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REVIEW

Non-adrenergic control of lipolysis and thermogenesis in adipose tissues

Katharina Braun[1,2,3], Josef Oeckl[1,2,3], Julia Westermeier[1,2], Yongguo Li[1,2] and Martin Klingenspor[1,2,3,*]

ABSTRACT

The enormous plasticity of adipose tissues, to rapidly adapt to altered physiological states of energy demand, is under neuronal and endocrine control. In energy balance, lipolysis of triacylglycerols and re-esterification of free fatty acids are opposing processes operating in parallel at identical rates, thus allowing a more dynamic transition from anabolism to catabolism, and vice versa. In response to alterations in the state of energy balance, one of the two processes predominates, enabling the efficient mobilization or storage of energy in a negative or positive energy balance, respectively. The release of noradrenaline from the sympathetic nervous system activates lipolysis in a depot-specific manner by initiating the canonical adrenergic receptor–G_s-protein–adenylyl cyclase–cyclic adenosine monophosphate–protein kinase A pathway, targeting proteins of the lipolytic machinery associated with the interface of the lipid droplets. In brown and brite adipocytes, lipolysis stimulated by this signaling pathway is a prerequisite for the activation of non-shivering thermogenesis. Free fatty acids released by lipolysis are direct activators of uncoupling protein 1-mediated leak respiration. Thus, pro- and anti-lipolytic mediators are bona fide modulators of thermogenesis in brown and brite adipocytes. In this Review, we discuss adrenergic and non-adrenergic mechanisms controlling lipolysis and thermogenesis and provide a comprehensive overview of pro- and anti-lipolytic mediators.

KEY WORDS: Brown adipocytes, Hormones, Receptors, Signalling pathways, Uncoupling protein 1, Energy balance

Introduction

Lipids encompass a large variety of molecules with diverse functions, including simple lipids (triacylglycerols and waxes), compound lipids (e.g. phospholipids and sphingolipids), steroids, fatty acids and terpenes. The triacylglycerols, also known as fat, consist of three fatty acids esterified to a glycerol backbone molecule. On a quantity basis, fat makes up 90% of all lipids in the human body and constitutes the major storage form of chemical energy. In the so called 'reference man' with a body mass of 70 kg, the body fat compartment makes up >70% of the total body energy content (12 kg=470 MJ). These fats are stored in lipid droplets (fat droplets), which can be found in every cell type but are most abundant in adipocytes. In humans, more than 80% of total body fat is stored in adipocytes located in subcutaneous adipose tissue depots and less than 20% is stored in adipocytes in intra-abdominal depots.

Stored fat mainly originates from dietary fat absorbed in the intestine and from *de novo* lipogenesis in the liver. As transport vehicles for triacylglycerol, lipid-rich lipoproteins are secreted from the gut into the lymphatic system (chylomicrons) or from the liver into the sinusoidal hepatic capillaries (very-low-density lipoprotein, VLDL). Once in circulation, the fat-laden chylomicrons and VLDLs are hydrolyzed by lipoprotein lipase in the capillary endothelium of adipose tissues. Fatty acid transporters, such as platelet glycoprotein 4 (CD36) and fatty acid transport proteins (FATPs), deliver free fatty acids (FFAs) into adipocytes. Once in the cell, fatty acids are re-esterified with glycerol and deposited as triacylglycerol in lipid droplets. *De novo* lipogenesis in adipocytes is normally low but may rise significantly in nutritional states of limited fatty acid import and excess glucose supply to adipocytes. Insulin is the only endocrine signal that orchestrates these anabolic processes in fat metabolism and promotes fat storage in adipocytes. The enormous capacity for hypertrophy, i.e. cell expansion owing to fat storage, is a unique hallmark of adipocytes, as illustrated by maximal cell diameters in the range of 10–180 µm (Lafontan, 2012). The ability of adipose tissue to expand is further augmented by hyperplastic growth, which recruits adipocytes from a progenitor cell pool residing in stem cell niches of the tissue.

Adipocytes not only accumulate but also mobilize large amounts of fatty acids from triacylglycerol stored in lipid droplets. Therefore, the size and number of lipid droplets change dynamically, mostly in response to variations in dietary caloric intake and energy expenditure. Thus, the key function of adipocytes is energy storage and mobilization of stored energy according to the energy requirements of the major high-metabolic rate organs, such as the heart, skeletal muscle and liver. Like many other metabolic pathways, these opposing processes are finely tuned by futile cycling. The breakdown of triacylglycerols and re-esterification of fatty acids occur in parallel with only a fraction of the fatty acids released from triacylglycerols being exported into circulation. It is generally assumed that futile cycling will allow a more dynamic transition from anabolism to catabolism, or vice versa, enabling larger changes in net flux through the pathway. Moreover, futile cycling fine tunes the cellular control of FFA levels and, thereby, prevents lipotoxicity. In addition, futile cycles also represent adenosine triphosphate (ATP) sinks, driving additional need for the regeneration of adenosine diphosphate by mitochondrial oxidative phosphorylation, and result in higher metabolic flux rates associated with increased heat dissipation. Futile ATP sinks may contribute to thermoregulatory heat production in endotherms and the discussion about their contribution to whole-body heat production has been revitalized recently (Flachs et al., 2017; Kazak et al., 2015; Rohm et al., 2016).

In catabolic states, such as fasting, exercise or cold exposure, endogenous stores of energy are mobilized. In respect to

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carbohydrates, the storage capacity of the body is low. Approximately 75 g of glycogen can be mobilized from storage granules in hepatocytes worth ∼1275 kJ, which can only fuel the resting metabolic rate for a couple of hours (Van Itallie et al., 1953), and is depleted even faster during exercise. To meet energetic demand, lipolysis is activated; the rate of lipolysis largely exceeds the rate of re-esterification, thereby promoting a net increase in the export of fatty acids and glycerol out of the adipocyte. Once exported into the extracellular matrix, fatty acids can either reenter the adipocyte or they cross the endothelial barrier into the capillary lumen and are transported, bound to serum albumin, to the peripheral tissues with highest metabolic demands. At the same time, the turnover of proteins is reduced and amino acids are spared for gluconeogenesis.

Activation of lipolysis is conveyed by the sympathetic nervous system (SNS). Post-ganglionic sympathetic nerve fibers innervate adipose tissue depots that are mainly located at subcutaneous, intra-abdominal and intra-thoracic sites, whereas parasympathetic innervation is mostly negligible (Vaughan et al., 2014). The tone of the sympathetic innervation exerts master control on lipolysis by the release of noradrenaline (also known as norepinephrine) and from varicosities and synapses in the parenchyma of adipose tissues, as demonstrated by surgical and chemical denervation experiments and retrograde tracing of sympathetic nerve fibers innervating adipose tissues (Vaughan et al., 2014). Experiments with adrenodemedullated rats underline that the contribution of circulating noradrenaline and adrenaline (also known as epinephrine) is negligible (Paschoalini and Migliorini, 1990). The functionality of the sympathetic nerves to activate lipolysis was verified recently *in vivo* using optogenetic depolarization of the sympathetic nerves projecting into the inguinal subcutaneous white fat depot. Unilateral nerve depolarization stimulated phosphorylation of HSL and reduced fat depot mass when applied chronically (Zeng et al., 2015). Melanocortins signaling via the melanocortin 4 receptor (MC4R) in the central nervous system (CNS) play a key role in regulating SNS activity (Berglund et al., 2014). Central stimulation of melanocortin receptor signaling in the brain, which mimics the physiological neuroendocrine sensation of a positive energy balance, increases the activity of postganglionic sympathetic nerves in a differential depot-specific manner. Sympathetic drive is increased selectively in inguinal, retroperitoneal and dorsosubcutaneous white adipose tissue (WAT) depots, but not in the epididymal WAT depot (Brito et al., 2007). Thus, in general, there is convincing evidence that the CNS–SNS axis plays a role in modulating lipolysis in adipose tissue. Various hormones secreted by peripheral tissues, such as glucagon-like peptide-1 (GLP-1) and leptin, regulate fat metabolism via the CNS–SNS axis (Lockie et al., 2012; Zeng et al., 2015). Efforts to devise pharmacological treatments for metabolic disease by selectively targeting the CNS–SNS–adipose axis have turned out to be complex. Nonselective activators of the SNS successfully promoted negative energy balance and weight loss; however, the cardiovascular side-effects prevented their use in clinical settings (Yen and Ewald, 2012). The search for other activators of adipose tissue operating in an SNS-independent fashion may be a promising alternative.

