Hunting for new pieces to the complex puzzle of obesity

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Disentangling the neuroendocrine systems that regulate energy homeostasis and adiposity has been a long-standing challenge in pathophysiology, with obesity being an increasingly important public health problem. Adipose tissue is no longer considered a passive bystander in body-weight regulation. It actively secretes a large number of hormones, growth factors, enzymes, cytokines, complement factors and matrix proteins, at the same time as expressing receptors for most of these elements, which influence fuel storage, mobilisation and utilisation at both central and peripheral sites. Thus, an extensive cross talk at a local and systemic level in response to specific external stimuli or metabolic changes underpins the multifunctional characteristics of adipose tissue. In addition to the already-known adipokines, such as IL, TNFα, leptin, resistin and adiponectin, more recently attention has been devoted to 'newcomers' to the 'adipose tissue arena', which include aquaporin, caveolin, visfatin, serum amyloid A and vascular endothelial growth factor. While in vitro and in vivo experiments have provided extremely valuable information, the advances in genomics, proteomics and metabolomics are offering a level of information not previously attainable to help unlock the molecular basis of obesity. The potential and power of combining pathophysiological observations with the wealth of information provided by the human genome, knock-out models, transgenesis, DNA microarrays, RNA silencing and other emerging technologies offer a new and unprecedented view of a complex disease, conferring novel insights into old questions by identifying new pieces to the unfinished jigsaw puzzle of obesity.

Obesity: Aquaporin: Caveolin: Comorbidities: Cardiovascular risk factors

Over the last century nutrition science has evolved from an initial elucidation of the nutrients essential to life to a more sophisticated inquiry into how cells and systems work. Once the essential nutrients were identified, nutrition scientists started to uncover the metabolic pathways, gradually disentangling the detailed role of each nutrient in both physiological and pathological conditions. One of the more fascinating and rewarding aspects of nutrition is represented by the application of this huge body of knowledge to improve animal and human well-being. In particular, clinical nutrition aims to translate the scientific advances into changes in everyday practice. Sir David Cuthbertson carried out pioneering work in clinical metabolism, driving the knowledge concerning the catabolic response to injury and setting the basis for the therapeutic benefit of stress reduction after trauma. The currently-available technology is providing new impetus to further unravel the basic molecular mechanisms involved in metabolic processes and disease, fostering the progress made in clinical nutrition (Aitman, 2003; Müller & Kersten, 2003). In this context obesity represents an example worth considering in relation to the role of nutritional science (Trayhurn, 2005a).

During the last decade energy-balance control has been an extremely active and fruitful research topic (Flier, 2004). Despite the unprecedented advances in the knowledge and understanding of systems biology, the obesity epidemic shows no signs of abatement (Fry & Finley, 2005).
2005; Ogden et al. 2006). The incidence of obesity and its main associated comorbidities, such as type 2 diabetes mellitus and CVD, has increased in the last decades, reaching epidemic proportions (Mokdad et al. 2003; Gregg et al. 2005). Paradoxically, the perpetuation of the increases in the prevalences of overweight and obesity coincides with a scenario in which the preoccupation with body weight and diet pervades today’s society. The hazards of excess body weight have been clearly established by epidemiological and clinical studies (Kopelman, 2000; Calle et al. 2003; Fontaine et al. 2003; Frühbeck, 2005a). Obesity will be among the leading causes of death and disability in the coming years, thus threatening to reverse many of the health gains achieved in the last decades (National Audit Office, 2001; Olshansky et al. 2005). Growing awareness of these risks has highlighted the need for increased insight into the understanding of the links between adipose tissue and pathophysiology.

The puzzle analogy

The neuroendocrine messages controlling energy storage and release have been a long-standing challenge in nutrition research for the last 50 years. From a simple point of view, body-weight maintenance results from the delicate balance between energy intake and expenditure. This balance, however, is subject to a plethora of multifactorial aspects ranging from genetic to environmental influences. Thus, the multidisciplinary nature of obesity represents the participation and interaction of many different elements that are intimately interconnected and can be viewed as the numerous pieces of a complex puzzle. Different strategies can be followed to solve a jigsaw puzzle. The pieces can be grouped according to different characteristics. For instance, sharing the same colour may help to identify pieces of the picture that probably fit together. Another approach is based on looking for pieces with a particular shape, which is very helpful to get the corners, edges and the ‘frame’ of the whole scene. The same is true for the complex puzzle of obesity. The current knowledge on this topic has been gathered from different perspectives. Valuable information has been derived from several approaches, such as whole-body physiology and in vitro and in vivo experiments, as well as from the observations of common obesity-associated conditions in both animals and man (Hetherington & Ranson, 1940; Frisch & McArthur, 1974; Coleman, 1978; Bray & York, 1979; Frühbeck, 2001; Frühbeck & Gómez-Ambrosi, 2001a; Trayhurn & Beattie, 2001; Frayn et al. 2003; Hauner, 2005). Extremely infrequent monogenic diseases (Farooqi & O'Rahilly, 2005), as well as the completion of the human genome (Cummings & Schwartz, 2003; Bell et al. 2005; Clement, 2005; Rankinen et al. 2006), have also contributed to achieving a more detailed insight. Furthermore, knocking out or overexpressing certain genes represents a further means of deciphering the importance of a specific protein or signalling cascade relevant to energy balance (Frühbeck & Gómez-Ambrosi, 2003; Blüher, 2005). The findings obtained from the wide range of the ‘omic’ technologies is providing an amount of information not previously attainable, with nutrigenomics (Müller & Kersten, 2003), epigenetics (Oommen et al. 2005), microarrays (Nadler & Attie, 2001; Collier et al. 2002; Copland et al. 2003; Middleton et al. 2004; Sjöholm et al. 2005), in silico approaches (Borodina & Nielsen, 2005), RNA silencing (Ashrafi et al. 2003; Grunweller & Hartmann, 2005; Shankar et al. 2005) and other emerging technologies (Carella et al. 2003; Mobasher et al. 2004; Droit et al. 2005; Ruden et al. 2005; Viguerie et al. 2005) offering new and unprecedented tools in this area. Interestingly, all approaches can and should be combined to better understand the complex puzzle of obesity.

