Molecular pathways linking metabolic inflammation and thermogenesis

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Summary

Obesity is caused by chronic positive energy balance because of higher energy intake relative to energy expenditure. Thermogenesis, the capacity of an organism to produce heat, is an important component of energy expenditure. Thus targeting the molecular mechanisms controlling thermogenesis could be an effective strategy for the prevention or treatment of obesity. Thermogenesis is modulated by three major factors: environmental temperature, nutrient quantity and quality, and by systemic inflammation. Obesity is now recognized to be a state of chronic low-grade systemic inflammation, which has been proposed to play a major role in the pathogenesis of obesity and obesity-associated diseases. This review discussed the molecular pathways that are recruited during metabolic inflammation and that are also implicated in the control of thermogenesis and energy balance. It emerges that the complex signalling network recruited during metabolic inflammation exerts a balanced action on the modulation of thermogenesis and energy balance, with some pathways promoting weight gain whereas other pathways have opposite actions. It is thus concluded that immunomodulation of metabolic inflammation, rather than an anti-inflammatory intervention aiming at its suppression, may be a more promising strategy to increase thermogenesis for the treatment or prevention of obesity and its associated diseases.

Keywords: Cytokines, IKK, JNK, MAPK, PI3K.

Innate immunity and thermogenesis: basic concepts

Obesity is caused by chronic positive energy balance resulting from a higher energy intake relative to energy expenditure. Understanding the molecular mechanisms controlling energy balance is thereby fundamental to the development of novel interventions for the treatment or prevention of obesity. Homeotherm organisms, including humans and laboratory rodents, dissipate a large proportion of their ingested food energy to the environment in the form of heat (1). This capacity of living organisms to generate heat is generally referred to as thermogenesis (2). Thermogenesis is variable among different individuals and is modulated by environmental factors (2). There are essentially three major environmental factors that potently influence thermogenesis: environmental temperature (e.g. cold-induced thermogenesis), food quantity and quality (e.g. diet-induced thermogenesis), and systemic inflammation in response to infection or tissue damage (e.g. fever) (2,3). Thermogenesis is chiefly controlled by the sympathetic nervous system (SNS) and depends on β-adrenergic receptors (4,5), which mediate the effects of norepinephrine and other catecholamines (3,6,7). Increased norepinephrine turnover was reported in the heart, white adipose tissue (WAT), and brown adipose tissue (BAT) in response to thermogenic stimuli like diet and cold (8,9). Binding of catecholamines to brown adipocyte β-adrenergic receptors activates a thermogenic programme including induction of lipolysis and uncoupling protein (UCP)-1 activity and gene expression.
β-adrenergic signalling in white adipocytes and cardiomyocytes is likely to play an important role in sustaining thermogenesis by providing fatty acid substrates from lipolysis that are transported to the BAT via an increased blood flow (Fig. 1).

The adipocyte-derived hormone leptin plays a major role in sustaining thermogenesis (Fig. 1). Indeed, mutant mice with defective leptin signalling display dramatically decreased sympathetic tone in BAT (10,11), in addition to showing impaired thermogenic response to cafeteria diet (12) and cold intolerance (13).

The SNS and more generally the nervous system are tightly interconnected with the immune system. These systems influence each other in a bidirectional manner and share similar molecular mechanisms (14). Indeed, on the one hand, the SNS is known to play a powerful action on white blood cells to control immunity (15), and it has also been shown that leukocytes themselves produce catecholamines to modulate the immune reaction (16,17). Furthermore, macrophage-mediated immunity and reactive oxygen species production have been shown to be modulated by the UCP-2, a protein with high sequence similarity to UCP-1 (18). On the other hand, the innate-immune system exerts a powerful control on the SNS and thermogenesis. Fever can be seen as a paradigm of such a neuro-immune interaction where pathogens activate leukocytes and other cells leading to increased levels of pro-inflammatory mediators whose action on the central nervous system results in increased core body temperature (Fig. 1). The precise cellular and molecular mechanisms controlling fever is still not completely understood and different mechanisms are recruited in different models. However, three central molecular mediators of the febrile response to exogenous pyrogens such as bacterial endotoxin have been identified: the toll-like receptor 4 (TLR-4), the pyrogenic cytokines and the pyrogenic prostaglandins (19–22). Pathogen-associated molecular patterns (PAMPs) are sensed by specific receptors such as TLR-4, the sensor of bacterial lipopolysaccharides (LPS). Once activated, TLR-4 receptors lead to the activation of different kinases including mitogen-activated protein kinases (MAPK) and the inhibitor of kappa-B kinase (IKK) complex, which are responsible, respectively, for the activation of the transcription factors activator protein 1 (AP-1) and nuclear factor kappa-B (NF-kB), leading to increased expression of inflammatory mediators (23). Pyrogenic cytokines, most notably the interleukins (IL) IL-1 and IL-6, are the primary endogenous pyrogens and are produced in response to either PAMPs or damage-associated molecular patterns (DAMPs) (19–22). Both endogenous pyrogenic cytokines and exogenous pyrogens