In isolated adipocytes, adrenaline and noradrenaline have dual effects on lipolysis owing to the cell surface expression of different adrenergic receptor (AR) paralogs with either anti- or pro-lipolytic action. At low ligand concentrations, their high affinity to α2-ARs triggers anti-lipolytic action, whereas at higher concentrations, the pro-lipolytic action mediated by highly abundant β-ARs prevails (Bousquet-Melou et al., 1995). These G-protein coupled β-receptors activate adipose triglyceride lipase (ATGL) through a signaling pathway targeting 5′-adenosine monophosphate-activated protein kinase and hormone-sensitive lipase (HSL) through the canonical AR–G_s–adenylyl cyclase–cyclic adenosine monophosphate (cAMP)–protein kinase A (PKA) pathway by phosphorylation.

Beyond their enormous capacity for hypertrophy and hyperplasia, adipose tissues are characterized by morphological and functional plasticity (Cannon and Nedergaard, 2012). Adipocytes comprise three subtypes: white adipocytes, brown adipocytes and inducible brown-like adipocytes found interspersed in WAT depots (Kajimura et al., 2015; Wang and Seale, 2016). The latter are most commonly referred to as brite (brown-in-white adipocytes) or beige adipocytes (Klingenspor et al., 2012). White adipocytes contain few mitochondria and their intrinsic metabolic rate contributes little to whole-body energy expenditure. In contrast, brown adipocytes are packed with mitochondria and show the highest respiration capacity among mammalian cells (Klingenspor et al., 2017). This extraordinary respiration capacity is employed to dissipate heat. In a cold-acclimated rodent, thermogenesis in brown adipose tissue (BAT) can contribute up to 50% of the total body heat production at rest, despite the tissue wet weight only representing 5% of total body mass (Foster and Frydman, 1979; Klingenspor et al., 2017). Brite adipocytes are brown-like adipocytes in respect to their cytoarchitecture, high respiration capacity and molecular signature; however, their contribution to whole-body thermogenesis may have been overestimated.

Like white adipocytes, lipolysis in brown and brite adipocytes is controlled by the neuronal release of noradrenaline from the sympathetic innervation of the tissue (Klingenspor et al., 2017). However, the physiological range of circulating catecholamine levels are insufficient to activate brown fat metabolism (Girardier and Seydoux, 1986). In response to cold exposure, a well-characterized somatosensory reflex relayed in the hypothalamic preoptical area translates cold sensation in the periphery to increased efferent sympathetic drive in BAT and WAT depots (Nakamura and Morrison, 2011). In contrast, fasting decreases sympathetic drive in BAT while increasing sympathetic drive in WAT (Brito et al., 2008).

Upon cold exposure, the neurotransmitter noradrenaline stimulates the same canonical AR–G_s–adenylyl cyclase–cAMP–PKA pathway, resulting in the lipolytic mobilization of fatty acids. In brown and brite adipocytes, these fatty acids activate uncoupling protein 1 (UCP1), which enables maximal mitochondrial oxidation rates without ATP synthesis, and serve as fuel to maintain high rates of thermogenesis. In brown and brite adipocytes, the mobilization of FFAs by lipolysis is essentially required for thermogenesis. Indeed, pharmacological inhibition of ATGL and HSL, which catalyze the first two steps in the hydrolysis of triglycerides, completely diminishes adrenergic stimulation of thermogenesis (Li et al., 2014). Moreover, the addition of FFAs stimulates thermogenesis in brown adipocytes in the absence of adrenergic stimulation.

After a brief summary of the present knowledge on adrenergic control, we will address non-adrenergic mechanisms in the control of lipolysis and thermogenesis in adipocytes. Without putting the dominant role of adrenergic signaling aside, a closer inspection and functional evaluation of other ligands, receptors and intracellular signaling pathways in the control of lipolysis in adipocytes seems to be a rewarding exercise, promising new insights into lipid metabolism and energy balance physiology. Our reviews of the published literature revealed several non-adrenergic biomolecules that have been identified to effect lipolysis in white adipocytes, only

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a few of which have been examined for their lipolytic action in brown and brite adipocytes (Duncan et al., 2007; Lafontan, 2012). As a working hypothesis, any stimulus affecting the balance of lipolysis and re-esterification potentially attenuates or activates thermogenesis in brown and brite adipocytes (Li et al., 2017) (Fig. 1). In this Review, we aim to provide a comprehensive coverage and discussion of biomolecules that modulate lipolysis in adipocytes by either receptor-dependent or independent mechanisms. Information on comparative aspects, species differences and effects on brown and brite adipocyte lipolysis and thermogenesis is provided where available.

Dual role of catecholamines in the adrenergic control of lipolysis

Adrenaline and noradrenaline

Adrenaline and noradrenaline both elicit distinct adrenergic signaling pathways in adipocytes by activating α- and β-ARs, which belong to the family of G-protein coupled receptors (GPCRs). The fat-laden cells express several paralogs of adrenergic GPCRs, mainly ADRA1, ADRA2 and ADRB1/2/3, which couple to G_s-, G_i - or G_q-dependent intracellular signaling modules. Although adrenaline and noradrenaline have greater affinity to α- than to β-receptors, the receptor abundance largely determines which signaling modules are activated. The β-3-AR ADRB3 is the predominant AR paralog expressed in rodent adipocytes. Noradrenaline released as a neurotransmitter from sympathetic nerves is the prime driver of lipolysis in adipose tissues by activating ADRB3, which signals through the canonical G_s–adenylyl cyclase–cAMP–PKA pathway (Fig. 2). Previous pharmacological studies of lipolysis comparing white adipocytes from different mammalian species concluded that guinea pigs and primates (humans and macacus monkeys) are hyporesponsive to ADRB3 agonists, whereas rodents (rats, golden hamsters and dormice) are hyperresponsive (Lafontan and Berlan, 1993). However, the lack of lipolytic action in guinea pigs and primates is likely owing to lower ADRB3 expression and the poor cross-species pharmacology of ligands available at that time.

Other α- and β-ARs differ regionally in their abundance between depots and between mammalian species. In white adipocytes, α2-AR (ADRA2) signaling opposes the stimulation of lipolysis by ADRB3. ADRA2 couples to G_i signaling, which reduces cAMP levels by inhibiting adenylyl cyclase (Lafontan and Berlan, 1993). Upon stimulation of adipocytes with adrenaline or noradrenaline, the effect on lipolysis largely depends on the balance of ADRA2 and ADRB1/2/3 expression. For example, adrenaline has anti-lipolytic effects in human subcutaneous fat where ADRA2 is more abundant than ADRB1/2/3. In contrast, lipolysis in omental fat with low ADRA2 expression is stimulated by adrenaline. A comparison of mammalian species revealed a large variation in the anti-lipolytic response to ADRA2 stimulation. Strong inhibition of lipolysis was observed in white adipocytes isolated from hamsters, humans, rabbits and dogs, whereas inhibition was low in jerboas, dormice, guinea pigs and rats. The different anti-lipolytic action observed in these species is associated with high and low ADRA2 expression in adipocytes of these species (Castan et al., 1994). Thus, species differences exist in the relative expression of ADRA2 and ADRB1/2/3. This α2-AR–β-AR balance is the cause of the anti-lipolytic effects of adrenaline in human but not in rat adipocytes.

ADRA2 is also expressed in brown adipocytes. Activation results in G_i-mediated inhibition of adenylyl cyclase and a reduction of cAMP levels and, hence, binding of noradrenaline to ADRA2 exerts an anti-lipolytic action. Indeed, pharmacological inhibition of ADRA2 increases the lipolytic and the thermogenic action of noradrenaline. Like white adipocytes, noradrenaline has a dual role in brown adipocytes, both inhibiting as well as stimulating lipolysis and thermogenesis. However, owing to the much higher expression levels of ADRB3 compared with ADRA2, the stimulatory action prevails.

