Adipose tissue oxygen tension: implications for chronic metabolic and inflammatory diseases

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Purpose of review
The present review aims to address the role of adipose tissue oxygen partial pressure (PO_2) in the metabolic and endocrine derangements in conditions characterized by insulin resistance.

Recent findings
The balance between adipose tissue oxygen supply and its metabolic rate seems to determine adipose tissue PO_2. Studies in *ob/ob* and dietary-induced obese mice have provided evidence for adipose tissue hypoxia in obesity, which has been explained by insufficient adipose tissue angiogenesis during the massive and rapid weight gain in these animals. However, conflicting data have been reported in humans, showing both increased and decreased adipose tissue PO_2 in obese compared with lean individuals. Both low and high adipose tissue PO_2 may induce a proinflammatory phenotype in (pre)adipocytes, but most studies have been performed under rather extreme PO_2 levels, not reflecting human adipose tissue physiology. Furthermore, adipose tissue PO_2 may affect glucose and lipid metabolism as well as adipogenic differentiation, but many issues still need to be addressed.

Summary
Adipose tissue hypoxia has been demonstrated in animal models of obesity, but findings in humans are controversial and require further investigation. Although adipose tissue PO_2 seems to be involved in metabolic and endocrine derangements in human adipose tissue, future studies should investigate how low and high adipose tissue PO_2 within the human physiological range (3–11% O_2) relates to adipose tissue blood flow and oxygen consumption, cellular metabolic responses, and the inflammatory phenotype.

Keywords
adipose tissue, hypoxia, inflammation, metabolism, oxygen tension

INTRODUCTION

Research of the past decade has substantially increased our understanding of adipose tissue function in health and disease. The pathophysiological aspects of adipose tissue expansion are becoming increasingly recognized with the increase in caloric intake and sedentary behavior that underlie the obesity epidemic in Western cultures. It has now become a plausible concept that an impaired function of adipose tissue in obesity, rather than total fat mass *per se*, plays a prominent role in the pathophysiology of type 2 diabetes mellitus, fatty liver disease and cardiovascular disease [1–3]. This is highlighted by the fact that obesity does not necessarily translate into increased risk for chronic metabolic and inflammatory diseases, since a subgroup of obese individuals (~10–25%) remains metabolically healthy [4]. Therefore, a better understanding of the events causing the metabolic and endocrine derangements in adipose tissue may lead to novel strategies to prevent and treat chronic metabolic and inflammatory diseases. In the present review, recent advances in the putative role of altered adipose tissue oxygen tension (PO_2) in adipose tissue dysfunction will be described and interpreted in the context of related metabolic and inflammatory disorders. The consequences of systemic alterations in oxygen tension on metabolism and inflammation (e.g. during intermittent hypoxia in

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Curr Opin Clin Nutr Metab Care 2012, 15:539–546
DOI:10.1097/MCO.0b013e328358fa87

KEY POINTS

- Insufficient angiogenesis during the massive and very rapid body fat gain in animal models of obesity may be a more important determinant of adipose tissue oxygen tension than oxygen consumption, whereas the opposite might hold true for human obesity.
- Adipose tissue hypoxia has been demonstrated in animal models of obesity, but findings on adipose tissue oxygen tension in humans are controversial and require further investigation.
- Adipose tissue oxygen tension seems to be involved in the regulation of metabolism and inflammatory processes in adipose tissue, but many issues still need to be addressed.
- Future cell culture studies should investigate the metabolic and endocrine effects of chronic exposure to oxygen tensions that reflect normal human adipose tissue physiology (3–11% O_2), rather than acute exposure to extremely low oxygen tension (1% O_2).

obstructive sleep apnea, severe chronic obstructive pulmonary disease and oxygen therapy) will not be discussed in this review.

ADIPOSE TISSUE OXYGEN TENSION: BALANCE BETWEEN BLOOD FLOW AND METABOLIC RATE

Since a constant supply of oxygen to organs is essential to sustain life, organisms have evolved multiple mechanisms to ensure maintenance of a delicate balance between oxygen supply and consumption. Adipose tissue PO_2 is the result of the balance between blood flow to adipose tissue (ATBF) and its metabolic rate (Fig. 1). However, perturbations in this homeostatic balance may occur in pathophysiological conditions that are characterized by impairments in ATBF and/or an altered metabolic rate.