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**Figure 1** Essential elements in the regulation of thermogenesis. Thermogenesis is modulated essentially by three factors: (i) environmental temperature; (ii) food quality and quantity; and (iii) systemic inflammation. It is well established that the induction of thermogenesis by cold and diet depends on the sympathetic nervous system (SNS). Increased catecholamine turnover was observed in brown adipose tissue and in the heart in response to cold and cafeteria diet, and in retroperitoneal white adipose tissue in response to dietary glucose or fructose. Some studies indicate that the immune system also modulate thermogenesis via the sympathetic nervous system. Systemic inflammation can lead to either fever or hypothermia, depending on the experimental conditions. The thermogenic outcome of systemic inflammation depends on the balanced action of pyrogenic cytokines (e.g. interleukin [IL]-1β and IL-6) and anti-inflammatory cytokines (e.g. IL-1Ra and IL-10). Leptin signalling is known to be required for an efficient induction of thermogenesis by cold and food. cAMP, cyclic adenosine monophosphate; COX-2, cyclooxygenase 2; LPS, lipopolysaccharides; PAMPs, pathogen-associated molecular patterns; PGE2, prostaglandin E2; PKA, protein kinase A; UCP-1, uncoupling protein.
(e.g. LPS) induce the expression of the gene encoding for the cyclooxygenase 2 (COX-2), the enzyme responsible for the production of pyrogenic and inflammatory prostaglandins such as PGE2 (prostaglandin E2) (19–22). Overall, LPS and pyrogenic cytokines can induce a complex physiological response leading to higher core body temperature by a mechanism that depends on COX-2 activity (24,25) and that involves increased thermogenesis partly because of the activation of SNS (26,27) (Fig. 1).

It is important to consider that, in addition to fever, systemic inflammation can also cause hypothermia depending on the experimental conditions (28,29). Indeed, intraperitoneal injection of a low dose of LPS (30 µg kg⁻¹) is known to cause fever without hypothermia, whereas a higher dose of LPS (2.5 mg kg⁻¹) causes a ‘multiphase’ response with hypothermia preceding fever, and finally, a lethal dose of LPS (10 mg kg⁻¹) was reported to induce sustained hypothermia (28). Furthermore, a hypothermic phase preceding fever was also observed in the caecal–ligation puncture model, which reproduces sepsis (28). Our current understanding of the mechanisms of hypothermia in systemic inflammation is poor compared with our understanding of fever. Systemic inflammation-induced hypothermia may be at least in part explained by increased heat loss, although the precise mechanism remains to be established and suppression of thermogenesis may also play an important role. Overall, it is evident that during systemic inflammation, several cytokines and other signalling molecules are released: some cytokines display clear pyretic effects (e.g. IL-1, and IL-6) whereas others are antipyretic (e.g. IL-1Ra and IL-10) (30) (Fig. 1). Thus, the final effect of systemic inflammation on body core temperature and thermogenesis is likely the outcome of the integrated action of the specifically recruited signalling network. Interestingly, the pro-inflammatory cytokine tumour necrosis factor (TNF) α is reported to display both pyretic and antipyretic activity depending on the specific model in which its action is tested. TNFα can be considered a pyrogen as intravenous injection of TNFα causes fever in different species by a mechanism involving SNS-dependent induction of thermogenesis (31–33). However, the pyrogenic action of TNFα depends on the specific experimental model as TNFα was also reported to act as antipyretic and promote LPS-induced hypothermia (34–36). Interestingly, one study showed an association between TNFα-induced hypothermia and decreased sympathetic outflow to BAT (37), suggesting that at least in some conditions, TNFα signalling may decrease thermogenesis. Thus, considering the powerful actions of different cytokines in the regulation of SNS-mediated thermogenesis, it is possible that signalling pathways implicated in innate immunity may also be involved in the control of energy balance and body weight homeostasis.

**Obesity is a state of low-grade chronic systemic inflammation**

Obesity is an inflammatory condition characterized by elevated levels of pro-inflammatory cytokines, accumulation of leukocytes within adipose tissue and other organs, activation of macrophages in liver and fat, and activation of pro-inflammatory signalling pathways in multiple organs (38–43). Inflammation can be triggered by pathogens or by tissue damage, and although the precise mechanism causing inflammation during obesity is still under investigation, two main mechanisms have been proposed: one focused on metabolic endotoxemia and the other one on metabolic stress. Concerning the former hypothesis, it was reported that the gut microbial population is altered in obese mice independently of the diet (44), and that feeding mice on a high-fat diet (HFD) causes a state of chronic low-grade endotoxemia, which was proposed to be an initiating factor of obesity-induced inflammation (45). The second mechanism proposed to underlie metabolic inflammation focuses on metabolic stress caused by excess nutrients. During positive energy balance, excess calories are safely stored in adipocytes as triglycerides. However, when the adipose tissue expandability limit is achieved, toxic lipids accumulate in non-adipose tissues causing metabolic stress and activating pro-inflammatory signalling cascades (38–42,46–48). At the same time, the hypertrophic adipocytes die by necrosis causing leukocyte infiltration into WAT with activated macrophages accumulating around the lipid vacuole from dead adipocytes to form typical crown-like structures (49). In support for the idea of a crucial role for adipose tissue expandability in metabolic inflammation is the demonstration that a similar inflammation to the one observed in obesity models is also observed in lipodystrophic mice, where adipocyte hypertrophy and fat storage saturation are achieved in non-obese mice on a low-fat diet (49,50).

