Lilly Lecture 2005

Adipose Tissue

From Lipid Storage Compartment to Endocrine Organ

Philipp E. Scherer

Adipose tissue, when carried around in excessive amounts, predisposes to a large number of diseases. Epidemiological data show that the prevalence of obesity has significantly increased over the past 20 years and continues to do so at an alarming rate. Here, some molecular aspects of the key constituent of adipose tissue, the adipocyte, are reviewed. While the adipocyte has been studied for many years and remarkable insights have been gained about some processes, many areas of the physiology of the fat cell remain unexplored. Our understanding of how cellular events in the adipocyte affect the local environment through paracrine interactions and how systemic effects are achieved through endocrine interactions is rudimentary. While storage and release of lipids are major functions of adipocytes, the adipocyte also uses specific lipid molecules for intracellular signaling and uses a host of protein factors to communicate with essentially every organ system in the body. The intensity and complexity of these signals are highly regulated, differ in each fat pad, and are dramatically affected by various disease states. *Diabetes* 55: 1537–1545, 2006

We have appreciated for a long time that excess adipose tissue predisposes toward the development of insulin resistance. It is less well known, but equally important, that loss of selective fat pads (or absence of adipose tissue altogether) is also associated with severe forms of insulin resistance (1–3). This is in part due to the absence of the compartment that is specialized for the storage of lipids under normal conditions. This leads to a dysregulation of triglyceride and free fatty acid levels, as well as a dysregulation of specific adipocyte-derived secretory proteins, a group of proteins that we refer to as adipokines. As the master regulator of systemic lipid storage and through secretion of a number of these adipokines, adipose tissue has an influence on many processes, including energy metabolism, inflammation, and pathophysiological changes such as cancer and infectious disease (4). At the interface of energy metabolism and inflammation, adipose tissue also plays a key role in the development of the metabolic syndrome. As such, our views of adipose tissue have changed significantly over the past 20 years. Initially considered an inert storage compartment for triglycerides, pioneering work from the Spiegelman and Flier (5) laboratories in the mid-1980s highlighted for the first time that adipocytes are an abundant source of a specific secretory protein, called adipsin or complement factor D. In 1995, Jeffrey Friedman's (6) group identified leptin as a fat cell–specific secretory factor deficient in the *ob/ob* mouse that mediates the hormonal axis between fat and the brain.

Around the same time, we and others described a protein that we initially termed Acrp30, which later became known as adiponectin (7–10). Additional proteins have joined this exclusive club of adipocyte-specific secretory proteins since then, including adipokines such as resistin (11,12) and acylation-stimulating protein (13), as well as the recently described visfatin (14,15) and retinol-binding protein-4 (16). Enzymes such as lipoprotein lipase are also abundantly produced and released from adipocytes. Finally, many proinflammatory cytokines and acute-phase reactants originate in the adipocyte. These include α1 acid glycoprotein, serum amyloid A, the C-reactive protein homolog pentraxin-3, the lipocalin 24p3, and a host of cytokines (17).

We are all painfully aware of the fact that adipose tissue is the only organ with unlimited growth potential at any stage of our life. These adipocytes can release protein and lipid derivatives that are highly proangiogenic and have an impact on the preexisting vasculature. Finally, the unique extracellular matrix environment of adipose tissue hosts a number of additional cells such as macrophages and offers unique growth potential for transformed cells such as breast cancer cells (18,19).

Using adipokines as one of the major communication tools, adipocytes affect a large number of other tissues, such as the liver, muscle, the brain, the reproductive system, pancreatic β-cells, and, as mentioned above, the vasculature.

The power of mouse genetics. Using a variety of mouse models that we have generated over the past couple of years, I would like to illustrate how powerful an impact some of these adipokines can have on overall body energy homeostasis. While the 2005 Banting Lecture focused on the physiology of *ob/ob* mice that lack leptin, I will describe variants of these *ob/ob* mice that span a wide range of body weights. These mice teach us valuable lessons about the function of specific adipokines, as well as the systemic impact of adipose tissue as a whole.

From the Departments of Cell Biology and Medicine, Diabetes Research and Training Center, Albert Einstein College of Medicine, Bronx, New York.

Address correspondence and reprint requests to Departments of Cell Biology and Medicine, Diabetes Research and Training Center, Albert Einstein College of Medicine, 1300 Morris Park Ave., Bronx, NY 10461. E-mail: scherer@aecom.yu.edu.

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AMPK, AMP-activated protein kinase; ER, endoplasmic reticulum; HMW, high molecular weight; IL, interleukin; PPAR, peroxisome proliferation–activated receptor; RELM, resistin-like molecule; ROS, reactive oxygen species; TZD, thiazolidinedione.