Adipose tissue blood flow

Adipose tissue possesses a relatively dense capillary network that ensures adequate delivery of nutrients and oxygen to the tissue. It could be anticipated that impairments in both the structural and functional properties of the adipose tissue vasculature may affect ATBF. We and others have previously shown that both fasting ATBF and the postprandial increase in ATBF are decreased in obese, insulin resistant and type 2 diabetic patients compared with lean, healthy controls [5▪,6–8]. The impaired ATBF response to nutrient intake in obesity seems to be closely associated with insulin resistance [5▪,8], suggesting that ATBF is of great importance in the regulation of metabolism. The adipose tissue vasculature serves to transport circulating lipids to their storage depot (adipocytes), and macrophages utilize the microcirculation to reach their targets. Furthermore, the vasculature transports adipokines and free fatty acids (FFAs) that have been released by adipose tissue. A decreased ATBF may negatively affect the lipid buffering capacity of adipose tissue via reduced clearance of circulating triglycerides [9] and increased re-esterification of FFAs [10], which may result in an excessive accumulation of lipids in the circulation and in non-adipose tissues (ectopic fat storage) and, as a consequence, insulin resistance [1].

Adipose tissue development and vascularization seem to be closely linked, as indicated by studies showing that antiangiogenic agents influence adipose tissue expansion and metabolism [11▪▪,12–15]. Nevertheless, it has been proposed that the expansion of adipose tissue mass during the progressive development of obesity may lead to a relative oxygen deficit in certain parts of adipose tissue, because angiogenesis is insufficient to maintain normoxia in the entire adipose tissue depot [16]. Indeed, several human studies have demonstrated reduced angiogenesis and capillary density in adipose tissue in obesity [5▪,17▪,18], which may contribute to lower ATBF. In addition, we have shown that both pharmacological and physiological manipulation of ATBF induced concomitant alterations in adipose tissue PO_2 in humans, suggesting that ATBF is an important regulator of adipose tissue PO_2 [5▪]. Thus, decreased ATBF in obesity reduces adipose tissue oxygen supply. Importantly, however, whether impaired adipose tissue oxygen supply in obesity may lead to decreased adipose tissue PO_2 and a relative oxygen deficit is also dependent on adipose tissue oxygen consumption, as outlined below (Fig. 1).

Adipose tissue oxygen consumption

Animal studies have shown that mitochondrial morphology is abnormal, that mitochondrial biogenesis and mass are reduced, and that oxygen consumption is lower in both white and brown adipose tissue of obese Zucker rats [19], *ob/ob* mice [20], *db/db* and high-fat diet-fed mice [21]. Although experimental data on the bioenergetics and oxidative capacity of human adipocytes are scarce, it has been reported that oxygen consumption per adipocyte is higher but oxygen consumption per gram of adipose tissue is lower in obese humans [22]. Interestingly, DNA microarray-based gene