The author’s work combines a clinical and whole-body physiology approach, together with the complementary information provided by molecular biology techniques, to the study of the pathophysiological mechanisms contributing to obesity and its associated comorbidities from the integrated perspective provided by everyday work in the clinical setting and basic research in experimental animals and cells. The efforts of the author’s group are concentrated on three interconnected research lines (Fig. 1): (1) the impact of adiposity on the development of comorbidities; (2) the intracellular signalling pathways activated in adipocytes and vascular smooth muscle cells (VSMC); (3) adipose tissue gene expression profiling.

Adipose tissue as an extremely-active endocrine–paracrine organ

Among the different functions of adipose tissue the findings have focused on its participation in lipolysis stimulation, blood pressure regulation and satiety control. White adipose tissue is no longer considered a passive bystander entirely devoted to energy storage. During the last decade adipose tissue has emerged as an active participant lying at the heart of a complex autocrine, paracrine and endocrine network that is implicated in the regulation of a variety of quite diverse pathophysiological functions, with its multifunctional nature being based on the ability of its cellular constituents to secrete a large number of products (Trayhurn, 2005b). These products can be categorised as hormones, growth factors, enzymes, cytokines, complement factors and matrix proteins, collectively termed adipokines; adipose tissue also expresses receptors for most of these factors (Trayhurn & Beattie, 2001; Frühbeck & Salvador, 2004; Trayhurn & Wood, 2004; Arner, 2005a; Berg & Scherer, 2005; Yu & Ginsberg, 2005; Klein et al. 2006). Adipose tissue is a special loose connective tissue that encompasses not only adipocytes but also other cell types (termed the stromavascular fraction), including blood cells, endothelial cells, macrophages and pericytes, as well as adipose precursor cells, which warrant an extensive cross talk at a local (Fig. 2) and systemic level in response to specific external stimuli or metabolic changes (Frühbeck & Gómez-Ambrosi, 2005). Glucocorticoids, sex steroids, PG, adipins, leptin, resistin and adiponectin are now among the better known secretions of adipose tissue. Growth factors include insulin-like growth factor I, macrophage colony-stimulating factor, transforming growth factor β, vascular endothelial growth factor (VEGF),
Peripheral effects of adipocyte-derived factors

Gene expression analysis in obesity and comorbidities

Cell-culture studies (adipocytes, VSMC, EC)

Clinical road map to comorbidity development

Signalling pathways; physiopathological implications

Integrative

Genomics
Proteomics
Metabolomics

Gene expression analysis
Peripheral effects of adipocyte-derived factors
Cell-culture studies
Clinical road map to comorbidity development
Signalling pathways; physiopathological implications

(a)
(b)

Fig. 1. Schematic representation of the selected research lines in obesity. (a), an image of a microarray; (b), a protein spectrum; VSMC, vascular smooth muscle cells; EC, endothelial cells.

Triacylglycerol overload → Activation and infiltration of macrophages → Cross talk and feedback loops

Adipocytes

Metabolites
Enzymes
Others
Hormones
Matrix proteins
Vasoactive factors

Adipokines
Classic cytokines
Chemokines
Complement factors
Growth factors

C3
NEFA
Others

MCP-1
VEGF
Others

TNFα
Leptin
IL-1β
Others

IL-6
Others

Fig. 2. Active cross talk between the numerous mediators secreted by the different cellular components of adipose tissue, which are enhanced in states of obesity, accompanied by recruitment of macrophages. Adipocytes and other cell types secrete inflammatory cytokines and immune factors exerting relevant autocrine, paracrine and endocrine influences. EC, endothelial cell; VSMC, vascular smooth muscle cell; MCP-1, monocyte chemoattractant protein-1; VEGF, vascular endothelial growth factor; C3, complement factor.
heparin-binding epidermal growth factor, leukaemia inhibitory factor, nerve growth factor and bone morphogenetic protein. In the category of non-secreted factors, the prominent factors are perilipin, adiponutrin, adipophilin, uncoupling proteins and the membrane channel proteins GLUT-4, caveolins and aquaporin (AQP)-7. Special attention has been devoted to adipose-derived factors, which have been shown to be implicated either directly or indirectly in the regulation of vascular homeostasis through effects on blood pressure, inflammation, atherogenesis, coagulation, fibrinolysis, angiogenesis, proliferation, apoptosis and immunity (Fruhbeck, 2004a; Fantuzzi, 2005; Lau et al. 2005; Sjoholm & Nystrom, 2005; Permana et al. 2006). A key group is represented by proinflammatory–proliferative–prothrombotic–atherosclerotic–vascular factors such as TNFα, plasminogen activator inhibitor-1, tissue factor, angiotensinogen, metallothionein, C-reactive protein and IL (in particular IL-6, -1, -10 and -8). Interestingly, weight loss is associated with short-term beneficial effects on blood pressure, lipid metabolism, insulin sensitivity, susceptibility to thrombosis, inflammatory markers and sympathetic activity, which can be observed even with a modest 5–10% weight reduction (Fruhbeck, 2004a). However, it has to be taken into consideration that in subjects with established risk factors for CVD and diabetes it is necessary to achieve initial weight losses of >10% in order to maintain longer-term losses of ≥5%, if the associated health benefits are the target (Krebs et al. 2002).

Regulation of lipid metabolism

In vertebrates adipose tissue represents the most important energy depot, which is stored in the form of triacylglycerols in a large lipid droplet that accounts for most of the adipocyte volume. Lipolysis of the stored triacylglycerols results in the release of glycerol and fatty acids from adipocytes and constitutes a key event in energy homeostasis (Arner, 2005b). Fat cell lipolysis is subject to precise regulation by nutritional conditions, neuroendocrine influences and pathophysiological circumstances. A number of hormones, cytokines and enzymes control the lipolytic activity, with catecholamines and insulin being the most important elements regulating human fat cell lipolysis.

