Diet, obesity and diabetes: a current update

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ABSTRACT

The prevalence of obesity has been increasing at a rapid rate over the last few decades. Although the primary defect can be attributed to an imbalance of energy intake over energy expenditure, the regulation of energy balance is now recognized to be complex. Adipose-tissue factors play a central role in the control of energy balance and whole-body fuel homeostasis. The regulation of adipose-tissue function, in particular its secretion of adipokines, is impaired by increases in adipose mass associated with obesity, and with the development of insulin resistance and Type 2 diabetes. This review analyses adipose-regulated energy input and expenditure, together with the impact of dietary macronutrient composition on energy balance in relation to susceptibility to the development of obesity and Type 2 diabetes, and how these metabolic conditions may be exacerbated by the consequences of abnormal adipose function. By gaining a greater understanding of how energy balance is controlled in normal, and in obese and diabetic states, a more practical approach can be employed to prevent and better treat obesity and metabolic disorders.

INTRODUCTION

Obesity is an accumulation of excess body fat such that health might be impaired. A quantitative definition of obesity is a BMI (body mass index) exceeding 30 kg/m². Obesity is one of the most prevalent disorders of developed countries, and its incidence is increasing rapidly. In England, the percentage of individuals classified as obese increased from 13.2% to 23.6% in men and 16.4% to 23.8% in women between 1993 and 2004. In English children aged 2–10 years, the incidence of obesity (using appropriate BMI for age and gender defined by the UK National BMI percentile classification) increased from 9.9% to 13.1% between 1995 and 2003 (reviewed in [1]). Obesity, in particular central (visceral) obesity, is associated with physiological changes which cause or contribute to the development of diseases, including Type 2 diabetes, hypertension and cardiovascular disease, as well as non-alcoholic fatty liver disease, gall bladder disease, osteoarthritis and some cancers [2–4]. Thus the increasing incidence of obesity is particularly detrimental because of its associated diseases which, in addition to the...
concerns posed to the individual, cause a serious drain on health systems as they require long-term expensive treatment. It is estimated that the cost of treating obesity-related diseases contributes 2–7 % of total national health care costs in developed countries.

As the incidence of obesity rises rapidly, so does that of Type 2 diabetes. In 2000, the incidence of diabetes worldwide was 171 million; this figure is predicted to rise to 366 million by 2030 (World Health Organization; http://www.who.int/diabetes/). Although Type 2 diabetes is currently more prevalent in developed countries, affecting 5–7 % of the UK population, its incidence is also increasing in developing countries [5], perhaps linked to a switch towards a more affluent lifestyle. Although in developed countries the incidence of Type 2 diabetes is greatest in people over the age of 65, its incidence is rapidly increasing between the ages of 35–64. Since many of the health and financial costs to society of obesity are via the long-term treatment of diabetic complications [6], the development of diabetes at this earlier age is important as the likelihood of developing long-term complications increases.

Although family, twin and adoption studies suggest that the genetic contribution to BMI ranges from 60–84 % [7], this alone is unlikely to account for the recent rise in the incidence of overweight and obesity and its accompanying disorders. Several changes resulting from modern lifestyle have contributed to the rapid increase. These relate to both increased energy intake and decreased energy expenditure. In addition, other factors related to modern lifestyle (such as altered dietary composition) may affect the physiology of energy balance and contribute further to the increased development of obesity [8,9]. The expansion of the adipose-tissue mass results in deleterious effects on energy balance regulation. To prevent the incidence of obesity accelerating at its current rate, increasing emphasis is placed on understanding the mechanisms by which long-term exposure of major tissues and organs to high concentrations of nutrients, including glucose and lipids (particularly in combination), cause tissue malfunction and obesity-related disease.

**Genotype and phenotype contribute to the predisposition to obesity and Type 2 diabetes**

Throughout history, animals (including man) have evolved to cope with intermittent or inadequate food supply. Energy is stored efficiently as esterified lipid [TAG (triaclyglycerol)] in (white) adipose tissue when food is abundant; adipocyte TAG is mobilized when energy intake is insufficient to meet energy expenditure. Evolutionary pressure would have promoted effective storage of energy when food was plentiful, together with physiological adaptations to promote survival when energy intake was inadequate [10,11]. The evolutionary selection of a specific genotype promoting these responses has been termed the ‘thrifty genotype’ [10,12]. The occurrence and apparent predominance of a thrifty genotype in humans has resulted in a genetic predisposition which results in easy excess energy storage as fat. The system controlling body weight homoeostasis which evolved to cope with food scarcity appears to be less effective at resisting the challenge of excessive abundance of energy-dense food, together with a lesser requirement for energy expenditure because of reduced physical activity.

It has also been proposed that factors that impair nutrient supply in early life induce permanent changes in tissue and organ function that conserve glucose and prioritize development of key fetal organs such as the brain and heart. This has been termed the ‘thrifty phenotype’ hypothesis [13]. Such alterations appear to be advantageous for survival providing that the individual remains on a poor diet throughout life. However, they may become detrimental by altering the balance of nutrient disposition towards storage away from disposal, and therefore predisposing to obesity when dietary intake is increased relative to expenditure. Evidence supporting early life origins of obesity and insulin resistance have been recently reviewed [14,15]. Some of these effects may be achieved by epigenetic mechanisms that exert a stable influence on gene expression, as reviewed elsewhere [16,17]. Epigenetic mechanisms offer one plausible explanation for the recent epidemic of obesity, which has occurred during a relatively short period over which substantial changes in genes pools are unlikely.

**ROLE OF ADIPOSE TISSUE IN FUEL HOMEOEOSTASIS**

Adipose tissue is an energy-dense reservoir: in humans, 15 kg of body fat supplies sufficient calories for survival for approx. 2 months [18]. Adipose tissue also has a remarkable capacity for expansion. As well as the ability to increase cell numbers, individual adipocytes can alter their volumes up to a 1000-fold, becoming engorged with TAG [18]. In the fed state, insulin increases adipocyte TAG storage. This is achieved by augmenting adipocyte glucose uptake (allowing the production of glycerol 3-phosphate for FA (fatty acid) esterification to form TAG as well as FA synthesis) [18] and by direct effects to stimulate enzymes in the pathway for esterification of incoming FA, generated locally via adipocyte LPL (lipoprotein lipase). FA transport into cells is achieved via diffusion across the plasma membrane and also via FA transporter proteins, of which two major classes have been described: CD36 [FAT (FA translo-

[case)]/GPIV (glycoprotein IV)] and FATPs [(FA transporter proteins) FATP1–6]. Adipocyte lipid accretion is enhanced by activation of the lipogenic transcription factor PPAR (peroxisome-proliferator-activated receptor) γ
Enlarged adipocytes release increased amounts of FAs; as they are insulin-resistant, they are unable to respond effectively to insulin with inhibition of lipolysis [27–30] (Figure 1). There may also be a ‘spillover effect’ resulting from saturation of the capacity for TAG storage [31,32]. Increased circulating FAs and ectopically stored fat reduce the insulin sensitivity of non-adipose tissues (Figure 1). In addition, although acute exposure to FAs facilitates GSIS (glucose-stimulated insulin secretion) by the pancreatic β-cells, more long-term overexposure (partially in combination with elevated glucose) (glucolipotoxicity) impairs GSIS [33] (Figure 1). However, insulin resistance in adipose tissue may not be as detrimental as might be predicted. Mice with an adipose-tissue-specific knockout of the insulin receptor (FIRKO mice) resist diet-induced obesity, are whole-body insulin-sensitive and show increased longevity [34].