Overall, obesity is an inflammatory state where signals reported to exert powerful effects on thermogenesis are chronically recruited. As described earlier, acute systemic immune reactions can have powerful effects in either increasing or decreasing body core temperature depending on the specific model. Such powerful and opposing effects of the immune system on core body temperature may in part be explained by the specific signalling network recruited in the specific context. It is therefore important to note that metabolic inflammation is characterized by a distinct cytokine-signalling network. The obese adipose tissue is characterized by the accumulation of pro-inflammatory leukocytes and polarization of the local macrophage population from M2
microbiota has been identified as an environmental factor thermogenic depending on the experimental conditions. Gut enogenous pyrogens, which paradoxically can also cause hypo-

PAMPs from gram-negative bacteria are most potent exog-

teriated inflammation and thermogenesis molecular patterns receptors in obesity- associated with obesity, it is important to note that among these cytokines may control thermogenesis via the sympathetic nervous system (SNS). However, possible peripheral actions should also be considered. Intact leptin signalling is known to be a basic requirement for efficient thermogenesis in response to cold and food. Thus, insufficient leptin signalling (e.g. leptin resistance) may lead to defective thermogenesis promoting positive energy balance and obesity.

(typically anti-inflammatory) towards an M1 phenotype (typically pro-inflammatory) (51,52). This is paralleled by a low-grade but chronic elevation of the production of pro-inflammatory pyrogenic cytokines, and decreased expression of the anti-inflammatory cytokine IL-10 (51,52). Despite the clear pro-inflammatory 'cytokine signature' associated with obesity, it is important to note that among the cytokines displaying the largest induction of gene expression in the obese adipose tissue one can find the IL-1 receptor antagonist (IL-1Ra), a most powerful anti-inflammatory and antipyretic cytokine whose function is to antagonize IL-1 signalling (30,53–55) (Fig. 2).

Exogenous pyrogens and pathogen-associated molecular patterns receptors in obesity-induced inflammation and thermogenesis

PAMPs from gram-negative bacteria are most potent exogenous pyrogens, which paradoxically can also cause hypothermia depending on the experimental conditions. Gut microbiota has been identified as an environmental factor implicated in energy balance (56). The gut microbial population is altered during obesity (44), and it has been shown that HFD increases the circulating levels of LPS (45). In a study investigating the effects of metabolic endotoxemia on obesity, LPS levels were chronically elevated to the concentra-

tion observed in HFD-fed mice for 4 weeks by continuous LPS infusion using an osmotic pump (45). Strikingly, LPS-infused mice kept on low-fat diet displayed higher weight gain than control saline-infused mice on low-fat diet, with the greater weight gain being similar to mice kept on HFD. However, whereas the increased weight gain on HFD-fed mice compared with low-fat diet-fed mice was largely due to increased energy intake, in LPS-infused mice energy intake was significantly lower than HFD-fed mice and similar to the one observed in low-fat diet-fed mice. This observation suggests that chronic low-grade endotoxemia may promote feed efficiency by reducing apparent energy expenditure.

TLR-4 was identified as a major sensor for bacterial LPS, and it was proposed that TLR-4 might be a sensor for saturated fatty acids (57). Nonetheless, the role of TLR-4 in diet-induced obesity is less obvious as different studies investigating TLR-4 loss of function in mice models of diet-induced obesity have reported different results, ranging from increased predisposition to obesity to a dramatic protection from diet-induced obesity compared with wild-type (WT) control mice (57–59). Whereas such discrepancies suggest that the impact of TLR-4 signalling on energy balance may depend on the specific experimental conditions, it should be noted that when loss of functional TLR-4 was linked to increased weight gain, this was largely due to increased food intake (57), whereas when resistance to diet-induced obesity was observed, this was explained by increased thermogenesis (58). Thus, it cannot be excluded that TLR-4 signalling might have opposing effects on food intake and thermogenesis in models of diet-induced obesity and might therefore either promote or antagonize positive energy balance depending on the specific experimental condition. Still, the precise role of TLR-4 on thermogenesis and energy balance in body weight homeostasis and obesity remains to be established.

