of growth hormone secretagogue receptor increases thermogenic capacity and EE in both mice and rats (Mano-Otagiri *et al.*, 2010; Lin *et al.*, 2011; Ma *et al.*, 2011). The absence of this receptor under both standard and high-fat diets leads to a lean mouse phenotype as well as increased EE, BMR, BAT mass and UCP1 gene expression (Mano-Otagiri *et al.*, 2010; Lin *et al.*, 2011; Ma *et al.*, 2011). Lack of this receptor also leads to increased insulin sensitivity and resistance to the effect of ghrelin on BAT especially in older mice (Mano-Otagiri *et al.*, 2010; Lin *et al.*, 2011; Ma *et al.*, 2011).

Aldehyde dehydrogenase 1 family member A1 – expressed more in WAT than BAT – irreversibly converts retinaldehyde dehydrogenase 1 (Rald) to retinoic acid resulting in a lower WAT functional plasticity (Kiefer *et al.*, 2012). Indeed, in aldehyde dehydrogenase 1 family member A1 deficient mice as well as human white adipocytes, Rald was not converted into retinoic acid and, thus, it could promote transcription of BAT markers in WAT [including UCP1, PPAR-γ, PGC-1α, PR (positive regulatory domain 1 binding factor 1 and retinoblastoma protein-interacting zinc-finger protein) domain containing 16 (PRDM16), and fatty acid-binding protein 3] (Kiefer *et al.*, 2012). This finding demonstrates the key role of Rald in human brite cell development. In contrast to all aforementioned positive- and negative-effect genes, α_1-AMP-activated protein kinase is a metabolic regulator but does not play a role in mouse NST and body mass regulation (Bauwens *et al.*, 2011).

(2) Other studies

To synthesise the available evidence systematically and highlight the critical advances of the evaluated studies, the papers included in the 'other studies' category were separated into four groups: 'Molecule treatment', 'Sex and polymorphisms', 'Temperature', and 'Diet and reproductive state' in which the effects of molecules, sex, UCP1 gene polymorphisms, temperature, litter size and diet were analysed, respectively, in terms of metabolism and NST (Table 2).

(*a*) *Molecule treatment group*

Circulating hormones such as insulin and T3 as well as β_3-adrenoceptor agonist CL 316243 influence NST activity and stimulate brite cell development in WAT (Lehr *et al.*, 2009). Such effects, however, are not exerted by circulating oleoyl-oestrone (Romero del Mar *et al.*, 2009) and by the β_3-adrenoceptor agonist zinc-α_2-glycoprotein (Wargent *et al.*, 2013). Mouse WAT stroma vascular cells that are cultured in brown adipocyte medium containing T3 and β_3-adrenoceptor agonist CL 316243 undergo brite cell transformation. However, natural substances such as L-menthol in mice (Ma *et al.*, 2012) and *Pinellia ternata* extract in rats (Kim *et al.*, 2006), as well as the administration of the thyroid hormone β-selective

agonist 2-[4-[(4-hydroxy-3-propan-2-ylphenyl)methyl]-3,5-dimethylphenoxy]acetic acid (GC-1) in diabetic rats (Matsen *et al.*, 2013) increase NST and EE and reduce adiposity, but are not involved in brite cell development. Moreover, treatments with specific neuropeptides lead to similar results on NST and EE. Indeed, the VGF-derived peptide TLQP-21 increases NST and EE, limiting mass gain in rats (Bartolomucci *et al.*, 2006). By contrast, a different neuropeptide, pyroglutamylated arginine-phenylalanine-amide peptide (QRFP43), reduces mouse BAT-induced NST and results in obesity phenotype development (Moriya *et al.*, 2006).