Adipocytes can rapidly transform from actively storing to actively releasing energy [35–37]. In response to lipolytic stimulation, adipocytes hydrolyse stored TAG. Until recently, HSL (hormone-sensitive lipase) was presumed to be the rate-limiting enzyme involved in adipose-tissue lipolysis; however, recent observations using HSL-null mice led to the characterization of a newly discovered adipose TAG lipase (ATGL), an enzyme expressed predominantly in (white and brown) adipose tissue and specifically localized in the adipose-tissue lipid droplet [38,39] (Figure 1). A lipid-droplet-associated protein CGI-58/ABHD5 is responsible for efficient ATGL activity; antisense RNA-mediated suppression of CGI-58 expression in 3T3 adipocytes inhibits TAG mobilization.

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**Figure 1** Impact of enlarged adipocytes on the regulation on nutrient handling

+ activation. FAO, FA oxidation.
HSL hydrolyses DAG (diacylglycerol) generated by ATGL to mono-acylglycerol and subsequently to FAs and glycerol. FAs and glycerol are released into the circulation. In a recent study [41], the relative contributions of ATGL and HSL to adipose tissue lipolysis was examined by comparing samples from obese and non-obese subjects. The authors concluded that HSL mediates catecholamine-stimulated lipolysis in white adipose tissue, whereas ATGL could be responsible for basal TAG lipolysis [42].

FAs are used for oxidative ATP production in a range of tissues; glycerol is used for hepatic gluconeogenesis, a process involving its conversion into glycerol 3-phosphate by hepatic glycerol kinase (Figure 1). FAs also act as ligands for transcription factors which regulate lipid breakdown, the lipo-oxidative transcription factors PPARα and PPARβ/δ [43] (Figure 1). Circulating NEFA concentrations therefore affect substrate utilization by altering gene expression to favour FA oxidation. For example, hepatic expression of PPARα is increased during fasting, directly enhancing expression of genes involved in FA uptake (FATP), β-oxidation (acyl-CoA oxidase) and ω-oxidation [44]. PPARα also enhances PDK4 (pyruvate dehydrogenase kinase 4) expression which, through suppressing pyruvate oxidation, facilitates gluconeogenesis [45]. A study using the pharmacological PPARα agonist WY14643 and PPARα-null mice demonstrated that PPARα activation up-regulates expression of hepatic genes involved in gluconeogenesis from glycerol, including cytosolic and mitochondrial glycerol 3-phosphate dehydrogenase, glycerol kinase and AQP (aquaglyceroporin) 3 and 9 [46] (Figure 1).

The discovery of the glycerol transporter AQP7 highlighted the previously underappreciated role of glycerol transport in the development of obesity [47,48]. AQP7 is highly expressed in adipose tissue and is prerequisite for efficient glycerol release from adipose tissue during fasting. Adipocyte glycerol content is elevated 3-fold, and measurements in adipocytes of comparable size showed a 65% reduction in glycerol permeability in AQP7-deficient adipocytes [48]. Rates of TAG lipolysis and FA synthesis are not impaired, indicating that the accumulation of intracellular glycerol does not feedback to inhibit TAG lipolysis [48]. AQP7 mRNA expression in adipose tissue is enhanced by insulin deficiency and suppressed by elevating insulin on refeeding after starvation [49] AQP7 mRNA expression in mesenteric adipose tissue is increased in insulin-resistant db/db mice [49], which exhibit impaired leptin receptor function. Thus removal of the restraint on lipolysis imposed by insulin under conditions of insulin deficiency or resistance is coupled with increased adipocyte AQP7 expression. A negative insulin-response element has been identified in the mouse [49] and human [50] AQP7 gene promoter.

In the fed state, AQP7-knockout mice become progressively obese with aging, with TAG-engorged adipocytes [47,48]. The development of obesity caused by AQP7 deficiency was accompanied in one AQP7−/− mouse line by severe insulin resistance and glucose intolerance [47]. Nevertheless, these mice have increased susceptibility to the rapid development of obesity in response to a high-fat/high-sucrose diet [47]. Thus glycerol retention within adipose tissue promotes expansion of the adipose tissue mass in the fed state and adequate AQP7 expression/function is important to restrict excessive TAG accumulation in adipocytes under anabolic conditions. The mechanism by which glycerol accumulation promotes adipocyte hypertrophy appears to involve enhancement of adipocyte glycerol kinase activity [47]. Both glycerol kinase activity and oleic acid uptake are increased by AQP7-knockdown in 3T3-L1 adipocytes [47].

AQP7 is a direct PPARγ target gene [19,49]. Differentiated mouse and human adipocytes respond to TZDs in culture with an increase in adipocyte AQP7 mRNA expression [46,50]. AQP7 mRNA expression in differentiated adipocytes is also increased by the exposure to the PPARβ/δ agonist L165041 in culture [46], but the overall physiological significance of PPARβ/δ signalling in adipocytes has not been elucidated.

ADIPOSE-REGULATED ENERGY BALANCE

Adipokines, produced and released from adipose tissue, regulate a range of metabolic, endocrine and immune functions that affect energy intake and expenditure and nutrient utilization [18,35,51]. Excess visceral fat is specifically associated with Type 2 diabetes, and insulin resistance is proportional to visceral fat mass [52,53], suggesting that adipokines secreted disproportionately from visceral fat are, in part, responsible for the loss of adipose-regulated energy balance.

An adequate amount of adipose tissue is also essential for metabolic homeostasis [36,54]: although obesity is intimately linked to insulin resistance and Type 2 diabetes, these conditions also develop in people with lipodystrophies [54] and occur in rodent models of lipodystrophy [24,54,55]. These findings have confirmed adipose tissue and its secreted adipokines in the axis between the detection of nutrient status and the maintenance of energy balance [18,35,56].