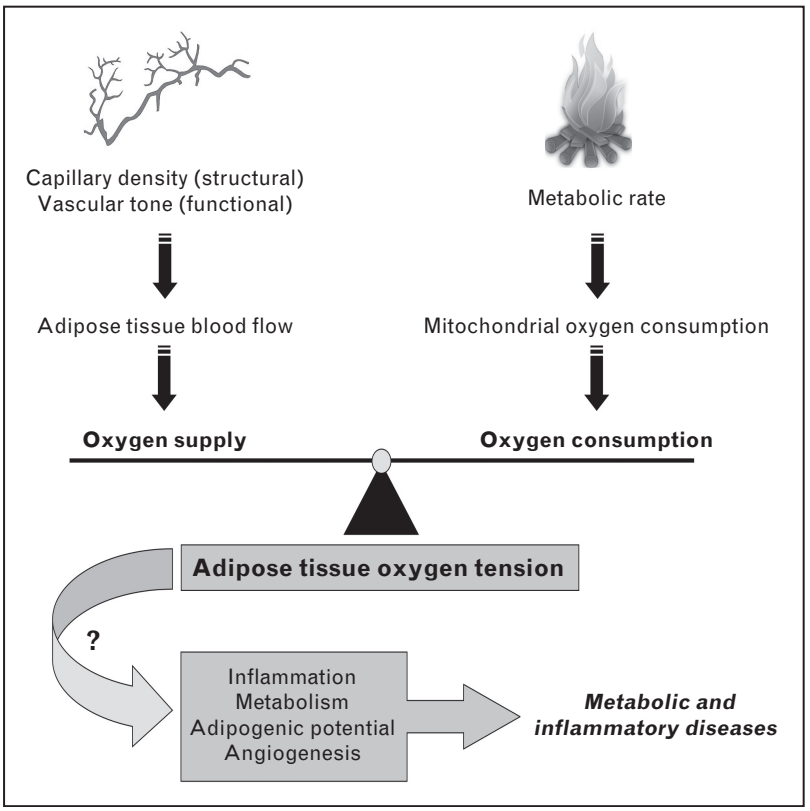

FIGURE 1. Determinants and putative effects of adipose tissue oxygen tension (AT PO_2). AT PO_2 is the result of a delicate balance between adipose tissue blood flow (oxygen supply) and its metabolic rate (oxygen consumption). Perturbations in this homeostatic balance may occur in pathophysiological conditions that are characterized by impairments in adipose tissue blood flow and/or altered metabolic rate. In obese patients, both adipose tissue blood flow and adipose tissue oxygen consumption are decreased compared with lean, healthy individuals. The extent of these impairments in obesity determines whether changes in AT PO_2 occur. Available evidence suggests that alterations in AT PO_2 may affect inflammation, metabolism, adipocyte differentiation and angiogenesis in adipose tissue.

expression profiling has revealed that increases in adipose tissue mass were paralleled by progressive down-regulation of metabolic pathways, including mitochondrial energy metabolism, in both visceral and abdominal subcutaneous adipose tissue [23▪]. In accordance, we have recently confirmed and extended these findings, showing that adipose tissue expression of mitochondrial function markers and *in vivo* adipose tissue oxygen consumption were markedly reduced in obese compared with lean individuals [5▪]. The expression of mitochondrial function markers was inversely associated with adipose tissue PO_2, suggesting that decreased mitochondrial oxygen consumption in obesity may be an important determinant of adipose tissue PO_2 [5▪].

ADIPOSE TISSUE OXYGEN TENSION IN OBESITY: ANIMAL AND HUMAN STUDIES

Animal studies have provided convincing evidence for adipose tissue hypoxia in obesity. First, the expression of several hypoxia-responsive genes, such as glucose transporter (GLUT)-1 and hypoxia-inducible factor (HIF)-1α, was higher in white adipose tissue of obese compared with lean mice [24–26]. Secondly, pimonidazole staining of white adipose tissue has demonstrated more hypoxic areas in obese KKAy, *ob/ob* and dietary-induced obese mice [24–26]. Thirdly, direct measurements of adipose tissue PO_2 have been performed using needle-type optic fiber oxygen sensors, showing lower adipose tissue PO_2 in white adipose tissue of *ob/ob* and dietary-induced obese mice [25–27]. Thus, multiple lines of evidence indicate that adipose tissue PO_2 is lower in animal models of obesity. These findings have been explained by insufficient angiogenesis in adipose tissue during the massive and rapid weight gain under these experimental conditions, as has been postulated by Trayhurn and Wood [16].

It is, however, important to note that adipose tissue hypoxia in human obesity has not been convincingly demonstrated in humans. Until now, two studies have investigated PO_2 in abdominal

subcutaneous adipose tissue in humans, with conflicting results. Pasarica *et al.* [18] have found that adipose tissue PO_2, as measured using commercially available polarographic micro Clark electrodes, was significantly lower in overweight/obese than in lean individuals. Although very exciting, these data should be interpreted with some caution, since groups were very heterogeneous with respect to age, ethnicity, sex and the presence of type 2 diabetes mellitus. We have recently developed, validated and applied a novel optochemical measurement system for the continuous monitoring of adipose tissue PO_2 *in vivo* in humans using microdialysis [5▪]. Using both pharmacological and physiological approaches to manipulate ATBF, we first demonstrated that ATBF is an important regulator of abdominal subcutaneous adipose tissue PO_2 in humans. Surprisingly, obese insulin resistant patients showed significantly higher adipose tissue PO_2 (~9%) than lean insulin sensitive controls (~6%), despite lower ATBF (~40% reduction). This seemed to be explained by the markedly lower (~60% reduction) *in vivo* adipose tissue oxygen consumption in obese individuals, since adipose tissue expression of mitochondrial function markers was inversely correlated with adipose tissue PO_2 [5▪]. Our findings have clearly challenged the concept of adipose tissue hypoxia in obesity. Likewise, adipose tissue hypoxia in obesity could not be confirmed by assessment of PO_2 in subcutaneous adipose tissue of the upper arm [28]. Obviously, more clinical studies in humans, phenotyped in detail, are warranted to investigate adipose tissue PO_2 in human obesity and other pathophysiological conditions.