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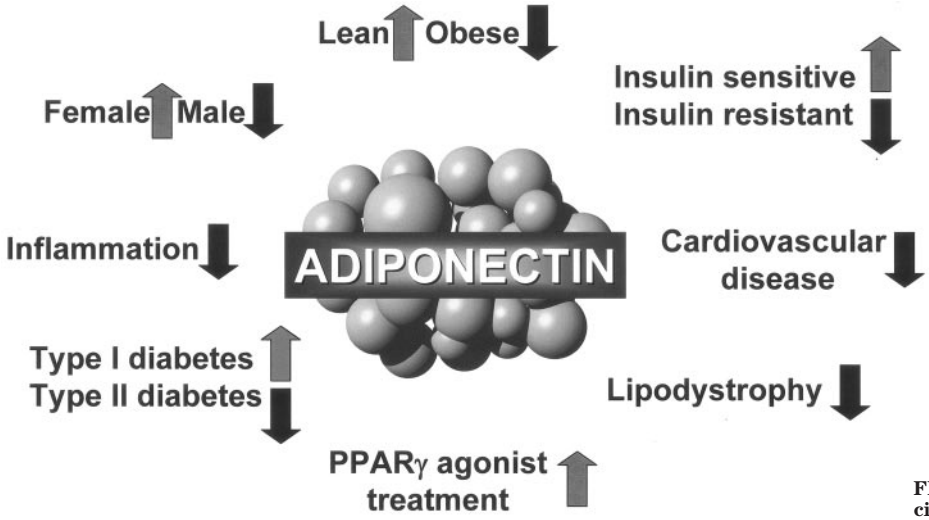

FIG. 1. Summary of established effects influencing circulating adiponectin levels.

Adiponectin. An adipokine that has been the focus of a large number of studies over the last couple of years is the protein adiponectin. Early studies using euglycemic clamp techniques indicated that adiponectin, when injected in a recombinant form, has a profound impact on the liver (20). Specifically, it reduces the need to infuse glucose by enhancing the insulin-mediated repression of glucose production in the liver. Similar observations can be made in vitro using primary hepatocytes exposed to varying levels of insulin in the presence or absence of adiponectin (21). Increasing levels of insulin repress the glucose output of hepatocytes. The presence of adiponectin at physiological concentrations maximizes the insulin effects even at low concentrations, suggesting that adiponectin functions as a highly effective insulin sensitizer at the level of the liver.

Since the initial report of these findings, >1,000 articles have been published on the topic and are summarized in Fig. 1. Despite the fact that adiponectin is produced exclusively in adipocytes, its serum levels tend to be lower in patients with increased fat mass. Some of the best correlations can be seen with insulin sensitivity, whereby higher levels of serum adiponectin are associated with increased insulin sensitivity. Patients with cardiovascular disease and other states associated with increased inflammation tend to have decreased levels of adiponectin. Consistent with the improved insulin sensitivity generally observed in female compared with male subjects, adiponectin levels are higher in women than in men. Patients with type 2 diabetes and other diseases associated with reduced insulin sensitivity, such as generalized or HIV-induced lipodystrophies, have decreased levels. Frequently, decreased adiponectin levels are not only observed in association with type 2 diabetes and cardiovascular disease but also serve as powerful predictors for the future development of these syndromes even in the absence of any other manifestations of the disease.

Genetic evidence has highlighted the region around 3q27 encoding the adiponectin locus as a susceptibility region for syndrome X, and many studies have associated mutations and polymorphisms in the gene encoding adiponectin with an increased prevalence of diabetes (22,23). Finally, other than weight loss, the only other viable approach to significantly improve adiponectin levels is through the use of pharmacological activators of the nuclear receptor peroxisome proliferatior–activated receptor (PPAR)-γ by thiazolidinediones (TZDs), which are widely used as insulin sensitizers in diabetes clinic (24). Work from many laboratories, among them Takashi Kadowaki's and Yuji Matzusawa's group (25), have studied the underlying molecular mechanisms for the antiatherosclerotic effects of adiponectin, which has anti-inflammatory properties as well as effects on smooth muscle cell proliferation and the suppression of the conversion of macrophages to foam cells. The absence of adiponectin leads to increased neointimal proliferation in response to vascular cuff injury in the absence of adiponectin.

Adiponectin circulates in several different size complexes in serum (Fig. 2). Its basic unit is a homotrimer. These homotrimers can assemble into higher-order structures, such as a hexamer, and several of these hexamers can assemble into a high–molecular weight (HMW) complex. All three forms can be found in serum (26). These structural variations have important biological implications. For instance, the higher levels of adiponectin in female subjects are primarily due to increased levels of the HMW complex. A metabolic challenge, such as a glucose or insulin infusion, results in a selective and transient reduction of the HMW form in circulation. The lower–molecular weight hexamer, in contrast, is not affected under those conditions. Very little information about the physiological changes related to the trimer is available at this stage.

A prominent role for the HMW form has been highlighted particularly well in the context of studies with PPAR-γ agonists. Almost invariably, treatment of mice or patients with various PPAR-γ agonists results not only in an improvement in insulin sensitivity but also in a robust increase in circulating adiponectin levels. This increase is primarily due to the induction of the HMW form, if the adiponectin complex distribution is compared before and after treatment in diabetic mice. In fact, we can generalize this statement and argue that type 2 diabetic individuals tend to have lower levels of the HMW form in circulation compared with insulin-sensitive individuals and that the development of type 2 diabetes in an individual is associated with a progressive decrease of the HMW form (27).