Participation of leptin in lipolysis

A decade ago the identification of functional leptin receptors (OB-R) in white adipose tissue suggested the involvement of leptin in the direct regulation of adipocyte metabolism at a peripheral level. In fact, leptin was shown to participate in lipid metabolism control through lipogenesis inhibition and lipolysis stimulation (Fruhbeck, 2001). At a local level, leptin has the ability to repress acetyl-CoA carboxylase gene expression, fatty acid synthesis and lipid synthesis, which are biochemical reactions leading to lipid accumulation (Bai et al. 1996; Wang et al. 1998). Additionally, leptin has been shown to exert an autocrine–paracrine lipolytic effect on white adipose tissue both


A functional relationship between leptin and NO has been established in several physiological processes (Fruhbeck et al. 2001a; Fruhbeck, 2006). Given the observed co-localisation of both factors in adipocytes and their involvement in lipolysis, the potential role of NO in the leptin-induced lipolytic effect has been investigated (Fruhbeck & Gomez-Ambrosi, 2001b). A dose-dependent increase in both serum NO concentrations and basal adipose tissue lipolytic rate was observed 1 h after exogenous leptin administration, with simple linear regression analysis demonstrating that 27% of the variability taking place in lipolysis is attributable to the changes in NO concentrations. Inhibition of NO synthesis by using Nω-nitro-L-arginine methyl ester pretreatment was shown to be followed by a reduction in leptin-mediated lipolysis stimulation compared with leptin-treated control animals. In contrast, in adipocytes obtained from rats under acute ganglionic blockade by chlorisondamine administration it was found that the leptin-induced lipolytic effect is not different from the lipolytic rate achieved by leptin in control rats treated with saline (9 g NaCl/l; Fig. 3).

It is well known that a rise in cAMP as a result of either adenylyl cyclase activation or phosphodiesterase inhibition stimulates lipolysis. In order to gain further insight into the potential mechanisms involved, lipolysis has been stimulated in isolated adipocytes using a number of agents acting at different levels of the lipolytic pathway (Fig. 4): (1) at the β-adrenergic receptor (isoproterenol); (2) at adenylyl cyclase (forskolin); (3) at phosphodiesterase E (isobutylmethylxanthine); (4) at protein kinase A

![Fig. 3. Lipolysis under nitric oxide synthase (NOS) inhibition and acute ganglionic blockade. Wistar rats under pharmacological pretreatment consisting of intravenous administration of vehicle (saline (9 g sodium chloride/l); control group), NOS inhibition (Nω-nitro-L-arginine methyl ester (L-NAME); 30 mg/kg) or acute ganglionic blockade (chlorisondamine; 30 mg/kg) were further injected with either saline (□) or leptin (100 mg/kg; ◆) to study the effect on basal lipolysis of fat cells. The lipolytic activity was measured as the amount of glycerol released by isolated adipocytes after 1 h. Results are expressed as the percentage of basal lipolysis of fat cells from saline-treated control animals and are mean values with their standard errors represented by vertical bars for eight animals per group (lipolytic experiments were performed in duplicate). Mean values were significantly different from those for the saline-treated animals: †P<0.01, ***P<0.001. Mean values were significantly different from those for the control leptin-treated animals: †P<0.05. Statistical analyses were performed using ANOVA and post hoc pair-wise comparisons.](https://doi.org/10.1079/PNS2006510)
Aquaporin-7

Recently, AQP7 has emerged as a new piece in the complex puzzle of obesity and insulin resistance. Over eons, mammalian evolution has selected for genes that allow for survival during famine or drought periods. The primary role of fat cells is to store triacylglycerols during periods of energy excess and to mobilise this reserve when expenditure exceeds intake. Among the complex mechanisms underlying energy balance control, AQP7 has emerged as a novel pathway critical to the modulation of lipid metabolism (Frühbeck 2005b; Frühbeck et al. 2006). AQP are a family of integral membrane proteins favouring water movement across cell membranes (King et al. 2004). AQP transport water, in some cases together with small molecules such as glycerol and other small solutes. The identification of AQP has allowed the recognition of the relevance of water channels for homeostatic control, and their true contribution to human health and disease has started to unfold (Verkman, 2005). AQP7 belongs to the subcategory termed aquaglyceroporins, which comprise channels known to permeabilise glycerol as well as water (King et al. 2004; Hara-Chikuma & Verkman, 2006). Originally named AQPPap, this water–glycerol pore was cloned from human adipose tissue (Kuriyama et al. 1997; Kishida et al. 2001; Kondo et al. 2002). AQP7 deficiency in adipocytes has been associated with adult-onset obesity in mice; thus, providing evidence that AQP7 functions as a glycerol channel in vivo whereby adipocyte glycerol permeability exerts a key role in the regulation of fat accumulation (Maeda et al. 2004; Hara-Chikuma et al. 2005; Hibuse et al. 2005; Wintour & Henry 2006). Fat cells of AQP7-knock-out mice exhibit an increase in glycerol kinase activity, which stimulates triacylglycerol synthesis, ultimately leading to adipocyte hypertrophy and subsequent development of obesity. The control exerted by AQP7 over the efflux of glycerol from fat cells further highlights its role in aged mice as a determinant of whole-body glucose homeostasis and insulin sensitivity (Hibuse et al. 2005). Interestingly, the coordinated regulation of
fat-specific AQP7 and liver-specific AQP9 may be key to determining glucose metabolism in physiology and insulin resistance (Kuriyama et al. 2002). Furthermore, the expression of AQP7 in fat cells has been reported to be sensitive to nutritional and neuroendocrine factors (Fig. 5) such as fasting, insulin, TNFα, steroids, adrenergic agonists and PPARγ (Kishida et al. 2001; Kondo et al. 2002; Kuriyama et al. 2002; MacDougald & Burant, 2005).

The potential contribution of AQP7 to human obesity development has not been completely elucidated. The AQP7 gene, which resides in chromosome 9p13, has not been previously identified as a candidate gene for obesity (Ishibashi et al. 1998; Kishida et al. 2001; Rankinen et al. 2006). The loss of function associated with mutation of the AQP7 gene has been identified only in a single human subject who showed no signs of development of obesity or diabetes (Kondo et al. 2002). The only observed alteration associated with the homozygous mutation is an impaired increase in plasma glycerol in response to exercise. The extent, as well as the true influence, of aquaglyceroporins in human energy balance remains to be fully established. A microarray analysis (Sjöholm et al. 2005) has shown increased AQP7 expression levels in the adipose tissue of obese women compared with obese men, while no differences were observed between the subcutaneous and omental depots. Elucidating the exact contribution of AQP7 and its role in the regulation of glycerol permeability, together with the potential impact on the current worldwide ‘diabetes’ epidemic, will provide a fertile area of research.