The nucleotide-binding oligomerization domain-like receptors (NLR) are a family of intracellular PAMPs and DAMPs receptors implicated in pathogen and damage sensing (60). A study reported that mice lacking two NLR family members NOD1 and NOD2 display reduced adiposity when placed on HFD compared with WT mice (61). However, this leaner phenotype could be partially explained by increased spontaneous physical activity and decreased food intake. Thus, at this stage, it is not clear whether NOD1 and NOD2 may also play a role in the control of thermogenesis. Another NLR family member that was recently implicated in metabolic inflammation and energy balance is the NOD-like receptor NLRP-3.
Obesity-induced inflammation and thermogenesis: possible roles for pyrogenic cytokines

Interleukin-6 and interleukin-1

The major pyrogenic cytokines IL-6 and IL-1 are elevated in adipose tissue and blood during obesity and were proposed to play an important role in obesity-induced glucose intolerance. However, as IL-6 and IL-1 activate thermogenesis during fever (19,20), it is possible that IL-6 and IL-1 could play a protective role in models of diet-induced obesity by promoting thermogenesis, thus antagonizing positive energy balance and the development of obesity. Consistent with this view is the report that mice lacking IL-6 spontaneously develop mature-onset obesity on a standard low-fat diet (68), a phenotype that was partially explained by the central effects of IL-6 on thermogenesis (68,69). However, in another study, age-related obesity was not observed in mice lacking IL-6 (70), thereby suggesting that the presence of IL-6 is not an essential requirement for body weight homeostasis. Yet, a third study reported that mice lacking IL-6 and IL-1 display increased weight gain, although this could be largely explained by increased food intake (71). IL-1 signalling was proposed to play an important role in leptin-mediated control of energy balance and thermogenesis (72). Since then, several studies have supported the concept of a role for IL-1 as a thermogenic signal implicated in the control of body weight. Indeed it has been shown that mice that do not express IL-1Ra, a most potent antagonist of IL-1 signalling, display decreased weight gain and increased energy expenditure (73). This observation was confirmed by another laboratory, which also correlated the decreased weight gain and increased energy expenditure observed in mice lacking IL-1Ra with increased sympathetic tone (74). Consistently, it was reported that mice lacking IL-1 receptor develop mature-onset obesity (75), a phenotype that was partially explained by decreased leptin sensitivity. It is important to note that IL-1Ra gene expression is markedly induced in obese WAT (53,54). IL-1β expression is also induced in obese WAT, but to a lower extent than IL-1Ra (53,54). Overall these data suggest that during obesity, decreased IL-1 signalling because of increased IL-1Ra levels may promote feed efficiency and positive energy balance (Fig. 2).

Interleukin-18

The cytokine IL-18, which like IL-1β, is processed into its active form by the inflammasome complex, has also been implicated in the control of energy expenditure. Indeed, mice lacking IL-18 display increased feed efficiency and reduced oxygen consumption compared with WT control mice (76,77). These studies on the role of IL-1 and...
IL-18 on energy balance are paradoxical with the one on
the inflammasome complex described earlier, where mice
lacking key inflammasome components are resistant to
diet-induced obesity because of increased energy expendi-
ture despite defective IL-1 and IL-18 processing (65,66).
Again, it was recently reported that the inflammasome is
implicated in the control of intestinal microbiota, and
that lack of inflammasome function leads to alteration
of the gut microbiota predisposing to obesity (67). Thus,
resolving these apparent paradoxes will be important
to better understand the complex action of the inflam-
masome, IL-1 and IL-18 in metabolic homeostasis and
thermogenesis.

Tumour necrosis factor-α
Another pro-inflammatory cytokine whose expression is
induced during obesity is TNFα. Blocking TNFα signalling
either using neutralizing antibodies or by genetic deletion of
the TNFα gene or both its receptors TNFR1 and TNFR2
genes was reported to improve glucose homeostasis in
rodent models of obesity without affecting body weight
(78,79). However, it was also complex action that genetically
obese ob/ob mice, which also lack TNFR1 or both TNFR1
and TNFR2, display increased β3-adrenergic receptor and
UCP-1 mRNA levels in BAT, reduced brown adipose tissue
apoptosis and increased number of multilocular brown adipocytes compared with ob/ob mice expressing TNFRI
(80). Furthermore, incubation of cultured brown adipocytes with TNFα caused decreased β3-adrenergic recep-
tor mRNA levels and defective cAMP (cyclic adenosine
monophosphate) production in response to β3-adrenergic receptor agonist (80). Collectively, these results suggest that
TNFα via TNFR1 decreases β3-adrenergic receptor signalling
in BAT and thus TNFR1 could be an important nega-
tive regulator of BAT activation. Most importantly, ob/ob
mice lacking TNFR1 and TNFR2 showed improved capacity
to maintain core body temperature when exposed to
cold (4°C) compared with ob/ob mice with intact TNFα
signalling, thus revealing an important role for TNFα sig-
nalling as negative modulator of cold-induced thermogen-
esis (80). Interestingly, in this study, body weight was
significantly reduced in TNFR-deficient female ob/ob mice
compared with ob/ob mice expressing the TNFα receptors,
suggesting that TNFα signalling may be implicated in body
weight regulation. Consistent with this view, another labora-
tory reported that mice specifically lacking TNFR1 sig-
nalling, but expressing a functional TNFRI were protected
from diet-induced obesity, a phenotype that was explained
by increased thermogenesis (81). Overall these studies on
TNFα signalling in cold-induced thermogenesis and energy
balance are consistent with the previously proposed role for
TNFα as antipyretic and negative modulator of the SNS-
BAT axis during fever (34–37) (Fig. 2). Whether TNFR2 is
also implicated in the control of thermogenesis and whether
there is a specific crosstalk between TNFR1 and TNFR2
signalling in thermogenesis remains to be established.