Leptin has been largely responsible for highlighting the role of adipose tissue in energy balance. Both ob/ob (leptin-deficient) and db/db (leptin-receptor-deficient) mice exhibit early hyperinsulinaemia and hyperphagia followed by obesity, hyperglycaemia and diabetes [57]. Leptin regulates appetite and energy expenditure, as well as altering substrate utilization [35,58]. In mice, insulin resistance associated with lipo-atrophy is reversed if adipose tissue is transplanted from wild-type mice [59], but not if it is obtained from ob/ob mice [60]. Exogenous leptin administered to severely lipo-atrophic humans and
mice also improves insulin sensitivity [55]. With the exception of the ob/ob mouse, systemic leptin concentrations are generally proportional to adipose tissue mass [61–66]. Leptin also responds to nutritional state, decreasing with fasting and restored by re-feeding. Glucose, insulin and increased adipocyte glucose metabolism stimulate leptin secretion [67–72]. Conversely, some FAs [73,74] and an increased rate of lipolysis [75–78] inhibit leptin secretion. All of these factors are altered in obesity and diabetes, therefore affecting the regulation of leptin levels.

Visfatin (discovered as a cytokine-like growth factor in lymphocytes, termed pre-B-cell colony-enhancing factor), as its name implies, was thought to be preferentially expressed and secreted by visceral fat, but is now known also to be secreted from subcutaneous adipose tissue [79,80]. Rather than inducing insulin resistance, as might be inferred from its expression in visceral fat, visfatin possesses insulin-mimetic activity [81]. The binding affinity of visfatin for the insulin receptor resembles that of insulin, and visfatin causes phosphorylation of various insulin signalling proteins, including IRS (insulin-receptor substrate) 1 and 2 (Figure 2). Visfatin does not compete with insulin for binding to the insulin receptor, so it is likely that these proteins interact with different receptor domains. Circulating visfatin levels are lower than those of insulin and are unaffected by feeding or fasting. Nevertheless, plasma visfatin concentrations correlate positively with body fat and thus increase during the development of obesity. Individuals with Type 2 diabetes have elevated plasma visfatin concentrations [82], as do pregnant women with gestational diabetes [83]. Mouse models of obese Type 2 diabetes and diet-induced obesity have higher visfatin levels and increased visfatin expression in visceral fat [80,81]. Further details of the actions of visfatin are reviewed elsewhere [84].

Decreased concentrations of adiponectin, another prominent adipokine, are associated with lowered insulin sensitivity [85,86]. Lipo-atrophic insulin-resistant mice are deficient in adiponectin [86]. Adiponectin-knockout mice develop severe diet-induced insulin resistance [87]. Replenishment of leptin in combination with adiponectin elicits a complete reversal of insulin resistance in lipoatrophic mice, compared with partial reversal observed with adiponectin or leptin individually [86], indicating that these two adipokines produce a protective effect on insulin sensitivity (Figure 2). Conversely to leptin, levels of adiponectin are reduced in obesity [88,89], an effect attributed to increased release of TNFα (tumour necrosis factor α) [90]. In vitro, adiponectin expression is suppressed by TNFα, which inhibits the adiponectin promoter [91].

In obese patients, increased levels of inflammation-related adipokines, including TNFα, led to the suggestion that obesity is a state of chronic low-grade inflammation [92,93]. Trayhurn and colleague [92,93] also developed the hypothesis that relative hypoxia associated with expansion of the adipose-tissue mass underlies the growing inflammatory response within the adipocyte. It is proposed that, as the adipose mass expands due to adipocyte hypertrophy with hyperplasia, adipocytes distinct from the vasculature become relatively hypoxic. Inflammation in white adipose tissue is also augmented through macrophage infiltration as the adipose mass expands. In
non-diabetic human subjects, fat accumulation correlates closely with markers of systemic oxidative stress. Similar responses are observed in adipose tissue of mouse models of obesity, including obese KKAy mice, which show enhanced mRNA expression of components of the NADPH oxidase complex and down-regulation of expression and activities of antioxidant enzymes. Exposure of fully differentiated adipocytes to ROS (reactive oxygen species) decreases PPARγ and adiponectin mRNA expression, while increasing mRNA expression of inflammatory cytokines [PAI1 (plasminogen-activator inhibitor 1) and IL6 (interleukin 6)]. These effects are reversed by antioxidant treatment. Chronic treatment of KKAy mice with the NADPH oxidase inhibitor apocynin reduces markers of oxidative stress in white adipose tissue, increases adiponectin and decreases TNFα [94].

A key consequence of expansion of the adipose-tissue mass may be its inability to detect metabolic status and release cytokines inappropriately. An impaired ability of the adipocyte to sense nutritional state in obesity affects the acute regulation of adipokine secretion, which in turn regulates energy balance. For example, fasting ordinarily produces a dramatic decline in leptin levels: this response is blunted in obese rodents [61,69,95]. When nutritional stimulation is mimicked by euglycaemic/hyperinsulinaemic clamp conditions, a loss in whole-body glucose tolerance is associated with a decreased leptin response [96–98]. Adiponectin responses to a mixed meal are unchanged in lean subjects, but increased from a lower baseline in obese subjects to a level higher than in the lean subjects [99]. When nutritional stimulation is achieved under hyperinsulinaemic/euglycaemic clamp conditions, serum adiponectin is again unaltered in lean subjects, but decreased in obese subjects [100]. It is yet to be determined why adiponectin secretion is more responsive in the obese state, and why the responses vary with different methods of nutritional stimulation.

Central regulation of energy intake

Specific feeding centres in the brain control food intake by stimulating appetite or feelings of satiety. These feeding centres are regulated by hormonal and nutritional signals from the body that, in turn, are altered by nutritional state [56]. The accelerated growth of obesity in modern societies indicates that the homeoeostatic mechanisms that maintain weight stability may be malfunctioning, such that appetite controlling mechanisms are compromised.

The hypothalamus, located in the middle of the base of the brain, houses a dense population of receptors for hormonal and metabolite control of satiety and appetite [56,101]. The signalling pathways work rapidly using agonist and antagonist systems in which there is redundancy [10]. Several neuropeptides within the ARC (arcuate nucleus) and PVN (paraventricular nucleus) of the hypothalamus stimulate (orexigenic) or inhibit (anorexigenic) feeding [56,102] (Figure 3). Orexigenic peptides include NPY (neuropeptide Y), AgRP (agouti-related peptide) and MCH (melanin-concentrating hormone) [103,104] (Figure 3). The NPY/AgRP neurons respond to afferent signals reflecting energy deficit, such
as hypoglycaemia. Levels of orexigenic peptides increase during fasting, where systemic glucose levels can fall by 1–2 mmol/l [104–107], and are chronically elevated in obese hyperglycaemic rodents (e.g. ob/ob mice) [105]. In rodents, central administration of both NPY and MCH stimulates hyperphagia, and overexpression of MCH results in obesity [105,108–110], whereas food intake decreases the expression of NPY, MCH and AgRP [105,106,108,109]. Genetic variation in the human AgRP gene (Ala67Thr) is associated with inherited leanness [111]. NPY-null mice have normal body weight and adiposity, suggesting that signalling via AgRP (or other orexigenic pathways) can compensate.