What could explain the differences between findings on adipose tissue PO_2 in animal models of obesity and obese humans? Two aspects may be of critical importance in this comparison. First, the relative amount of body fat is much higher in mouse models of obesity compared with that in the average obese individual. In fact, body fat content of the *ob/ob* mice that were studied by Yin *et al.* [27] at 6 weeks of age was about 50% (five-fold higher compared with age-matched lean mice). Of note, data from lean mice showed unchanged adipose tissue PO_2 when body fat content increased from 10 to 18% over 6 weeks [27]. Thus, the exact threshold at which adipose tissue PO_2 decreases in these animals remains unclear, but might be close to 50% body fat. Secondly, and perhaps even more important, it usually takes many years for obesity to develop in humans, whereas the rate of body fat gain is much higher in mouse models of obesity. Thus, impaired angiogenesis might be a more important determinant of adipose tissue PO_2 in animal models of obesity than in obese humans.

OXYGEN TENSION AND ADIPOSE TISSUE DYSFUNCTION

Enlargement of abdominal subcutaneous adipocytes is present in obese, metabolically unhealthy individuals [1,5▪,29], and appears to be an independent determinant of insulin resistance and type 2 diabetes [29,30]. The underlying mechanisms are thought to be an increased production of pro-inflammatory cytokines by hypertrophic adipocytes, as well as impairments in lipid metabolism, leading to lipid overflow in the circulation and lipid accumulation in nonadipose tissues (ectopic fat storage) [1].

Several cell culture experiments have been performed to address the question whether oxygen tension is involved in the regulation of metabolic and inflammatory processes at the cellular level in adipose tissue. It is very important to mention that most, if not all, studies have compared acute exposure to extremely low oxygen tensions (1% O_2) with conventional *in vitro* culturing at ambient air (21% O_2). Recent studies from two independent laboratories [5▪,18], including ours [5▪], have demonstrated that O_2 levels in human abdominal subcutaneous adipose tissue range between 3 and 11%, indicating that 1% O_2 does not reflect normal human adipose tissue physiology. This should be kept in mind when interpreting the studies described in the following sections.

Adipose tissue inflammation

An impaired endocrine function of adipose tissue has been linked to peripheral insulin resistance and obesity-associated diseases [1]. Many cell culture experiments have been performed over the past few years to examine the putative effects of adipose tissue PO_2 on adipokine expression and secretion. It has been demonstrated that acute exposure to extremely low oxygen tension vs. normoxia (1 vs. 21%) may induce a pro-inflammatory response and a reduction in adiponectin expression in 3T3-L1 [24,26,31▪,32–34] and human adipocytes [35]. Comparable results have been reported using higher oxygen tensions in human adipocytes [36], and small changes in oxygen tension seem to modulate responses. Responses of 3T3-L1 adipocytes to low oxygen tension may be mediated by HIF-1α but are also dependent on peroxisome proliferator-activated receptor gamma (PPARγ) [37▪]. In this context, it has been shown that HIF-1 DNA-binding activity, HIF-1α protein and HIF-1β protein each increased exponentially as cells were subjected to decreasing O_2 concentrations, with a half maximal response between 1.5 and 2% O_2 and a maximal response at

0.5% O_2 [38]. In other words, it can be questioned whether the HIF-1 pathway is responsive to physiological PO_2 levels (3–11%) in human adipose tissue. This, however, does certainly not exclude a role for HIF-1α signaling in AT inflammation, since HIF-1 can be activated under normoxic conditions by other factors such as insulin and nitric oxide [39▪,40].