Although ADRB3 is quantitatively the most abundant AR in the BAT of rodents, this is not the case in humans. In human brown adipocytes, ADRB3 is far less abundant than ADRB1 and ADRB2

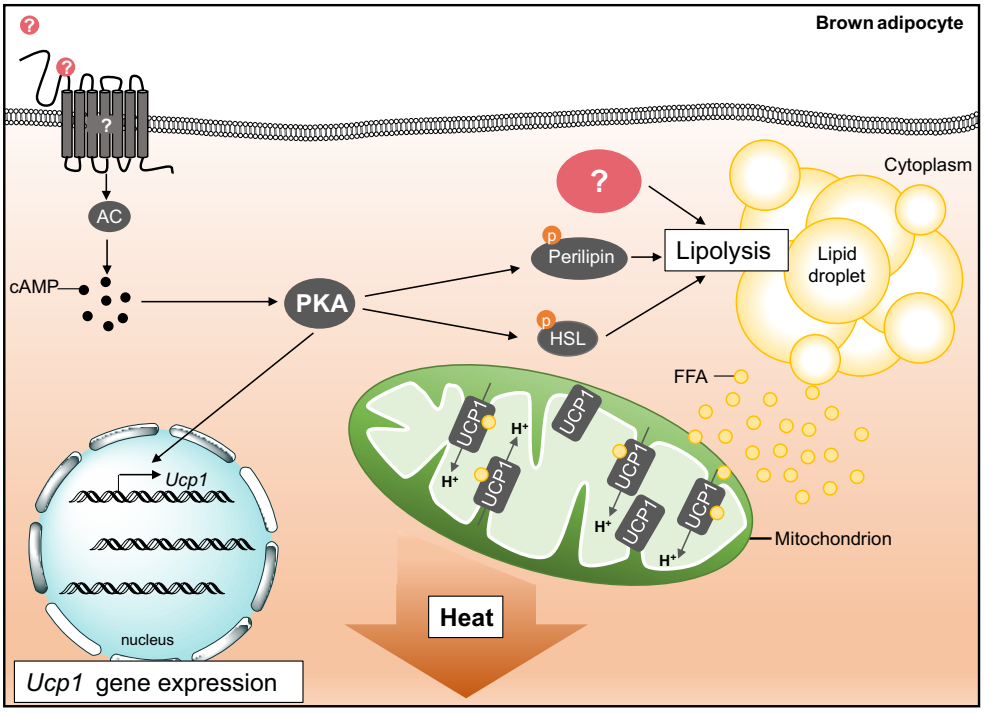

Fig. 1. Working hypothesis: lipolytic agents are potential activators of thermogenesis in brown adipocytes. Lipolysis is an essential prerequisite for thermogenesis in brown and brite adipocytes. We therefore hypothesize that any pro-lipolytic stimulus potentially activates thermogenesis in these cells. Free fatty acids (FFAs) released from lipid droplets as a result of lipolysis act as both fuel for mitochondrial β-oxidation and activators of the uncoupling protein 1 (UCP1). UCP1 is a unique feature of brown and brite adipocytes located in the inner mitochondrial membrane. Upon activation by FFAs, UCP1 uncouples oxygen consumption from adenosine triphosphate (ATP) synthesis by allowing protons (H^+) to reenter the mitochondrial matrix without generating ATP. Thus, the chemical energy of nutrients is dissipated as heat. Extracellular stimulation of lipolysis via the canonical adenylyl cyclase–cyclic adenosine monophosphate–protein kinase A (AC–cAMP–PKA) pathway not only leads to the phosphorylation of perilipin and hormone-sensitive lipase (HSL) but also induces *Ucp1* gene expression.

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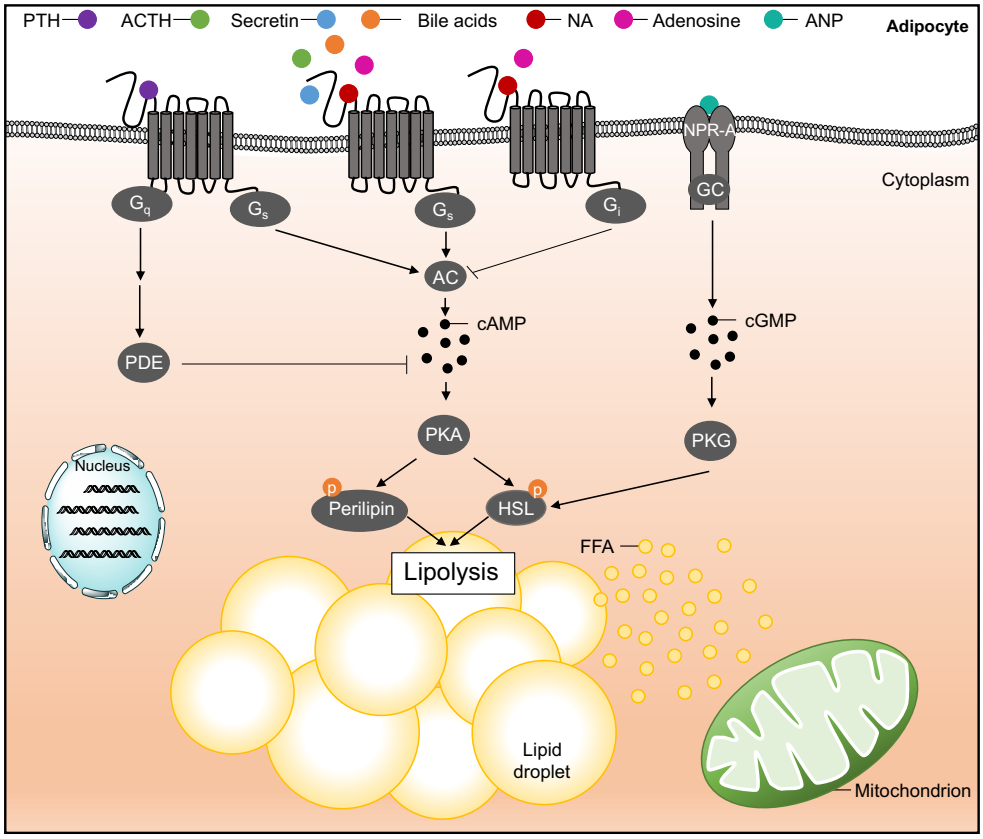

Fig. 2. Receptor-dependent pathways regulating lipolysis in adipocytes. Ligand binding of a G_s protein-coupled receptor leads to the activation of adenylyl cyclase (AC) and a rise in cyclic adenosine monophosphate (cAMP) levels, which in turn activates protein kinase A (PKA). Activated PKA phosphorylates perilipin and hormone-sensitive lipase (HSL) leading to lipolysis. Examples of such G_s protein-coupled receptors and their respective ligands are: membrane-bound bile acid receptor (TGR5) and bile acids; melanocortin receptor 2 (MC2R) and adrenocorticotropic hormone (ACTH); adenosine receptor 2a (ADORA2A) and adenosine; β-adrenergic receptor (β-AR) and noradrenaline (NA). Gi protein-coupled signaling inhibits AC and, thereby, exerts anti-lipolytic effects. Thus, activation of α-AR by NA or activation of adenosine receptor 1 (ADORA1) by adenosine attenuates lipolysis. Binding of parathyroid hormone (PTH) to parathyroid hormone receptor 1 (PTHR1) activates Gs- and Gq-coupled signaling. Whereas Gs activates the AC–cAMP–PKA pathway, Gq signaling events lead to increased sequestration of cAMP by phosphodiesterase (PDE) activation, which counteracts the lipolytic effects of Gs-coupled signaling elicited by PTHR1. Atrial natriuretic peptide (ANP) binds to natriuretic peptide receptor A (NPR-A), leading to the activation of the guanylyl cyclase (GC) domain of NPR-A and the rise of cyclic guanine monophosphate (cGMP) levels, with the subsequent activation of protein kinase G (PKG). PKG phosphorylates and thereby activates HSL.

(see RNA-Seq data SAMEA2563965 at https://www.ebi.ac.uk/ena/data/view/SAMEA2563965 by Shinoda et al., 2015; Revelli et al., 1993). The species-related differences may partly explain the poor responsiveness of human fat cells to β−3 agonists compared with murine adipocytes. The low β3-AR abundance does not inspire confidence that ADRB3 would be a suitable molecular target in humans and, hence, it is remarkable that a recently developed human ADRB3 agonist acutely increased serum FFA levels and activated brown fat thermogenesis in human subjects (Cypess et al., 2015).

Anti-lipolytic effectors

Adenosine

Adenosine is a purine nucleoside generated in cellular adenine nucleotide metabolism that activates purine receptors in the plasma membrane. Extracellular adenosine in the tissue can arise from different sources. Adipocytes liberate adenosine into the extracellular space where it acts in an autocrine/paracrine fashion. Moreover, extracellular ecto-5′-nucleotidase (CD37) generates adenosine from ATP released by either parenchymal cells or sympathetic neurons as a purinergic co-transmitter of noradrenaline. Four adenosine receptors of the GPCR1 family are known, of which the two paralogs ADORA1 and ADORA2A are of functional relevance in adipose tissues. These two adenosine receptors have opposing roles in the regulation of cAMP levels. ADORA1 inhibits adenylyl cyclase through G_i, whereas ADORA2A activates adenylyl cyclase through G_s (Fig. 2). Adenosine has anti-lipolytic action because ADORA1 expression predominates in adipocytes.