The melanocortin system, which promotes negative energy balance, is inhibited by high levels of AgRP and MCH and activated by anorexigenic peptides, the most potent of which is α-MSH (α-melanocyte-stimulating hormone), derived from POMC (pro-opiomelanocortin) by post-translational processing and released from POMC neurons [103,104]. Melanocortin peptides, such as α-MSH and corticotropin (adrenocorticotropic), bind to melanocortin receptors (MC3R and MC4R) expressed within the VMN (ventromedial nucleus) of the hypothalamus. Many POMC neurons also produce a neuropeptide precursor protein, CART (cocaine- and amphetamine-regulated transcript), which is processed to yield anorectic peptides. The action of CART appears to be mediated by neurons within brain areas that process stress and reward signals, namely the third and forth ventricles. The synthesis of α-MSH and other anorexigenic peptides, including CART and CRH (corticotropin-releasing hormone), is decreased during fasting [112,113]. Mutations causing a deficiency in POMC or the melanocortin receptor MC4R are the most common cause of genetic obesity in humans [114]. MC4R agonists are in development as an obesity therapy [1].

**REGULATION OF ENERGY INTAKE BY NUTRIENTS**

The hypothalamus responds to varying circulating concentrations of nutrients, in particular glucose and FAs. The hypothalamus also responds to insulin, leptin and the predominantly stomach-derived orexigenic peptide ghrelin (Figure 3). All of these signals interact with many of the orexigenic and anorexigenic signalling systems that translate a chemical response to food intake into a neural response, which then regulates subsequent food intake [103,104]. Glucostatic and lipostatic mechanisms have been formulated: the former proposed that circulating glucose is the main signal to the hypothalamus of the body’s nutritional status, whereas the latter proposed that lipids are of primary importance. When glucose is administered via ICV (intracerebroventricular) injection, blood glucose levels decrease, indicating a direct central action [115]: glucose also modulates both insulin secretion from the pancreatic β-cell and leptin secretion from the adipocyte [67], and so direct effects are reinforced. The glycaemic index, a measure of the ability of nutrients to elevate glycaemia, is inversely proportional to the effect on subsequent food intake in the short term [116]. By virtue of its rapid conversion into fat (see below), fructose has a low glycaemic index (5-fold lower than that of glucose) and thus induces satiety more slowly than consumption of isocaloric amounts of glucose, resulting in increased food intake.

The lipostatic hypothesis, which originally proposed that circulating lipids were of primary importance for hypothalamic nutrient sensing, has evolved into the adipostat hypothesis. Here lipid storage, rather than the systemic lipid concentration, is proposed as the major regulator (reviewed in [117]). Nevertheless, central administration of oleic acid inhibits food intake [118] and, in the short term, FAs potentiate GSIS [119].

Substrate competition is integral to energy homeostasis. The Randle (glucose/FA) cycle [120] is conventionally viewed as the dominance of FAs over glucose as the preferred energy substrate, with suppression of glucose oxidation via inhibition of the PDC (pyruvate dehydrogenase complex), inhibition of glycolysis via inhibition of PFK1 (phosphofructo-1-kinase) and feedback inhibition of glucose uptake/phosphorylation by glucose 6-phosphate. When glucose is abundant and insulin concentrations are high, a ‘reverse glucose/FA cycle’ operates whereby glucose utilization blocks FA oxidation by its effect to increase malonyl-CoA. This reverse cycle, developed by McGarry and colleagues [121], has been comprehensively reviewed [122]. By analogy to the operation of the glucose/FA cycle and the ‘reverse glucose/FA cycle’ in muscle, substrate-led interactions also may influence hypothalamic nutrient sensing to modify feeding behaviour. Both inhibition of hypothalamic lipid oxidation and central inhibition of the lipogenic enzyme FAS (FA synthase), the enzyme complex that utilizes malonyl-CoA to form FAs, suppress feeding (reviewed in [117]). Recently, it has been demonstrated that molecular disruption of the hypothalamic nutrient-sensing mechanism induces obesity [123]. Administration of adeno-associated virus expressing MCD (malonyl-CoA decarboxylase), which depletes malonyl-CoA and decreased long-chain FA-CoA in the mediobasal hypothalamus, blunted the hypothalamic response to increased lipid availability, caused hyperphagia and induced a progressive increase in fat mass.

Within the context of the aetiology of Type 2 diabetes, hypothalamic sensing of both glucose (via hypothalamic pyruvate metabolism) [115] and FAs is required to optimize glucose homeostasis [124], with major actions on hepatic glucose fluxes. Interventions such as exercise, which modify systemic lactate+pyruvate and FA levels, may therefore not only influence energy expenditure (see...
below) but also energy input. Understanding the biochemical steps involved in hypothalamic nutrient sensing is fundamental to evaluating whether abnormalities in this mechanism contribute to obesity. Chronic activation of ACC (acetyl-CoA carboxylase) within the hypothalamus in obesity, perhaps because of chronic exposure to high levels of circulating glucose, via long-term suppression of FA oxidation secondary to increased malonyl-CoA levels, could cause hypothalamic insulin resistance due to the accumulation of lipid intermediates in a manner reminiscent of that seen in skeletal muscle.

Central actions of insulin and leptin

Taking nutrient control to a secondary level, the interplay between POMC/CART and NPY/AgRP neurons in controlling energy homeostasis, and thus obesity levels, is emphasized by their responses to insulin and leptin, whose secretion from the pancreatic β-cell and the adipocyte respectively, occurs in response to nutrient abundance. Central insulin administration decreases food intake [125]; conversely, a reduction in hypothalamic insulin receptor expression increases food intake [126]. A small-molecular-mass insulin–receptor agonist suppressed food intake when given either centrally or peripherally [127]. Insulin resistance in obesity can be accompanied by islet compensation, resulting in insulin hypersecretion [128]. Long-term exposure of the hypothalamus to high insulin levels could down-regulate or desensitize the hypothalamic insulin receptor, resulting in increased food intake. Since visfatin binds to insulin receptors, it is possible that visfatin could influence appetite control and body weight regulation. Mice homozygous null for the visfatin gene die during embryogenesis, but heterozygotes with 30% lower circulating visfatin concentrations have high plasma glucose levels [81], suggesting a physiological role in energy homeostasis.