In light of these observations, we proposed that the correlations seen by measuring the total levels of adiponectin could be further strengthened in some cases by taking the complex distribution into account. The adiponectin sensitivity index (S_A) reflects the fraction of adiponectin found in the HMW form. This index has

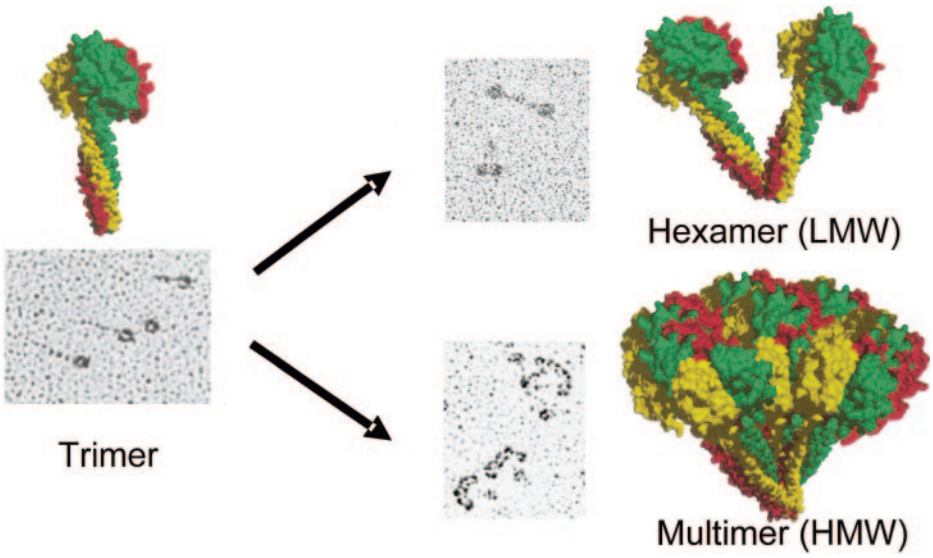

FIG. 2. Adiponectin multimerization. Shown are the three major forms of adiponectin found in circulation. Electronmicrographs of purified complexes are shown (26). Schematic representations of the different complexes are shown as well, with each adiponectin subunit in the basic trimeric building block represented in a different color.

become particularly useful to assess the efficacy of PPAR-γ agonist treatment. Comparison of changes in S_A versus changes in total adiponectin treatment upon PPAR-γ agonist shows that S_A is a far superior indicator of improvements in hepatic insulin sensitivity. These correlations have been observed in a number of different studies involving a number of different PPAR-γ agonists, including TZD and non-TZD–based agonists. These studies are highly suggestive of a direct involvement of adiponectin in mediating the improvements in insulin sensitivity induced by PPAR-γ agonists (27) (Fig. 3).

Under other circumstances, it may be the absolute amount of the HMW form that is most relevant. Regardless of whether S_A or total HMW is more relevant, a number of recent articles highlight the importance of focusing on the differential complex distribution and show that many correlations initially established for total adiponectin levels can be further strengthened by taking the levels of the HMW forms into account (28–32). To date, applications of the measurements of the different adiponectin complexes in plasma have been quite limited, mostly due to the lack of high-throughput assays, which would make these measurements feasible for some of the larger clinical studies. Relief is on the way, though, since a number of companies will soon offer high-throughput assays that can selectively measure the HMW form. This is a very exciting development. However, we should also use utmost caution with respect to applying these assays to clinical studies until they have been fully cross-validated under a number of different clinically relevant conditions.

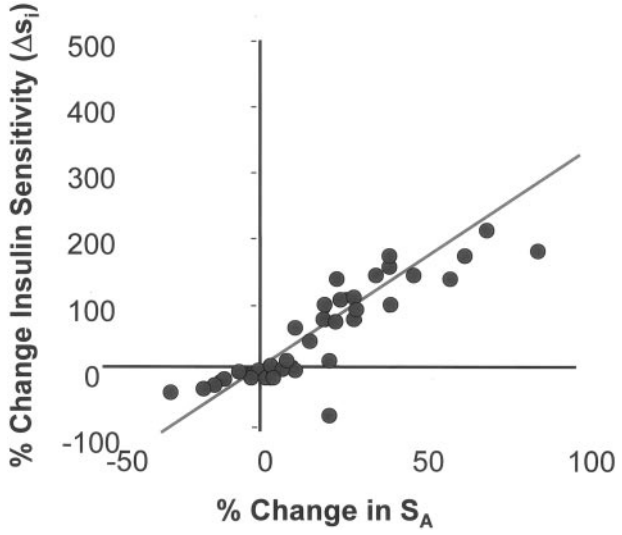

FIG. 3. Improvements in insulin sensitivity in response to PPAR-γ agonist treatment are proportional to the changes induced in the HMW-to-total adiponectin ratio (S_A). From ref. 27.

All of the above data on adiponectin originate from epidemiological studies. This is very powerful but, by its very nature, strictly correlational. We can probe the mechanistic impact of this protein on metabolism more directly by using rodent models amenable to genetic manipulation. We now have an array of these mouse models available that display a wide range of adiponectin levels. An early example is a mouse that overexpresses adiponectin from an adipose tissue–specific promoter (33). This is a modest overexpression in the same physiological range that can be achieved with PPAR-γ agonist treatment. These mice display increased hepatic insulin sensitivity and are resistant to the negative physiologic impact of a high-fat Western diet. They also show substantial improvements in their lipid profile. These and many additional effects seen in the mice are similar to effects that can be achieved by prolonged treatment with PPAR-γ agonists. In addition, just like during PPAR-γ agonist treatment, magnetic resonance imaging reveals a significant increase in adipose tissue mass, particularly in the interscapular region, which contains brown adipose tissue, the tissue responsible for nonshivering thermogenesis.

