The adipocyte is emerging as a major drug target due to its central role in a vast array of pathophysiological processes that include obesity, diabetes, inflammation and cancer.

Keynote review:

The adipocyte as a drug discovery target

Andrea R. Nawrocki and Philipp E. Scherer

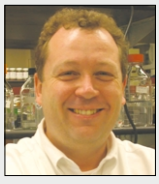
The adipocyte has pleiotropic functions beyond the storage of energy in times of nutrient abundance. Considerable efforts in adipocyte biology within the past ten years have emphasized the important role of adipose tissue in processes as diverse as energy metabolism, inflammation and cancer. Adipocytes are able to communicate with the brain and peripheral tissues implementing metabolic signals such as satiety, food intake and energy expenditure. Despite its huge pharmacological potential, only a small number of clinical applications interfere directly with adipocyte physiology. Here, we want to highlight various areas of adipocyte physiology that have not yet been explored pharmacologically and emphasize some of the limitations associated with these pharmacotherapies.

Excess adipose tissue is accompanied by a dramatically increased risk for the development of insulin resistance and type 2 diabetes mellitus. The higher propensity of obese individuals towards the development of dyslipidemia, hypertension, coronary heart disease and stroke is well appreciated [1]. In addition, many large-scale epidemiological studies highlight a relationship between body mass index and the incidence of some cancer types. Breast cancer is particularly susceptible to this interaction [2,3]. Some of the critical adipocyte-derived molecules that stimulate breast cancer growth have recently been identified [4] and offer great potential for therapeutic intervention, such as collagen VIα3.

Paradoxically, not only an excess of adipose tissue, but also the total absence of fat or the accumulation of adipocytes at the wrong places is associated with an increased risk for these complications [5]. This implies that the adipose tissue itself and the lipid and protein derivatives originating from it offer an attractive avenue to treat diseases related to abnormal adipose tissue mass. Currently available drugs

Andrea R. Nawrocki
Department of Cell Biology, Albert Einstein College of Medicine, 1300 Morris Park Ave., Bronx, NY 10461, USA
Philipp E. Scherer
Departments of Cell Biology and Medicine, Diabetes Research and Training Center, Albert Einstein College of Medicine, 1300 Morris Park Ave., Bronx, NY 10461, USA
e-mail: scherer@aecom.yu.edu

ANDREA R. NAWROCKI
Andrea R. Nawrocki is a postdoctoral fellow in the laboratory of Philipp Scherer at the Albert Einstein College of Medicine. Her work is focusing on the impact of adipocyte-derived factors on cardiovascular disease and the response of these factors to various pharmacological interventions, such as PPARγ agonist treatment. Andrea Nawrocki obtained her PhD from the University of Bern, Switzerland, studying the transcriptional regulation of 11β-HSD2 (11β-hydroxysteroid dehydrogenase type 2). She is currently funded by a fellowship from the American Heart Association.



PHILIPP E. SCHERER
Philipp E. Scherer is an Associate Professor in the Departments of Cell Biology and Medicine (Division of Endocrinology) at the Albert Einstein College of Medicine and a member of the Diabetes Research and Training Center and the NCI designated Cancer Center at the same institution. The Scherer laboratory has been involved for several years in the study of adipocyte-derived secretory products, with adiponectin and resistin proteins featuring as the main focus of the laboratory. Additional interests include the role of local inflammatory processes in adipose tissue as well as the specific contributions of adipocytes to early tumor growth in breast cancer. He obtained his PhD from the University of Basel, Switzerland, focusing on mitochondrial biogenesis and spent several years as a postdoctoral fellow at the Whitehead Institute at the Massachusetts Institute of Technology in Cambridge. His research is funded by several grants from the National Institutes of Health, the American Diabetes Association, the Komen Foundation, the Yamanouchi USA Foundation and he is the recipient of an Irma T. Hirschl Career Scientist Award.

1359-6446/05/$ – see front matter PII: S1359-6446(05)03569-5

TABLE 1

Areas of adipocyte physiology and their therapeutic potential for drug development

Therapeutic target area	Putative drugs	Advantage	Disadvantage	Therapeutic potential	Refs
Angiogenesis within adipose tissue	Vascular endothelial growth factor (VEGF) peptide inhibitors	Might inhibit growth of stromal tumors (e.g. breast cancer), Might be effectively inducing weight loss	Lipotoxicity: might result in hepatic steatosis and accumulation of lipids in muscle	High	[30,129]
Apoptosis of adipocytes		Might be effectively inducing weight loss	Might result in hepatic steatosis and accumulation of lipids in muscle	High	
Adiponectin administration	Recombinant protein	Acute and chronic insulin sensitization	Requires large amounts of recombinant protein	Moderate	
Leptin administration	Recombinant protein	Treatment of acquired and inherited leptin deficiency	Not useful to treat general obesity	High	[34]
Anti-inflammatory strategies in adipocytes	TZDs	Selective targeting of a major contributor of systemic inflammation	Weight gain when used in the context of PPARγ agonists	High	[54]
Cholesterol synthesis within adipocytes	Statins	Might have a positive impact on signaling and the release of inflammatory cytokines	Might have a negative impact on insulin sensitivity	Unknown	[106]
Lipid metabolism	Antisense oligos against SCD1 or PTP1B	Might reduce fat mass and increase insulin sensitivity	Stability and treatment modality of antisense oligos	High	[115,117]
Glucocorticoid metabolism	11β-HSD1 inhibitors	Might reduce fat mass and increase insulin sensitivity	Specificity: difficulty to target specifically to adipocytes	High	[60]
ROS production	Antioxidants	Will improve insulin sensitivity	Specificity: difficulty to target specifically to adipocytes	High	[119]

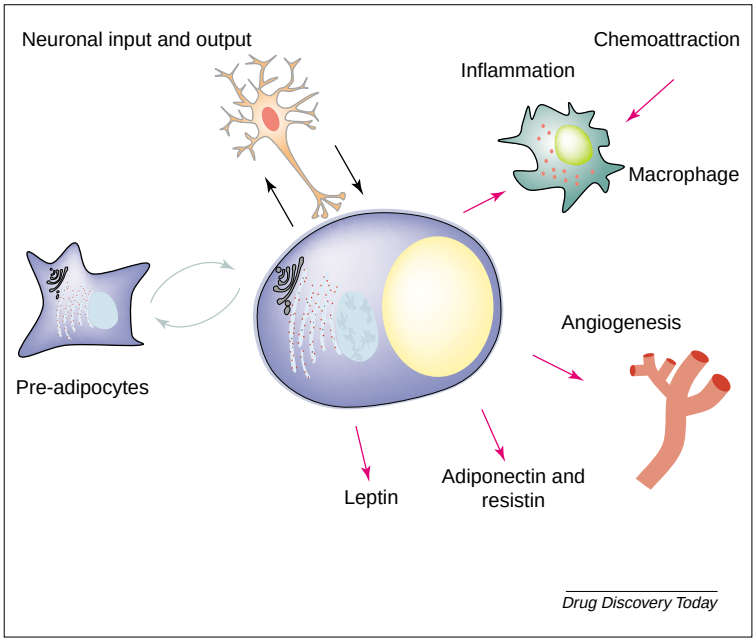


FIGURE 1

Extracellular, adipocyte-derived targets. Partial overview of areas with potential for pharmacological intervention: adipokines leptin, adiponectin and resistin as protein therapeutics or targets for neutralization; local angiogenesis inhibitors as anti-obesity therapeutics and anti-cancer therapeutics; attraction of macrophages into the growing fat pads and interference with local paracrine crosstalks between adipocytes and macrophages; selective neuronal input and output into adipose tissue as an area leading for a better understanding of adipose tissue crosstalk; preadipocyte differentiation as a pharmacological target.

stimulate anorexigenic signals in the central nervous system to suppress appetite or they inhibit nutrient absorption in the gut [6]. In spite of the growing understanding of the relationship between fat mass, diabetes and cardiovascular risk factors, only a few of the medications on the market interfere with the adipose tissue directly. The most prominent examples are the thiazolidinediones (TZDs), a class of peroxisome proliferator activated receptor γ (PPARγ) agonists that target the adipocyte at least with some specificity. Targeting the adipocyte seems particularly challenging because of its many overlapping functions and its integration into complex networks that are necessary for a proper fine-tuning of energy homeostasis (Table 1, Figure 1 and 2). As sensors of the body's metabolic status, adipocytes and the surrounding stromal cells produce and secrete a variety of hormonally active factors termed adipokines. These factors influence many metabolic processes such as the mobilization of lipids from fat, glucose production in the liver, as well as glucose and lipid utilization in skeletal muscle and β-cell function. Possible new therapies could involve adipogenesis, differentiation and proliferation of pre-adipocytes, adipose tissue vascularization, modulation of lipid metabolism, the release of inflammatory cytokines as well as the secretory pathway, mitochondrial energy dissipation and the production of reactive oxygen species by adipocytes (Table 1). Alternatively, a subset of adipokines secreted by adipocytes, such as

leptin and adiponectin, could be attractive in recombinant forms as therapeutic modalities for obesity-related complications (Table 1).