Leptin, administered as a continuous subcutaneous infusion, as repeated intraperitoneal injections or as a single intravenous injection, decreases food intake and body weight in lean mice in a dose-dependent manner [129–131]. As the highest density of leptin receptors exists in the hypothalamus, this area of the brain is believed to co-ordinate the major functions of leptin [132]. A single leptin dose administered directly into the lateral ventricle of mice by ICV cannulation decreases food intake [129]. Increases in hypothalamic leptin, through central administration or feeding, decrease the expression of NPY, MCH and AgRP [103–106,109], whose levels increase during fasting [104–107], but leptin administration during fasting prevents activation of orexigenic neurons and increases expression of orexigenic peptides [107,113]. Leptin administered during fasting also normalizes the decreased expression of anorexigenic peptides, including CART and CRH [113,133,134]. A loss of leptin receptors in the hypothalamus, as seen in db/db mice or through the use of aurothioglucose [135], causes hyperphagia and increased weight gain [57,136]. This control may be mediated by NPY, as central administration of both insulin and leptin activate NPY neurons [107]. The neuronal signalling events triggered by fasting can be opposed by the administration of leptin, insulin or glucose which prevent activation of orexigenic neurons and increased expression of such orexigenic peptides [107,113].

Interactions between insulin, leptin and ghrelin

Plasma ghrelin levels increase during fasting, decrease during food ingestion and have been proposed to contribute to the aetiology of human obesity. Ghrelin secretion and circulating ghrelin levels are reciprocal to those of insulin in relation to food intake, glucose loading and glucose infusion [137–140]. Hyperphagia associated with diabetes is rapidly reversed by the administration of a ghrelin-receptor antagonist [141]. The postprandial decrease in systemic ghrelin levels is slower in obese than in lean subjects, and this may contribute to differences in food intake [142]. Grossly elevated ghrelin levels are found in Prader–Willi syndrome, which is associated with hyperphagia and obesity [143]; reviewed in [144]). Human subjects that receive ghrelin have a 28% increase in food intake [145]. These findings suggest that failure to suppress ghrelin levels postprandially may lead to hyperphagia and obesity. Ghrelin and leptin are suggested to regulate energy homeostasis as mutual antagonists on hypothalamic neurons that regulate feeding behaviour, but ablation of ghrelin in ob/ob mice fails to rescue the obese hyperphagic phenotype, indicating that the ob/ob phenotype is not a consequence of ghrelin action opposed by leptin [146].

Regulation of hypothalamic AMPK (AMP-activated protein kinase) activity

AMPK is a prominent cellular ‘fuel gauge’ (reviewed in [147]). AMPK is activated by depletion of ATP: allosteric activation when it binds AMP and/or through AMP-facilitated phosphorylation by activation of the protein kinases, including LKB1 and CaMKK2β (Ca²⁺/calmodulin-dependent protein kinase 2β), or by inhibition of PP2C (protein phosphatase 2C) [148]. AMPK exists as a heterotrimeric complex of a catalytic α-subunit (encoded by two genes, α1 and α2), a regulatory β-subunit (also encoded by two genes, β1 and β2) and a γ-subunit (encoded by three genes, γ1, γ2 and γ3).

Increased glucose, insulin and leptin, which curtail feeding behaviour, all decrease hypothalamic AMPK activity, whereas fasting increases hypothalamic AMPK activity [149]. Hypothalamic activation of the α2 isoform of AMPK decreases in response to glucose, insulin and leptin, and bidirectional changes in hypothalamic α2-AMPK activity are sufficient to modulate feeding behaviour [149]. AMPK may therefore co-ordinate nutritional
signals from the periphery to neuronal signalling to feeding behaviour (reviewed in [150]). An involvement of α2-AMPK is particularly compelling for the anorexigenic action of leptin (see [150]). Systemic or central leptin administration decreases α2-AMPK activity selectively in the ARC and PVN and, when this decrease is abrogated by hypothalamic delivery of an adenovirus expressing a constitutively active form of AMPK, food intake is no longer decreased by leptin. Overexpression of hypothalamic AMPK increases expression of NPY, AgRP and MCH [149]. It is not yet known how leptin decreases AMPK activity in the ARC, but signalling events may include stimulation of STAT3 (signal transducer and activator of transcription 3), PI3K (phosphoinositide 3-kinase) and MAPK (mitogen-activated protein kinase) [151,152], and/or inhibition of the protein/lipid phosphatase PTEN (phosphatase with tensin homology; a potent inhibitor of PI3K signalling) [153]. Nevertheless, decreased ARC activity of AMPK could lead to increased active (dephosphorylated) ACC, increased malonyl-CoA concentrations, decreased FA oxidation and accumulation of long-chain acyl-CoA. Chronic increases in glucose, insulin and leptin, as seen in obesity and diabetes, are not associated with the decreases in orexigenic and increases in anorexigenic peptides seen in response to acute increases in these agents [96-98,103,154,155], suggesting that signalling to AMPK may be affected.

FoxO1 (forkhead box-containing protein of the O subfamily 1) regulates metabolism in a PI3K-dependent manner [156]. Interestingly, delivery of adenovirus encoding a constitutively mutant FoxO1 to the hypothalamic ARC results in a loss of ability of leptin to curtail food intake, and FoxO-1 and STAT3 exert opposing actions on expression of Agrp and Pomc [157]. As FoxO1 is considered to mediate stress resistance, it was suggested that mechanisms to preserve body weight might be part of an ancestral stress resistance pathway, which might explain why mammals are better predisposed to preserve body weight than to lose it [157].

ADIPOSE-REGULATED ENERGY EXPENDITURE

Energy is expended in supporting the body’s basic cellular functions (resting metabolic rate), in digesting and assimilating food, by physical activity and during diet-induced thermogenesis [158]. Alterations in food intake are mostly compensated by subsequent adjustments in energy expenditure and changes in appetite [12]. The modern lifestyle is often characterized by a decrease in voluntary movement (exercise and incidental activity) [9]. Approx. 25% of American children are classified as completely sedentary [159]. This clearly contributes to increased incidence of obesity and is largely influenced by environmental factors directly resulting from the modern lifestyle. However, a genetic component also influences an individual’s activity level; genetic factors account for 78% of variance in physical activity [160]. In addition to exercise altering the energy intake to energy expenditure ratio, exercise increases insulin sensitivity which has additional beneficial effects on the regulation of body weight homeostasis and on the prevention of Type 2 diabetes. However, these improvements in insulin action occur independently of changes in adiponectin levels or of other adipokines, including leptin and TNFα, and consequently their effects on energy balance by central and peripheral mechanisms. Involuntary energy expenditure encompasses all other forms of energy expenditure. This can be influenced by environmental factors (e.g. temperature and dietary modification) and genetic factors, which can alter the response or the capacity to respond to environmental change. Sympathetic neural control, as well as signalling molecules, can alter the rate of thermogenesis in response to altered environment. Genetic differences in energy expenditure have been demonstrated in animal models. The obese ob/ob mouse, when restricted to the same energy intake as a wild-type mouse, will exhibit increased weight gain [57]. Factors governing this difference in energy expenditure are an attractive target for weight-loss drugs. Although many monogenic forms of obesity have been identified in humans, including defects in the genes for leptin, the leptin receptor, POMC and the melanocortin system [114], all involve genes controlling appetite and satiety (energy intake). Few gene defects have as yet been discovered which affect energy expenditure [114]. MCH-null mice are lean, hyperactive, hypermetabolic, resistant to diet-induced and aging-associated obesity and have a reduced visceral fat mass [161].