**Caveolins**

Caveolae are 50–100 nm flask-shaped cell-surface plasma-membrane invaginations implicated in a wide range of cellular functions such as endocytosis, cholesterol homeostasis and cell signalling control (Cohen et al. 2004). The discovery of caveolin (Rothberg et al. 1992; now termed caveolin-1) as a protein marker and main component of caveolae has provided more insight into the roles of these organelles. Caveolins are integral membrane proteins that serve as the structural elements of caveolae. They function as scaffolding as well as being capable of recruiting numerous signalling molecules to the plasma membrane lipid rafts and regulating their activity (Stan, 2005; Parton et al. 2006). Interestingly, caveolins are cholesterol-binding proteins with a unique hairpin topology that allows both the amino and carboxy terminals to face the cytoplasm (Fig. 6). Three different caveolin isoforms have been identified so far, exhibiting a unique tissue distribution pattern. While caveolin-3 is expressed in skeletal, cardiac and smooth muscle cells (Tang et al. 1996), caveolin-1 is expressed in the majority of cells, excluding those of the muscular lineage, with expression being particularly high in adipocytes, fibroblasts and epithelial and endothelial cells (Parton et al. 1994; Scherer et al. 1997). Expression of caveolin-2 most closely follows the distribution pattern of caveolin-1, although it has been recently shown that caveolin-2 is also found in cardiac myocytes (Rybin et al. 2003).

The study of the phenotypes of caveolin-knock-out mice has considerably enhanced the understanding of the functional contribution of these proteins in diverse tissues and cellular processes (Le Lay & Kurzchalia, 2005). An in vivo role for caveolin-1 in the development of obesity and insulin resistance through a direct participation in lipid homeostasis has been clearly established (Razani et al. 2002; Cohen et al. 2003a,b). Despite an evident hyperphagia, caveolin-1-knock-out mice show a lean phenotype with overt resistance to diet-induced obesity resulting from
an impaired adipocyte functioning as a consequence of altered lipid handling. Adipocytes of caveolin-1-null mice lack caveolae, a derangement that with increasing age translates into a systemic inability for lipid accumulation, resulting in a near complete ablation of fat depots accompanied by histological alterations, including reduced fat cell number and diameter. The difference in weight of caveolin-1-knock-out mice when compared with wild-type mice becomes even more striking when the rodents are challenged with a high-fat diet. Caveolin-1-deficient mice show normal circulating glucose, insulin and cholesterol concentrations at the same time as exhibiting increased triacylglycerol and NEFA levels. The impaired triacylglycerol clearance takes place in the setting of normal total and hepatic lipoprotein lipase activity. It has further been shown that caveolin-1 is essential for adequate non-shivering thermogenesis in brown adipose tissue (Cohen et al. 2005). Since caveolin has been previously reported to operate as a positive regulator of insulin signalling (Yamamoto et al. 1998), the involvement of caveolin-1 in this process has been examined. No changes in insulin receptor gene expression are evident in caveolin-1-deficient mice (Cohen et al. 2003b). However, there is >90% reduction in insulin receptor protein levels in adipose tissue of these knock-out rodents, with ectopic expression of caveolin-1 being sufficient to restore expression to that of wild-type mice. The in vivo metabolic consequences of the genetic ablation of caveolin-3 in mice also affect glucose metabolism and lipid homeostasis, with caveolin-3-null mice displaying a marked postprandial hyperinsulinaemia and impaired glucose tolerance that progresses to whole-body insulin resistance with decreased insulin-stimulated whole-body glucose uptake. Even in white adipose tissue, which does not express caveolin-3, an approximately 70% decrease in insulin-stimulated glucose uptake is observed that also suggests an insulin-resistant state for this tissue (Capozza et al. 2005). Interestingly, caveolin-3-null mice have also been reported to develop whole-body insulin resistance associated with an impaired glucose tolerance (Oshikawa et al. 2004; Capozza et al. 2005). Caveolae act as communication platforms, serving as a concentrating point for numerous signalling cascades. Effective insulin signalling in adipocytes is dependent on the localisation in caveolae of insulin-responsive elements. Caveolin-1 seems to play a role in the regulation of glucose homeostasis, both through a direct interaction with the insulin receptor and as an agent for GLUT4-mediated glucose uptake (Cohen et al. 2003a). Caveolin-3 appears to attenuate the insulin-stimulated activation of insulin receptors and downstream molecules, such as insulin receptor substrate 1 and Akt, in skeletal muscles (Oshikawa et al. 2004; Capozza et al. 2005).
Vasoactive factors secreted by adipose tissue

Mature adipocytes are characterised by the ability to synthesise a pleiad of proteins, which operate as relevant bioactive mediators. Recently, there has been particular interest in the vasoactive factors that exert an impact on the control of blood pressure, inflammation, atherogenesis, coagulation, fibrinolysis and angiogenesis (Wellen & Hotamisligil, 2003; Frühbeck, 2004a; Chaldakov et al., 2005; Lau et al., 2005; Trayburn, 2005b; Matsuzawa, 2006). The study of the effects of some adipokines, such as leptin, resistin, adiponectin, visfatin, ghrelin and other well-known cardiovascular risk factors, has provided a new insight into the molecular links between obesity and CVD.