(*b*) *Sex and polymorphisms group*

The different gene expression of steroid receptors (i.e. oestrogen receptor α and androgen receptor) in BAT of male and female rats explains sex hormone effects on BAT physiology (Rodriguez-Cuenca *et al.*, 2007*a*). Progesterone activates mitochondrial biogenesis by modifying the mRNA expression of several mitochondrial transcription factors. For instance, when combined with norepinephrine, progesterone increases the mRNA levels of PPAR-γ, but testosterone and 17β-estradiol down-regulate its expression (Rodriguez-Cuenca *et al.*, 2007*b*). Therefore, these sex hormones are responsible, at least in part, for the differences in mitochondrial biogenesis between the sexes (Rodriguez-Cuenca *et al.*, 2007*b*). Additional human evidence shows that methylation of the cytosine–phosphate–guanine (CpG) islands in the promoter region of UCP1 does not play a role in UCP1 protein expression (Shore *et al.*, 2012), but haplotypic variation in the 5′-enhancer region of this gene affects NST and metabolism by altering UCP1 protein levels (Rose *et al.*, 2011). When this variation is present, 17-βestradiol and progesterone produce opposing effects on UCP1 transcription (Rose *et al.*, 2011). Specifically, UCP1 transcription activity is increased by progesterone and retinoic acid and decreased by 17-βestradiol in the presence of polymorphisms A-3826-G and, primarily, C-3737-A in the UCP1 gene sequence (Rose *et al.*, 2011). On the other hand, overexpression of cyclic guanosine monophosphate-dependent protein kinase I protects female but not male mice against diet-induced obesity by stimulating lipolysis in WAT as well as NST in BAT (Nikolic *et al.*, 2011).

(*c*) *Temperature group*

The recent identification of active BAT in protoendothermic mammals (Oelkrug *et al.*, 2013) supports the prevailing hypothesis that BAT evolved to defend body temperature for migration to cold environments. This hypothesis is also supported by the recent discovery of higher UCP1 expression in the human longus colli and carotid sheath compared to superficial BAT depots. Indeed, the anatomical localisation of these depots, which are adjacent to the sympathetic chain and the carotid arteries, permits a rapid response to cold stimuli as well as effective heating of the cerebral vasculature (Cypess *et al.*, 2013). The recent finding that cold acclimation leads to increased EE and BAT activity in humans – without an effect on brite cell development in abdominal subcutaneous WAT (van der Lans *et al.*, 2013; Yoneshiro *et al.*, 2013) – further supports the involvement of BAT in body temperature regulation. Indeed, cold exposure in mice stimulates NST and induces the expression of clock genes, such as brain and muscle arnt-like 1 (Li *et al.*, 2013) and period2 (Chappuis *et al.*, 2013), that are involved in lipid catabolism and co-activation of UCP1 transcription factors leading to increased EE. Despite these findings demonstrating a link between BAT and EE, it is important to mention that the presence of the mitochondrial regulator function optic atrophy 3 gene mutation leads to an increase of lipid storage in mouse BAT without NST activation (Wells *et al.*, 2012).

Different lines of evidence suggest that low temperature increases EE, BMR and NST in BAT in non-acclimated Brandt's voles (*Lasiopodomys brandtii*) (Liu *et al.*, 2009; Zhang, Jing & Wang, 2009) as well as in tree shrew (*Tupaia belangeri*) (Zhang *et al.*, 2012). Interestingly, the effect of cold also has been confirmed in cold-acclimated mammals. For instance, prolonged cold exposure of Arctic ground squirrels (*Urocitellus parryii*) generates significant increases in UCP1 mRNA and protein levels in BAT (Barger *et al.*, 2006). The same study demonstrated that this cold-induced NST is not produced by up-regulation of UCP3 in the muscle. A study on Syrian hamsters (*Mesocricetus auratus*) showed that the ability of cold exposure to induce BAT thermogenesis and reduce body mass is stronger in hibernating animals due to their greater BAT mass compared to cold- or warm-acclimated animals (Kitao & Hashimoto, 2012). On the other hand, the effect of cold on body mass loss appears to be linked with photoperiod. Specifically, the combined effect of cold and short photoperiod (typically occurring in the winter and potentially serving as a seasonal cue for the acclimation of energy balance) leads to reduced body mass in Maximowiczi's voles (*Microtus maximowiczii*) (Chen, Zhong & Wang, 2012).