Targeting adipocyte differentiation: a promising approach?

Blocking the differentiation of adipocytes seems at first sight a very attractive and straightforward concept to regulate the amount of adipose tissue. This idea is based on the assumption that the presence of excess amounts of fat represents a health hazard. Many studies focusing on *in vitro* differentiation paradigms have described specific ways by which adipogenesis can be inhibited [7]. However, two fundamental issues curb the enthusiasm for this type of approach. First, adipogenesis is required to maintain the 'healthy' functions of adipose tissue. Clinically, it is well established that not only an excess of fat but also the lack (lipoatrophy) or an abnormal regional distribution of adipose tissue (lipodystrophy) is closely associated with insulin resistance, diabetes and cardiovascular disease [8]. Therefore, the localization and amount of adipose tissue is an important determinant of related metabolic complications. Excessive accumulation of visceral fat in the abdominal cavity is thought to be more harmful than the deposition of metabolically less active subcutaneous fat [9]. The presence of adipose tissue helps to sustain an insulin-sensitive state. This observation has been confirmed in rodent models of generalized lipoatrophy where the development of adipose tissue is blocked at the embryonic stage [10,11]. Such fatless mice are severely insulin resistant with defects in insulin signaling in the liver and skeletal muscle [12]. When these mice receive a transplant of fat tissue from a normal mouse, the phenotype is seemingly reversed. Detailed studies of fatless mouse models led to the conclusion that the absence of adipose tissue causes deleterious lipid depositions in liver, muscle and pancreas. The consequences are liver steatosis and fibrosis, insulin resistance and possibly β-cell dysfunction [13–15]. Triglycerides and free fatty acids (FFAs) have a negative impact on insulin sensitivity when they accumulate in tissues other than fat, a phenomenon that has been described as lipotoxicity. The lack of a sufficient number of adipocytes during excess caloric intake might, therefore, be detrimental. The issue is further complicated in constitutively fatless mice because of the lack of adipocytes during late embryonic development and in the early postnatal phase [10]. A more recent mouse model that we have developed in our laboratory permits the inducible ablation of adipocytes, thereby allowing an assessment of adipocyte function at a more acute time scale. This model will be discussed more extensively below [16].

The second reason why targeting adipogenesis might be of limited practicality is the very slow turnover of adipocytes in adults. Whereas estimates of adipocyte life span vary, there is a general consensus that adipocytes are very long-lived cells under normal physiological conditions. They might turn over within months or years [17,18], or might in fact not turn over at all. This suggests that during stable weight maintenance, interfering with adipogenesis could have a minimal impact within the limited time span, during which such a pharmacological approach could be sustained. However, during periods of significant and acute weight gain, interfering with *de novo* adipogenesis could be effective. Adipocyte mass is defined by the average size and the overall number of adipocytes. Long-term changes in adipocyte mass involve both the size and number of cells. Whereas existing adipocytes become engorged with lipids and increase in size, further exposure to caloric excess prompts *de novo* differentiation of adipocytes, a process that occurs throughout adult life [19]. However, as mentioned before, blocking deposition of lipids in adipose tissue might partition these lipids to tissues such as skeletal muscle and liver, which is not desirable due to their toxic effects on insulin sensitivity [20].

Targeted apoptosis of adipocytes

De novo differentiation of adipocytes from precursor cells is virtually irreversible. One of the reasons for the slow or non-existing turnover is the adipocyte's remarkable resistance to apoptosis [21]. The underlying mechanisms for the hardiness of this cell type are not clear, but can be explained in part by the very high Akt/protein kinase B levels in mature adipocytes. Furthermore, the levels of the anti-apoptotic factors Bcl-2 and neuronal apoptosis-inhibitory

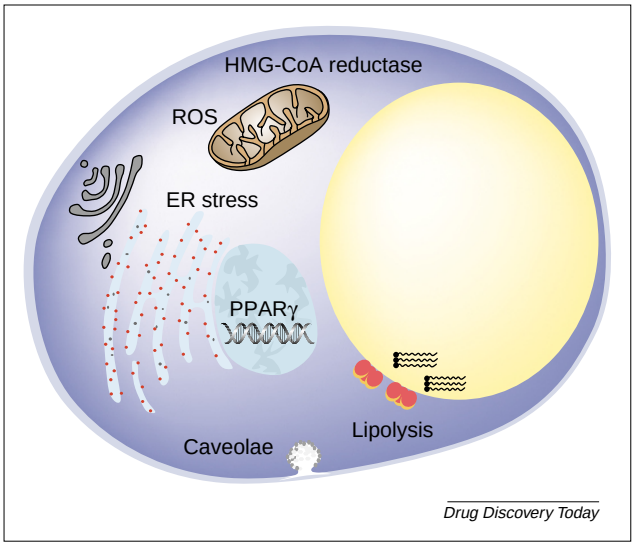


FIGURE 2

Intracellular targets for pharmacological intervention in the adipocyte. A better understanding of PPARγ agonist action will continue to contribute greatly to our understanding of adipocyte physiology. Statin action (HMG-COA inhibitors) remains vastly unexplored in adipocytes. Therapeutic approaches towards an improvement of mitochondrial dysfunction and lowering ROS levels are likely to have a great impact on local insulin sensitivity. Stress in the endoplasmic reticulum might not only affect pancreatic β cells, but also the adipocyte. Processes enhancing lipolysis might be targets to reduce adipose tissue mass. Caveolae and their major structural and regulatory components, caveolins, are attractive targets for the manipulation of a vast number of processes (e.g. signaling, FFA uptake, protein trafficking).

TABLE 2

Function and therapeutic potential of adipokines released from adipose tissue

Adipokine	Function	Therapeutic potential
Adiponectin	Hepatic insulin sensitizer	Protein therapeutic
Resistin, RELMβ	Hepatic insulin desensitizers	Therapies targeted toward neutralization
Leptin	Lipostat	Protein therapeutic
IL-6	Inflammatory cytokine, inducer of lipolysis	Unknown
TNFα	Inflammatory cytokine	Therapies targeted toward local neutralization
PAI-1	Hemostasis	Unknown
SAA3	Acute phase reactant	Unknown
α1-Acid Glycoprotein	Acute phase reactant	Unknown
24p3	Lipocalin	Unknown
Pentraxin-3	Acute phase reactant	Unknown
Collagen VIα3	Extracellular matrix protein, mitogen	Anti-cancer therapies

protein increase during adipogenesis, imparting resistance to cell death [22,23]. Caloric restriction leads to reduction of fat mass, but it does not necessarily involve a reduction of the number of fat cells. However, conditions of pathological fat wasting can involve loss of adipocytes through apoptotic mechanisms. For example, apoptotic events were observed in fat tissue of patients with tumor cachexia. Apoptosis was also implicated in the fat remodeling processes associated with highly active antiretroviral therapy in HIV infected patients with lipodystrophy [24–27]. Targeted and moderate induction of apoptosis in adipocytes could be an attractive approach to reduce the number of fat cells, particularly when coupled with a reduction of caloric intake to avoid spill over of lipids into other tissues. However, at this time little is known about the apoptotic machinery in adipocytes and thus therapeutic modalities relying on a selective induction of apoptosis within this cell type remain a distant prospect.

Prevention of adipose tissue vascularization

Is it possible to regulate adipose tissue mass by targeting its vasculature? This type of approach is based on the trials to inhibit tumor growth by inhibiting its neovascularization [28]. Angiogenesis inhibitors could be deployed to ablate the blood vessels that supply adipose tissue with the necessary nutrients [28]. Adipose tissue is highly vascularized and expansion involves the formation of new capillaries. Initially, Folkman and colleagues demonstrated that systemic treatment of obese mice with anti-angiogenic agents induced a loss of white adipose tissue [28]. These results suggested that neovascularization is required for adipose tissue maintenance and that there might be a constant remodeling process sustaining the viability of the tissue [29]. Therefore, adipose tissue vasculature might be responsive to anti-angiogenic agents in the absence of additional growth. These results (systemic exposure to anti-angiogenic agents) provided a proof of principle for the feasibility of the approach, but are clearly not practical as a therapeutic modality because of their lack of specificity. To address this problem, Kolonin *et al.* [30] used the phage-display technique to identify selective targets within the adipose tissue endothelium. They delivered proapoptotic molecules specifically to the vasculature of white adipose tissue and managed to induce a highly significant weight loss in obese mice that was accompanied by a reduction of lipid depositions in liver and muscle. These results are very promising and represent examples of potential new classes of future therapeutic agents.