In a more recent (unpublished) model, we overexpressed adiponectin in a similar fashion in the classical *ob/ob* mouse, which is deficient in functional leptin. Remarkably, a modest overexpression of adiponectin results in a complete normalization of metabolic parameters. We can demonstrate dramatic improvements in glucose clearance during an oral glucose tolerance test. Concomitant with that, improvements in fasting insulin levels, islet morphology, and β-cell function can be seen as well. These effects are not limited to carbohydrate metabolism. *Ob/ob* mice overexpressing adiponectin display a more efficient clearance of triglycerides during lipid ingestion, likely mediated at least in part through higher levels of lipoprotein lipase in all fat pads examined. Furthermore, they have a decreased deposition of lipids in the liver. A closer

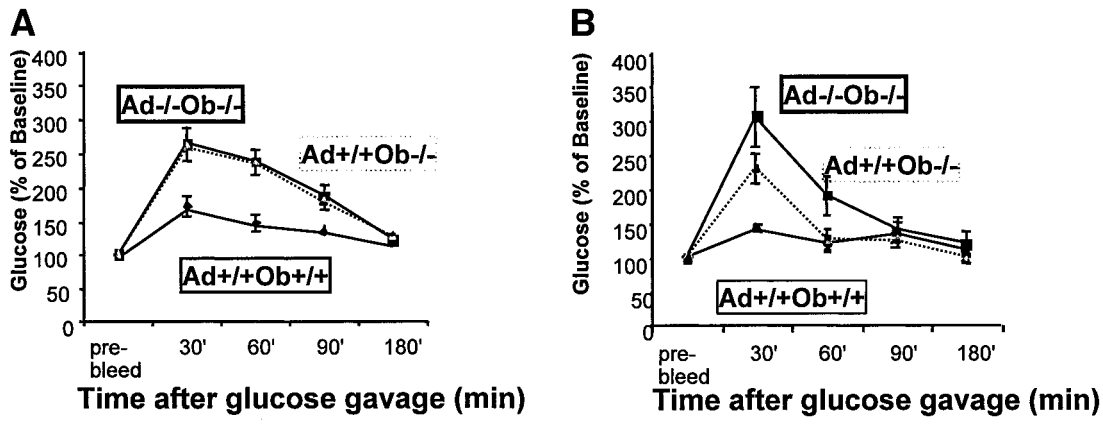

FIG. 4. Glucose tolerance in obese *ob/ob* mice lacking adiponectin after treatment with PPAR-γ agonists (TZDs). *A*: Before TZD treatment. *B*: After TZD treatment. Twelve-week-old male mice were treated with rosiglitazone (10 mg · kg body wt^{-1} · day^{-1}) over 10 days and subjected to an oral glucose tolerance test. Blood glucose was measured at 0 (prebleed), 30, 60, 90, and 180 min after glucose dosage. The curves for adiponectin and leptin double-null mice (Ad-/-Ob-/-, gray), obese adiponectin wild type-mice (Ad+/+Ob-/-, dashed), and lean control mice (Ad+/+/Ob+/+, black) are shown. Values are percentage induction from basal glucose levels. *$P < 0.05$. From ref. 34.

look at the adipose tissue of these mice shows that most of the adipocytes are considerably smaller in these mice, consistent with the widespread believe in the field that smaller adipocytes have a more positive impact on the metabolic profile than larger adipocytes. Overall, modest overexpression of adiponectin results in a normalization of almost all metabolic parameters examined, including the lipid levels deposited in the liver. Surprisingly, it is quite apparent that *ob/ob* mice overexpressing adiponectin are markedly bigger than their *ob/ob* littermates, the current gold standard for an obese mouse. This increase in body weight is mostly due to an increase in total adipose tissue mass.

Adiponectin-mediated weight gain can be augmented even further. If a more active version of adiponectin (a protein carrying a mutation at position cys39) is expressed as a transgene at very low steady-state levels, we obtain massively obese mice, weighing more than four times the weight of their wild-type littermates. Compared with *ob/ob* littermates, they are nearly twice as heavy. Again, despite this massive obesity, these mice are metabolically remarkably healthy, with reduced circulating lipids and a reduced accumulation of lipids in the liver (J.-Y. Kim, T. Schraw, P.E.S., unpublished results).

What should we conclude from these studies? Does this suggest that the more adipose tissue we manage to accumulate, the better off we are? Under some circumstances, this may be true. Since lipid accumulation in tissues such as liver and muscle has a dramatic negative impact on the insulin sensitivity of these tissues, during times of caloric excess, depositing lipids into adipocytes is far more desirable than depositing lipids into these other tissues. In this context, we could view adiponectin as a starvation signal produced and released from the adipocyte, mediating a redistribution of lipid deposition away from tissues such as liver and muscle into adipose tissue where these triglycerides can be stored in a more inert fashion. As a result, decreased lipid levels in muscle, and particularly in the liver, cause improvements in insulin sensitivity. Overall, the adiponectin-mediated redistribution of triglycerides is remarkably similar to the actions of PPAR-γ agonists. The proposed mechanism of action of these insulin-sensitizing compounds rely to a large extent on the ability to redistribute triglycerides to adipose tissue and to partition triglycerides within adipose tissue into an increased number of smaller adipocytes.

Our models of adiponectin overexpression have provided meaningful insights into transgene-mediated chronic overexpression of adiponectin in the obese state (a condition usually associated with a downregulation of adiponectin levels in circulation) triggering a lipid deposition in adipose tissue.