(*d*) *Diet and reproductive state group*

It is well known that nutrition is considered an important aspect in both metabolism and NST playing a key role in obesity and energy balance. In this light, while BAT serves to maintain body temperature during exposure to cold, leading to excess EE (Kozak, 2010), research has shown that BAT thermogenesis can be also influenced by dietary factors. Specifically, early postnatal over-feeding in rats leads to sympathetic nervous system-mediated permanent changes in BAT thermogenesis resulting in reduced NST rates during adulthood (Xiao *et al.*, 2007). However, this effect on BAT activity is dependent on nutritional fibre content. Indeed, high-fibre diet acclimation in adult Mongolian gerbils (*Meriones unguiculatus*) decreases BMR and NST, but these changes are reversed after restoration to a low-fibre diet (Zhao & Wang, 2009).

Adaptation to diet of different fibre content occurs mainly *via* phenotypic plasticity in digestive tract morphology

which counteracts changes in body mass (Zhao & Wang, 2009). Another example of organ adaptation involves high thoracic spinal cord injury which triggers a prolonged post-injury loss of body mass and a reduction in WAT mass in rats (Primeaux, Tong & Holmes, 2007). Interestingly, these changes occur despite a decrease in BAT UCP1 gene expression and a higher cumulative caloric intake (Primeaux *et al.*, 2007). Therefore, this phenotype is not due to hypophagia or an increase in sympathetic nervous system-induced thermogenesis, but is caused by permanent changes in gastrointestinal transit and absorption, as well as whole-body homeostatic mechanisms (Primeaux *et al.*, 2007). This is also confirmed in lactating Brandt's voles that show a reduction in body fat mass and UCP1 content despite an increase in energy intake compared to non-reproductive animals. The fact that NST was not augmented during lactation despite a rise in BMR may be an energy-conservation strategy for milk production (Zhang, Li & Wang, 2008).

Other dietary factors such as the inclusion of food additives and/or the removal of specific branched-chain amino acids can also influence NST and metabolism. Siberian hamsters (*Phodopus sungorus*) treated with monosodium glutamate (a molecule used as a food additive) showed a reduction in BAT activity associated with obesity phenotype development (Leitner & Bartness, 2009). This effect on NST and metabolism can also result from feeding animals diets rich in leucine (Cheng *et al.*, 2010), valine and isoleucine (Du *et al.*, 2012). Mice fed with leucine or valine or an isoleucine deprivation diet show an increase in EE, expression of β-oxidation genes, and lipolysis (Cheng *et al.*, 2010; Du *et al.*, 2012). In addition, lipogenic gene expression and fatty acid synthesis in WAT are diminished, together with a reduction in food intake primarily due to isoleucine and valine. These findings together with a higher UCP1 activity in BAT – despite a reduction in BAT mass – could explain the WAT loss observed in these isoleucine- or valine-deprived mice compared to controls (Cheng *et al.*, 2010; Du *et al.*, 2012). Finally, exposure to high-protein-content diets or leucine supplementation does not affect energy homeostasis and UCP1 expression (Noatsch *et al.*, 2011).