In vitro responses of (pre)adipocytes may also depend on the donor, since mature adipocytes of diabetic *db/db* and TallyHo mice did not efficiently respond to hypoxia [41]. Furthermore, depot differences in the response of purified stromal vascular cells (SVF) from obese and lean adipose tissue to hypoxia have been found, with absence of a pro-inflammatory response in SVF derived from subcutaneous adipose tissue [42▪]. More conflicting data with respect to the effects of oxygen tension on adipose tissue inflammation have been reported. Microarray analysis has revealed that hypoxia (1% O_2) did not alter the expression of hypoxia-regulated genes involved in inflammation in human Simpson-Golabi-Behmel syndrome (SGBS) adipocytes [43]. Furthermore, hypoxic human primary adipocytes showed lower secretion of monocyte chemoattractant protein (MCP)-1 under basal conditions and displayed an impaired response to inflammatory stimuli, resulting in reduced nuclear factor kappa B (NF-κB) signaling and decreased MCP-1 secretion [44▪]. In line, treatment of 3T3-L1 adipocytes with 95% O_2 induced an up-regulation of proinflammatory adipokines, which may be mediated by increased release of reactive oxygen species (ROS) [45]. Finally, we have recently demonstrated that *in vivo* adipose tissue PO_2 was positively correlated with adipose tissue expression of several proinflammatory markers in humans [5▪].

In summary, the effects of oxygen tension on the inflammatory response in adipose tissue appear highly complex. Since adipose tissue consists of different cell types, including preadipocytes, macrophages, leukocytes, fibroblasts and endothelial cells, additional experiments are needed to examine the effects of physiological oxygen tensions in these cell types. Furthermore, future studies should take into account duration of the O_2 exposure (e.g. acute exposure vs. chronic exposure during differentiation), adipose tissue depot and donor differences in the response to altered oxygen tension, and should employ oxygen tensions that better reflect human adipose tissue physiology.

Adipose tissue glucose metabolism

In vitro experiments in 3T3-L1 and human adipocytes have indicated that 1% O_2 increased the expression of several but not all glucose transporters [24,35,36,42▪,44▪,46], and increased basal glucose uptake [36,46–48]. In line, hyperoxia (95% O_2) decreased basal glucose uptake [45]. Two studies have also assessed the effect of hypoxia (1% O_2, 16–24 h) on insulin-mediated glucose uptake [47,48]. Exposure to hypoxia decreased protein expression of insulin receptor (IR)-β and insulin receptor substrate (IRS)-1 in 3T3-L1 adipocytes, which was accompanied by reduced insulin-mediated glucose uptake and absence of Akt Ser^{473} phosphorylation [48]. In line with these findings, hypoxia impaired insulin signaling in both 3T3-L1 and human adipocytes, as indicated by reduced insulin receptor and IRS tyrosine phosphorylation, decreased protein kinase B and AS160 phosphorylation, and impaired glucose uptake [47]. These effects, mediated by HIF-1 and HIF-2 proteins, were reversible under normoxic conditions (15–30 min reoxygenation before insulin stimulation) [47]. Taken together, it seems that hypoxia increases basal glucose uptake, whereas insulin-mediated glucose uptake decreases during exposure to extremely low oxygen tension in adipocytes.

Adipose tissue lipid metabolism

In addition to impairments in the endocrine function of adipocytes, obese subjects with enlarged adipocytes show impairments in lipid metabolism, which may contribute to lipid accumulation in nonadipose tissues (ectopic fat storage) and insulin resistance [1,49,50]. Studies that have investigated the effects of oxygen tension on lipid metabolism are scarce and have yielded conflicting results. Exposure to 1% O_2 has been shown to stimulate lipolysis in 3T3-L1 adipocytes [27,47], but exposure of 3T3-L1 adipocytes to hyperoxia (95% compared with 21% O_2) also increased lipolysis [45].

The expression and secretion of angiopoietin-related protein 4 (ANGPTL4), which reduces triacylglycerol clearance by inhibition of lipoprotein lipase (LPL), has been found to be increased by hypoxia in human adipocytes [51] and decreased by hyperoxia in 3T3-L1 adipocytes [45].

Finally, a reduced FFA uptake during hypoxia has been reported in 3T3-L1 adipocytes, which may be related to inhibition of fatty acid transport proteins [27]. Both increased lipolysis and decreased FFA uptake by adipocytes may have contributed to increased plasma FFA concentrations during restriction of blood flow evoked by clamping of the femoral artery in lean rats [27]. Thus, although more studies are certainly needed, there is some evidence that oxygen tension may be involved in the regulation of adipose tissue lipid metabolism.