Leptin

The role of leptin in the development of cardiovascular complications, beyond its effects on energy balance, only started to unfold some years after the identification of the hormone (Correia & Haynes, 2004; Matsuzawa, 2005). Leptin has been shown to contribute to blood pressure homeostasis by inducing a pressor response attributable to sympatheoactivation (Correia & Haynes, 2004) and a depressor response attributable to the vasodilation of conduit and resistance vessels (Frühbeck, 1999; Beltowski et al., 2006; Beltowski, 2006a). In relation to this role, the author’s group was the first to identify NO as the key molecule responsible for the depressor response induced by leptin (Frühbeck, 1999). Subsequent studies have further shown that leptin acts on the endothelium, inducing the synthesis of NO via the activation of endothelial NO synthase, which induces an endothelium-dependent vasodilation (Kimura et al., 2000; Lembo et al., 2000; Winters et al., 2000; Beltowski et al.; Vecchione et al., 2002; Beltowski, 2006a). Since OB-R are also expressed in the underlying smooth muscle layer, it is considered that VSMC also represent an important target for the vascular actions of leptin (Fortuño et al. 2002; Rodríguez et al. 2006). It has been shown that leptin acts on VSMC, inhibiting the increase in cytosolic Ca induced by angiotensin II, thus blunting the contractile response caused by this potent vasoactive peptide. However, the intracellular mechanisms underlying this endothelium-independent vasodilation induced by leptin have not been completely elucidated. Given that the main signalling cascades activated by OB-R include the Janus kinase and signal transduction and activator of transcription cascades as well as the phosphoinositol-3 kinase/Akt pathways (Frühbeck, 2006; Peelman et al., 2006), it is hypothesised that an up-regulation of inducible NO synthase underlies the inhibitory effect of leptin on the angiotensin II-induced response in VSMC via the Janus kinase/signal transduction and activator of transcription and phosphoinositol-3 kinase/Akt pathways, as illustrated in Fig. 7.

Leptin has been postulated to be one of the potential links between adiposity and inflammation (Berg & Scherer, 2005; Fantuzzi, 2005; Härle & Straub, 2006). Although the increase in the expression of leptin mRNA in white adipose tissue and the elevation in circulating concentrations triggered by inflammatory stimuli in experimental animals have not been consistently observed in human subjects (Fantuzzi & Faggioni, 2000; Gómez-Ambrosi et al. 2004a), in general a proinflammatory role has been attributed to leptin. In this sense, the effects of leptin on inflammation and immunity need to be considered in a more broad and complex setting. In endothelial cells leptin has been shown to up regulate endothelin-1 and NO synthase, and stimulate the expression of adhesion molecules and monocyte chemoattractant protein-1, at the same time as inducing oxidative stress (Lau et al. 2005; Beltowski, 2006b). Leptin has also been observed to stimulate angiogenesis, platelet aggregation and atherothrombosis, and to have a direct effect on macrophages, promoting their accumulation of cholesterol at the same time as leading to an increased release of monocyte colony-stimulating factor. Leptin operates as a chemoattractant devoid of secretagogue properties but capable of inhibiting neutrophil chemotaxis to classical neutrophil chemo-attractants (Montecucco et al. 2006). This effect is reportedly dependent on the activation of intracellular kinases and, in particular, via p38 mitogen-activated protein kinase and Src kinase (Montecucco et al. 2006).

Fibrinogen, C-reactive protein and von Willebrand factor represent well-known markers of inflammation and endothelial dysfunction, which are increased in obese patients (Hansson, 2005). A positive association has been observed between these markers and body fat, which in the case of fibrinogen and von Willebrand factor is higher than the correlation observed with BMI (Gómez-Ambrosi et al. 2002; Gómez-Ambrosi et al. 2006b). Despite its wide use, BMI does not provide an accurate measure of body composition. In this context more precise indicators need to be incorporated into the clinical diagnosis of obesity in order to better estimate the cardiovascular-related risk (Frühbeck, 2004b). Although these markers are significantly correlated with leptin concentrations (P<0.001 for C-reactive protein; Fig. 8), the statistical significance is lost after adjusting for body fat, suggesting that they are
Fig. 8. Scatter diagram showing the highly significant positive correlation (n 553; r 0.144; P<0.001) between the circulating concentrations of log leptin and log C-reactive protein (CRP). ( ), Men; ( ), women.

not regulated by leptin itself. Recent studies (Gómez-Ambrosi et al. 2005, 2006b) have provided evidence of direct associations between adipose tissue stores and circulating concentrations of fibrinogen, C-reactive protein and von Willebrand factor that are not dependent on the influence of leptin.

Resistin

The seminal observation of the induction of insulin resistance in mice by a novel adipokine led to it being termed resistin (Steppan et al. 2001). Increased circulating resistin concentrations have been observed in genetically-obese rodents (ob/ob and db/db mice) as well as in diet-induced obesity. Immunoneutralisation of resistin ameliorates hyperglycaemia and insulin resistance in these obese mice, while recombinant resistin administration results in impaired glucose tolerance and insulin action in normal mice (Steppan et al. 2001; Steppan & Lazar, 2004). Glucose, growth hormone (GH), glucocorticoids, insulin, β₂-adrenoreceptor stimulation and thiazolidinediones reportedly increase the expression of resistin (Koerner et al. 2005). However, insulin, TNFα, adrenaline, β-adrenergic receptor stimulation and thiazolidinediones have also been observed to decrease resistin gene expression (Steppan & Lazar, 2004; Koerner et al. 2005). Moreover, the real contribution of resistin to human pathophysiology still remains controversial (Arner, 2005a).

Adiponectin

In pathological conditions such as obesity and the characteristic insulin resistance accompanying both the pre-diabetic state and overt type 2 diabetes mellitus, a clear hyperadiponectinaemia has been observed (Fröhbeck & Salvador, 2004; Arner, 2005a; Berg & Scherer, 2005; Koerner et al. 2005). Adiponectin (also termed Acrp30, AdipoQ, apM1 or GBP28) operates completely differently from other known adipokines, since it has been shown to improve insulin sensitivity, inhibit vascular inflammation and exhibit a cardio-protective effect. White adipose tissue adiponectin expression has been observed to be increased in lean individuals and women, exhibiting an association with lower extents of insulin resistance and TNFα expression (Yamauchi et al. 2003). Glucocorticoids, TNFα and IL-6 inhibit the gene expression of adiponectin in human visceral adipose tissue, while insulin, insulin-like growth factor 1 and PPARγ agonists increase its expression. To date three putative adiponectin receptors have been cloned. Adiponectin receptor 1 has been shown to be abundantly expressed in skeletal muscle and adiponectin
receptor 2 is predominantly present in liver, while a third receptor has been identified in endothelial cells and smooth muscle (Kadowaki & Yamauchi, 2005).

