An update on visfatin/pre-B cell colony-enhancing factor, an ubiquitously expressed, illusive cytokine that is regulated in obesity

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Purpose of review

The aim of this article is to summarize all of the recent studies on pre-B cell colony-enhancing factor (PBEF)/visfatin, a ubiquitously expressed secreted protein that has been implicated in obesity and insulin resistance. Although PBEF was discovered over 10 years ago, there are many remaining questions about the regulation and function of this protein.

Recent findings

Studies in the last decade have revealed the endocrine properties of fat cells. One of the most recent proteins shown to be highly expressed in adipose tissue is visfatin, originally identified as PBEF. Visfatin/PBEF appears to be preferentially produced by the visceral adipose tissue and has insulin mimetic actions. Studies by many groups indicate that obesity-related diabetes and accompanying metabolic disorders in humans have been specifically linked to increased visceral adipose tissue mass. The different roles of various adipocyte depots, however, are still poorly understood. It has been hypothesized that understanding the differences in the biology of visceral and subcutaneous human adipose tissue may hold the key to therapeutic strategies aimed at reducing obesity-induced insulin resistance and alleviating symptoms of the metabolic syndrome. Interestingly, some observed actions of visfatin indicate that this secreted protein may be an interesting therapeutic target. Several recent studies, however, indicate that our understanding of visfatin is still speculative.

Summary

This review summarizes all of the papers in the last year on the expression and function of visfatin/PBEF and highlights inconsistent observations from various investigators studying this protein. It also highlights previous observations on the role of PBEF. We suggest that that pathophysiologic role of visfatin/PBEF in humans remains largely unknown.

Keywords

adipokine, adipose tissue, diabetes, insulin mimetic, obesity, PBEF, visfatin, visceral

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Abbreviations

OLETF Otsuka Long-Evans Tokushima fatty
PBEF B-cell colony-enhancing factor

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Introduction

Adipocytes are important endocrine cells and secrete several hormones and cytokines that are involved in metabolic diseases such as obesity and type 2 diabetes. The endocrine properties of adipocytes continue to be an active and forthcoming area of investigation. Ten years after the identification of a novel protein secreted from lymphocytes [1], it was shown that this protein is also secreted from adipose tissue [2••]. This may come as little surprise given that immune cells and adipocytes are known to express many of the same genes. In the past 11 years, several groups have studied the functions of pre-B-cell colony-enhancing factor (PBEF). These studies reveal that PBEF has some interesting roles in several tissues and has two fairly unique features including the lack of a signal sequence and the ability to localize in the nucleus. The recent discovery that PBEF, now also termed visfatin, is highly expressed in visceral fat and circulating levels correlate with obesity [2••] is of great interest to many researchers. Although a recent study confirmed the modulation of visfatin expression in obesity [3••], these observations are not without controversy [4••]. Moreover, it has been suggested that visfatin can act as an insulin analog on the insulin receptor [2••], but no follow-up studies have confirmed this original observation. Clearly, our understanding of PBEF/visfatin is in its infancy and its pathophysiologic role in humans remains largely unknown.

Visfatin is not a fat-specific protein

PBEF was originally identified as a 52 Kd protein that is primarily expressed in bone marrow, liver, and muscle [1]. PBEF is also upregulated in neutrophils by IL-1β and functions as a novel inhibitor of apoptosis in response to a variety of inflammatory stimuli [5••]. Both genomic and genetic studies have identified PBEF as a novel

candidate gene and biomarker in acute lung injury [6•], and recent studies indicated that PBEF is critically involved in thrombin-induced lung endothelial cell barrier dysregulation [7•]. Other studies showed that PBEF is constitutively expressed by fetal membranes during pregnancy and is expressed throughout gestation in the amniotic epithelium and mesenchymal cells as well as in the chorionic cytotrophoblast and parietal deciduas [8] and it has been hypothesized that PBEF has a central role in the mechanism of infection-induced preterm birth [9]. PBEF has also been shown to regulate nicotinamide adenine dinucleotide (NAD+)-dependent protein deacetylase activity and promote vascular smooth muscle cell maturation. In this study, the authors suggested that PBEF did not have standard attributes of a cytokine but instead imparted the cell with increased nicotinamide phosphoribosyltransferase activity that was involved in the acquisition of a mature smooth muscle cell phenotype [10•]. Previous studies had revealed that PBEF was a nicotinamide phosphoribosyltransferase, a cytosolic enzyme involved in NAD biosynthesis [11]. Overall, these studies indicated that PBEF is ubiquitously expressed and is associated with a variety of functions in different cell types.