What about the opposite situation: a mouse model that completely lacks adiponectin due to a disruption of the genetic locus that encodes the protein? Several groups have shown that lack of adiponectin leads to insulin resistance, particularly in the context of a high-fat diet. We have recently shown that *ob/ob* mice that lack adiponectin display a reduced response to PPAR-γ agonist treatment, as judged by the lack of improvements of glucose clearance after TZD treatment compared with *ob/ob* mice that have adiponectin available (34). Similar observations can be seen for the activation of AMP-activated protein kinase (AMPK) in the liver, an important local mediator of PPAR-γ agonist action. Wild-type mice respond to PPAR-γ agonists through a marked induction of AMPK activity. The response in adiponectin-null mice is sharply reduced. However, even though the induction of adiponectin is an intrinsic component of the mechanism of action of PPAR-γ agonists, it is clear that additional adiponectin- and AMPK-independent mechanisms are contributing to PPAR-γ agonist effects (Fig. 4).

The various forms of adiponectin continue to draw a lot of attention, with many groups studying the effects on tissues such as muscle, the vasculature, the brain, liver, and adipose tissue. We have mostly focused on the effects on the liver, notably due to the privileged access that the visceral adipose tissue has to this organ. In lean individuals, visceral depots are a major production site for adiponectin. In the obese insulin-resistant state, the combination of a drop in the adiponectin production in these depots combined with the increased release of free fatty acids from the visceral fat pads is thought to have a major negative impact on hepatic insulin sensitivity.

In summary, adiponectin has gained widespread acceptance as a marker in the context of obesity and diabetes. Increased evidence points to an involvement in cardiovascular disease. Due to the impact of inflammation on

adiponectin levels, adiponectin is emerging as an important link among type 2 diabetes, cardiovascular disease, and the metabolic syndrome. Many additional studies suggest that adiponectin may have an even broader influence on the development of certain cancers, wound healing, and a number of additional processes.

Additional adipokines and the secretory pathway of adipocytes. Adiponectin is not the only adipokine that attracted our attention. A novel family of cytokines has emerged over the last couple of years, initially reported by Mitch Lazar's (11,35) group at the University of Pennsylvania. These are the resistin and resistin-like molecules, the so-called RELMs. In mice, resistin is a protein that is primarily released from adipocytes. When we infused resistin and one of the RELMs (RELM-β) in a recombinant form under euglycemic clamp conditions, rather than having a positive impact on hepatic insulin sensitivity, as seen for adiponectin in similar experiments, resistin has in fact a negative impact on the same process (36). When we reported the structures of resistin and RELM-β in a collaborative effort with Larry Shapiro's (37) lab at Columbia, we noticed a highly unusual feature of these proteins. They have externally exposed disulfide bonds that glue a dimer of trimers together. Due to the high level of exposure to the aqueous environment, these disulfide bonds are prime candidates for reduction. Usually, such disulfide bonds tend to be buried and protected inside proteins. The disulfide bonds found in the resistin and RELM-β structures are among the most highly exposed disulfides reported to date for any naturally occurring protein complex for which the structure has been solved at atomic resolution.

In the case of resistin, there is mounting evidence that a version of the protein unable to form these three disulfide bonds displays higher bioactivity. Similar observations have been made for adiponectin. In both cases, the formation and disruption of critical disulfide bonds within the quaternary structure has a major impact on the bioactivity of the protein.

Decisions as to when and how such disulfide bonds are formed are made in the secretory pathway, within the lumen of the endoplasmic reticulum (ER) of the adipocyte. The redox potential within the secretory pathway plays a major role in this process, and this redox potential is governed by the levels of glutathione. Furthermore, the presence of critical chaperones enable secretory proteins in the ER and the Golgi lumen to fold properly. One of the critical factors known to affect cellular glutathione levels is reactive oxygen species (ROS). During times of excess intracellular nutrient availability, ROS levels increase as a by-product of mitochondrial respiration. This has been extensively studied in many cell types, including endothelial cells. Adipocytes are no different in this respect. Upon exposure to hyperglycemic conditions, they contain increased levels of ROS, and this phenomenon can effectively be reduced by inhibitors of mitochondrial respiration. This is due to the fact that adipocytes share the same hallmark feature with all other cell types susceptible to this phenomenon: they are unable to downregulate glucose uptake under conditions of enhanced extracellular glucose levels. This leads to a reduced insulin sensitivity of adipocytes, as judged by the reduced ability of adipocytes to increase glucose uptake in response to insulin (38). Similar to the situation in the endothelium, hyperglycemia-induced ROS also trigger the activation of the proinflammatory transcription factor nuclear factor κB in adipocytes. As a result, a major proinflammatory cascade is activated, leading to the induction of several cytokines, such as interleukin (IL)-6.

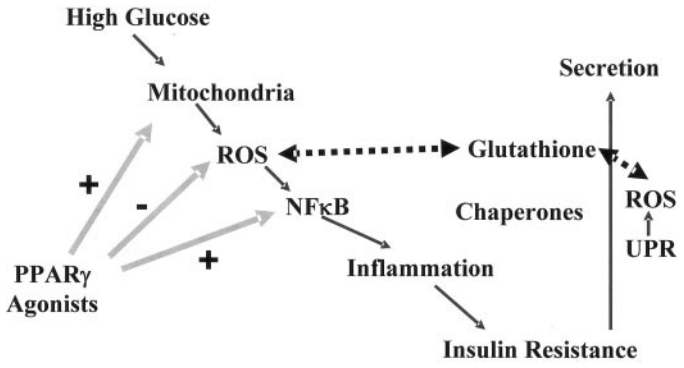

FIG. 5. Hyperglycemia-induced increased production of ROS and subsequent induction of a proinflammatory cascade and insulin resistance. Increased levels of ROS also negatively impact on the redox potential in the ER lumen and consequently may affect a subset of proteins critically dependent on proper disulfide bond formation. PPAR-γ agonist alleviate these effects at a number of different levels, inducing mitochondrial biogenesis, reducing ROS levels, displaying potent anti-inflammatory properties, inducing critical chaperones for the secretory pathway, and reducing the unfolded protein response (UPR). NFκB, nuclear factor κB.