Adipokines: viable drug targets?

The therapeutic potential of adipose tissue-derived factors was initially recognized upon the discovery of leptin. Leptin is an adipocyte-specific protein that acts as satiety signal from fat to the brain [31]. In addition, several hormonally active factors have been discovered that are produced uniquely in adipocytes and might serve directly as drug targets (Table 2) [32]. Many of these proteins, referred to as adipokines, play a role in obesity-related metabolic complications. The release of adipokines from fat is regulated as a function of the system's metabolic state and in response to inflammatory signals. Therefore, conditions of abnormal adipose tissue mass result in the dysregulation of adipokines [33]. Replenishment of recombinant forms of adipokines that are downregulated in obesity or the depletion of selective adipokines that are produced in excess in the obese state might have a powerful therapeutic impact. Alternatively the stimulation or the reduction of the release of these proteins from adipocytes could represent an additional approach to alter circulating levels of these proteins.

Leptin

To date, the success of adipokine therapy has been somewhat limited [34]. Leptin supplementation is currently used clinically to treat patients with congenital leptin deficiency. These patients are hyperphagic and morbidly obese and the administration of recombinant leptin leads to a dramatic reduction of body weight [35]. Leptin deficiency is also found in acquired and inherited lipodystrophy that is accompanied by fat loss in selective areas of the

body. These rare conditions are associated with severe insulin resistance, dyslipidemia and hepatic steatosis [14]. Leptin-replacement therapy in these patients leads to a correction of the hyperglycemia and a reduction in plasma lipids, thus helping to minimize the risk of metabolic complications [36]. Nevertheless, the results of clinical trials with recombinant leptin as anti-obesity drugs remained disappointing. In generalized obesity, administration of leptin is ineffective because leptin levels increase in proportion to adipose mass and are relatively high in obese patients. Leptin resistance rather than lack of circulating leptin is the underlying reason, such that leptin replacement therapy is of limited value in this context [37].

Adiponectin

A promising therapeutic adipokine is adiponectin. Adiponectin (also known as Acrp30) is a complex protein secreted specifically from adipocytes. Low serum levels of adiponectin are causally linked to insulin resistance and are predictive for the development of diabetes and cardiovascular disease [38]. A sexual dimorphism exists for plasma adiponectin levels with significantly higher concentrations in the serum of females [39]. Obesity is associated with decreased adiponectin levels [40]. Promising results have been obtained for adiponectin as a therapeutic agent in numerous animal experiments and human epidemiological studies. Adiponectin is a multifunctional protein with protective roles against the development of insulin resistance, dyslipidemia, nonalcoholic fatty liver disease, atherosclerosis, cardiac hypertrophy and ischemic injury [32,41,42]. Beyond that, adiponectin has been shown to act in the brain to decrease body weight [43]. However, the understanding of the molecular mechanisms by which adiponectin achieves these effects is still poor. The production of recombinant adiponectin is challenging because of the complex tertiary and quaternary structure of the protein and the distinct activities of the different isoforms. Adiponectin, like other adipokines (such as resistin), is s ecreted from the adipocyte as distinct complexes [44]. Adiponectin can form trimers (the basic building block for the higher-order complexes), hexamers consisting of two trimers, and higher molecular-weight forms (HMW) consisting of up to 12–18 subunits [45]. Proper folding and assembly into these higher-order structures depend on posttranslational modifications that can be achieved only in mammalian production systems. Adiponectin contains a collagenous tail domain and a globular head domain. The subunits are linked through intermolecular cysteine bonds. A globular form of adiponectin produced in *Escherichia coli* possesses biological activity distinct from the mammalian produced full-length protein. Whereas a pharmacological dose of the latter improves hepatic insulin sensitivity in lean mice and even more effectively in obese animals [46], the former was shown to activate fat oxidation in skeletal muscle [47]. Interestingly, a mutant version of adiponectin that lacks a critical cysteine residue in the collagen domain is considerably more bioactive than wild-type adiponectin [45]. Mice overexpressing this mutant form of adiponectin (Cys39Ser) in a genetically obese background (leptin deficient *ob/ob* mice) display massive adipose tissue accumulation (unpublished observations from our laboratory). These morbidly obese animals are however metabolically very healthy with nearly normal glucose and lipid levels. These observations suggest a processing step in the activation cascade for adiponectin that converts the HMW form into the smaller short-lived trimer, possibly involving a serum reductase or protease. Clearly, many fundamental questions remain to be answered on the relationship between structure and function of adiponectin [45].

Resistin and resistin-like molecules

Resistin has been proposed as another adipokine involved in the complex etiology of insulin resistance [33]. In rodents the cysteine-rich 10 kDa protein resistin is secreted mainly from adipocytes, whereas in humans its main source is peripheral-blood mononuclear cells [48]. In contrast to adiponectin, resistin levels increase approximately twofold in mice fed a high-fat diet, leading to severe hepatic insulin resistance [49]. The neutralization of resistin with a specific antibody or the downregulation of resistin to normal levels via antisense technology is effective in re-establishing normal insulin sensitivity in these rodent models [49, 50]. Although the unusual hexameric tail-to-tail conformation of resistin and its closest family member resistin-like molecule (RELM) β suggests a receptor clustering mechanism of activation, the discovery of corresponding receptors remains elusive [44]. Intriguingly, infusion of the intestinally produced RELMβ has similar effects on hepatic insulin sensitivity as does the infusion of the adipocyte-derived resistin, suggesting related signaling mechanisms between nutrient absorbing tissues and nutrient-storing tissues [51]. Although the functional role of resistin in humans is not well established, this small hormone represents an interesting candidate from a pharmacological perspective. As it is the case for adiponectin, minor variations in the circulating levels of these adipokines might have potent therapeutic effects.

Unraveling the thiazolidinediones

The most prominent examples of drugs favoring a 'healthy' adipokine profile are the TZDs. TZDs are the first class of agents that directly target the adipocyte. These drugs (rosiglitazone and pioglitazone) are widely used to ameliorate insulin sensitivity in patients with type 2 diabetes. They are potent inducers of adipogenesis, and many metabolically relevant adipocyte-derived proteins are regulated in response to TZDs (adiponectin, resistin, tumor necrosis factor α (TNFα), 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1), the fatty acid-binding protein aP2, and others). TZDs are selective ligands of the nuclear

transcription factor PPARγ. PPARγ is expressed at highest concentrations in adipocytes and it is considered the 'master switch' of adipocyte differentiation [52,53]. Several endogenous, mostly hydrophobic ligands for PPARγ have been identified with various affinities for the receptor. Although the precise mechanisms of the insulin sensitizing action of TZDs are not well understood, it is clear that adipose tissue is the critical tissue for the glucose-lowering effects of TZDs. Most certainly, a reduction of lipotoxicity in other tissues by partitioning lipids into adipose tissue is one aspect of TZD action [54]. However, an indirect mechanism, i.e. through the induction or repression of adipokines such as adiponectin and resistin, is another likely mechanism of action. Clinical studies have shown that improvements in insulin sensitivity after treatment with rosiglitazone are strongly associated with an increased release of the HMW form of adiponectin [55,56]. The ratio between the HMW over the total amount is directly related to the biological activity of adiponectin [55]. The hypothesis that adiponectin might be a mediator of TZD action is underlined by the observation that a chronic elevation of adiponectin in a mouse model has many features similar to prolonged TZD treatment in diabetic patients [57]. These might include wanted and unwanted effects such as increased weight gain or, on the beneficial side, reduced liver fat content, increased lipid clearance, reduction of inflammatory markers and protection of the vascular endothelium from the development of atherosclerotic plaques. TZDs as well as adiponectin might exert direct effects on lipid metabolism and the vascular wall. Low levels of PPARγ are found in macrophages, endothelial cells, vascular smooth muscle cells and other cell types. These low levels can change under pathophysiological conditions. For instance, PPARγ is elevated in macrophage foam cells of atherosclerotic lesions and appears to be directly involved in the regulation of both the anti- and pro-atherogenic genes [58,59]. The only FDA-approved clinical indication for the prescription of TZDs is type 2 diabetes, however the range of TZD effects might be considerably wider than just regulating insulin sensitivity [54].