The cardio-protective characteristics of adiponectin have been attributed to its involvement in the prevention of atherosclerotic plaque formation through the inhibition of monocyte adhesion to endothelial cells, by decreasing NFκB signalling via a cAMP-dependent pathway (Ouchi et al. 2000; Kawajiri et al. 2004). Interestingly, in rodent models of atherosclerosis, such as ob/ob and apoE-deficient mice, adiponectin reportedly exerts a protective effect on the development of both atherosclerosis and type 2 diabetes mellitus (Frühbeck, 2004a; Lau et al. 2005). The findings in animals have a certain clinical parallel in human subjects, evidenced by a negative correlation between adiponectinaemia and markers of inflammation (Pischon et al. 2004; Xydas et al. 2004). Circulating adiponectin levels have been observed to be inversely correlated with insulin resistance and C-reactive protein concentrations (Ouchi et al. 2003). In line with the cardio-protective properties of the adipokine, patients with CHD present with hypoadiponectinaemia compared with age- and BMI-adjusted controls, and a lower risk of myocardial infarction in men has been reported to be associated with high plasma adiponectin concentrations (Wells & Hotamisligil, 2003). Furthermore, adiponectin has also been observed to regulate vascular inflammation via a direct effect on endothelial cells, as well as by decreasing VSMC proliferation and migration via a reduction in the effects of certain growth factors such as platelet-derived growth factor and heparin-binding epidermal growth factor (Frühbeck, 2004a). The detrimental effects of hypoadiponectinaemia in obesity, CVD and type 2 diabetes mellitus may be further related to an anti-inflammatory activity of the adipokine on macrophages. An inhibition of the endothelial inflammatory response by decreasing VSMC proliferation and vascular cell adhesion molecule-1 expression seems to underlie the anti-atherogenic effects of adiponectin (Lau et al. 2005).

**Visfatin**

Among the more recently identified adipokines, visfatin is mainly produced and secreted by visceral white adipose tissue. It has putative anti-diabetogenic properties through its binding to the insulin receptor and the exertion of an insulin-mimetic effect both in vitro and in vivo (Fukuhara et al. 2005; Sethi & Vidal-Puig, 2005). Originally identified as pre-B-cell colony-enhancing factor, visfatin is a cytokine that is abundantly present in the broncho-alveolar lavage fluid of animal models of acute lung injury as well as in the neutrophils of patients with sepsis (Fantuzzi, 2005). In spite of the descriptive name, plasma concentrations of visfatin and visceral visfatin mRNA expression have been reported to correlate with measures of obesity but not with the visceral fat mass or the waist:hip ratio. Furthermore, no differences have been observed in visfatin mRNA expression between the visceral and subcutaneous fat depots (Berndt et al. 2005). IL-6 has been reported to exert an inhibitory effect on visfatin expression, which is in part mediated by the p44/42 mitogen-activated protein kinase (Kralisch et al. 2005). A 2-fold increase in circulating concentrations of visfatin has been observed in patients with type 2 diabetes mellitus (Chen et al. 2006). Nonetheless, after adjusting for BMI and waist:hip ratio the independent association between visfatin and diabetes disappears. The pathophysiologic relevance of visfatin deserves further investigation in order to clarify the paradoxical effects of simultaneously favouring fat accretion and promoting insulin sensitivity (Arner, 2006). It is still not clear whether visfatin actively participates in the feedback mechanisms regulating fat accretion in the intra-abdominal depot and its accompanying insulin resistance, or merely represents an epiphenomenon that might be useful as a surrogate marker of increased omental adipose tissue.

**Vascular endothelial growth factor**

Microarray technology has been applied to the analysis of gene expression profiles in adipose tissue obtained from lean and obese individuals in order to identify differential expression patterns and key genes involved in obesity. Consequently, attention has been focused on the changes observed in angiogenic factors (Gómez-Ambrosi et al. 2004b). VEGF is known to promote angiogenesis, inducing migration and proliferation of vascular endothelial cells (Berg & Scherer, 2005). Although VEGF is encoded by a single gene, four isoforms are produced by alternative splicing, which have been implicated in both normal blood vessel development and in pathogenic neovascularisation and atherosclerosis. In obese patients serum concentrations of one of the isoforms have been observed to be dependent on the intra-abdominal fat depot (Miyazawa-Hoshimoto et al. 2003). VEGF mRNA expression has been identified in various cell types, including endothelial, epithelial and mesenchymal cells. Interestingly, one of these microarray studies (Gómez-Ambrosi et al. 2004b) has provided evidence for increased expression of VEGF-B mRNA in omental adipose tissue of obese patients, in accordance with the need for enhanced vascularisation to support adipose mass enlargement. In particular, the mRNA of VEGF-B186 and VEGF-B167, the two known isoforms of VEGF-B, were shown to be upregulated 1.6-fold and 2.1-fold respectively. This finding suggests a potential link in the involvement of VEGF-B in angiogenesis and obesity-related endothelial dysfunction. This aspect needs to be explored further in relation to the implication that VEGF is involved in vascular inflammation and remodelling through increased subendothelial macrophage accumulation and intima media thickening in the context of atheroma initiation and restenosis episodes.

**Serum amyloid A**

Recognition of obesity as a chronic low-grade inflammatory state has driven recent productive research efforts. Adipose tissue is considered an extremely active immune organ that secretes numerous immunomodulatory factors, and it has emerged as an important source of inflammatory signals known to be related to comorbidity development (Trayhurn & Wood, 2004; Sjöholm & Nyström, 2005).
Thus, inflammation within white adipose tissue represents a crucial step, contributing to the appearance of many of the pathological features accompanying increased adiposity. The mounting evidence of the relevance of inflammation to vascular disease and insulin resistance has orientated plentiful research efforts towards molecules that modulate leukocyte migration from the bloodstream to the vessel wall. Serum amyloid A (SAA) is an acute-phase reactant protein secreted by diverse cell types, including adipocytes. It has been associated with systemic inflammation, as well as being linked to atherosclerosis and serving as a predictor for coronary disease and cardiovascular outcome (Lau et al. 2005). Circulating SAA concentrations are increased in obese patients and those with diabetes (Berg & Scherer, 2005). Under physiological circumstances white adipose tissue is known to express low levels of SAA, which have been shown to be strongly up-regulated in obesity (Clémont et al. 2004). From a mechanistic perspective, the detrimental effects of augmented SAA levels appear to be related to the displacement of apoA1 from HDL-cholesterol, increasing its binding to macrophages, therefore decreasing the availability of the cardio-protective HDL-cholesterol (Lau et al. 2005). In addition, SAA functions as a chemoattractant, an inducer of remodeling metalloproteinases and a stimulator of T-cell cytokine production (Berg & Scherer, 2005).