Leptin-deficient ob/ob mice not only have increased energy intake, but also decreased body temperature and energy expenditure [57]. This suggests that neuronal control of energy intake and energy expenditure is closely linked. Pair-feeding experiments indicate that the effects of leptin on weight reduction cannot be explained by decreased food intake alone [162,163]. Leptin treatment of ob/ob mice corrects the abnormally low body temperature of these mice, but does not affect body temperature of lean mice [130,164]. Similarly, leptin treatment increases energy expenditure in ob/ob, but not lean, mice. In each case, the increase in energy expenditure observed in ob/ob mice returns their rate to that of control mice [164,165]. Conversely, central administration of leptin to lean mice prevents the decrease in energy expenditure normally associated with fasting [166]. Whereas normal fasting reduces lean body mass as well as adipose-tissue mass, leptin treatment specifically targets adipose tissue [130,163,166,167]. Experimental hyperleptinaemia increases glycerol, but not FA, levels. This suggests that FAs are metabolized at the same rate as they are generated by lipolysis, whereas fasting- or
starvation-induced lipolysis are associated with an increase in circulating glycerol levels and increasing circulating FA levels [58]. Leptin levels therefore manipulate whole-body substrate utilization.

A recent study [168] demonstrated that a single alteration in adipose-tissue physiology can alter the character of the leptin/body weight regulation system. Overexpression of UCP1 (uncoupling protein 1), which increases thermogenesis, in epididymal adipose tissue of obese mice produces a rapid (3 day) reduction in adipose-tissue leptin expression and circulating leptin concentrations. This was associated with a significant decrease in NPY expression and a trend for increased POMC expression and decreased food intake. A leptin-tolerance test showed that UCP1-overexpressing mice exhibited increased sensitivity to leptin. Thus the characteristics of the leptin signalling system in obese mice can be reset by changing a single aspect of adipose physiology.

The role adiponectin plays in the regulation of energy balance is less clear than that of leptin. Despite the opposing regulation of leptin and adiponectin, adiponectin injections decrease fat pad weight, however, unlike leptin, without a change in energy intake [86]. Direct ICV administration of adiponectin reduces body weight through an increase in energy expenditure, suggesting that the latter is partially centrally controlled [169]. Adiponectin administration lowers plasma glucose levels by suppressing hepatic glucose production and increasing glucose utilization [85,86,170], and also enhances both FA oxidation and glucose utilization by skeletal muscle via AMPK activation [171]. Increased substrate utilization is associated with increased expression of genes involved in energy dissipation and an increased body temperature, leading to the increased energy expenditure [86].

Not all adipokines may promote positive effects on insulin sensitivity and whole-body metabolism. These include TNFα and IL6 produced from the enlarged and inflamed adipose tissue mass (reviewed in [32,172]). It remains to be determined whether visfatin has a role in energy expenditure.

**INFLUENCE OF DIETARY MACRONUTRIENT COMPOSITION ON SUSCEPTIBILITY TO OBESITY AND DIABETES**

Dietary recommendations are that between 45–65% of energy input should be derived from carbohydrates, 20–30% from fat and 10–35% from protein, with a maximum of 25% of total energy from added sugars (Institute of Medicine of the National Academies; http://www.iom.edu/report.asp?id = 4340). As the cause of obesity is energy intake in excess of expenditure, the role of diet in the increased incidence of obesity is to an extent clear. However, the role that changes in diet have played in the increase in incidence of obesity, insulin resistance and Type 2 diabetes is more complex. In susceptible individuals, alterations to the constituents of the diet (increased simple sugars, saturated fats and energy density) may promote the development of insulin resistance, diabetes and obesity independently of an increased energy intake [173–175]. A clearer understanding of this aspect of regulation may lead to the more logical design of diets to help manage and prevent obesity and diabetes.

**Dietary glucose and fructose and consequences of their excess**

The predominant dietary sugars are glucose, fructose, sucrose, lactose and maltose. The monosaccharides glucose and fructose are absorbed directly and enter cells via specific transporters (GLUT1, GLUT2 and GLUT4 for glucose, and GLUT5 for fructose). GLUT1 is ubiquitous, GLUT 2 is found in liver and the pancreatic β-cell, and GLUT4 is abundant in skeletal muscle and adipose tissue. Although GLUT5 is expressed at low levels in several tissues, it is expressed at high levels in liver, the most important site of fructose breakdown.

Metabolic fates of glucose and fructose, in addition to degradation for ATP production, include storage as glycogen or conversion into FAs. Under conditions of a sustained surplus of glucose, acetyl-CoA production from glucose via the PDC may exceed the requirements for ATP production via the tricarboxylic acid cycle, with resultant accumulation of citrate. Citrate, on exit from the mitochondria, will allosterically activate the regulatory lipogenic enzyme ACC. Cleavage of citrate by ATP-citrate lyase provides carbon to fuel rates of malonyl-CoA formation. If malonyl-CoA synthesis exceed malonyl-CoA disposal via FAS and MCD, the resultant elevation of malonyl-CoA concentration blocks oxidation of dietary FA at the level of CPT1 (carnitine palmitoyltransferase 1), the expression of which in muscle is regulated by PPARα. Suppression of CPT1 promotes FA partitioning from oxidation towards esterification. Muscle malonyl-CoA concentrations are elevated in models of rodent obesity [176,177], suggesting that obesity favours the operation of the ‘reverse glucose/FA cycle’. In obesity or situations of nutrient excess, elevated malonyl-CoA levels, possibly in conjunction with impaired FA oxidation, can cause intracellular accumulation of lipid-derived compounds that interfere with the mechanisms that optimize energy homoeostasis. Prolonged exposure to excess glucose also up-regulates the lipogenic transcription factor SREBP1c (sterol-regulatory-element-binding-protein 1c) and key genes encoding lipogenic enzymes in muscle [178,179], permitting an increased capacity for FA synthesis. Knockout mice lacking the ACC isoform found in muscle (ACC2) exhibit decreased muscle malonyl-CoA levels and increased FA oxidation [180].

An inability to increase reliance on fat oxidation during fasting, despite elevated plasma FA, may be a hallmark of
insulin resistance associated with obesity [181]. Similarly, the increase in FA oxidation that normally occurs in response to exercise or \( \beta \)-adrenergic stimulation is diminished in obese and/or diabetic subjects (reviewed in [182]). The inability of muscle to switch between metabolic fuels (glucose compared with lipid) as oxidative substrate has been termed ‘metabolic inflexibility’ [181].