Inhibition of 11-β hydroxysteroid dehydrogenase type 1

11-β hydroxysteroid dehydrogenase enzymes (11β-HSD) type 1 and 2 regulate the activity of glucocorticoids and mineralocorticoids at the pre-receptor level [60]. The type 1 isoform (11β-HSD1) has received increasing attention recently thanks to its putative role in the development of the metabolic syndrome. 11β-HSD1 expression is rather ubiquitous throughout the body, with highest levels in the liver, adipose tissue and the brain, and protein levels are subject to regulation by inflammatory mediators such as TNFα and interleukin (IL) 1β [61,62]. The enzyme functions primarily as a reductase converting hormonally inactive cortisone into the high-affinity ligand cortisol. Glucocorticoids are potent inducers of adipocyte differentiation in *in vitro* systems and 11β-HSD1 activity increases during adipogenesis. Accordingly, an excess of glucocorticoids is associated with the development of obesity. This becomes apparent in patients with the Cushing's syndrome, a condition of pathophysiologically elevated glucocorticoids resembling the metabolic syndrome [63]. Transgenic mice overexpressing 11β-HSD1 selectively in adipose tissue also display the features of the metabolic syndrome [64]. Interestingly, 11β-HSD1 knockout mice are resistant to high-fat diet-induced obesity and have reduced resistin and elevated adiponectin levels, suggesting a possible indirect insulin-sensitizing effect of a pharmacological inhibition of this enzyme [65]. Clinical studies to test selective 11β-HSD1 inhibitors are to be expected in the near future and will help to further elucidate the role of this enzyme in regulating fat mass and insulin sensitivity.

Paracrine roles of adipocytes: therapeutic implications

The recognition that adipocytes are active endocrine entities requires reconsideration of the role of adipocytes residing within tissues that are not conceptually designed as fat depots. The effects of adipocyte-derived hormonal factors on neighboring cells in the local environment is of particular interest in obesity and, as appreciated only recently, in bone related diseases as well [66]. In most conditions associated with bone loss, adipogenesis occurs at the expense of osteogenesis and the amount of adipocytes within the bone marrow is reciprocally related to bone mass [67]. This is true for all conditions with an imbalance between osteoblast formation and bone resorption, including age-related osteoporosis, treatment with glucocorticoids, prolonged immobility or menopause [68–71]. Bone marrow adipocytes and bone-forming osteoblasts originate from common multipotent mesenchymal stem cells [72]. PPARγ is a major regulator committing these cells towards adipogenesis while inhibiting osteogenesis and promoting osteoblasts apoptosis [73]. In this context, the safety of TZD treatment has to be considered as it might accelerate bone loss in some cases [74,75]. By contrast, the statin drugs that are widely used to lower cholesterol in the circulation have been shown to prevent bone loss possibly by suppressing PPARγ activity [76]. Mesenchymal stem cells are precursors not only for fat and bone but also for muscle cell lineages [72]. PPARγ ligands were shown to promote transdifferentiation of myoblasts towards an adipocyte-like phenotype, most probably by inhibiting the myogenic factor MyoD1 [77,78]. Adipocyte cells themselves can disperse and expand within skeletal muscle fibers [79]. This is of pharmacological interest, because intra-muscular adipocytes might modulate the insulin sensitivity of neighboring myocytes through their local release of adipokines, cytokines and FFAs, emphasizing the potential and the limitations of interfering with adipogenesis in specific areas of the body [80,81].

Selective targeting of inflammation in adipose tissue

Inflammation and atherosclerosis

Recent research efforts have reinforced the concept that inflammation is a common component of many obesity-related co-morbidities [33]. There is a subtle but significant elevation of baseline inflammation in patients with obesity and diabetes [82]. This chronic increase in the inflammatory tone is strongly associated with an increased risk for the development of cardiovascular disease [83]. Chronically elevated lipid and glucose levels present in obese and diabetic patients pose a permanent stress on the vascular endothelium that is aggravated by inflammatory proteins and ultimately promotes the development of atherosclerosis. In recognition of this phenomenon, the American Heart Association recently examined whether the measurement of inflammatory markers is useful and applicable for routine risk assessment of cardiovascular complications in public health practice [84]. Adipose tissue is most certainly a major contributor to systemic inflammation and the expression of inflammatory cytokines is increased in obesity. Among the many other cytokines, adipose tissue produces and secretes TNFα, IL-6 and IL-1β (Table 2). In addition, adipocytes secrete high levels of acute-phase proteins including serum amyloid A3 (SAA3), plasminogen activator inhibitor-1 (PAI-1), C-reactive protein (CRP), the lipocalin 24p3, pentraxin-3, and α1-acid glycoprotein [85,86]. It remains to be demonstrated whether increases in baseline levels of these proinflammatory factors are directly causative of or merely correlative to cardiovascular problems. The correction of lipid abnormalities either through dietary changes or using lipid-lowering drugs, such as statins or fibrates, is the primary goal to control the risk of cardiovascular disease. However, the reduction of inflammation is likely to prevent the progression of already established atherosclerotic lesions and the fatal event of plaque rupture and artery occlusion.

Inflammation and insulin resistance

Targeting inflammation also might be an attractive approach for the treatment of insulin resistance. There is no doubt that low-grade inflammation plays a key role in the development of insulin resistance [87]. In recent years, great progress has been made towards understanding the mechanisms that link inflammation with metabolic pathways. Proinflammatory signals can interfere with early steps of the insulin signal-transduction cascade at the level of the insulin receptor and its downstream target insulin receptor substrate-1 (IRS-1) [88]. Intriguingly, deletion of distinct proinflammatory mediators primarily results in a phenotype affecting the adipose tissue. For instance, c-Jun N-terminal kinase knockout mice gain less weight than the wild-type controls and are more resistant to high-fat diet-induced insulin resistance [89]. Furthermore, mice lacking TNFα display insulin-sensitive adipocytes and are also protected against obesity-induced insulin resistance [90]. Increased insulin sensitivity at the level of the adipocyte is beneficial in several aspects. Beyond the insulin-dependent inhibition of lipolysis with a subsequent reduction of serum FFAs, the release of proinflammatory proteins into the circulation is reduced as well. High doses of aspirin targeted towards inhibition of the nuclear factor kappa B (NFκB) pathway might be useful to prevent obesity-induced insulin resistance [91]. NFκB signaling is enhanced in the livers of genetically obese mice and mice with diet-induced obesity [92,93]. Any pharmacological approach targeted towards inhibiting inflammation selectively in adipose tissue has the potential to improve systemic insulin sensitivity. The most prominent examples are once again the TZDs. These drugs, in addition to their antidiabetic effects, have potent anti-inflammatory properties, although the precise mechanisms are still unknown. The reduction of the local inflammatory environment within the adipose tissue might alter the expression profile of adipokines. Adiponectin transcription is highly susceptible to some types of inflammatory stimuli [38]. The reduction of local levels of TNFα and IL-6 might therefore significantly upregulate the secretion of adiponectin complexes.

In addition, inhibition of intrinsic adipose tissue inflammation might lead to decreased levels of systemic inflammation. The overall efficacy and potential risks of targeting systemic inflammation with respect to improving insulin sensitivity remain to be further evaluated. For example, although blocking of TNFα in the circulation of mice has beneficial effects on insulin sensitivity, two early studies in humans using either a single injection of a TNFα antibody or a TNFα receptor fragment to block its activity have failed to improve insulin sensitivity [94,95]. Similarly, a study in morbidly obese subjects found little effect on insulin sensitivity, presumably because of the ineffective neutralization at the local level in adipose tissue and muscle [96]. More recent observations with the TNFα antibody infliximab, currently prescribed to treat rheumatoid arthritis, point to a possible benefit regarding insulin sensitivity. These studies suggest that a chronic treatment rather than a single administration with TNFα blocking agents is required to affect insulin sensitivity [97].

Involvement of the adipocyte in selective infectious disease states

Previous studies have indicated that hyperglycemia significantly increases parasitemia and mortality in mice infected with *Trypanosoma cruzi* [98]. We recently specifically focused on the consequences of adipocyte infection *in vitro* and *in vivo* [99]. Cultured 3T3-L1 adipocytes can be infected at high efficiency with *T. cruzi*. Similarly, high levels of parasites can be found in primary adipocytes *in vivo*. The intracellular parasites cluster around lipid droplets, suggesting that the parasites have a high affinity for the surface of these lipid droplets. The adipocyte is therefore an important target cell during acute Chagas' disease. Infection of adipocytes by *T. cruzi* has an effect on the expression pattern of adipokines [99]. During

chronic infection, adipocytes are an important long-term reservoir for parasites from which relapse of infection can occur. The acute infection manifests a unique metabolic profile, with a high degree of local inflammation in adipose tissue, hypoadiponectinemia, hypoglycemia and hypoinsulinemia [99]. The adipocyte could therefore be a significant player in the initial proliferative phase of parasite infection, in the chronic phase of infection as well as an integral contributor to the host inflammatory response.