IV. DISCUSSION

The objective of this paper was to evaluate critically, using a systematic approach, recent evidence on the molecular pathways linking NST and obesity in mammals, focusing on the mechanisms involved in BAT development. Our results, summarised in Fig. 2, demonstrated that molecules such as fatty acid-binding protein 3 (Yamashita *et al.*, 2008), cardiac natriuretic peptides (Bordicchia *et al.*, 2012), *Pinellia ternate* extract (Kim *et al.*, 2006), L-menthol (Ma *et al.*, 2012), progesterone (Rose *et al.*, 2011), bone morphogenetic protein 7 (Tseng *et al.*, 2008), PPAR-γ (Gray *et al.*, 2006), placenta-specific 8 (Jimenez-Preitner *et al.*, 2011), T3 (Lehr *et al.*, 2009), Sirt3 (Giralt *et al.*, 2011), period2 (Chappuis *et al.*, 2013) as well as cold stimuli (Barger *et al.*, 2006; Liu *et al.*, 2009; Zhang *et al.*, 2009; Chappuis *et al.*, 2013; van der Lans *et al.*, 2013; Yoneshiro *et al.*, 2013) can increase UCP1 expression in existing brown adipocytes (Fig. 2: mechanism A). Specific molecules such as PPAR-γ (Gray *et al.*, 2006), placenta-specific 8 (Jimenez-Preitner *et al.*, 2011), Rald (Kiefer *et al.*, 2012), T3 (Lehr *et al.*, 2009), cardiac natriuretic peptides and cold exposure (Bordicchia *et al.*, 2012) are implicated in brite cell development (Fig. 2: mechanism B). In turn, this leads to enhanced numbers of brite cells (i.e. brite mass) in WAT that contribute to increased UCP1 expression in WAT. It is important to note that the effect of cold exposure on white-to-brite transdifferentiation has been confirmed in rodents (Bordicchia *et al.*, 2012), yet further research is required to assess its efficacy in humans (van der Lans *et al.*, 2013). On the other hand, cold exposure as well as molecules such as Sirt3 (Giralt *et al.*, 2011), bone morphogenetic protein 7 (Tseng *et al.*, 2008), PPAR-γ (Gray *et al.*, 2006), placenta-specific 8 (Jimenez-Preitner *et al.*, 2011), and T3 (Lehr *et al.*, 2009) stimulate brown adipocyte differentiation (Fig. 2: mechanism C), leading to augmented BAT mass. Interestingly, the increase in BAT mass can also emerge from hibernation (Kitao & Hashimoto, 2012) (Fig. 2: mechanism C), a concept discussed further below. The up-regulation of UCP1 gene and protein expression in brown and brite adipocytes through the aforementioned mechanisms A and/or B, respectively, leads to augmented NST and EE. Finally, frequent NST stimulation can result, eventually, in a rise of BMR and a reduction in adiposity levels (Koutedakis *et al.*, 2005; Feldmann *et al.*, 2009; Shabalina *et al.*, 2010; Carrillo & Flouris, 2011).

It is worth noting that the evaluated studies suggest that specific molecules such as fatty acid-binding protein 3 (Yamashita *et al.*, 2008), *Pinellia ternate* extract (Kim *et al.*, 2006), L-menthol (Ma *et al.*, 2012), and progesterone (Rose *et al.*, 2011) are involved in the activation of UCP1 in the existing brown adipocytes (Fig. 2: mechanism A) but do not contribute to BAT mass development. Thus, their effects on NST and metabolism depend on the available number of brown adipocytes. This is confirmed by the finding that cold exposure generates greater NST response and body mass reduction in hibernating animals that demonstrate larger BAT mass compared to warm- or cold-acclimated animals that demonstrate lower BAT mass (Kitao & Hashimoto, 2012).

The transdifferentiation of white adipocytes to brite adipocytes (i.e. increasing brite mass) in mammals is a crucial step in the process towards increasing NST and metabolism, given the key role of brite mass in UCP1 expression. Stimulating a larger mass of cells to express UCP1 results in a more prominent increase of NST (Kitao & Hashimoto, 2012) thus augmenting total EE by a magnitude proportional to the number of available brown and brite adipocytes. Moreover, frequent NST stimulation, without food intake compensation, may lead to body mass loss. Several early studies showed that BAT is present in adult humans (Heaton, 1972; Huttunen, Hirvonen & Kinnula,