Ciliary neurotropic factor
The cytokine ciliary neurotropic factor (CNTF), which so
far, was not directly implicated in metabolic inflammation,
emerged for its potential as antiobesogenic drug (82,83).
CNTF is a pleiotropic cytokine that was identified as
neuron-survival factor (84) and was later found to be an
endoogenous pyrogen (85), although its physiological role in
systemic inflammation and fever remains to be established.
During a clinical trial for the treatment of amyotrophic lateral sclerosis with recombinant CNTF it was observed that
obese patients receiving CNTF significantly lost weight
compared with placebo-treated patients, suggesting an
important role for CNTF in energy balance (86,87). Mouse
studies show that the antiobesogenic action of CNTF is
largely leptin independent (88). Similarly to leptin, CNTF
acts on hypothalamic neurons to decrease food intake and
increase thermogenesis and UCP-1 gene expression in BAT
(88,89), and peripherally to promote AMPK (5' adenosine
monophosphate-activated protein kinase)-dependent fatty
acid oxidation (90). Differently from leptin, CNTF was
also shown to induce COX-2 gene expression in brain
vascular tissue (91). Thus, it is possible that CNTF, like
other pyrogens, induces thermogenesis via COX-2-
dependent activation of the SNS. Indeed, the CNTF recep-
tor CNTFRα, similarly to IL-6 receptor, signals via gp130
protein and CNTF was also shown to bind to the IL-6
receptor (92–94); thus IL-6 and CNTF are recruiting dis-
tinct, but overlapping signalling networks.

A possible role for leukocytes in thermogenesis
and energy balance
Overall the studies above suggest that the specific cytokine
network recruited during obesity might have profound
effects on energy balance. The obese WAT is characterized
by leukocyte accumulation, polarization of resident mac-
rophages towards a so-called M1 ‘pro-inflammatory’ phe-
notype, and induction of pro-inflammatory cytokine gene
expression including TNFα, IL-1 and IL-6. The specific
impact of M1-activated pro-inflammatory macrophages on
energy balance and thermogenesis during obesity remains
to be established. However, it was recently reported that
cold exposure induces alternative activation of macro-
phages towards an ‘M2 anti-inflammatory’ phenotype (95).
Most notably, the authors show that mice with defective
macrophage alternative M2 activation display cold intoler-
ance, which was explained by defective lipolysis in WAT,
depletion of BAT lipid storage, and decreased BAT activa-
tion. Thus the study mentioned earlier suggests an impor-
tant role for alternatively activated M2 macrophages in the
promotion of lipolysis and thermogenesis. By contrast, another study reported that during caloric restriction, a condition characterized by increased lipolysis and suppressed thermogenesis, macrophages accumulate to WAT to decrease glycerol and FFA efflux from lipolysis (96). Thus it will be important to test whether classically activated M1 ‘pro-inflammatory’ macrophages and alternatively activated M2 ‘anti-inflammatory’ macrophages play opposing roles in lipolysis and thermogenesis.

In addition to a possible role for macrophages in thermogenesis, it was shown that genetic deficiency or pharmacological stabilization of mast cells leads to decreased weight gain in a mouse model of diet-induced obesity (97). Whereas this study suggests a role for mast cells in the control of energy balance during obesity, it remains to be established whether mast cells modulate thermogenesis and feed efficiency rather than digestible energy intake.

Overall, the major pro-inflammatory cytokines induced during obesity have somehow been implicated in the control of thermogenesis and energy balance. A more detailed understanding of the specific cytokine network recruited during obesity and its action on thermogenesis and energy balance may thus be fundamental for novel strategies to treat or prevent obesity.

The cyclooxygenase-2 in thermogenesis and energy balance

Pyrogenic cytokines and exogenous pyrogens such as bacterial LPS are believed to activate the SNS and thermogenesis by a mechanism involving COX-2-mediated production of pyrogenic prostaglandins. A possible role for COX-2 in energy balance was suggested by a study showing that mice that are heterozygous for a COX-2 null mutation, with reduced COX-2 levels, display increased weight-gain and adiposity compared with control mice (98). Curiously, the same authors reported that mice bearing the COX-2 null mutation on both alleles, which did not express COX-2, showed similar body weight and adiposity compared with WT control mice. Furthermore, another study reported that whereas mice lacking COX-2 display similar body weight to WT control mice until the age of 6 months, by the age of 9 months mice lacking COX-2 gained much less weight than WT controls despite similar food consumption (99). These studies suggest that COX-2 may be implicated in the control of energy balance by a dose- and age-dependent mechanism.