Proliferation and adipogenic differentiation

Adipocyte turnover has recently been shown to be a dynamic process by which mesenchymal stem cells (MSCs) undergo lineage commitment, preadipocyte proliferation, growth arrest and terminal differentiation into mature adipocytes. Approximately 10% of adipocytes are renewed annually at adult ages and at all levels of BMI [52]. The enlargement of adipocytes during the development of obesity may result from an imbalance between the rate of lipid accumulation within adipocytes (determined by lipid turnover [53▪▪]) and the recruitment of new adipocytes. Uncommitted MSCs have been characterized by a stable undifferentiated phenotype, as well as by the ability to proliferate extensively while retaining the potential to differentiate along osteogenic, chondrogenic and adipogenic lineages *in vitro* [54].

Increased proliferation of human bone marrow and adipose tissue-derived MSCs, while preserving stemness, has been shown under hypoxic conditions (1–5% O_2) [55–58]. Furthermore, it has been demonstrated in 3T3-L1 preadipocytes, mouse embryonic fibroblasts and human bone marrow and adipose tissue-derived MSCs that hypoxia diminished adipogenic differentiation [55,58–61], which might be mediated via the HIF-1 pathway [59,61]. However, contrasting results have also been reported. Preexposure of adipose tissue-derived MSCs to hypoxia (2% O_2) increased their adipogenic potential [62,63], and 8% O_2 accelerated bone marrow-derived MSC differentiation [64]. Taken together, extremely low PO_2 seems to increase proliferation but no consensus has been reached at the moment regarding its effect on differentiation towards the adipogenic lineage.

CONCLUSION

It has now been recognized that adipose tissue dysfunction in obesity plays a prominent role in the pathophysiology of chronic metabolic and inflammatory diseases. The inciting event causing the metabolic and endocrine derangements in adipose tissue of obese individuals remains to be established, but alterations in adipose tissue PO_2 may be involved. Since both ATBF (oxygen supply) and adipose tissue oxygen consumption (metabolic rate) are decreased in obesity, the balance between these perturbations determines adipose tissue PO_2. The concept of adipose tissue hypoxia in human obesity is actually based on very limited scientific proof. Although animal studies have provided convincing evidence for the presence of adipose tissue hypoxia in obesity, both decreased and increased adipose tissue PO_2 have been reported in obese humans. Reduced oxygen supply due to insufficient angiogenesis during the massive and very rapid body fat gain in animal models of obesity may be a more important determinant of adipose tissue PO_2 than oxygen consumption, whereas the opposite might hold true for human obesity.

Cell culture experiments have shown that both low and high adipose tissue PO_2 may induce a pro-inflammatory phenotype in (pre)adipocytes, but most studies have been performed under rather extreme PO_2 levels, not reflecting human adipose tissue physiology. Furthermore, low adipose tissue PO_2 has been found to increase basal glucose uptake, whereas it decreases insulin-stimulated glucose uptake in adipocytes. Finally, conflicting data have been reported regarding the effects of oxygen tension on lipid metabolism and adipogenic differentiation.

Thus, more clinical studies in well phenotyped humans are needed to further investigate adipose tissue PO_2 in human obesity. Although many issues still need to be addressed in this exciting field of research, alterations in adipose tissue PO_2 may underlie adipose tissue dysfunction and related chronic metabolic and inflammatory diseases. Different experimental conditions (e.g. duration of O_2 exposure, cell type, adipose tissue depot and donor differences) may explain the controversial findings with respect to the effects of oxygen tension on adipose tissue inflammation and metabolism. Future studies should employ chronic exposure to oxygen tensions that reflect normal human adipose tissue physiology (3–11% O_2), rather than acute exposure to extremely low oxygen tension (1% O_2). A more complete understanding of the cellular responses to oxygen tension may provide new approaches to prevent or treat chronic metabolic and inflammatory diseases.

Acknowledgements

The authors are grateful to the colleagues who have contributed to the studies performed by our research group. The authors thank the Dutch Diabetes Research Foundation (grant no. 2008.11.010 to G.G.) for financial support.

Conflicts of interest

There are no conflicts of interest.

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Papers of particular interest, published within the annual period of review, have been highlighted as:

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