The action of hyperglycaemia to suppress fat oxidation in myotubes derived from human quadriceps is variable, as is the ability of myotubes to respond to increased FA concentrations with increased FA oxidation, with a positive relationship between metabolic flexibility and insulin sensitivity [183]. Expression of SCD (stearoyl-CoA desaturase) 1, which catalyses the synthesis of monounsaturated FAs, is enhanced in skeletal muscle from obese humans [184]. High expression and activity of SCD1 correlates with low rates of FA oxidation and increased TAG synthesis, together with increased relative abundance of monounsaturated compared with saturated FAs. Importantly, elevated SCD1 expression and abnormal lipid partitioning are retained in primary skeletal myocytes derived from obese compared with lean donors, implying that these traits might be driven by epigenetic and/or heritable mechanisms. Overexpression of human SCD1 in myotubes from lean subjects reproduces the obese phenotype.

Although glucose and fructose share the same empirical formula, their metabolism is quite different. Fructose is unable to stimulate insulin secretion in the absence of stimulatory glucose concentration [185], and has a low glycaemic index. A potential association between increased fructose consumption and the prevalence of obesity, insulin resistance and Type 2 diabetes has been highlighted (reviewed in [186]). Dietary fructose is converted into fructose-1-phosphate by fructokinase, abundantly expressed in liver, the organ primarily responsible for extraction of absorbed fructose (reviewed in [186,187]). Fructokinase activity in the liver is usually higher than glucokinase and hexokinase activities combined and, whereas glucose metabolism is negatively regulated by PFK1, fructose by-passes this critical regulatory step, thereby serving as an unregulated hepatic acetyl-CoA generator (reviewed in [186,187]). Fructose-derived acetyl-CoA serves as a precursor for FA synthesis. Fructose can also provide carbon for glycerol 3-phosphate synthesis. Glycerol 3-phosphate and FAs are used to form hepatic TAG, thereby facilitating high rates of hepatic VLDL (very-low-density lipoprotein) production. An increase in lipoprotein assembly, enhanced expression of hepatic microsomal TAG protein and reduced Apo (apolipoprotein) B degradation are observed on ingestion of a high-fructose diet [188]. Fructose also lowers the ApoE to ApoC ratio, thereby reducing VLDL-TAG removal by peripheral tissues [189]. As a consequence, fructose often induces hypertriglyceridaemia in the postprandial state. Chronic hyperinsulinaemia and increased plasma FAs, commonly seen with insulin resistance and central obesity, exacerbate this action.

Diet high in fructose induce systemic insulin resistance, with reduced tyrosine phosphorylation of the insulin receptor and IRS1 ([190]; reviewed in [174]). Normal weight and/or insulin-sensitive subjects may be relatively resistant to the insulin-desensitizing effect of sustained fructose, whereas fructose may be more likely to exacerbate insulin resistance in subjects where insulin action is already impaired, as in obesity [186]. However, the addition of relatively modest amounts of fructose to the diet may have beneficial effects on glucose handling by subjects with Type 2 diabetes during periods of neutral energy balance (see e.g. [191,192]), possibly because of improved hepatic glucose uptake through hepatic glucokinase activation [193].

Fructose consumption attenuates postprandial suppression of ghrelin [194] and, in this way, high dietary fructose may contribute to the accelerated rates of obesity in modern societies [186]. Different mouse strains have differing susceptibilities to the metabolic derangements induced by high-fructose diets (reviewed in [187]), indicating a genetic component. Postprandial hyperinsulinaemia, hypertriglyceridaemia and visceral fat accumulation following the ingestion of a high-fructose diet can be associated with increased hepatic expression of SREBP1, and susceptibility to metabolic perturbations following a high-fructose diet may be determined by polymorphisms in the SREBP1 gene [187]. In mice lacking SCD, a 60% fructose diet failed to induce SREBP1 and lipogenic gene expression ([195]; reviewed in [187]). Conversely, mice deficient in the FA transport protein CD36 are more responsive to fructose-induced impairments in insulin action than wild-type mice [196].

### Dietary lipid and consequences of its excess

Dietary lipid is presented mainly in the form of TAG, with some NEFAs, cholesterol and sterols. Lipids enter the blood stream as chylomicrons. LPLs, located on the luminal surface of capillary endothelial cells, hydrolyse the TAG component to FA and monoacylglycerol. NEFAs then enter cells or bind to albumin for distribution in the circulation [53]. Mice lacking CD36 exhibit decreased FA uptake in muscle (cardiac and skeletal) and adipose tissue [197]; decreasing FA uptake into skeletal muscle improves insulin sensitivity [198]. Similarly, FATP1-knockout mice are resistant to lipid-induced insulin resistance in muscle [199]. In adipocytes, translocation of FATP1 from the intracellular perinuclear compartment to the plasma membrane is induced by insulin, and this coincides with increased adipose-tissue FA uptake [200]. Thus the uptake of FAs in adipose tissue is regulated by insulin via FATP1 and, as a consequence,
FATP1-null mice are protected against obesity induced by the provision of a high-saturated-fat diet [200]. Within cells, FAs are sequestered by tissue-specific binding proteins [FABPs (FA-binding proteins), also known as LBPs (lipid-binding proteins)]. Adipocytes contain ALBP (adipocyte LBP) and KLBP (keratinocyte LBP). ALBP complexes with HSL and may sequester FAs as they are released by lipolysis [201]. Compared with wild-type controls, ALBP-null mice exhibit decreased FA release from adipose tissue, increased epididymal fat mass and increased glucose oxidation relative to fat oxidation in the fasting state [202]. Mice deficient in the perilipin (which coats the lipid droplet and which, when phosphorylated, facilitates translocation of HSL to the surface of the lipid droplet) have a reduced adipose-tissue mass and resistance to diet-induced and genetic obesity [203].

Some controversy exists over the quantity and type of dietary fats involved in the development of insulin resistance, obesity and diabetes [204]. The specific type of dietary fat, rather than the amount, appears to be more significant [205]. Thus despite a reduction in dietary fat as a percentage of energy intake in developed countries such as U.S., there has nevertheless been a dramatic rise in the prevalence of obesity and Type 2 diabetes. The consensus view is that diets high in saturated fats favour the development of insulin resistance and, in susceptible individuals, obesity. In rats, diets high in saturated fats or rich in linoleic acid (an $n-6$ FA) lead to insulin resistance [206] and inhibit liver and muscle PI3K activity [207]. In adipose tissue, this is associated with decreased GLUT4 expression and PI3K activity [208]. Conversely, consumption of diets rich in polyunsaturated and monounsaturated FAs improve insulin sensitivity, as well as having a beneficial effect on obesity [209]. The role of FAs, particularly polyunsaturated FAs of the $n-6$ and $n-3$ series, as regulators of adipogenesis and in relation to the development of obesity has been reviewed recently [210].