A positive correlation between BMI and circulating SAA concentrations has been reported (Urieli-Shoval et al. 2000). However, whether serum SAA levels are increased in obese patients in relation to the body fat compartment was not directly addressed. In a recent study (Gomez-Ambrosi et al. 2006a) obese patients were found to exhibit a 6-fold increase in circulating SAA concentrations compared with lean individuals. Furthermore, increased expression of SAA mRNA in the omental fat depot of obese patients was established. Interestingly, it was found that weight loss following bariatric surgery was able to reduce SAA concentrations, which may play a role, in part, in the beneficial effects that accompany weight reduction following bariatric surgery. It can be concluded that the elevated SAA levels in both serum and omental adipose tissue observed in obese patients may contribute to the obesity-associated CVD risk, which can be beneficially influenced by weight loss.

**Ghrelin**

Ghrelin is a potent GH-releasing peptide that was originally isolated from the stomach and subsequently identified as an endogenous ligand for the GH secretagogue receptor. It has been shown to be involved in the regulation of food intake by exerting an orexigenic effect (Kojima et al. 1999; Inui et al. 2004). Similarly to leptin, other sources of ghrelin production have been located, providing evidence for a physiological role of the hormone beyond energy balance (Ghigo et al. 2005). The almost universal distribution of GH secretagogue receptors, including in adipose tissue and the cardiovascular system (Papotti et al. 2000; Gnanapavan et al. 2002), supports the plausibility of vascular actions of ghrelin (Cao et al. 2006). In particular, GH secretagogue receptors are expressed in both blood vessels and cardiomyocytes, providing evidence for direct cardiovascular effects of ghrelin. Exogenous administration of ghrelin has been shown to exert beneficial haemodynamic effects via a vasodilatory effect, reducing mean arterial pressure and increasing cardiac index and stroke volume without elevating heart rate in human subjects and different rodent models (Cao et al. 2006). Circulating ghrelin concentrations are reportedly decreased in obese individuals. Interestingly, ghrelin has been shown to induce vasorelaxation, acting via an endothelium-independent mechanism, which reverses the effect of endothelin-1 on isolated human arteries (Wiley & Davenport, 2002), and at the same time exerts an effect on the endothelium by increasing endothelial NO bioavailability (Shimizu et al. 2003). Recent studies (Kawczynska-Drozdz et al. 2006) have shown that ghrelin counteracts vascular oxidative stress through the inhibition of vascular superoxide production.

A direct cardio-protective effect of ghrelin has also been observed, with ghrelin operating as a trophic local factor to inhibit apoptosis of cardiomyocytes and endothelial cells *in vitro* through the activation of extracellular signal-regulated kinase-1/2 and Akt serine kinases (Baldanzi et al. 2002). The signalling pathways underlying the vascular effects of ghrelin are being studied in more detail, extending to CD36, a multiligand scavenger receptor related to macrophage foam cell formation and the pathogenesis of atherosclerosis (Bodart et al. 2002), as well as addressing the impact of the hormone on cell adhesion molecule expression (Skilton et al. 2005). Taken together these findings highlight the involvement of ghrelin in the etiopathogenesis of hypertension as well as atherosclerosis.

**Ghrelin changes following bariatric surgery**

The last decade has witnessed major advances in the understanding of the basic metabolic pathways, brain circuitry, humoral responses and energy-consuming and -conserving processes, as well as psycho-social determinants of obesity (Horvath, 2005; Badman & Flier 2005; Fruhbeck, 2005c; Vaidya, 2006). A sustained positive energy balance over a prolonged period of time, resulting from imbalances between food intake and energy expenditure, results in weight gain. The apparent simplicity of the laws of thermodynamics contrasts with the intricate mechanisms involved in guaranteeing energy homeostasis. In fact, appetite control is governed by a complex interaction of multiple processes, which include the participation ofafferent signals from the gastrointestinal tract to provide information to the central nervous system.

In the current unabating overweight and obesity epidemic, bariatric surgery has been proven to be an effective therapeutic option for morbidly-obese carefully-selected patients with previous failure on conventional treatment (Buchwald et al. 2004; Steinbrook, 2004; Crookes, 2006; Hansen et al. 2006; O’Brien et al. 2006). One of the effects of bariatric surgery is to enhance satiety and reduce subjective hunger. Adjustable gastric banding represents a purely restrictive intervention. It is primarily designed to decrease food intake through the placement of a silicone...
band around the upper part of the stomach to produce a pouch of reduced dimensions. Restrictive procedures increase oesophageal and gastric distension in response to small amounts of food, eliciting an early satiety sensation. The proximal gastric bypass (RYGB) and bilo-pancreatic diversion are both mixed techniques. They combine a restrictive effect derived from a small gastric reservoir and rapid transit via the gastrointestinal system with an added malabsorptive component resulting from undigested food being quickly shunted into the large intestine in the bilo-pancreatic diversion. Circulating ghrelin concentrations have been reported to be suppressed in morbidly-obese patients following RYGB (Cummings et al. 2002), while no marked changes have been observed after adjustable gastric banding (Hanush-Enserer et al. 2003). This discrepancy has been attributed to the reduction in insulin secretion, which occurs as a result of improved insulin sensitivity and weight loss following adjustable gastric banding (Hanush-Enserer et al. 2003). However, the decrease in insulin resistance is even more evident in patients undergoing the RYGB. For this reason, the explanation of the discrepancies observed in circulating ghrelin concentrations after performing either a merely restrictive or a mixed procedure has been approached from a different perspective.