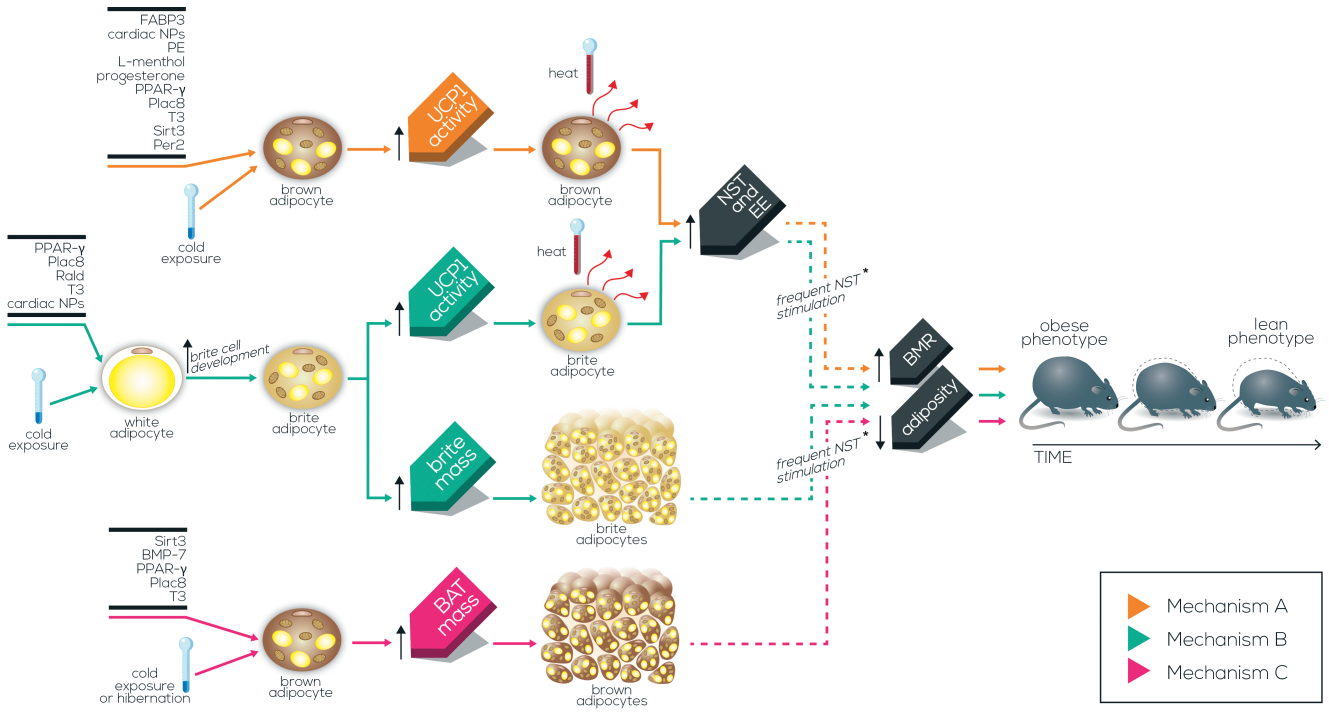

Fig. 2. Schematic representation of the effect of specific molecules, hibernation, and cold on brown adipose tissue (BAT)/brite development, non-shivering thermogenesis (NST) stimulation and adiposity based on the results of the present systematic analysis. In mechanism A, cold stimuli or specific molecules activate uncoupling protein-1 (UCP1) in the existing brown adipocytes. In mechanism B, certain molecules or cold exposure stimulate brite cell development, increasing the number of brite cells (i.e. brite mass) and the expression of UCP1 in brite adipocytes. In mechanism C, specific molecules, or hibernation, or cold exposure lead to increased BAT mass. The up-regulation of UCP1 expression in brown (mechanism A) and brite (mechanism B) adipocytes enhances NST and energy expenditure (EE). Finally, all three mechanisms can lead to augmented BMR and reduced adiposity levels in mammals when frequent NST stimulation without food intake compensation occurs. * = without food intake compensation; ↑ = increase; ↓ = decrease; BMP-7 = bone morphogenetic protein 7; BMR = basal metabolic rate; FABP3 = fatty acid-binding protein 3; NPs = natriuretic peptides; PE = *Pinellia ternate* extract; Per2 = Period2; Plac8 = placenta-specific 8; PPAR-γ = peroxisome proliferator-activated receptor γ; Rald = retinaldehyde dehydrogenase 1; Sirt3 = silent mating type information regulation 2, homolog 3; T3 = tri-iodothyronine.