The addition of adenosine deaminase (ADA) in cell-based assays of lipolysis sequesters extracellular adenosine in the medium and, thereby, attenuates the anti-lipolytic action. For example, in white adipocytes isolated from murine epididymal WAT, basal lipolysis was increased more than sevenfold in the presence of ADA (0.1 U ml^{-1}), and >18-fold in a combined treatment with ADA and noradrenaline (10 nmol l^{-1}). In the absence of ADA, a more than ninefold increase was observed in response to noradrenaline (Johansson et al., 2008). These pro-lipolytic effects of ADA are owing to the clearance of adenosine, which inhibits adenylyl cyclase activity via ADORA1. Experimental variation (technical and biological) in the extracellular adenosine concentrations challenges the robustness of lipolysis assays in cell culture systems. Therefore, the addition of both ADA and the ADORA1-specific agonist phenylisopropyladenosine (PIA) was introduced to clamp a defined state of anti-lipolysis in the experimental set-up (Honnor et al., 1985), and was recommended as state-of-the-art (Lee and Fried, 2014). Pharmacological activation and genetic ablation

demonstrated that ADORA1 inhibits lipolysis in adipocytes isolated from diverse mammalian species, including hamsters (Rosak and Hittelman, 1977; Schimmel and McMahon, 1980), rats (Honnor et al., 1985), mice (Johansson et al., 2008) and humans (Heseltine et al., 1995). The anti-lipolytic action of adenosine was therefore assigned as an obligatory paracrine mechanism in mammals (Castan et al., 1994). Although the anti-lipolytic action of adenosine potentially extends the dynamic range of lipolysis rates in adipocytes, the physiological relevance was repeatedly questioned. In human subcutaneous adipose tissue, interstitial adenosine concentrations, as assessed by microdialysis in the unstimulated state, ranged from 25 to 300 nmol l^{-1} (Lonnroth et al., 1989). In isolated human white adipocytes, noradrenaline-stimulated lipolysis was inhibited by stable analog 2-chloroadenosine concentrations equipotent to 150–300 nmol l^{-1} adenosine. Thus, adenosine concentrations in the intercellular space of human adipose tissue are sufficiently high to counteract sympathetic stimulation of lipolysis (Lonnroth et al., 1989). In germline knockout mice, although loss of ADORA1 function ablated the anti-lipolytic effects of adenosine, phenotypically the ADORA1 knockout mice did not show increased lipolysis and decreased triglyceride storage in WAT. Thus, a putative physiological function of adenosine as a negative modulator of lipolysis could not be established in this model, possibly owing to pleiotropic functions of ADORA1 expressed in other tissues (Johansson et al., 2008).

In 1981, Bukowiecki and colleagues proposed that '*mitochondrial respiration would principally be regulated by the activity of the hormone sensitive lipases that would represent the flux generating step controlling brown adipose tissue oxidative metabolism*' (Bukowiecki et al., 1981). In line with this proposal, an anti-lipolytic effector should put a break on thermogenesis in brown adipocytes. Adenosine was therefore studied early on as a putative inhibitor of brown fat thermogenesis. In brown adipocytes isolated from golden hamsters, the anti-lipolytic action of adenosine is associated with a pronounced attenuation of noradrenaline-stimulated thermogenesis, an effect that can be reversed by adding ADA (Szillat and Bukowiecki, 1983). These observations were later confirmed in brown adipocytes isolated from rat interscapular BAT (Woodward and Saggerson, 1986).

Unexpectedly, a recent study revealed pro-lipolytic action of adenosine at low concentrations ranging from 10 to 100 nmol l^{-1} in human and mouse brown adipocytes (Gnad et al., 2014). Analysis of gene expression revealed ADORA2A as the most abundantly expressed adenosine receptor in BAT of humans and mice. Based on subsequent pharmacological analysis and genetic ablation, the pro-lipolytic action of adenosine was conveyed by stimulating adenylyl cyclase activity through G_s-coupled ADORA2A signaling. In brown adipocytes from ADORA2A-ablated mice, the pro-lipolytic action required higher concentrations of adenosine. Treatment of murine brown adipocytes, either with adenosine or with a specific ADORA2A agonist, increased cAMP levels, lipolysis and the oxygen consumption rate (Gnad et al., 2014). The anti-lipolytic effect of adenosine in hamster brown adipocytes is most likely owing to higher levels of expression of ADORA1 relative to ADORA2A (Gnad et al., 2014). Nevertheless, the physiological relevance of potentially opposing adenosine effects on brown adipocytes in different mammalian species remains elusive and merits further investigation.

It is conceivable that other ligands binding to Gi-coupled GPCRs will inhibit adenylyl cyclase and decrease cAMP production, therefore harboring anti-lipolytic effects. Organic acids, such as lactate, succinate and short-chain fatty acids (acetate and propionate), and the ketone body β-hydroxybutyrate (β-OHB), through binding to their respective Gi-coupled receptors GPR81 (Liu et al., 2009), GPR91 (Regard et al., 2008), GPR43 (Ge et al., 2008) and HM74a (Taggart et al., 2005), fall into this category. A comprehensive review of these anti-lipolytic effectors has been published (Nielsen et al., 2014).

Pro-lipolytic effectors and signaling pathways

Melanocortins

The POMC gene encodes for prepro-opiomelanocortin. Posttranslational cleavage of POMC by prohormone convertases generates several biologically active peptides classified as melanocortins. Beyond their central action via melanocortin receptors in the CNS, some melanocortins mediate their effects via melanocortin receptors in the periphery, of which α-melanocyte-stimulating hormone (αMSH) and corticotropin (adrenocorticotropic hormone, ACTH) are known for their significant pro-lipolytic action in adipocytes of rodents, as first reported in 1958 (Lafontan, 2012). In rodent adipocytes, ACTH binds to the melanocortin 2 receptor, which stimulates lipolysis via Gs-coupled cAMP–PKA-mediated phosphorylation of HSL (Cho et al., 2005) (Fig. 2). However, in human adipocytes, ACTH and αMSH lack lipolytic activity, which is likely owing to differential expression of melanocortin receptors in primates and rodents (Kiwaki and Levine, 2003; Lafontan and Langin, 2009).

Shortly after the initial demonstration of BAT function as a heater organ (Smith, 1961), the potential role of ACTH, glucocorticoids and steroids for non-shivering thermogenesis was explored (Jansky et al., 1969). In the European hedgehog, pharmacological inhibition of adrenal glucocorticoid synthesis stimulated resting energy expenditure, possibly owing to an accumulation of the corticosterone precursor 11-deoxycorticosterone or an increase of pituitary ACTH secretion (Werner and Wunnenberg, 1980). However, the crucial role of pituitary hormones and their effector hormones for brown fat recruitment during cold acclimation was questioned (Fellenz et al., 1982), and inhibitory actions of corticosterone as the effector hormone of the hypothalamus–pituitary–adrenal (HPA) axis on brown fat were reported (Galpin et al., 1983). In dietary or genetically obese rats, BAT function was enhanced by ACTH, an effect opposed by corticosterone and dietary status (Rothwell and Stock, 1985; York and Al-Baker, 1984).

The role of the HPA axis for brown fat function in mice was recently revisited and further specified. Cold exposure for one day activates the HPA axis resulting in increased levels of circulating ACTH and increased fecal corticosterone excretion (van den Beukel et al., 2014). Like white adipocytes, in murine immortalized brown adipocytes, ACTH stimulates intracellular cAMP concentrations in a dose-dependent manner, even exceeding the effect of noradrenaline. ACTH at a dose of 50 nmol l^{-1} increases glycerol release by ∼50% of noradrenaline-stimulated lipolysis at 1 µmol l^{-1} (van den Beukel et al., 2014). Further analysis revealed that ACTH also increased UCP1 messenger ribonucleic acid and protein levels, which is mediated by p38 mitogen-activated protein kinase (MAPK) signaling (Iwen et al., 2008). Notably, ACTH stimulated oligomycin-insensitive oxygen consumption in brown adipocytes by 40%, suggesting activation of a UCP1-dependent proton leak. This observation needs further validation because the experimental design did not control for a possible uncoupling effect of fatty acids (Li et al., 2014). *In vivo*, positron-emission tomography (PET) demonstrated that ^{18}fluorodeoxyglucose (^{18}FDG) uptake was stimulated by ACTH. Interestingly, increased basal glucose

uptake and an enhanced level of stimulation by ACTH were observed in mice treated with a glucocorticoid receptor antagonist. These results further manifest the conclusion from previous studies that the enhancing effect of ACTH on BAT function is attenuated by corticosterone in rodents. Acute stress may lead to a transient activation of BAT thermogenesis that is downregulated by the subsequent rise of corticosterone. However, it remains to be clarified whether the physiological peak concentrations of ACTH in plasma in response to stress are sufficiently high to activate BAT thermogenesis *in vivo* (van den Beukel et al., 2014).