Recently, COX-2 was implicated in cold-induced thermogenesis. Indeed two different laboratories reported that cold exposure leads to increased COX-2 expression in adipose tissue of mice, which correlate with the expression of markers for BAT differentiation (100,101). This induction of COX-2 gene expression was recapitulated by treatment of mice with a β3-adrenergic receptor agonist suggesting that cold-induced COX-2 gene expression is mediated by increased sympathetic activity. Mice lacking COX-2 activity were less able to maintain core body temperature in the cold and showed defective induction of markers for BAT differentiation in inguinal WAT compared with control mice expressing COX-2 (100,101). Importantly, mice treated with indomethacin, a non-selective COX inhibitor displayed increased weight gain and adiposity, despite the fact that energy intake was not increased compared with control mice (100). Furthermore, it was shown that mice overexpressing COX-2 in WAT gained less weight than control mice kept on either low-fat or HFD. Consistently, compared with WT control mice, the transgenic mice overexpressing COX-2 in WAT displayed elevated thermogenesis and induction of BAT differentiation within intra-abdominal WAT pads (101). Collectively, these studies strongly suggest that the sympathetic action in cold-induced thermogenesis is in part mediated by COX-2. Furthermore, COX-2 levels may play an important role in energy balance and body weight homeostasis.

Obesity-activated pro-inflammatory signal transducers, their role in thermogenesis and diet-induced obesity

Metabolic inflammation, a possible mechanism for obesity-induced leptin resistance

Obesity is characterized by hyperleptinaemia. While this suggests that excess adiposity may cause leptin resistance (102), the elevated leptin levels may also be part of a homeostatic reaction to the excessive caloric intake. In either case, insufficient leptin signalling may promote positive energy balance and diet-induced obesity via increased food consumption and impaired thermogenic response to excess caloric intake. Two major negative regulators of leptin signalling are the protein tyrosine phosphatase 1B (PTP1B) and the suppressor of cytokine signalling 3 (SOCS3). Remarkably, mutant mice that do not express functional PTP1B in the brain or heterozygote mice for SOCS3 loss of function mutation are protected from diet-induced obesity and insulin resistance (103–107). Furthermore, a study showed that compared with control mice, double mutant mice, which do not express PTP1B and SOCS3 in neurons are resistant to diet-induced obesity largely because of increased thermogenesis (108). Metabolic inflammation emerged as a molecular mechanism for obesity-induced leptin resistance. Indeed, it was reported that PTP1B expression during obesity correlates with inflammation, and that TNFα increases PTP1B mRNA levels via activation of the transcription factor NF-kB (109). Similarly, it was shown that SOCS3 induction during obesity depends on NF-kB activation in neurons (110). These studies strongly suggest that the expression of major
negative regulators of leptin signalling, PTP1B and SOCS3, is induced by pro-inflammatory pathways recruited during obesity (Fig. 3).

**Myeloid differentiation primary response gene 88 (MYD88)**

Myeloid differentiation primary response gene 88 (MYD88) is an adapter protein that functions as scaffold recruiting pro-inflammatory signal cascades downstream to IL-1R and TLR receptors. It was reported that mice lacking neuronal MyD88 are protected from diet-induced obesity and leptin resistance, and display decreased food intake and increased thermogenesis compared with control mice (111). The precise pathway linking neuronal MyD88 signalling to leptin resistance during obesity remains to be identified but it was proposed to be mediated by the protein kinase IKKβ, the pro-inflammatory signal transducer responsible for the activation of NF-κB (Fig. 3).

**Inhibitor of nuclear factor kappa-B kinase-β (IKKβ)**

IKKβ is part of the IKK complex, comprising the catalytic subunits IKKα and IKKβ, and the regulatory protein IKKγ (112). IKKβ is activated in response to several pro-inflammatory signals and is considered to play a major role in obesity-induced inflammation and insulin resistance (39). In acute inflammation, IKKβ acts as a sensor and a signal amplifier where cytokines and PAMPs such as IL-1, TNFα, and LPS potently induce IKKβ activity resulting in increased NF-κB nuclear translocation and consequent induction of the expression of several inflammatory mediators including IL-1 and TNFα (112). It was shown that genetically modified mice that do not express IKKβ in neurons are protected from diet-induced obesity and leptin resistance compared with WT control mice (110). The resistance to diet-induced obesity in mice lacking neuronal IKKβ could be largely explained by decreased food intake consequent to improved leptin sensing within the hypothalamus. However, given the key role of leptin in diet-induced thermogenesis, it is possible that an improved thermogenic response to excess calories also contributes to the obesity-resistant phenotype of mice with neuronal IKKβ inactivation. At the molecular level, it was proposed that in the obese hypothalamus, excess adiposity causes endoplasmic reticulum stress response activating the IKKβ NF-κB signalling pathway, which induces SOCS3 gene expression and thus leptin resistance (110,113) (Fig. 3).