1981; Astrup *et al.*, 1985) and that it is highly correlated with body mass index (Vijgen *et al.*, 2011). Individuals with a larger BAT mass demonstrate greater cold-induced thermogenesis, EE, and BAT activity following cold exposure (Vijgen *et al.*, 2011). Therefore, the increase of BAT/brite mass (through mechanisms B and C in Fig. 2) as well as frequent NST stimulation (through mechanisms A and B) may be promising in obesity treatment.

The current analysis produced an interesting concept regarding human brite cell development: one of the analysed studies showed that Rald (a molecule included in mechanism B of Fig. 2) induces white-to-brite transdifferentiation in human white adipocytes. This occurs by stimulating the WAT expression of the zinc-finger protein PR domain containing 16 (Kiefer *et al.*, 2012). The latter is known to increase directly – or indirectly through the activation of PGC-1α – the type 2 deiodinase expression (Seale *et al.*, 2007) that catalyses the conversion of thyroxine in T3 (Hall *et al.*, 2010). Interestingly, several animal studies analysed herein suggested that T3 (Fig. 2: mechanism B) augments brite cell development, UCP1 transcription, NST, and EE resulting in lower adiposity levels (Lehr *et al.*, 2009; Hall *et al.*, 2010; De Jonghe *et al.*, 2011; Chen *et al.*, 2012). It is, therefore, logical to suggest that Rald may induce brite cell development in human WAT though the stimulation of thyroid hormone T3. However, the validity of this hypothesis remains to be confirmed by future research.

Systematic reviews determine the information to be obtained from a literature analysis using explicit inclusion and exclusion criteria (e.g. key words, years, and languages). Thus, the advantage of using a systematic analysis lies in reducing the likelihood of bias in identifying, selecting, and aggregating individual studies (Wieseler & McGauran, 2010). While the systematic approach is likely to produce more accurate results regarding the effects of a specific phenomenon across a wide range of settings and empirical methods, it incorporates the risk of not including relevant studies due to inadequate or incorrect methodology. In the present review we made considerable efforts to minimise these shortcomings by adopting published guidelines (Wieseler & McGauran, 2010), using appropriate key words, and searching two of the largest databases available.

Our systematic review is not a catalogue of everything that has been done, but rather a systematic synthesis of the work conducted since 2006 – the year when UCP1 was suggested to be the only mediator of NST in mammalian BAT (Barger *et al.*, 2006). We attempted to ensure that our work provides concrete and unbiased added value and substance, draws together knowledge and highlights critical advances, thus creating new knowledge. In this light, our analysis suggests that future research in both animals and humans should address the interplay between body mass, BAT mass, as well as the main molecules involved in brite cell development.

V. CONCLUSIONS

(1) Specific molecules can enhance NST and metabolism by: (*i*) stimulating brite cell development in white adipose tissue, (*ii*) increasing UCP1 expression in brite adipocytes and in existing brown adipocytes, and (*iii*) augmenting BAT/brite mass.

(2) BAT mass also can be increased through low temperature, hibernation, and/or molecules involved in brown adipocyte differentiation.

(3) Cold stimuli and/or certain molecules activate UCP1 in existing brown adipocytes, thus increasing total energy expenditure by a magnitude proportional to the number of available brown adipocytes.

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