Glucocorticoids

In contrast to the findings in rodents, glucocorticoids can activate BAT in humans. Administering healthy male subjects with three doses of prednisolone (the first dose was administered 24 h before visiting the research facility) had no effect on basal BAT activity; however, PET and infrared thermography of skin temperature in the supraclavicular region revealed that cold-induced glucose uptake in BAT and skin temperature were enhanced by prednisolone (Ramage et al., 2016). An independent study using infrared thermography demonstrated that infusing healthy male subjects with hydrocortisone for 24 h had no effect at room temperature; however, a cold-induced increase in skin temperature of the supraclavicular region was enhanced (Scotney et al., 2017). Results obtained in cell culture are in line with these observations. In respiration experiments directly comparing human and mouse brown adipocytes, cortisol stimulated the metabolic rate in human brown adipocytes but inhibited isoproterenol-induced respiration in murine brown adipocytes. For this species comparison, brown adipocytes were pretreated with cortisol for 24 h. Importantly, these thermogenic effects are likely owing to genomic effects of glucocorticoids as indicated by corresponding changes in *Ucp1* gene expression. To date, acute non-genomic effects of glucocorticoids on brown fat thermogenesis have not been reported.

The biological significance for the differential effects of ACTH and glucocorticoids in the control of lipolysis and thermogenesis in WAT and BAT of human and mouse is not understood. In humans, higher doses of cortisol, and more chronic elevations of glucocorticoids inhibit BAT activity, as concluded from cell culture data and clinical observations reporting a lower prevalence of BAT negative patients during chronic glucocorticoid therapy (Ramage et al., 2016).

Natriuretic peptides

Natriuretic peptides comprise a family of three structurally related peptides mediating a wide range of physiological functions centered around blood pressure control and volume homeostasis. Atrial natriuretic peptide (ANP), the first member of this family, was discovered in 1981 by de Bold (de Bold et al., 1981). B-type natriuretic peptide (BNP) and C-type natriuretic peptide (CNP) were subsequently characterized in 1989 and 1991, respectively (Sudoh et al., 1988, 1990).

All natriuretic peptides are synthesized as preprohormones and further processed to prohormones. ProANP is the major form of ANP stored in atrial granules (Oikawa et al., 1984). Upon its release, proANP is readily cleaved into the biologically active form of ANP (Yan et al., 2000). The sequence of mature ANP is highly conserved across different species; it is identical in humans, chimps, dogs, pigs, horses and sheep (Potter et al., 2009). ANP release is triggered by atrial distension and by neurohumoral stimulation (Dietz, 1984; Mukoyama et al., 1991). In general, natriuretic peptides are either enzymatically degraded or removed from circulation by binding to their clearance receptor (Nussenzveig et al., 1990; Stephenson and Kenny, 1987).

BNP was initially purified from porcine brain, thus, it was originally termed ‘brain natriuretic peptide’ (Sudoh et al., 1988). However, after much higher BNP concentrations were detected in atrial ventricles, the neutral terminology ‘B-type’ was adopted (Mukoyama et al., 1991). BNP is produced in response to states of high pre- and afterload pressures (Thuerauf et al., 1994). In addition to the effects of BNP on blood pressure, studies suggest that BNP might act as a paracrine regulator of cardiac remodeling (Tamura et al., 2000).

CNP is mainly expressed in vascular endothelial cells, neurons and in testicles; however, there are no convincing data to support the expression of CNP in cardiomyocytes (Herman et al., 1993; Middendorff et al., 1996; Suga et al., 1992b; Takahashi et al., 1992). CNP is not stored in granules and its release is triggered by growth factors, sheer stress and in response to vascular injuries (Brown et al., 1997; Chun et al., 1997; Suga et al., 1992b). CNP is primarily known to stimulate long bone growth (Mericq et al., 2000). The half-life times of natriuretic peptides in the human circulation are ∼2–3 min for ANP and CNP (Hunt et al., 1994; Yandle et al., 1986) and 20 min for BNP (Mukoyama et al., 1990).

The various effects of natriuretic peptides are mediated by the natriuretic peptide receptors A, B and C (NPR-A/-B/-C). NPRs are expressed in a broad range of tissues, including kidney (Goy et al., 2001), lung (Lowe et al., 1989), adipose (Jeandel et al., 1989), brain (Herman et al., 1996), heart (Lin et al., 1995), testis, adrenal, bone, liver (Sarzani et al., 1996) and vascular smooth muscle tissues (Schiffrin et al., 1986). The intracellular domain of NPR-A as well as NPR-B has guanylyl cyclase activity, thereby catalyzing the synthesis of the second messenger cyclic guanine monophosphate (cGMP; Miyagi and Misono, 2000). NPR-C does not exhibit cyclase activity; thus, it was initially considered to be a clearance receptor only. However, studies have revealed that NPR-C mediates intracellular effects through G_i-signaling and the consequent inhibition of adenylyl cyclase and phospholipase C activation (Rose and Giles, 2008). NPR-A is the principal receptor for ANP and BNP, whereas NPR-B preferentially binds CNP (Bennett et al., 1991; Suga et al., 1992a). Among NPRs, NPR-C is the most widely and abundantly expressed receptor (Anand-Srivastava, 2005). Beyond the regulation of blood pressure and fluid homeostasis, an important role for ANP, BNP and CNP in the control of adipose tissue metabolism has emerged over the past decades (Sengenès et al., 2000).

Almost 30 years ago, the first studies demonstrated that ANP stimulated cGMP levels in rat adipocytes, although ANP failed to increase lipolysis (Jeandel et al., 1989; Okamura et al., 1988). In 2000, the first report demonstrated that ANP as well as BNP, and to a lesser degree CNP, can induce lipolysis in human subcutaneous fat in a cGMP-dependent fashion (Sengenès et al., 2000). ANP has a lipolytic potency comparable to that of catecholamines. The order of potency is: ANP>BNP>>CNP. ANP and isoproterenol have additive effects at low concentrations; however, the maximal lipolytic effect of isoproterenol is not significantly amplified by ANP (Moro et al., 2004b; Sengenès et al., 2000). The precise lipolytic pathway was discovered only a few years later. ANP binds to NPR-A, guanylyl cyclase is activated, cGMP levels are increased and protein kinase G is activated, leading to the subsequent phosphorylation of HSL (Sengenès et al., 2003) (Fig. 2). Nevertheless, lipolytic pathways mediated by β- and α2-ARs and the ANP-dependent pathway do not interact. In contrast to the adrenergic pathway, insulin does not modulate the lipolytic effect of

ANP. Similar to isoproterenol-stimulated lipolysis, the ANP-dependent lipolytic pathway exhibits homologous desensitization; however, there is no cross-interaction between isoproterenol and ANP (Moro et al., 2004b; Sengenès et al., 2000, 2002). Moreover, the effect of natriuretic peptides on lipid metabolism was confirmed in healthy humans. Infusion of ANP triggered peripheral lipid mobilization and increased circulating FFA levels (Birkenfeld et al., 2005). Although the physiological relevance of the ANP-mediated lipolytic pathway in humans is still not clear, further studies have shown that increased circulating ANP concentrations may trigger exercise-induced lipolysis (Moro et al., 2004a). However, natriuretic peptides can only elicit a lipolytic response in primates: for instance, the amount of NPR-C is much higher in the adipose tissue of rodents than in humans, thereby diminishing the pro-lipolytic stimulus (Sengenès et al., 2002). Notably, it was demonstrated that this effect can be completely reversed by the ablation of NPR-C in mice (Bordicchia et al., 2012). Consequently, ANP as well as BNP can induce a brown thermogenic signature in the white adipocytes of NPR-C$^{-/-}$ mice and humans by activating p38-MAPK, triggering mitochondrial biogenesis, and increasing the expression of UCP1 and PGC1-α. Likewise, treatment of NPR-C$^{-/-}$ mice with BNP caused browning of white fat and the activation of already existing brown fat (Bordicchia et al., 2012). In wild-type mice, an increase in ANP and BNP expression and a shift in the adipose tissue NPR-A/NPR-C ratio, favoring adipose tissue activation, was observed during cold exposure (Bordicchia et al., 2012). A similar change in adipose tissue NPR patterns was reported in rats upon food deprivation (Sarzani et al., 1996).