Targeting adipose tissue macrophages

Even though the adipocyte itself expresses many proinflammatory cytokines and acute phase reactants, macrophages residing within the adipose tissue play a considerable role in the inflammatory response observed in obesity [100]. There is great plasticity between adipocytes and macrophages [101]. Adipose tissue of obese individuals is subject to increased infiltration of macrophages [102,103]. Whereas the signals responsible for the infiltration of macrophages into adipose tissue remain to be analyzed in further detail, there is little doubt that these macrophages have a profound impact not only on the local proinflammatory environment but also on the net levels of systemic inflammation. We have recently developed a mouse model of inducible lipoatrophy [16]. Interestingly, in these mice we observe a significant drop in systemic inflammatory markers after the acute ablation of adipocytes. This effect is even more pronounced after stimulation with endotoxin, revealing that the lipoatrophic state is associated with a significantly reduced systemic inflammatory response. This leads to the provocative, yet untested, hypothesis that in the context of acute sepsis a repression of local inflammation in adipose tissue might be therapeutically beneficial.

Adipose tissue macrophages have a unique expression profile compared to macrophages in other tissues because of their proximity with surrounding adipocytes. Upon acute ablation of the adipocytes in our inducible lipoatrophic model, the remnant adipose pad is infiltrated with a large number of macrophages. These macrophages do not manifest the proinflammatory characteristics of their counterparts in intact adipose tissue [16]. Within the limits of an *in vitro* system this can be mimicked efficiently by exposing macrophages to conditioned medium from adipocytes [104]. Adipocyte-conditioned medium has unique proinflammatory properties on macrophages that are not observed with the medium from other cell types. The components responsible for this inflammatory response remain to be characterized, but these observations suggest that conceptually the inhibition of inflammation within the adipose tissue might be a powerful pharmacological strategy to lower systemic inflammation.

Statins and inflammation: is the adipocyte in the picture?

Statins are widely used for the management of plasma cholesterol levels in patients at risk for cardiovascular disease. This class of drugs comprises inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, an enzyme involved in cholesterol synthesis primarily in the liver. The scope of statin action however is not limited to the lowering of blood cholesterol [105]. Several recent studies highlight the importance of anti-inflammatory aspects of statin action [106]. As outlined above, the reduction of general inflammatory markers is advisable for the reduction of cardiovascular events along with an improvement of the lipoprotein profile. The levels of CRP correlate with the risk for the development of cardiovascular disease [107]. While statins have been shown to efficiently lower CRP, it is still a matter of debate whether the reduction of CRP *per se* leads to a reduction of cardiovascular events or whether it merely serves as a sensitive but indirect indicator for other inflammatory factors that have a more direct impact on disease progression [108]. CRP is expressed in adipose tissue, but adipose tissue is unlikely to be a major systemic source for this protein. An alternative explanation is that statins target adipocytes directly and might achieve some of their beneficial effects by altering the composition of adipocyte plasma membranes or adipokines. This is an area that has remained relatively unexplored to date. Hauner and colleagues analyzed differentiated adipocytes *in vitro* after statin treatment and found a reduction of IL-6 secretion [106]. It is possible that lowering the local production of cholesterol in adipocytes can have an impact on adipocyte signaling. Lipid raft structures that form caveolar invaginations in the plasma membrane are highly abundant in adipocytes (making up to 20% of the cell surface of adipocytes) [109]. These membrane domains critically depend on cholesterol, which is highly enriched in raft structures [110]. Lipid rafts serve as important relay stations for signal transduction events by concentrating signaling receptors. In addition, lipid rafts are the major sites for the translocation of FFAs, as suggested recently by Stralfors and colleagues [111], and because of their unique lipid composition they might help to maintain the stability and integrity of the plasma membrane while FFA travel across the plasma membrane. The translocation of FFA is facilitated by binding to transport proteins such as caveolin-1, fatty acid binding protein (FABP-1) and fatty acid translocase (FAT/CD36) [112]. These proteins are recruited to the lipid rafts and regulate the storage and the mobilization of FFA from the adipocytes. Adipocytes isolated from caveolin-1 deficient mice have decreased insulin receptor levels and an impaired response to β3 adrenergic stimulation of lipolysis [113]. The role of lipid rafts in the pathogenesis of insulin resistance remains to be further investigated. Perturbations within these caveolar structures have a profound impact on many signaling pathways and therefore they offer attractive pharmacological targets. It remains to be examined whether statins have an effect on adipocytes, and if they do, whether the effects can be linked to local cholesterol levels.

Modulating adipocyte lipid metabolism by antisense oligonucleotides

Adipocytes provide a unique machinery to respond to metabolic challenges by rapidly providing energy in the form of FFAs during fasting and exercise and preserving excess energy through re-esterification of FFA and deposition in lipid droplets. Recently, pharmacologic approaches to modulate lipid metabolism have received intense interest from the pharmaceutical industry. Emerging drug targets within adipocytes include enzymes involved in lipolysis (hormone-sensitive lipase and perilipin) and lipogenesis (diacylglycerol acyltransferase, DGAT). Besides natural and synthetic small molecule inhibitors, the pharmacologic application of antisense oligonucleotides (ASOs) has evolved as a novel strategy to treat obesity and associated metabolic diseases. Improved chemistry and modifications of the backbone enhance stability *in vivo* and provide good tissue distribution and decreased toxicity of these molecules, thus effectively inhibiting the expression of target genes [114]. In animal models, ASOs were used successfully to reduce fat mass, liver steatosis and increase insulin sensitivity in mice treated with ASOs specifically inhibiting stearoyl-CoA desaturase -1 (SCD-1) [115]. Furthermore, the treatment of obese and hyperglycemic *ob/ob* mice with ASOs directed against protein tyrosine phosphatase-1B (PTP1B), an important regulator of insulin signaling, resulted in reduced adiposity and a reduced expression of lipogenic genes in fat [116,117]. These ASOs (ISIS-113715) are undergoing initial clinical trials for the treatment of diabetes and hyperlipidemia [118].

Nutrient excess and the role of reactive oxygen species in the cellular homeostasis of the adipocyte

The secretory pathway

Adipocytes fail to downregulate basal glucose uptake in response to hyperglycemic conditions. Excess nutrients that enter adipocytes under these conditions lead to the production of reactive oxygen species (ROS). Like in endothelial cells, oxidative stress induces an inflammatory response in adipocytes and is associated with reduced insulin sensitivity [119,120]. Decreasing the levels of ROS leads to a decrease in the inflammatory status of the cell and consequently is associated with improved insulin sensitivity. A pharmacological approach targeted towards the prevention of ROS production or aimed at the quenching of excess ROS levels in adipocytes might therefore be relevant for generalized systemic improvements of metabolic parameters in obesity. In addition, excessive ROS disturbs the cellular glutathione homeostasis, which might have an impact on the efficiency of the secretory apparatus. The secretory apparatus of adipocytes is very active and therefore quite susceptible to such disturbances. Furthermore, metabolic changes that alter the efficiency of protein assembly by the secretory apparatus tend to affect highly active secretory cells like adipocytes. These include changes in the levels of chaperones, the reduced ability to reshuffle disulfide bonds or additional changes that lead to the induction of the unfolded protein response (UPR) in the secretory pathway [121]. Adipokines, such as adiponectin and resistin, critically depend upon proper folding and assembly of critical disulfide bonds. Clearly, the rate at which the various isoforms of these proteins are generated and released from the adipocyte is governed by chaperones, some of which are among the most highly induced proteins within the cell upon exposure to TZDs [122]. Mutations in the UPR pathway as well as in thioredoxin-interacting protein lead to phenotypes in the liver and in insulin-secreting pancreatic β cells, but they have also an adipocyte-specific phenotype [123,124]. A pharmacological approach enabling the secretory pathway in the endoplasmic reticulum (ER) to sustain a healthy environment for protein folding under conditions of stress is, therefore, a promising option. It is known that obesity *per se* triggers enhanced ER stress in liver and in the adipocytes [123].