Only moderate modification of the type of FAs included in the diet is necessary to prevent adverse effects of dietary fat on insulin action. The substitution of $n-3$ long-chain FAs [mainly EPA (eicosapentaenoic acid) and DPA (docosahexaenoic acid)] for only a small percentage (6–7%) of saturated FAs prevents the development of whole-body insulin resistance elicited in response to high-saturated-fat feeding [206,211]. Furthermore, when fish oil, rich in long-chain $n-3$ FA is substituted for one-third of the lipid fraction of the diet, the decrease in muscle PI3K activity is completely prevented, as is decreased adipose-tissue SLC2A4 (GLUT4) gene expression [207]. However, suppression of hepatic PI3K activity remains unaffected [207]. Long-chain $n-3$ FA enrichment of a high-saturated-fat diet leads to hepatic insulin resistance with respect to suppression of glucose output despite improved peripheral insulin sensitivity [211]. Thus $n-3$ FAs influence insulin signalling introduced by high-saturated fat in a tissue-specific manner. Supplementation of a low-fat diet with a low level of long-chain $n-3$ FA increases PI3K activity in adipose tissue [208]. It was suggested that this enhancement of PI3K activity, if accompanied by increased glucose uptake, could compensate for impaired insulin signalling in other tissues, including liver [208].

Polyunsaturated FAs are more potent activators of PPAR$_{\alpha}$ than saturated FA or monounsaturated FAs [212]; nevertheless, PPAR$_{\alpha}$ is activated during chronic high-saturated-fat feeding and mediates the effect of high-saturated-fat feeding on hepatic gene expression [213]. In PPAR$_\alpha$-null mice, the effect of high-fat feeding on PPAR$_\alpha$ target genes in liver may be mediated by PPAR$_\gamma$, which is up-regulated, as PPAR$_\gamma$ does not appear to possess any intrinsic property that prevents it from activating classical PPAR$_\alpha$ target genes [213]. Up-regulation of hepatic PPAR$_\alpha$ expression in response to high-saturated-fat feeding in C57BL/6 mice occurs only in those developing obesity, but not in those remaining lean [214]. Thus, as for fructose, there may be genetic (or epigenetic) predisposition to become insulin resistant in response to dietary lipid. Indeed, there is considerable variability between individuals in their abilities to respond to increased dietary lipid; some individuals increase FA oxidation, others (for example those with obesity or a family history of obesity) do not, and instead accumulate intracellular lipid [215].

**Involvement of lipid derivatives in insulin resistance induced by diets high in lipids or fructose**

Parallels exist between the effects of fructose- and lipid-enriched diets to induce insulin resistance, as both may be due to increased lipid delivery to insulin-sensitive tissues. Many studies have shown an association between tissue insulin resistance and intracellular lipid accumulation in high-fat-fed animals [216,217]. Intramuscular TAG content correlates more closely with systemic insulin resistance than BMI or total adiposity [217]. Candidate mediators of insulin resistance include ceramide (a lipotoxic derivative of FAs), lipid metabolites involved in the synthesis of TAG (e.g. FA-CoA) or generated by TAG hydrolysis (e.g. DAG). These lipid metabolites can alter insulin action by impairing tyrosine phosphorylation of the insulin receptor and IRS1 (reviewed in [218,219]). FAs can activate a serine kinase cascade, culminating in serine phosphorylation of IRS1 at Ser632, leading to decreased IRS1 tyrosine phosphorylation and IRS1-associated PI3K activity, and inhibition of downstream components of the insulin signalling pathway, such as PKB (protein kinase B)/Akt [220]. Ceramide also impairs activation of IRS1, PI3K and PKB/Akt [221,222]. This mechanism appears to depend...
on the type of FA, and is inoperative when muscle is exposed to mono- or poly-unsaturated FAs [223]. More generally, the FA signalling cascade may also involve IKK (inhibitor of NF-κB kinase) [216] and/or persistent translocation and activation of DAG-sensitive isoforms of PKC (protein kinase C). Inhibition of these kinases by pharmacological or genetic means can reverse insulin resistance in rodents (e.g. [224]). Insulin resistance induced by diets high in either fructose or saturated fat is also ameliorated by lowering of circulating lipids and dissipation of tissue lipids by, for example, low-fat feeding, fasting and exercise or PPARα activation [225–228].

AMPK phosphorylates and inactivates ACC, and FA oxidation consequently rises through relief of malonyl-CoA-induced suppression of CPT1 activity. AMPK would therefore be predicted to relieve insulin resistance secondary to lipid accumulation. Dietary polyunsaturated FAs have been shown to enhance hepatic AMPK activity [229]. Interestingly, both leptin and adiponectin activate AMPK [230]. Mice lacking the α2 subunit display increased insulin sensitivity [231,232], whereas the α3 isoform has been suggested to have a role in the regulation of carbohydrate metabolism in muscle (reviewed in [233]). Transgenic mice overexpressing a mutant (Tg-Prkag3<sup>γ25Q</sup>) form of AMPK, AMPKγ3, which increases ACC phosphorylation, are protected against insulin resistance induced by high-fat feeding [234]. Clearly, AMPK dysregulation has a significant association with obesity and Type 2 diabetes.

**CONCLUSIONS AND NEW DIRECTIONS**

Obesity is increasing rapidly in prevalence, but the effects of obesity on the regulation of energy balance is only now being recognized fully. Emerging research has revealed that adipose-tissue function and adipose-tissue factors play an important role in the control of energy balance. The massive expansion of adipose tissue that accompanies obesity is associated with a loss of normal regulation of adipose-tissue function and adipokine secretion. Many adipokines are regulated by nutritional factors which are also altered by the development of insulin resistance and diabetes. The alterations to adipose-tissue function and adipokine regulation can, in turn, alter the regulation of energy balance.

Extending the lipostatic hypothesis for hypothalamic nutrient sensing, an exciting new finding is that restoration of hypothalamic lipid-sensing normalizes energy and glucose homoeostasis in Sprague–Dawley rats presented with a high-palatable lard-supplemented diet [235]. These overfed rats fail to respond either to the systemic administration of leptin [236] or to the central administration of oleic acid [237]. Overfed rats failed to respond to doubling of concentrations of long-chain FAs in the circulation with doubling of concentrations of MBH (mediobasal hypothalamus), due to decreased MBH malonyl-CoA concentrations [235]. Inhibition of MBH CPT1 activity by infusing a sequence-specific ribozyme against CPT1A or an isoform-selective inhibitor of CPT1 normalized hypothalamic levels of long-chain CoA and markedly inhibited feeding behaviour despite leptin resistance [235]. Thus MBH levels of long-chain FAs may act downstream of leptin signalling, perhaps directly at the level of AMPK. Importantly, this manipulation also normalized the excessive hepatic glucose output observed in the overfed animals [235]. These new studies indicate that improved nutrient sensing may hold the key for improving insulin resistance in obesity and Type 2 diabetes.

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