Taken together, the ability of natriuretic peptides to initiate lipolysis, induce browning of WAT, and to activate UCP1 might bring further clinical benefits. However, the basic role of natriuretic peptides in metabolism and their full range of effects has yet to be discovered.

Bile acids

Bile acids are essential factors in dietary lipid absorption and end products of cholesterol catabolism (Lefebvre et al., 2009; Watanabe et al., 2006). They are synthesized from cholesterol in the liver, stored in the gallbladder, and secreted after meals to promote absorption of fat from the intestine. Bile acids are then either excreted or reabsorbed into the enterohepatic circulation. Beyond this well-established role, functions for bile acids as signaling molecules have emerged in recent years. Bile acids are natural ligands for the nuclear hormone receptor farnesoid X receptor (FXR) (Kawamata et al., 2003; Maruyama et al., 2002), which controls the synthesis and enterohepatic circulation of bile acids by adjusting the expression of essential gene products involved in bile acid synthesis, transport, conjugation and detoxification (Houten and Auwerx, 2004; Russell, 2003). In addition to FXR, bile acids signal through another pathway involving the G_s-coupled receptor TGR5 (also GBAR1, M-Bar, BG37) (Fig. 2). In both rodents and humans, BAT is targeted by bile acids. In C57BL/6 mice, dietary supplementation with cholic acid increased the thermogenetic capacity of BAT even in a thermoneutral environment and prevented diet-induced obesity (Teodoro et al., 2014; Watanabe et al., 2006; Zietak and Kozak, 2016). Mechanistically, the binding of bile acids to the TGR5 receptor increased the intracellular concentrations of the second messenger cAMP, which activates expression of the gene encoding for type 2 deiodinase (DIO2). This enzyme converts the inactive thyroid hormone thyroxine (T4) to active 3-5-3′-triiodothyronine (T3). Increased saturation of thyroid hormone receptors with T3 enforces the expression of Pgc1-α, a transcriptional coactivator of mitochondrial biogenesis and Ucp1. As a result, bile acids boost the thermogenic capacity of BAT. In humans, two oral ingestions of the primary bile acid chenodeoxycholic acid (CDCA) within 24 h enhanced cold-induced glucose uptake into BAT. In support of this thermogenic action, CDCA also triggered increased uncoupled leak respiration in primary brown adipocytes upon acute treatment (Broeders et al., 2015). The latter observation was also reported for human skeletal muscle cells (Watanabe et al., 2006); however, to date, such acute activation has not been demonstrated in mice. Moreover, all these findings have not been substantiated in TGR5 knockout mice, which are readily available (Maruyama et al., 2002). It is reasonable to postulate that bile acids are pro-lipolytic given that TGR5 is a G_s-coupled receptor. However, direct evidence is lacking. Bile acid deoxycholate has no pro-lipolytic activity in 3T3L-1 adipocytes (Klein et al., 2009). Therefore, further studies are needed to decipher the pro-lipolytic effects of bile acids.

Parathyroid hormone

The parathyroid hormone (PTH) is a peptide hormone controlling minute-to-minute levels of ionized calcium in the blood and in the extracellular fluid. It is released by the parathyroid gland in response to low calcium plasma levels detected by calcium-sensing receptors and counteracts calcitonin. PTH binds to cell surface receptors in bone and kidney tissue, triggering responses that increase blood calcium. PTH also increases renal synthesis of calcitriol, the hormonally active form of vitamin D, which then acts on the intestine to augment absorption of dietary calcium, in addition to promoting calcium fluxes into blood from bone and kidney tissue. The resulting increase in blood calcium and in calcitriol feeds back on the parathyroid glands to decrease the secretion of PTH. The parathyroid glands, bones, kidney and gut are, thus, the crucial organs that participate in PTH-mediated calcium homeostasis.

The pro-lipolytic nature of PTH was first demonstrated by Werner and Löw in 1973 (Werner and Löw, 1973). The authors showed that PTH stimulated lipolysis three- to fivefold in rat epididymal adipose tissue measured *in vitro* as glycerol release. PTH was then shown to also stimulate lipolysis in human adipocytes. The N-terminal 1–34 fragment of the peptide hormone was shown to be sufficient to elevate the intracellular cAMP level and thereby mediate the lipolytic action of PTH (Sinha et al., 1976).

In murine primary brown adipocytes, PTH also induces the activation of the cAMP–PKA pathway; however, the lipolytic action of PTH is quite low compared with the non-selective β-adrenergic agonist isoproterenol. The enzyme that degrades cAMP is phosphodiesterase 4 (PDE4). PDE4 inhibitors block the degradative action of the enzyme and thereby increase cAMP levels. Whereas the lipolytic action of isoproterenol is not affected by PDE4 inhibition, the lipolytic action of PTH is strongly potentiated (Larsson et al., 2016). Thus, isoproterenol predominantly induces lipolysis by increasing cAMP, whereas PTH stimulation to a much larger extent leads to anti-lipolysis by sequestering cAMP. This suggests that the balance between lipolytic and anti-lipolytic actions is quite distinct for isoproterenol and PTH. The PTH receptor type 1 (PTHR1) is a family B GPCR that binds and is activated by the endocrine ligand PTH, as well as the paracrine ligand PTH-related protein (PTHrP) to mediate divergent functions in different tissues. Other than isoproterenol binding to the ADRB receptors, PTH binding to PTHR1 activates multiple intracellular signaling pathways, including coupling to Gs and Gq signaling. Whereas Gs activates the adenylyl cyclase–cAMP–PKA pathway, Gq activates the

phospholipase C (PLC)-dependent formation of diacylglycerol and β-inositol triphosphate, resulting in a rise of cytosolic Ca^{2+} and protein kinase C activity. These Gq signaling events may lead to increased sequestration of cAMP by PDE4 activation and thereby counteract Gs signaling elicited by PTHR1 (Fig. 2). Therefore, the application of a PDE4 inhibitor is recommended to attain the full lipolytic potential of PTH. Compared with physiological PTH concentrations of 1–10 pmol l^{-1} (10–65 pg ml^{-1}) in healthy adults, effective doses of 10 nmol l^{-1}–1 µmol l^{-1} are considered as supraphysiological.

The molecular basis in terms of specific interactions of ligands with PTHR1 and the post-binding events triggering downstream signals controlling entirely different functions needs further exploration (Cheloha et al., 2015; Gardella and Vilardaga, 2015). In comparison to synthetic $hPTH_{3–34}$ (human PTH), $hPTH_{1–34}$ was reported to significantly stimulate lipolysis in human adipose tissue, indicating that amino acids at positions 1 (serine) and 2 (valine) are crucial for the lipolytic action of PTH. Costimulation with $hPTH_{3–34}$ and $hPTH_{1–34}$, or with isoproterenol or forskolin revealed a dose-dependent inhibition of $hPTH_{1–34}$-stimulated lipolysis but had no effect on forskolin- and isoproterenol-stimulated lipolysis (Taniguchi et al., 1985). This indicates that the truncated peptide does competitively bind to the receptor but does not activate cAMP signaling, which normally results in lipolysis. Thus, PTH_{3-34} could be considered as an antagonist for the PTH receptor. Given that the β-blocker propranolol dose-dependently inhibited isoproterenol-induced lipolysis, but had no effect on PTH-stimulated lipolysis, it has been suggested that PTH causes lipolysis after binding to receptors distinct from β-ARs (Taniguchi et al., 1985).

PTH and the PTHrP share one common function. Kir et al. (2014) demonstrated that tumor-derived PTHrP plays an important role in tumor cachexia and adipose tissue browning. Treatment with both PTH and PTHrP results in increased basal respiration and maximal respiration in primary white adipocytes, and is accompanied by increased thermogenic gene expression (Kir et al., 2014). Consistent with these results and given that PTHrP binds the same receptor as PTH, PTHrP might exert similar lipolytic action in adipose tissue as PTH.

Secretin

Secretin is synthesized predominantly by enteroendocrine S cells in the duodenum and proximal jejunum. Gastric acid, bile salts and luminal nutrients stimulate secretin, and somatostatin inhibits its release. Secretin stimulates pancreatic and biliary hydrogen carbonate and water secretion, and it may regulate pancreatic enzyme secretion. Secretin also stimulates the gastric secretion of pepsinogen and inhibits lower esophageal sphincter tone, postprandial gastric emptying, gastrin release and gastric acid secretion.