The hexosamine pathway

Excess nutrients also cause the induction of the hexosamine biosynthetic pathway. This pathway has been proposed to act as a nutrient-sensing pathway, protecting cells against abundant fuel supply [125]. The accumulation of hexosamines is one of the biochemical mechanism by which hyperglycemia and hyperlipidemia induce insulin resistance [126,127]. The acetylated aminosugar nucleotide uridine 5′-diphospho-N-acetylglucosamine (UDP-Glc-NAc) is the end product of this pathway and the donor substrate for N-acetylglucosamine (O-GlcNAc) modification of nucleocytoplasmic proteins at serine and threonine residues. Transgenic overexpression of the rate limiting enzyme for hexosamine synthesis (glutamine:fructose-6-phosphate aminotransferase, GFAT) in adipose tissue results in skeletal muscle insulin resistance and altered regulation of key adipokines [128]. The cellular effects are primarily mediated through O-GlcNAc modification of key proteins in the insulin signal-transduction cascade, such as IRS-1, Akt and glycogen synthase. Approaches that impair the O-GlcNAc modification of proteins or enhance the deglycosylation of modified polypeptides in adipocytes can be considered. However, it is not clear whether these modifications have a role in normal cellular homeostasis and whether inhibition of this process can have other side effects.

Conclusions

The adipocyte as a drug target has tremendous potential due to its intricate involvement in a vast array of physiological processes (Table 1). There is overwhelming evidence that the adipocyte is at the center of dysfunctional regulatory cascades in many disease states, ranging from obesity, diabetes, cancer, infectious disease and inflammation. Many aspects of this involvement can be explained on the basis of adipocyte-derived secretory proteins. Some of the extracellular, adipocyte-derived targets are highlighted

in Figure 1. Targeting of the adipocyte as a cell remains more challenging. Possible intracellular targets are highlighted in Figure 2. In the case of TZDs high expression levels of PPARγ in adipocytes is taken advantage of. The presence of triglycerides will concentrate hydrophobic agonists preferentially in adipose tissue. However, this is frequently more of a disadvantage than an advantage because these lipid-soluble agonists might be unavailable within the aqueous remainder of the cell. The relative paucity (in fact, the complete absence) of adipocyte-specific cell surface markers makes the targeting through antibodies very difficult. Perhaps most importantly, there are no markers that will identify a given fat pad specifically. The gene expression pattern between a subcutaneous and a visceral fat pad shows distinct differences, however, they are quantitative rather than qualitative in nature. These issues remain major challenges for the development of cell- and fat pad-specific approaches to drug therapy in the adipocyte. Neverthrless, the large number of possible target molecules with important physiological impact continues to make the adipocyte and its products an attractive area of research.

Acknowledgements

A.R. Nawrocki is supported by a postdoctoral fellowship from the American Heart Association. P.E. Scherer is supported by NIH grants R01-DK55758, R01-CA112023, and R03 EY014935–01.

References

1 Glass, C.K. and Witztum, J.L. (2001) Atherosclerosis. the road ahead. *Cell* 104, 503–516
2 Calle, E.E. and Kaaks, R. (2004) Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms. *Nat. Rev. Cancer* 4, 579–591
3 Iyengar, P. *et al.* (2003) Adipocyte secreted factors synergistically promote mammary tumorigenesis through induction of anti-apoptotic transcriptional programs and proto-oncogene Stabilization. *Oncogene* 22, 6408–6423
4 Iyengar, P. *et al.* (2005) Adipocyte-derived collagen VI affects early mammary tumor progression *in vivo*, demonstrating a critical interaction in the tumor/stroma microenvironment. *J. Clin. Invest.* 115, 1163–1176
5 Grinspoon, S. and Carr, A. (2005) Cardiovascular risk and body-fat abnormalities in HIV-infected adults. *N. Engl. J. Med.* 352, 48–62
6 Shi, Y. and Burn, P. (2004) Lipid metabolic enzymes: emerging drug targets for the treatment of obesity. *Nat. Rev. Drug Discov.* 3, 695–710
7 Harp, J.B. (2004) New insights into inhibitors of adipogenesis. *Curr. Opin. Lipidol.* 15, 303–307
8 Spiegelman, B.M. and Flier, J.S. (2001) Obesity and the regulation of energy balance. *Cell* 104, 531–543
9 Vidal, H. (2001) Gene expression in visceral and subcutaneous adipose tissues. *Ann. Med.* 33, 547–555
10 Moitra, J. *et al.* (1998) Life without white fat: a transgenic mouse. *Genes Dev.* 12, 3168–3181
11 Shimomura, I. *et al.* (1998) Insulin resistance and diabetes mellitus in transgenic mice expressing nuclear SREBP-1c in adipose tissue: model for congenital generalized lipodystrophy. *Genes Dev.* 12, 3182–3194
12 Kim, J.K. *et al.* (2000) Mechanism of insulin resistance in A-ZIP/F-1 fatless mice. *J. Biol. Chem.* 275, 8456–8460
13 Browning, J.D. and Horton, J.D. (2004) Molecular mediators of hepatic steatosis and liver injury. *J. Clin. Invest.* 114, 147–152
14 Garg, A. (2004) Acquired and inherited lipodystrophies. *N. Engl. J. Med.* 350, 1220–1234
15 Yamashita, T. *et al.* (2004) Role of uncoupling protein-2 up-regulation and triglyceride accumulation in impaired glucose-stimulated insulin secretion in a beta-cell lipotoxicity model overexpressing sterol regulatory element-binding protein-1c. *Endocrinology* 145, 3566–3577
16 Pajvani, U.B. *et al.* (2005) Fat apoptosis through targeted activation of caspase 8: a new mouse model of inducible and reversible lipoatrophy. *Nat. Med.* 11, 797–803
17 Strawford, A. *et al.* (2004) Adipose tissue triglyceride turnover, *de novo* lipogenesis, and cell proliferation in humans measured with 2H2O. *Am. J. Physiol. Endocrinol. Metab.* 286, E577–E588
18 Neese, R.A. *et al.* (2002) Measurement *in vivo* of proliferation rates of slow turnover cells by 2H2O labeling of the deoxyribose moiety of DNA. *Proc. Natl. Acad. Sci. U. S. A.* 99, 15345–15350
19 Ntambi, J.M. and Young-Cheul, K. (2000) Adipocyte differentiation and gene expression. *J. Nutr.* 130, 3122S–3126S
20 Unger, R.H. and Orci, L. (2000) Lipotoxic diseases of nonadipose tissues in obesity. *Int. J. Obes. Relat. Metab. Disord.* 24 (Suppl. 4), S28–S32
21 Sorisky, A. *et al.* (2000) Adipose cell apoptosis: death in the energy depot. *Int. J. Obes. Relat. Metab. Disord.* 24 (Suppl. 4), S3–S7
22 Xu, J. and Liao, K. (2004) Protein kinase B/AKT 1 plays a pivotal role in insulin-like growth factor-1 receptor signaling induced 3T3-L1 adipocyte differentiation. *J. Biol. Chem.* 279, 35914–35922
23 Magun, R. *et al.* (1998) Expression and regulation of neuronal apoptosis inhibitory protein during adipocyte differentiation. *Diabetes* 47, 1948–1952
24 Villarroya, F. *et al.* (2005) Lipodystrophy associated with highly active anti-retroviral therapy for HIV infection: the adipocyte as a target of anti-retroviral-induced mitochondrial toxicity. *Trends Pharmacol. Sci.* 26, 88–93
25 Domingo, P. *et al.* (1999) Subcutaneous adipocyte apoptosis in HIV-1 protease inhibitor-associated lipodystrophy. *AIDS* 13, 2261–2267
26 Fischer-Posovszky, P. *et al.* (2004) Inhibition of death-receptor mediated apoptosis in human adipocytes by the insulin-like growth factor I (IGF-I)/IGF-I receptor autocrine circuit. *Endocrinology* 145, 1849–1859
27 Prins, J.B. *et al.* (1994) Human adipocyte apoptosis occurs in malignancy. *Biochem. Biophys. Res. Commun.* 205, 625–630
28 Rupnick, M.A. *et al.* (2002) Adipose tissue mass can be regulated through the vasculature. *Proc. Natl. Acad. Sci. U. S. A.* 99, 10730–10735
29 Dallabrida, S.M. *et al.* (2003) Adipose tissue growth and regression are regulated by angiopoietin-1. *Biochem. Biophys. Res. Commun.* 311, 563–571
30 Kolonin, M.G. *et al.* (2004) Reversal of obesity by targeted ablation of adipose tissue. *Nat. Med.* 10, 625–632
31 Friedman, J.M. (2002) The function of leptin in nutrition, weight, and physiology. *Nutr. Rev.* 60, S1–14
32 Gimeno, R.E. and Klaman, L.D. (2005) Adipose tissue as an active endocrine organ: recent advances. *Curr. Opin. Pharmacol.* 5, 122–128
33 Rajala, M.W. and Scherer, P.E. (2003) Minireview: The adipocyte–at the crossroads of energy homeostasis, inflammation, and atherosclerosis. *Endocrinology* 144, 3765–3773
34 Gorden, P. and Gavrilova, O. (2003) The clinical uses of leptin. *Curr. Opin. Pharmacol.* 3, 655–659
35 Licinio, J. *et al.* (2004) Phenotypic effects of leptin replacement on morbid obesity, diabetes mellitus, hypogonadism, and behavior in leptin-deficient adults. *Proc. Natl. Acad. Sci. U. S. A.* 101, 4531–4536
36 Oral, E.A. *et al.* (2002) Leptin-replacement therapy for lipodystrophy. *N. Engl. J. Med.* 346, 570–578
37 Montez, J.M. *et al.* (2005) Acute leptin deficiency, leptin resistance, and the physiologic response to leptin withdrawal. *Proc. Natl. Acad. Sci. U. S. A.* 102, 2537–2542
38 Pajvani, U.B. and Scherer, P.E. (2003) Adiponectin: systemic contributor to insulin sensitivity. *Curr. Diab. Rep.* 3, 207–213
39 Combs, T.P. *et al.* (2003) Sexual differentiation, pregnancy, calorie restriction, and aging affect the adipocyte-specific secretory protein adiponectin. *Diabetes* 52, 268–276
40 Hotta, K. *et al.* (2000) Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. *Arterioscler. Thromb. Vasc. Biol.* 20, 1595–1599
41 Xu, A. *et al.* (2003) The fat-derived hormone adiponectin alleviates alcoholic and nonalcoholic fatty liver diseases in mice. *J. Clin. Invest.* 112, 91–100
42 Shibata, R. *et al.* (2004) Adiponectin-mediated modulation of hypertrophic signals in the heart. *Nat. Med.* 10, 1384–1389
43 Qi, Y. *et al.* (2004) Adiponectin acts in the brain to decrease body weight. *Nat. Med.* 10, 524–529
44 Patel, S.D. *et al.* (2004) Disulfide-dependent multimeric assembly of resistin family hormones. *Science* 304, 1154–1158
45 Pajvani, U.B. *et al.* (2003) Structure-function studies of the adipocyte-secreted hormone Acrp30/adiponectin. Implications fpr metabolic regulation and bioactivity. *J. Biol. Chem.* 278, 9073–9085
46 Berg, A.H. *et al.* (2001) The adipocyte-secreted