**The inhibitor of nuclear factor kappa-B kinase-ε (IKKe)**

The inhibitor of NF kappa-B kinase-ε (IKKe) is a protein kinase whose expression is induced by NF-κB during inflammation and that was implicated in the regulation of NF-κB activity (112). It was proposed that IKKe plays an important role in energy balance during diet-induced obesity. Indeed, one study showed that mice lacking IKKe were protected in a model of diet-induced obesity because of increased thermogenesis, which was explained by increased expression of UCP-1 in WAT (114). The relative importance of IKKe in the promotion of diet-induced obesity was questioned by another study, as the impact of IKKe inactivation on diet-induced obesity may depend on the specific experimental condition (115). Still, the latter study supported the role of IKKe in the control of UCP-1 gene expression in WAT. Overall these studies suggest that IKKe is a negative regulator of thermogenesis possibly by
inhibiting brown adipocyte differentiation within WAT depots. The specific mechanism by which IKKe regulates UCP-1 gene expression within WAT remains to be established, but it would be interesting to test whether IKKe controls COX-2 expression in WAT pads.

c-Jun N-terminal kinases and other mitogen-activated protein kinases

The c-Jun N-terminal kinases (JNK) is a member of the MAPK family, whose activity is induced in response to several pro-inflammatory and stress signals (116). JNK activity is chronically elevated during obesity in multiple tissues and the JNK1 and JNK2 isoforms emerged as major molecular links connecting obesity, inflammation and insulin resistance (39–41). Indeed mouse genetic studies showed that JNK1 and JNK2 play an important role in the promotion of diet-induced obesity (117,118). The role of JNK1 in diet-induced obesity is largely leptin independent as leptin-deficient ob/ob mice lacking a functional JNK1 display a leaner phenotype compared with ob/ob mice expressing JNK1 (118). Obesity resistance in mice lacking JNK1 is largely due to increased thermogenesis and depends on its activity within a non-haematopoietic compartment (119). Indeed, studies of mouse tissue-conditional JNK1 gene deletion showed that the brain is a main site for the obesogenic action of JNK1, and suggested that neuronal JNK1 negatively regulates hypothalamic-mediated induction of thyroid hormone release (120). Altogether, these studies support the hypothesis that neuronal JNK1 activity may promote positive energy balance and obesity by a mechanism involving negative regulation of thyroid hormone secretion.

JNK1 is not the only MAPK that has been implicated in diet-induced obesity. Indeed, it was reported that mice that do not express the extracellular-regulated kinase-1 (ERK1) are also resistant to diet-induced obesity and display exaggerated thermogenic response to food compared with control mice (121). As the loss of ERK1 did not reduce obesity in ob/ob mice, the mechanism of action of ERK1 in diet-induced obesity depends on leptin signalling (122).

Another study implicated the MAPK p38α in obesity-induced glucose intolerance (123). Interestingly, mice that do not express p38α are partially protected from diet-induced obesity (123), although it remains to be established whether the role of p38α in diet-induced obesity is to control thermogenesis.

The double-stranded RNA-dependent protein kinase

The double-stranded RNA-dependent protein kinase (PKR) was proposed to play a major role in lipid-induced inflammation, JNK activation and insulin resistance (124). Mice bearing a targeted deletion at the PKR gene are protected from lipid-induced insulin resistance and HFD-induced glucose and insulin intolerance. Interestingly, mice that do not express PKR are dramatically protected from diet-induced obesity compared with WT control mice (124). PKR activity may promote food intake and decrease rate of oxygen consumption by a mechanism involving JNK activation (124). However, the precise molecular mechanism by which PKR controls energy balance during diet-induced obesity is still to be identified.

The class IB phosphatidylinositol-3 kinase phosphatidylinositol-3 kinase (PI3Kγ)