The 27-amino acid peptide is initially synthesized as a larger precursor, composed of a signal peptide, an N-terminal peptide, secretin, a Gly-Lys-Arg amidation-cleavage sequence and a 72-amino acid C-terminal peptide, before it is cleaved proteolytically into the active hormone (Kopin et al., 1990). In solution, the secretin protein has a partial helical conformation (Bodanszky et al., 1969).

Approximately 80 years after the discovery of secretin, the presence of a high-affinity receptor in pancreatic acinar cells was reported (Jensen et al., 1983). The secretin receptor belongs to the superfamily of class B1 GPCRs. Depending on the cell type, this class of receptors can activate G_s- and G_q-protein-coupled signaling pathways (Siu et al., 2006).

Research investigating the metabolic role of secretin began shortly after its discovery. The involvement of the hormone in fatty acid metabolism, glucose homeostasis and food intake regulation were examined by different groups in various organisms (Bainbridge and Beddard, 1906; Butcher and Carlson, 1970; Dehaye et al., 1977; Frandsen and Moody, 1973; Glick et al., 1971; Grovum, 1981; Rodbell et al., 1970). However, drawing clear conclusions from these data was challenging owing to contradictory results. In rat white adipocytes, the lipolytic actions of secretin were associated with elevated intracellular cAMP levels (Butcher and Carlson, 1970; Rodbell et al., 1970); however, this lipolytic action of secretin was not observed in white adipocytes isolated from chicken and mice (Dehaye et al., 1977; Frandsen and Moody, 1973). More recent studies appear to confirm that secretin can directly impact adipocyte development and metabolism. In the murine 3T3-L1 preadipocyte cell line, secretin promotes the early phase of adipogenesis by stimulating preadipocyte proliferation, mitochondrial activity and cellular triglyceride content (Miegueu et al., 2013). In mature adipocytes, secretin further enhanced substrate cycling by stimulating the uptake of fatty acids and glucose into white adipocytes in parallel with the lipolytic release of fatty acids and glycerol (Miegueu et al., 2013). The acute lipolytic effect of secretin was confirmed recently in primary epididymal white adipocytes of mice (Sekar and Chow, 2014) (Fig. 2). The effect of secretin on lipolysis and thermogenesis in brown adipocytes is unknown.

Receptor-independent pro-lipolytic effectors

Besides the above-mentioned receptor-dependent lipolysis modulators, biomolecules that bypass receptor-mediated activation and directly stimulate lipolysis in adipocytes have also been identified. For example, α-β hydrolase domain-containing protein 5 (ABHD5), also known as CGI-58 (comparative gene identification 58), is a protein binding to perilipin on lipid droplets under basal conditions, preventing interaction with ATGL. Upon activation, perilipin is phosphorylated by PKA, and ABHD5 rapidly disperses into the cytoplasm, enabling lipase coactivation. Its synthetic ligands SR-4995 and SR-4559, which disrupt the interaction of ABHD5 with perilipin-1 (PLIN1) or perilipin-5 (PLIN5), rapidly stimulate lipolysis in cultured brown adipocytes (Sanders et al., 2015). Similarly, blocking protein serine/threonine phosphatase activity with potent inhibitors such as okadaic acid and calyculins promotes perilipin phosphorylation and increases lipolysis in primary rat adipocytes (He et al., 2006) (Fig. 3). These lipolytic effects occur independently of cAMP and PKA. Therefore, these studies showcase alternative strategies to modulate lipolysis while bypassing the canonical G_s-coupled signaling cascade. This may provide means of activating these processes under conditions where receptor signaling is compromised given that prolonged agonist stimulation results in down-regulation of most G protein-coupled receptors.

Additional pro-lipolytic effectors

As well as the aforementioned examples of pro-lipolytic effectors, additional regulators of lipolysis have been identified (listed in Table 1). Given that lipolysis activation represents the canonical pathway to stimulate thermogenesis, these pro-lipolytic molecules qualify as putative thermogenic mediators in brown and brite adipocytes. A stringent validation of their thermogenic potential using the recently developed microplate-based respirometry assay should shed new light on this aspect (Li et al., 2014). However, the lipolytic actions of some effectors may have a lag period of up to

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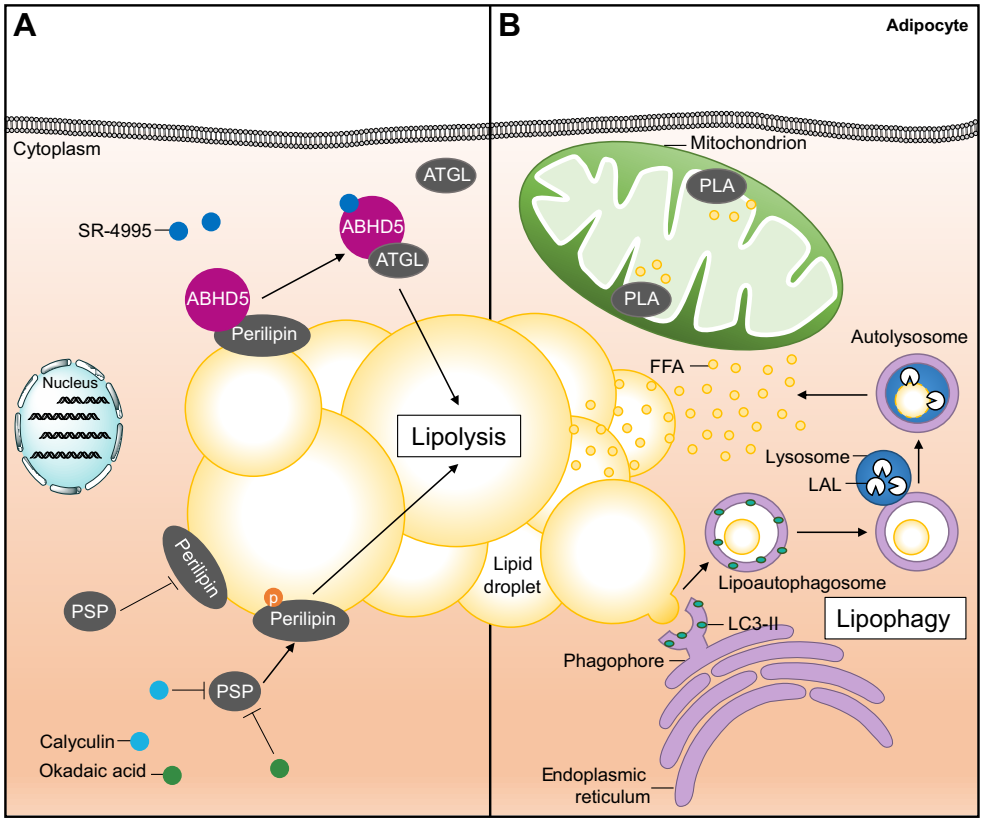

Fig. 3. Receptor-independent modulators of lipolysis and alternative sources of free fatty acids (FFAs). (A) α-β Hydrolase domain-containing protein 5 (ABHD5) binds to perilipin under basal conditions, preventing interaction with adipose triacylglyceride lipase (ATGL). Binding of synthetic ligands of ABDH5, such as SR-4995, leads to the dissociation of perilipin and ABDH5, enabling the binding of ABDH5 to ATGL, which translocates ATGL to the lipid droplet and to subsequent lipolysis. Protein serine/threonine phosphatase (PSP) dephosphorylates perilipin, leading to reduced lipolysis. Potent inhibitors blocking PSP activity such as calyculin or okadaic acid promote perilipin phosphorylation and, thereby, increase lipolysis. (B) Ca^{2+}-independent mitochondrial phospholipase (PLA) liberates FFAs from phospholipids of the inner mitochondrial membrane. In a process termed lipophagy, enabling the selective degradation of lipid droplets via autophagy, a portion of larger lipid droplets is sequestered or smaller lipid droplets are engulfed by LC3-phosphatidylethanolamine-bound phagophore membranes leading to the formation of a lipoautophagosome. Fusion of the latter with lysosomal acid lipase (LAL)-containing lysosomes results in the generation of an autolysosome, wherein LAL degrades the lipid droplet portion and FFAs are released into the cytoplasm.

hours, which is in contrast to virtually no lag period for catecholamines. For example, the lipolytic effect of growth hormone was seen only after a lag period of 1–2 h (Fain et al., 1971). A potential lag period for lipolysis activation by these regulators should be taken into account when testing their thermogenic activity.