We can summarize these events as follows: excess extracellular glucose results in excess intracellular glucose in adipocytes. The excess of nutrients triggers the production of ROS at the level of mitochondria. Excess ROS not only change the cellular glutathione pool, affecting the redox potential in the secretory pathway, but also activate nuclear factor κB, triggering a proinflammatory cascade. This local activation of the proinflammatory cascade can feed back on the adipocyte and cause decreased insulin sensitivity. Within the secretory pathway, the altered redox potential has an impact on proteins such as adiponectin and resistin, which are dependent on the formation of critical disulfide bonds, which are highly susceptible to redox chemistry. In extreme cases, this can lead to a more generalized disruption of the secretory pathway, ultimately inducing the ER-unfolded protein response. This in turn results in an additional production of ROS originating within the secretory pathway. PPAR-γ agonists can interfere with these processes at many different levels. They transcriptionally induce adipokines, such as adiponectin. They also regulate part of the machinery required for the correct assembly and release of proteins in the secretory pathway, such as some of the critical chaperones. Overall improvements in mitochondrial function can be achieved through the transcriptional induction of key mitochondrial constituents. In addition, ROS levels are effectively lowered through transcription-dependent and -independent mechanisms, leading to an overall healthier adipocyte, similar to the adipocytes from mice overexpressing adiponectin (Fig. 5).

The physiological impact of an acute loss of adipose tissue. I have mentioned the phenotypes of some of our massively obese mice, which manage to have an excellent metabolic profile because of (rather than in spite of) increased fat mass. How about the other extreme of the spectrum: mouse models that completely lack adipose tissue? Several lipodystrophic mouse models have been described in the literature. An early model from Bruce Spiegelman's (39) laboratory, models described in the Brown and Goldstein laboratory (40), and, most recently, the A/ZIP mice described by Vinson and Reitman (2) have

given us tremendous insights into the physiological role of adipose tissue. However, all of these models have the distinct disadvantage of being constitutively lipoatrophic, suffering from the complete absence of fat during development that results in severe insulin resistance and hepatic steatosis in early adolescence. To generate a mouse model that would allow us to probe the effects of fat loss in the adult at a more acute level, we generated and recently described "FAT-ATTAC" mice: *F*AT *A*poptosis *T*hrough *T*riggered *A*ctivation of *C*aspase-8 (3). These mice carry a transgene encoding a caspase 8 protein fused to a dimerization domain under the control of a fat cell–specific promoter. Caspase 8 is an upstream activator of controlled cell death (apoptosis). Apoptosis is usually initiated through dimerization of caspase 8, triggered by interactions of caspase 8 with death receptors on the cell surface. The activation of caspase 8 results in the activation of the rest of the proapoptotic cascade and ultimately results in cell death. Since we engineered a protein domain that allows us to induce this dimerization step with a small chemical that we can provide to the mice at any stage during their life, we are now at liberty to cause the complete loss of all adipocytes within a couple of days at any age. This allows us to examine the consequences of acute loss of fat tissue, without the secondary consequences that are invariably triggered by a more chronic absence of fat. The activation of the transgene indeed triggers the appearance of TUNEL (transferase-mediated dUTP nick-end labeling)–positive cells in adipose tissue. This is an excellent indicator that widespread cell death takes place. Upon removal of the adipocytes, we observe extensive infiltration of macrophages into the remnant tissue, as judged by the large number of cells that stain positive for the macrophage-specific marker F4/80. As a result of this induced adipocyte apoptosis, adipokines, such as adiponectin and resistin, are effectively reduced to near baseline levels. However, we do not destroy the precursor cells; upon cessation of the treatment with dimerizer, the levels of these adipokines are reconstituted and the adipose tissue returns to its normal appearance. When we perform a similar study by crossing this transgene into the leptin-deficient *ob/ob* background, *ob/ob* mice that carry the transgene do not show a significant weight difference before the initiation of dimerizer treatment. However, upon exposure to the dimerizer, we observe the onset of a massive weight loss that is the result of the loss of all functional adipocytes, while the dimerizer has no effect in mice not carrying the transgene.

These FAT-ATTAC mice show a number of very interesting metabolic phenotypes. Rather than going through the entire metabolic characterization of these mice, which we have published elsewhere (3), I will pick out a few salient points in an attempt to highlight the many yet-unexplored aspects of adipocyte physiology. For instance, we noticed that both fed and fasted insulin levels were lower in these mice after loss of fat, despite continued hyperglycemic conditions. The pancreatic β-cells remain fully functional. The ability to secrete insulin in response to the activation of the β3 adrenergic receptor in adipocytes is, however, completely lost in fatless mice. This is consistent with elegant work from Brad Lowell's (41) group concluding that the presence of the β3 adrenergic receptor on the surface of fat cells is an absolute requirement for the phenomenon of increased insulin secretion induced by β3-agonists. Since this effect is highly unlikely to be mediated by an increase in fatty acids caused by lipolysis, it remains to be determined how the fat cell achieves such an efficient communication with the β-cell.