The phosphatidylinositol-3 kinase-γ PI3Kγ is the only member of class IB phosphatidylinositol-3 kinase that, unlike the class IA PI3Ks, is not activated by receptor tyrosine kinases such as the insulin receptor, but is instead activated by several G-protein coupled receptors (125–128). PI3Kγ signalling is recruited by receptors implicated in inflammation such as chemokine receptors and receptors involved in the control of energy balance and thermogenesis such as the angiotensin II AT1 receptors and β-adrenergic receptors (128–131). Because of its importance in chemokine signalling, PI3Kγ plays a key role in inflammation by promoting leukocyte migration to the site of infection (128). Interestingly, PI3Kγ was also shown to be a negative regulator of β-adrenergic signalling in the cardiomyocytes (132,133). The latter may be relevant to thermogenesis, as cold-induced and diet-induced thermogenesis depend on β-adrenergic signalling (4,6) and both cold exposure and cafeteria diet increase norepinephrine turnover in the heart (9). It was recently proposed that PI3Kγ plays an important role in the link between obesity, inflammation and glucose intolerance (55,134). Most notably, mice bearing a targeted gene deletion at the PI3Kγ locus are protected from diet-induced obesity because of decreased feed efficiency consequent to enhanced diet-induced thermogenesis (55). The precise molecular mechanism linking PI3Kγ to diet-induced thermogenesis remains to be identified; however, it may involve control of sympathetic signalling in the heart and lipolysis in WAT (55,132,133). Furthermore, the effects of PI3Kγ inactivation on energy balance depends on leptin signalling as mice that do not express PI3Kγ are protected from obesity in dietary models, but not in the leptin-deficient ob/ob model (55,134). Consistent with the data mentioned earlier, it was recently reported that mice overexpressing the phosphatase PTEN (phosphatase and tensin homolog), which counteracts the activity of class I PI3Ks, display decreased adiposity because of increased thermogenesis (135). Overall, these results suggest that the class IB PI3Kγ promote positive energy balance and diet-induced obesity via negative regulation of thermogenesis.
Final considerations

Environmental temperature, diet (quality and quantity) and systemic inflammation are the three main factors modulating thermogenesis (2,3). It is now established that obesity is characterized by a low-grade systemic chronic inflammation, which was proposed by several authors to trigger pathways promoting positive energy balance (39,41,136,137). Here are reviewed the pathways that may be implicated in linking obesity-associated metabolic inflammation with thermogenesis and feed efficiency. Overall, a large body of evidence suggests that the cytokines and signalling molecules described in this review should be regarded as pleiotropic signal transducers which, on top of their function in innate immunity, also play an important role in body weight homeostasis (Figs 2 and 3, and Table 1). During systemic inflammation, complex signalling cascades are recruited, and each specific model of systemic inflammation is characterized by a specific signalling network. Obesity-induced inflammation is a peculiar inflammatory state characterized by chronic low-grade increased levels of several pro-inflammatory cytokines and a significant elevation of the levels of the anti-inflammatory IL-1Ra. Most inflammatory pathways recruited during obesity were proposed to promote positive energy balance and thus their inhibition may be beneficial for the treatment of obesity. However, some of the cytokines recruited during metabolic inflammation (e.g. IL-1, IL-18 and IL-6) as well as COX-2, may promote thermogenesis and negative energy balance, and thus could be protective towards the development of obesity (68,71–77,100,101). It is concluded that a specific modulation of metabolic inflammation, rather than its suppression, could be a promising strategy for the prevention and treatment of obesity and related diseases. In support of this concept are studies showing that pharmacological administration of the pyrogen CNTF to rodents and humans leads to reduced body weight compared with controls (86–89,138). CNTF can be seen as a paradigm of the pleiotropic nature of many cytokines; indeed it acts as a neuron-survival factor (84), an endogenous pyrogen (85), as well as an anorexigenic and thermogenic signal implicated in body weight homeostasis (88). There could be potential limitations and pitfalls in exploiting inflammatory pathways to treat obesity. The development of adverse effects may be a possible concern, and it is not clear to which extent it can be possible to achieve stable weight loss over a long period of time by such approaches. Thus, a better understanding of the role of metabolic inflammation in the control of body weight homeostasis is necessary. Of interest, some evidence suggests that metabolic inflamma-

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<td>IKKe</td>
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<td>PKR</td>
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<td>(55,134)</td>
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ERK, extracellular-regulated kinase-1; IKK, inhibitor of kappa-B kinase; JNK, c-Jun N-terminal kinases; PKR, protein kinase; PTP1B, protein tyrosine phosphatase 1B; UCP-1, uncoupling protein; WAT, white adipose tissue.
tion and thermogenesis could be modulated by nutrients (139,140). In particular long-chain saturated fatty acids are considered to be a major cause of metabolic inflammation whereas some unsaturated fatty acids have been shown to be protective (141–145). Thus, for the future, in addition to a pharmacological approach, it will be important to investigate whether specific pathways linking metabolic inflammation and thermogenesis could be targeted by a nutraceutical strategy aimed at the prevention of obesity in overweight individuals as well as obesity relapse after therapeutic dieting.

**Conflict of interest statement**

The author declares that there is no conflict of interest for the field covered by this review.

**Acknowledgements**

G.S. is supported by grants from the Swiss National Science Foundation 31003A_135684) and the European Foundation for the Study of Diabetes (Diabetes and Cancer). Because of space limitation, references are limited to the one more essential for the focus of this review. I apologize for the many excellent works that are not cited here. I am grateful to Dr. Barbara Becattini of the University of Fribourg for her feedback on this paper.

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