Conclusions and perspectives

Given that adipose tissue not only functions as the major organ for fat storage and mobilization but also as an endocrine and thermogenic organ, research on lipolytic modulators has intensified. Other than being a catabolic substrate in mitochondrial β-oxidation, fatty acids liberated by lipolysis are transformed into paracrine/autocrine and endocrine signaling molecules. Moreover, lipolysis is an essential prerequisite for thermogenesis in brown and brite adipocytes. Endogenous and xenobiotic biomolecules affecting the balance between lipolysis and re-esterification of fatty acids are therefore of pertinent interest. In this Review, we discuss an extensive assembly of pro- and anti-lipolytic biomolecules of various origins and physiological functions. For most of these molecules, our knowledge about their impact on the lipid metabolism of adipocytes is mostly limited to white adipocytes cultured *in vivo*, with little or no insights available on their physiological function *in vivo*. Moreover, to date, few of these modulators have been studied for their effects on lipolysis in brown or brite adipocytes. Given that lipolysis is an essential requirement for the activation of UCP1-mediated thermogenesis in these cells, the collection of modulators discussed in this Review can be regarded as a list of potentially pro- or anti-thermogenic modulators of metabolism. Importantly, pro-lipolytic effectors working through the cAMP–PKA pathway are very likely to also induce *Ucp1* gene expression in brown and brite adipocytes. In this respect, their biological activity remains to be determined. Beyond the single effects of individual molecules, it would be interesting to analyze their putative additive or synergistic effects on lipid metabolism in adipocytes and adipose tissues, respectively.

In attempting to investigate pro-lipolytic effectors for their potential to activate UCP1-mediated thermogenesis in brown adipocytes, one has to take into account additional sources of FFAs other than the hydrolysis of triacylglycerol in lipid droplets via the classical lipolytic pathway. For example, mitochondrial phospholipase 2 (PLA2) provides long-chain fatty acids within the inner mitochondrial membrane, which may serve as a physiological mechanism of UCP1 regulation, along with the generation of FFAs by lipolysis of cytoplasmic lipid droplets (Fedorenko et al., 2012).

Table 1. Biomolecules with pro-lipolytic effects on adipocytes categorized as protein, small molecules and plant extracts

Regulators	Mechanism	Model	References
Protein			
ANGPTL3	Unknown	Mice and 3T3-L1 adipocyte	Shimamura et al., 2003
ANGPTL4	cAMP–PKA	Mice and adipocytes	McQueen et al., 2017
ApoA-I Milano	Unknown but independent of cAMP–PKA	In mice and primary epididymal cells	Lindahl et al., 2015
β-Lipotropin	Possibly a melanocortin receptor	Rabbit adipocyte	Richter and Schwandt, 1985
Cardiotrophin-1	cAMP–PKA	3T3-L1 adipocyte	Lopez-Yoldi et al., 2014
Endorphins, enkephalins and naloxone	cAMP–PKA	Rabbit adipocyte	Baptiste and Rizack, 1980
Growth differentiation factor 15	TGF-β signaling	3T3-L1 adipocyte	Chung et al., 2017
Heptapeptide Met-Arg-His-Phe-Arg-Trp-Gly	Melanocortin receptor	Rabbit adipocytes	Draper et al., 1973
Growth hormone	cAMP–PKA	Human and 3T3-F442A adipocyte	Dietz and Schwartz, 1991; Ottosson et al., 2000
Lactoferrin	cAMP–PKA	Rat adipocyte	Ono et al., 2013
MSH (α, β, VA-β-MSH)	Melanocortin receptor	3T3-L1 adipocyte	Fricke et al., 2005
Thyroid-stimulating hormone	cAMP–PKA	Human and 3T3-L1 adipocyte	Gagnon et al., 2010
Zinc α2-glycoprotein	β3-adrenoceptor	Mice and epididymal adipocytes	Russell et al., 2004
Small molecules			
α-Lipoic acid	cAMP–PKA	3T3-L1 adipocyte	Fernández-Galilea et al., 2012
Flavonoids – quercetin	Phosphodiesterase inhibition	Rat adipocyte	Kuppusamy and Das, 1992
Hydroxytyrosol	PKA and ERK1/2 pathway	3T3-L1 adipocyte	Drira and Sakamoto, 2014
Pycnogenol	β-receptor-mediated activity	3T3-L1 adipocyte	Mochizuki and Hasegawa, 2004
Medium-chain enriched diacylglycerol oil	Unknown	Mice	Kim et al., 2017
Procyanidin	cAMP–PKA	3T3-L1 adipocyte	Pinent et al., 2005
Sphingosine-1-phosphate	cAMP–PKA	Rat adipocytes	Jun et al., 2006
Sulforaphane	HSL activation	3T3-L1 adipocyte	Lee et al., 2012
Ursolic acid	cAMP–PKA	3T3-L1 adipocyte	Li et al., 2010
YC-1	cGMP–PKG pathway	Rat adipocytes	Chin et al., 2012
Plant extracts			
Biflavones of *Ginkgo biloba*	Inhibition of cAMP-phosphodiesterase	3T3-L1 adipocyte	Dell'Agli and Bosisio, 2002
Constituents from the leaves of *Nelumbo nucifera*	β-AR pathway	Mice	Ohkoshi et al., 2007
Ethanolic extracts of *Brassica campestris* spp. *rapa* roots	β3-adrenoceptor	Mice and 3T3-L1 adipocyte	An et al., 2010

Potential mechanisms of action, experimental models and references are provided.
Abbreviations: β-AR, β-adrenergic receptor; cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanine monophosphate; ERK1/2; extracellular signal-regulated kinase 1/2; HSL, hormone-sensitive lipase; PKA, protein kinase A; PKG, protein kinase G; TGF-β, transforming growth factor β; VA-β-MSH, VA-β-melanocyte-stimulating hormone (where V is valine and A is alanine).

Furthermore, lipophagy, which is one form of macroautophagy, contributes to the hydrolysis of triacylglycerols stored in lipid droplets (Singh et al., 2009). The large size of lipid droplets impedes their recruitment into lipoautophagosomes, therefore lipophagy only recruits small portions of lipid droplets. Although the potential mechanisms of lipid droplet fragmentation remain unidentified, lipoautophagosomes fuse with lysosomes and within the resultant autolysosomes, lysosomal acid lipase (LAL) presumably hydrolyses triacylglycerols and FFAs are subsequently released into the cytoplasm (Fig. 3). A substantial contribution of lipophagy to the catabolism of triacylglycerols has been demonstrated in various cell types, including brown adipocytes (Saftig et al., 2008).

Storage, mobilization and dissipation of energy are essential for survival so we should not be too surprised that these key functions in energy balance are controlled by multiple redundant pathways. As outlined in this Review, these encompass neuronal control by the SNS, endocrine regulation and metabolic modulators. However, with regard to the number and diversity of endogenous non-adrenergic endocrine and metabolic modulators of lipolysis and thermogenesis in mammals, the physiological relevance of their impact on lipid storage and mobilization in relation to the dominant adrenergic control by the SNS remains to be addressed in more detail. The available studies mostly investigated single effects of molecules; however, to date, only a few of these studies have provided insights regarding additive or synergistic effects. Beyond this complexity within the organism, species-specific differences reported in the literature for some modulators, such as adrenergic agonists, natriuretic peptides, glucocorticoids and corticotropin, remain at the descriptive and mechanistic level without addressing the biological significance of such differences. A general question in this context, is whether differences between species are the result of divergent evolutionary histories and resulting physiological constraints. Traits such as body size and composition, metabolic rate, feeding habits and nutrient selection, energy partitioning in the body, tolerance to fasting, life style and activity behavior, as well as

Table 2. Pro-lipolytic effectors with species-specific differences in the regulation of lipolysis (see text for details and references)

Effectors	Rodents	Humans
β3 agonists	++++	+
Corticotropin (adrenocorticotropic hormone)	+++	–
Glucocorticoids	–	++
Natriuretic peptides	–	+++

torpor/hibernation are likely to have strongly influenced the mechanisms that evolved for the management of body fat stores. Indeed, in small rodents, rapid responses triggered by the SNS and the HPA axis seem to dominate; whereas in humans, endocrine regulation with a slower response time prevails, as exemplified by natriuretic peptides and glucocorticoids (Table 2). However, one may also conclude that more dedicated comparative studies are required to consolidate apparent species-specific differences.

Competing interests
The authors declare no competing or financial interests.

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