protein Acrp30 enhances hepatic insulin action. *Nat. Med.* 7, 947–953
47 Yamauchi, T. *et al.* (2003) Globular adiponectin protected ob/ob mice from diabetes and apoE deficient mice from atherosclerosis. *J. Biol. Chem.* 278, 2461–2468
48 Patel, L. *et al.* (2003) Resistin is expressed in human macrophages and directly regulated by PPAR gamma activators. *Biochem. Biophys. Res. Commun.* 300, 472–476
49 Muse, E.D. *et al.* (2004) Role of resistin in diet-induced hepatic insulin resistance. *J. Clin. Invest.* 114, 232–239
50 Steppan, C.M. *et al.* (2001) The hormone resistin links obesity to diabetes. *Nature* 409, 307-312
51 Rajala, M.W. *et al.* (2003) Adipose-derived resistin and gut-derived resistin-like molecule-beta selectively impair insulin action on glucose production. *J. Clin. Invest.* 111, 225–230
52 Forman, B.M. *et al.* (1995) 15-deoxy-prostaglandin J2 is a ligand for the adipocyte determination factor PPARγ. *Cell* 83, 803–812
53 Berger, J. and Moller, D.E. (2002) The mechanisms of action of PPARs. *Annu. Rev. Med.* 53, 409–435
54 Yki-Jarvinen, H. (2004) Thiazolidinediones. *N. Engl. J. Med.* 351, 1106–1118
55 Pajvani, U.B. *et al.* (2004) Complex distribution, not absolute amount of adiponectin, correlates with thiazolidinedione -mediated improvement in insulin sensitivity. *J. Biol. Chem.* 279, 12152–12162
56 Tonelli, J. *et al.* (2004) Mechanisms of early insulin-sensitizing effects of thiazolidinediones in type 2 diabetes. *Diabetes* 53, 1621–1629
57 Combs, T.P. *et al.* (2004) A transgenic mouse with a deletion in the collagenous domain of adiponectin displays elevated circulating adiponectin and improved insulin sensitivity. *Endocrinology* 145, 367–383
58 Hsueh, W.A. and Law, R.E. (2001) PPARgamma and atherosclerosis: effects on cell growth and movement. *Arterioscler. Thromb. Vasc. Biol.* 21, 1891–1895
59 Li, A.C. *et al.* (2000) Peroxisome proliferator-activated receptor gamma ligands inhibit development of atherosclerosis in LDL receptor-deficient mice. *J. Clin. Invest.* 106, 523–531
60 Tomlinson, J.W. *et al.* (2004) 11beta-hydroxysteroid dehydrogenase type 1: a tissue-specific regulator of glucocorticoid response. *Endocr. Rev.* 25, 831–866
61 Escher, G. *et al.* (1997) Tumor necrosis factor alpha and interleukin 1beta enhance the cortisone/cortisol shuttle. *J. Exp. Med.* 186, 189–198
62 Kostadinova, R.M. *et al.* (2005) Tumor necrosis factor alpha and phorbol 12-myristate-13-acetate down-regulate human 11beta-hydroxysteroid dehydrogenase type 2 through p50/p50 NF-kappaB homodimers and Egr-1. *FASEB J.* 19, 650–652
63 Seckl, J.R. *et al.* (2004) Glucocorticoids and 11beta-hydroxysteroid dehydrogenase in adipose tissue. *Recent Prog. Horm. Res.* 59, 359–393
64 Masuzaki, H. *et al.* (2001) A transgenic model of visceral obesity and the metabolic syndrome. *Science* 294, 2166–2170
65 Morton, N.M. *et al.* (2004) Novel adipose tissue-mediated resistance to diet-induced visceral obesity in 11 beta-hydroxysteroid dehydrogenase type 1-deficient mice. *Diabetes* 53, 931–938
66 Nuttall, M.E. and Gimble, J.M. (2004) Controlling the balance between osteoblastogenesis and adipogenesis and the consequent therapeutic implications. *Curr. Opin. Pharmacol.* 4, 290–294
67 Justesen, J. *et al.* (2001) Adipocyte tissue volume in bone marrow is increased with aging and in patients with osteoporosis. *Biogerontology* 2, 165–171
68 Ton, F.N. *et al.* (2005) Effects of low-dose prednisone on bone metabolism. *J. Bone Miner. Res.* 20, 464–470
69 Sivagurunathan, S. *et al.* (2005) Influence of glucocorticoids on human osteoclast generation and activity. *J. Bone Miner. Res.* 20, 390–398
70 Poole, K.E. *et al.* (2002) Falls, fractures, and osteoporosis after stroke: time to think about protection? *Stroke* 33, 1432–1436
71 Gao, Y. *et al.* (2004) Estrogen prevents bone loss through transforming growth factor beta signaling in T cells. *Proc. Natl. Acad. Sci. U. S. A.* 101, 16618–16623
72 Pittenger, M.F. *et al.* (1999) Multilineage potential of adult human mesenchymal stem cells. *Science* 284, 143–147
73 Ali, A.A. *et al.* (2005) Rosiglitazone causes bone loss in mice by suppressing osteoblast differentiation and bone formation. *Endocrinology* 146, 1226–1235
74 Akune, T. *et al.* (2004) PPARgamma insufficiency enhances osteogenesis through osteoblast formation from bone marrow progenitors. *J. Clin. Invest.* 113, 846–855
75 Rzonca, S.O. *et al.* (2004) Bone is a target for the antidiabetic compound rosiglitazone. *Endocrinology* 145, 401–406
76 Li, X. *et al.* (2003) Lovastatin inhibits adipogenic and stimulates osteogenic differentiation by suppressing PPARgamma2 and increasing Cbfa1/Runx2 expression in bone marrow mesenchymal cell cultures. *Bone* 33, 652–659
77 Holst, D. *et al.* (2003) Roles of peroxisome proliferator-activated receptors delta and gamma in myoblast transdifferentiation. *Exp. Cell Res.* 288, 168–176
78 Hunter, J.G. *et al.* (2001) Peroxisome proliferator-activated receptor gamma ligands differentially modulate muscle cell differentiation and MyoD gene expression via peroxisome proliferator-activated receptor gamma -dependent and -independent pathways. *J. Biol. Chem.* 276, 38297–38306
79 Asakura, A. *et al.* (2001) Muscle satellite cells are multipotential stem cells that exhibit myogenic, osteogenic, and adipogenic differentiation. *Differentiation* 68, 245–253
80 Dietze, D. *et al.* (2002) Impairment of insulin signaling in human skeletal muscle cells by co-culture with human adipocytes. *Diabetes* 51, 2369–2376
81 Nawrocki, A.R. and Scherer, P.E. (2004) The delicate balance between fat and muscle: adipokines in metabolic disease and musculoskeletal inflammation. *Curr. Opin. Pharmacol.* 4, 281–289
82 Pickup, J.C. (2004) Inflammation and activated innate immunity in the pathogenesis of type 2 diabetes. *Diabetes Care* 27, 813–823
83 Lau, D.C. *et al.* (2005) Adipokines: Molecular links between Obesity and Atheroslcerosis. *Am. J. Physiol. Heart Circ. Physiol.* 288, H2031–H2041
84 Pearson, T.A. *et al.* (2003) Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. *Circulation* 107, 499–511
85 Lin, Y. *et al.* (2001) Hyperglycemia-induced production of acute phase reactants in adipose tissue. *J. Biol. Chem.* 276, 42077–42083
86 Berg, A.H. and Scherer, P.E. (2005) Adipose tissue, inflammation, and cardiovascular disease. *Circ. Res.* 96, 939–949
87 Kim, J.K. *et al.* (2001) Prevention of fat-induced insulin resistance by salicylate. *J. Clin. Invest.* 108, 437–446
88 Hotamisligil, G.S. (2003) Inflammatory pathways and insulin action. *Int. J. Obes. Relat. Metab. Disord.* 27 (Suppl. 3), S53–S55
89 Hirosumi, J. *et al.* (2002) A central role for JNK in obesity and insulin resistance. *Nature* 420, 333–336
90 Uysal, K.T. *et al.* (1997) Protection from obesity-induced insulin resistance in mice lacking TNF-α function. *Nature* 389, 610–614
91 Yuan, M. *et al.* (2001) Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of Ikkbeta. *Science* 293, 1673–1677
92 Cai, D. *et al.* (2005) Local and systemic insulin resistance resulting from hepatic activation of IKK-beta and NF-kappaB. *Nat. Med.* 11, 183–190
93 Arkan, M.C. *et al.* (2005) IKK-beta links inflammation to obesity-induced insulin resistance. *Nat. Med.* 11, 191–198
94 Ofei, F. *et al.* (1996) Effects of an engineered human anti-TNF-α antibody (CDP571) on insulin sensitivity and glycemic control in patients with NIDDM. *Diabetes* 45, 881–885
95 Paquot, N. *et al.* (2000) No increased insulin sensitivity after a single intravenous administration of a recombinant human tumor necrosis factor receptor: Fc fusion protein in obese insulin-resistant patients. *J. Clin. Endocrinol. Metab.* 85, 1316–1319
96 Di Rocco, P. *et al.* (2004) Lowered tumor necrosis factor receptors, but not increased insulin sensitivity, with infliximab. *Obes. Res.* 12, 734–739
97 Yazdani-Biuki, B. *et al.* (2004) Improvement of insulin sensitivity in insulin resistant subjects during prolonged treatment with the anti-TNF-alpha antibody infliximab. *Eur. J. Clin. Invest.* 34, 641–642
98 Tanowitz, H.B. *et al.* (1988) *Trypanosoma cruzi* infection in diabetic mice. *Trans. R. Soc. Trop. Med. Hyg.* 82, 90–93
99 Combs, T.P. *et al.* (2005) The Adipocyte as an Important Target Cell for *Trypanosoma cruzi* Infection. *J. Biol. Chem.* 280, 24085–24094
100 Wellen, K.E. and Hotamisligil, G.S. (2003) Obesity-induced inflammatory changes in adipose tissue. *J. Clin. Invest.* 112, 1785–1788
101 Charriere, G. *et al.* (2003) Preadipocyte conversion to macrophage. Evidence of plasticity. *J. Biol. Chem.* 278, 9850–9855
102 Weisberg, S.P. *et al.* (2003) Obesity is associated with macrophage accumulation in adipose tissue. *J. Clin. Invest.* 112, 1796–1808
103 Xu, H. *et al.* (2003) Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J. Clin. Invest.* 112, 1821–1830
104 Berg, A.H. *et al.* (2004) Adipocyte differentiation induces dynamic changes in NF-kappaB expression and activity. *Am. J. Physiol. Endocrinol. Metab.* 287, E1178–E1188
105 Miida, T. *et al.* (2004) Cholesterol-independent