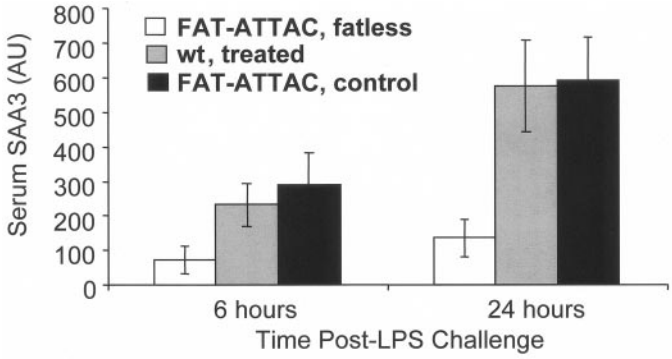

FIG. 6. Decreased response to endotoxin in fatless mice. Functional adipose is required for normal basal and lipopolysaccharide (LPS)-stimulated inflammatory tone. Decreased lipopolysaccharide-stimulated serum amyloid A3 in lipoatrophic FAT-ATTAC *ob/ob* mice relative to control *ob/ob* littermates or FAT-ATTAC *ob/ob* animals treated with vehicle. wt, wild type. From ref. 3.

Another interesting phenomenon relates to food intake and energy expenditure. Fatless *ob/ob* mice have a significant increase in food intake despite their reduced body weight. The loss of leptin may play an important role in fatless wild-type mice. However, in *ob/ob* mice, there is no leptin present at any stage. Therefore, there must be mechanisms by which the absence of fat triggers an increase in food intake in a leptin-independent fashion. Similarly, energy expenditure increases, as judged by an increase in core body temperature. Again, we do not understand how these metabolic changes are brought about by the absence of fat. The identification of the underlying mechanisms holds great potential for future therapeutic applications.

What we do know, however, is that upon ablation of adipose tissue, we see a reduction in systemic inflammation. Inflammatory markers, such as IL-6 and others, are reduced. Under more extreme conditions, as under conditions encountered during sepsis, injection of bacterial endotoxin leads to a markedly reduced inflammatory response in fatless mice compared with normal mice. This highlights the important systemic contributions of adipose tissue toward inflammation (Fig. 6).

In light of our current views that type 2 diabetes is in essence an inflammatory disease, this observation is very interesting. Data from the Ferrante and Leibel (42) laboratories have recently demonstrated that as we gain weight and expand our fat mass, there is an increase in the number of macrophages that infiltrate adipose tissue. There is more and more reason to believe that adipose tissue–borne macrophages are the primary source of inflammatory cytokines. What is the role of the adipocyte in this process, and why do we see a reduction of inflammatory markers upon removal of the adipocyte? This can be answered with a very simple experiment in which we harvest medium from cultured cells that were allowed to secrete factors into the supernatant for several hours. This conditioned medium can be transferred to cultured macrophages, and the degree of inflammation can then be monitored by examining the levels of inflammatory markers, such as IL-6 and tumor necrosis factor α in the supernatant of macrophages. Adipocyte-conditioned medium is enriched in factors that exert a potent proinflammatory stimulus on macrophages. This phenomenon

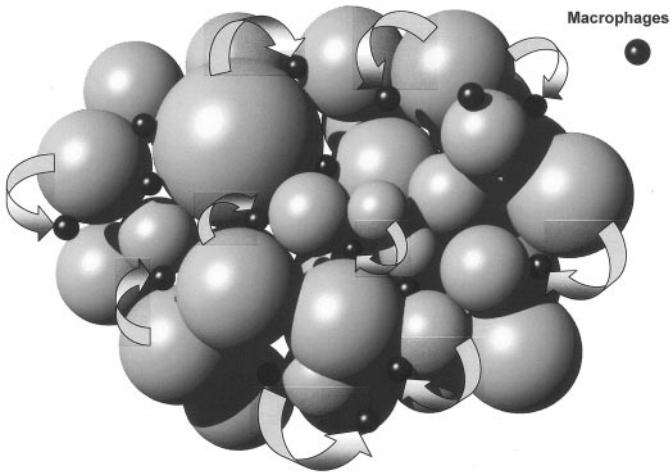

FIG. 7. Paracrine cross talk of adipocytes with macrophages. Macrophages serve as the major source of proinflammatory factors released from adipose tissue. However, macrophages critically rely on proinflammatory input from surrounding adipocytes to exert their full inflammatory potential.

is fairly unique to adipocytes, since other cell types (including preadipocytes) do not display such proinflammatory properties (43).

Combining these in vitro experiments with the in vivo results in the FAT-ATTAC mice, it appears that the local adipose tissue macrophages are in a constant state of inflammation and are the primary source of proinflammatory markers. This is primarily due to local stimuli released from adipocytes in a paracrine fashion. Upon ablation of the adipocytes, the macrophages in the remnant fat pads remain behind, but these cells are now no longer inflamed to the same degree due to the absence of the major instigator of the inflammation, the adipocyte. These observations demonstrate the complex nature of the local and systemic interactions of the adipocyte with its environment, which lead to an increased infiltration of macrophages into adipose tissue and, in a second step, to an independent activation of these resident macrophages (Fig. 7).

Outlook and conclusions. Where do we go from here? Let me highlight some of the areas that I believe bear great potential for our future understanding of adipocyte physiology.