The isolation of ghrelin from the stomach represents a hallmark finding not only in the GH field, but also in appetite and energy balance control (Kojima et al. 1999; Diéguez & Casanueva, 2000; Inui et al. 2004). In both animals and man the greatest amount of ghrelin-immunoreactivity has been found in neuroendocrine cells of the gastric fundus (Date et al. 2000; Tomasetto et al. 2000; Ariyasu et al. 2001; Gnanapavan et al. 2002; Sakata et al. 2002). Most gastric ghrelin cells are closed-type cells that have no continuity with the lumen (Ariyasu et al. 2001), suggesting that they respond to physical stimuli from the lumen or chemical stimuli from the basolateral site, or both. As the most-frequently-performed bariatric surgery procedures are based on different mechanistic approaches in relation to the functional conservation of the fundus, it is hypothesised that the decrease in circulating ghrelin concentrations may depend on the impact of the selected surgical technique on the anatomy and physiology of the ghrelin-producing cells.

In order to avoid potential confounding influences, obese patients in the study of Frühbeck et al. (2004a) were matched according to BMI, excess weight loss and percentage body fat. The study provides evidence that the abnormally-low ghrelin concentrations observed after RYGB do not only depend on surgically-induced weight loss, but on the extent of dysfunctionality of the fundus. Thus, the reduction in circulating ghrelin concentrations in patients undergoing diverse bariatric procedures depends on the extent to which the surgical technique excludes the fundus and the subsequent isolation of ghrelin-producing cells from direct contact with ingested nutrients, which regulate ghrelin concentrations. In agreement with this reasoning it has further been shown (Frühbeck et al. 2004b) that the decrease in ghrelin concentrations in patients undergoing the RYGB takes place within 24 h after the surgical intervention, when potential confounding effects of weight loss and insulin sensitivity can be disregarded. A short-term dissociation of the leptin–insulin relationship in obese men following a bariatric intervention has previously been described (Frühbeck et al. 2002).

A prospective study has addressed the reduction in circulating ghrelin concentrations in patients undergoing the RYGB at 6 months after surgery. The results indicate that ghrelin concentrations are not determined by an active weight loss or an improved insulin sensitivity, but rather depend on the surgically-induced bypass of the fundus (Frühbeck et al. 2004c). This observation is not in agreement with the findings of other studies with a longer follow-up period in which the initial reduction in circulating ghrelin concentrations attributable to the RYGB has not been observed (Faraj et al. 2003). Plausible explanations for this controversial finding can be related to subtle differences in the surgical technique applied, which include the actual size of the stomach remaining, whether or not a vagotomy was performed and the large interindividual variability in gastrointestinal hormones in response to nutritional challenges (Cummings & Shannon, 2003; Morinigo et al. 2004; Jebb et al. 2006). In order to gain an insight into the real contribution of ghrelin changes to body-weight reduction following RYGB, further long-term prospective studies are needed that address these issues and also consider potential individual compensatory mechanisms in relation to changes in the absorptive and secretory capacities of the remaining digestive tract. The differences in the extent to which bariatric surgery changes the anatomical and physiological conditions of the stomach, and the variations in the subsequent adaptive responses may contribute in the long term to the different average weight loss of excess body weight achieved by patients undergoing a RYGB.

Future perspectives

Nutrition pervades all branches of medicine (Allison, 2005). An excess, a lack or even subtle imbalances of nutrients are among the causes of ill health. At the same time, diseases themselves can cause important nutritional and metabolic alterations. The wide spectrum ranging from a lack of nutrients to an excess of nutrients seamlessly embraces human development from intrauterine influences until death, exerting an undeniable effect on life expectancy. For this reason, nutritional care cannot be satisfactorily practised in isolation from other aspects of management and treatment, since drugs, fluid replacement, electrolyte balance and surgery affect nutritional status. Conversely, nutritional treatment exerts beneficial or detrimental effects according to its amount, composition and way of delivery and the pathophysiological circumstances in which it is given. The need to integrate healthy nutrition and lifestyles with the acquired knowledge and scientific background becomes especially important in halting the obesity pandemic.

Traditional boundaries among basic, clinical and patient-oriented research are merging into a single continuous bidirectional spectrum. In this spectrum studies involving basic science provide a physiological and molecular
foundation for the development of novel experiments that lead to clinical investigations that synergistically open up new areas of knowledge and successful implementation (Fig. 9). However, despite the unprecedented molecular and technological advances, great concern about the obstacles encountered by translational research has recently been recognised (Hörig et al. 2005; Zerhouni, 2005). In this context, the obesity epidemic represents an example worth considering. During the last decade energy-balance control has been an extremely active and productive research topic. However, this increased knowledge has not translated into improved medical care of obesity or more effective prevention strategies, with childhood obesity deserving particular attention. On the contrary, a perpetuation of the increases in the prevalence of obesity parallels a scenario in which, paradoxically, opportunities for diagnosis and treatment appear to be missed (Galuska et al. 1999; Frühbeck et al. 2003). The final aim of alleviating human suffering will remain elusive as long as scientific advances fail to drive changes in everyday clinical practice fostered by a robust, bidirectional information flow between healthcare professionals and research scientists.

‘Knowing is not enough – we must apply; willing is not enough – we must do’

Johann Wolfgang von Goethe

Concluding remarks

Obesity research is like a dynamic puzzle; as more pieces of the puzzle are found, more questions arise and more pieces are needed. Some of the many pieces of the complex ‘obesity puzzle’ have been already identified and put together to explain some of the underlying mechanisms of adipose tissue regulation. However, energy homeostasis is a particularly active research topic. Barely 1 week passes without the spotlight falling on some new potential regulator of appetite or body weight. Most probably, certain pieces may have been misplaced, forced to fit where they do not really belong, thereby providing a distorted view or sometimes contradictory findings, as may be the case with resistin. It may also be necessary to change certain facets of an already known molecule so that it actually adapts to the surrounding space and forms of other related pieces (Fig. 10). Certainly, missing pieces that complete the complex puzzle still need to be found. Despite the growing understanding of adipose tissue biology, critical pathways have yet to be identified. Given its versatile and pleiotropic nature, additional and unexpected roles of adipose tissue are bound to emerge. In this sense, it is important to maintain an integral view, provided by complementary approaches with an open-minded and functional perspective. The inscription Sir David Cuthbertson chose for his coat of arms on receiving his knighthood in 1965 was ‘Understand and nourish’. ‘Understand’ represents the goal of science and ‘nourish’ stands for the importance of nutrition. This combination represents the exciting challenge in which researchers are taking part, trying to follow Sir David Cuthbertson’s noble motto.

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