effects of statins and new therapeutic targets: ischemic stroke and dementia. *J. Atheroscler. Thromb.* 11, 253–264
106 van Harmelen, V. *et al.* (2003) HMG-CoA reductase inhibitor cerivastatin inhibits interleukin-6 expression and secretion in human adipocytes. *Horm. Metab. Res.* 35, 466–470
107 Danesh, J. *et al.* (2004) C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease. *N. Engl. J. Med.* 350, 1387–1397
108 Willerson, J.T. and Ridker, P.M. (2004) Inflammation as a cardiovascular risk factor. *Circulation* 109 (21, Suppl. 1), II2–10
109 Cohen, A.W. *et al.* (2004) Role of caveolae and caveolins in health and disease. *Physiol. Rev.* 84, 1341–1379
110 Karlsson, M. *et al.* (2004) Colocalization of insulin receptor and insulin receptor substrate-1 to caveolae in primary human adipocytes. Cholesterol depletion blocks insulin signalling for metabolic and mitogenic control. *Eur. J. Biochem.* 271, 2471–2479
111 Ost, A. *et al.* (2005) Triacylglycerol is synthesized in a specific subclass of caveolae in primary adipocytes. *J. Biol. Chem.* 280, 5–8
112 Pohl, J. *et al.* (2005) FAT/CD36-mediated long-chain fatty acid uptake in adipocytes requires plasma membrane rafts. *Mol. Biol. Cell* 16, 24–31
113 Cohen, A.W. *et al.* (2004) Role of caveolin-1 in the modulation of lipolysis and lipid droplet formation. *Diabetes* 53, 1261–1270
114 Gleave, M.E. and Monia, B.P. (2005) Antisense therapy for cancer. *Nat. Rev. Cancer* 5, 468–479
115 Jiang, G. *et al.* (2005) Prevention of obesity in mice by antisense oligonucleotide inhibitors of stearoyl-CoA desaturase-1. *J. Clin. Invest.* 115, 1030–1038
116 van Huijsduijnen, R.H. *et al.* (2002) Selecting protein tyrosine phosphatases as drug targets. *Drug Discov. Today* 7, 1013–1019
117 Waring, J.F. *et al.* (2003) PTP1B antisense-treated mice show regulation of genes involved in lipogenesis in liver and fat. *Mol. Cell. Endocrinol.* 203, 155–168
118 Holmlund, J.T. (2003) Applying antisense technology: Affinitak and other antisense oligonucleotides in clinical development. *Ann. N. Y. Acad. Sci.* 1002, 244–251
119 Lin, Y. *et al.* (2005) The hyperglycemia-induced inflammatory response in adipocytes: the role of reactive oxygen species. *J. Biol. Chem.* 280, 4617–4626
120 Furukawa, S. *et al.* (2004) Increased oxidative stress in obesity and its impact on metabolic syndrome. *J. Clin. Invest.* 114, 1752–1761
121 Schroder, M. and Kaufman, R.J. (2005) The mammalian unfolded protein response. *Annu. Rev. Biochem.* 74, 739–789
122 Wilson-Fritch, L. *et al.* (2004) Mitochondrial remodeling in adipose tissue associated with obesity and treatment with rosiglitazone. *J. Clin. Invest.* 114, 1281–1289
123 Ozcan, U. *et al.* (2004) Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. *Science* 306, 457–461
124 Minn, A.H. *et al.* (2005) Thioredoxin-interacting protein is stimulated by glucose through a carbohydrate response element and induces {beta} cell apoptosis. *Endocrinology* 146, 2397–2405
125 Du, X.L. *et al.* (2000) Hyperglycemia-induced mitochondrial superoxide overproduction activates the hexosamine pathway and induces plasminogen activator inhibitor-1 expression by increasing Sp1 glycosylation. *Proc. Natl. Acad. Sci. U. S. A.* 97, 12222–12226
126 Brownlee, M. (2001) Biochemistry and molecular cell biology of diabetic complications. *Nature* 414, 813–820
127 Rossetti, L. (2000) Perspective: Hexosamines and nutrient sensing. *Endocrinology* 141, 1922–1925
128 Hazel, M. *et al.* (2004) Activation of the hexosamine signaling pathway in adipose tissue results in decreased serum adiponectin and skeletal muscle insulin resistance. *Endocrinology* 145, 2118–2128
129 Reitman, M.L. (2004) Magic bullets melt fat. *Nat. Med.* 10, 581–582