Adipocyte-specific secretory products remain a major area of interest to us. There is clearly a lot of work that remains to be done in the area of adiponectin physiology. The recent identification of several receptors for these adiponectin complexes potentially sheds more light on the signaling mechanisms of the molecule (44). However, there are huge gaps in our knowledge about the mechanism of action of adiponectin. Does it affect the same downstream targets in all tissues and cells, and do the different adiponectin complexes elicit differential responses? If the answer to the latter question is yes, which of these responses are physiologically relevant, and which effects are limited to in vitro readouts induced by nonphysiological forms of the protein? With respect to other adipokines: the resistin and RELM receptors remain to be identified, and major advances are still being made in the leptin field. The existence of additional, yet-unidentified adipocyte-specific or at least adipocyte-enriched factors, is highly likely. Genomic approaches may be useful for that purpose. However, the low-hanging fruit has already been picked with this approach. Both proteomic and metabolomic methods have the potential to identify additional physiologically relevant events at the posttranscriptional, posttranslational, and functional levels.

I have mentioned the important role that the macrophage plays in adipose tissue physiology. What are the underlying mechanisms responsible for the attraction of macrophages into adipose tissue? What are the critical proinflammatory mediators produced by the adipocyte responsible for the paracrine interactions between the adipocyte and the macrophage?

Local effects of angiogenesis are another exciting area. Several recent articles have provided the proof of concept that adipose tissue is highly sensitive to systemic angiogenesis inhibitors. Adipose tissue is highly vascularized, and the expansion of adipose mass involves the formation of new capillaries. Systemic treatment of obese mice with antiangiogenic agents induces a marked loss of white adipose tissue. These results suggest that neovascularization is required for adipose tissue maintenance even under steady-state conditions and that there may be a constant remodeling of the vasculature required to sustain the viability of adipose tissue. Combining local antiangiogenic approaches with reduced caloric intake may not only offer a therapeutic modality for obesity but may also be highly relevant to tumors embedded in adipose tissue. In this context, the extracellular matrix surrounding adipose tissue offers tremendous potential for intervention. Of particular interest are the specific interactions of transformed ductal epithelial cells with the mammary fat pad, relevant for the developmental aspects as well as for the well-established dependence of mammary tumors on adipose tissue during early stages of lesion growth. Our recent work on collagen VI has underlined the importance of this extracellular matrix component, which is highly enriched in adipose tissue for the growth of such mammary ductal epithelial tumors (18).

A better understanding of neuronal input into (and potentially output from) adipose tissue is becoming increasingly relevant in light of our current realization that many metabolic phenomena in peripheral tissues are directly controlled through central neuronal circuits, particularly through central pathways of nutrient sensing.

Finally, with respect to the differentiation of precursor cells, many questions remain to be answered. How are adipocyte stem cells recruited, what determines the location of fat deposition, and what are the signals that ultimately trigger de novo adipogenesis? In this context, what can we learn from extreme situations, such as the massive differentiation and lipid loading of fat cells in the mammary fat pad at the end of weaning during the process called involution, a process unparalleled in any other physiological setting? At the other extreme lies the wasting syndrome that we refer to as cancer cachexia (associated with some types of cancer), which results in the massive loss of adipose tissue (associated with inflammation and a high degree of insulin resistance). Our understanding of the underlying mechanisms here remains rudimentary.

With respect to intracellular targets: a better understanding of PPAR-γ agonist action will continue to shed light on adipocyte physiology in general. Questions relating to mitochondrial dysfunction in the context of type 2 diabetes become increasingly important, and related to that, curbing local ROS production in the adipocyte is likely to have a beneficial impact. We already widely

appreciate the issue of stress within the secretory pathway in the pancreatic β-cells. The adipocyte, with its highly active secretory pathway, which is geared to sustain constant levels of short-lived adipokines in circulation (some of which are quite abundant, such as adiponectin), is equally susceptible to events that can have a negative impact on protein folding and vesicular trafficking within the secretory pathway. Targeting the proinflammatory mechanisms within the adipocyte has great therapeutic potential, since it is increasingly appreciated that inflammation in the adipocyte has an impact on the surrounding macrophages and ultimately contributes significantly to systemic inflammatory responses.

Glucocorticoids are potent inducers of adipocyte differentiation in in vitro systems. 11-β hydroxysteroid dehydrogenase type 1 activity, the rate-limiting enzyme for glucocorticoid production, increases in adipocytes during differentiation. Accordingly, an excess of glucocorticoids is associated with the development of obesity, and the inhibition of this enzyme has the potential to have a positive impact on metabolism.

Caveolae, small flask-shaped structures within the plasma membrane involved in signal transduction and fatty acid transport, are highly abundant in adipocytes. The integrity of these organelles critically depends on the local cholesterol concentration. Athough the adipocyte is not a significant systemic source of cholesterol biosynthesis, intracellular synthesis of isoprene tails is thought to enable signaling components of the inflammatory cascade to insert into the cell membrane. Although the adipocyte has not been extensively studied in the context of statin action, local inhibition in adipocytes of the main target enzyme for statins, hydroxy-methylglutaryl coenzyme A reductase, may contribute significantly to the beneficial systemic effects that statins exert.

Events taking place on the surface of the lipid droplet relating to lipolysis and the complex proteinaceous machinery mediating this process also offer attractive pharmacological targets.

Last but not least, since adipocytes are a major source of proinflammatory activity both locally and systemically, curbing this activity within the adipocyte has the potential of having an impact on the acute systemic response to sepsis. In addition, it may also be an important target for reducing chronic subclinical inflammation, which is tightly epidemiologically linked to an increased propensity to develop cardiovascular disease.

In summary, I hope I have convinced you that fat is far more than just an inert storage compartment for triglycerides. The adipocyte remains a highly intriguing cell type, and many secrets remain to be unveiled.

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