A summary of the studies examining the expression of visfatin in obesity and non-insulin dependent diabetes mellitus

PBEF was also termed visfatin when the levels of this protein were shown to be increased in visceral adipose tissue of KKAy mice and in human female participants compared with its levels in subcutaneous adipose tissue [2••]. This original observation is supported by a recent study that demonstrated that visfatin levels are altered in patients with type 2 diabetes mellitus [3••]. In this study, visfatin, adiponectin and resistin levels were measured by enzyme-linked immunosorbent assay in type 2 diabetic and non-diabetic subjects. A decrease in adiponectin and an increase in visfatin was observed in type 2 diabetic patients and the authors suggested that the increasing concentrations of visfatin were independently and significantly associated with type 2 diabetes mellitus [3••]. Another study in which visceral fat mass was calculated from computed tomography scans indicated, however, that there was no correlation between plasma visfatin concentrations and visceral fat mass [4••]. In fact, no significant correlation between visfatin plasma concentrations and various parameters of insulin sensitivity, including fasting insulin, fasting plasma glucose concentrations, and the glucose infusion rate during the steady state of an euglycemic–hyperinsulinemic clamp that were independent of percent body fat were observed [4••]. The results from this study did indicate, however, a significant correlation between visceral visfatin gene expression and percent body fat, but no significant association between body mass index or percent body fat and subcutaneous visfatin mRNA expression. Overall, this study concluded that plasma concentrations of visfatin or visceral visfatin mRNA expression correlated with measurements of obesity but not with visceral fat mass or waist-to-hip ratio [4••]. An additional independent study in a rodent model of metabolic syndrome also observed that visfatin gene expression was not associated with the metabolic syndrome in diseased rats compared with lean controls [12•]. Since only a handful of studies have examined the expression of visfatin in obesity related disorders, the regulation of visfatin/PBEF under these conditions is still not entirely clear.

The modulation of visfatin expression by thiazolidinediones, adipocyte differentiation, and cytokines

Although the biological role of visfatin is still illusive, there are some studies that have investigated the regulation of this protein by other cytokines or by anti-diabetic thiazolidinediones. The expression of adipocytokines in visceral fat depots of Otsuka Long-Evans Tokushima fatty (OLETF) rats from early to advanced diabetic stage (28–40 weeks of age) was recently examined [13••]. As expected, serum glucose and insulin concentrations were significantly decreased in rosiglitazone treated OLETF rats compared with untreated OLETF rats. In addition, rosiglitazone significantly increased serum adiponectin concentration in these rats from 20 to 40 weeks of age. The expression of both visfatin and adiponectin mRNA in visceral fat deposits was elevated by rosiglitazone when compared with untreated OLETF rats [13••]. If visfatin can act as an insulin mimetic, then it makes some sense that an insulin-sensitizing drug like rosiglitazone might increase the expression levels of visfatin/PBEF. Another study on the expression and regulation of visfatin mRNA was performed by studying 3T3-L1 adipocytes during adipogenesis and after treatment with various hormones known to alter insulin sensitivity. In this study, visfatin expression was about sixfold higher in 3T3-L1 adipocytes *in vitro* as compared with epididymal fat *in vivo* [14•]. This is an interesting observation and an interesting comparison to the first visfatin paper which showed that visfatin levels in the liver of c57BL mice or KKAy mice were shown to be much higher than the levels in subcutaneous white adipose tissue of c57BL mice [2••]. Nonetheless, both studies indicate that visfatin expression is increased during adipogenesis of 3T3-L1 cells. It has also been shown that dexamethasone treatment of 3T3-L1 adipocytes significantly increased visfatin mRNA levels, whereas growth hormone, TNFα, and isoproterenol substantially downregulated visfatin mRNA in a dose and time dependent manner [14•]. Insulin did not influence the synthesis of visfatin in these cells [14•]. It should be noted that this study only examined visfatin mRNA and did not examine the secretion or sub-cellullar location of

this protein. Taken together, these studies demonstrate increased expression of visfatin during adipocyte differentiation and an increase of visfatin levels by a thiazolidinedione in diabetic rodents, and modulation by cytokines, which are known effectors of adipogenesis and insulin action.

In an amniotic epithelial cell line, lipopolysaccharide, IL-1β, TNFα and IL-6 all significantly increased the expression of PBEF after a 4-h cytokine treatment [9]. In these cells, the addition of dexamethasone to IL-1β and TNFα significantly reduced the response of PBEF to these cytokines, whereas in cultured adipocytes dexamethasone alone increased visfatin mRNA levels [14•]. Interestingly, PBEF expression is upregulated in amniotic epithelial cells by cytokines that promote insulin resistance such as lipopolysaccharide, IL-1β, TNFα and IL-6 [9]. In cultured adipocytes, however, TNFα [14•] and IL-6 [15] resulted in a decrease of visfatin mRNA levels. Overall, these studies do not greatly enhance our understanding of the modulation of visfatin/PBEF expression and do not clearly support many recent speculations that this cytokine might be an interesting novel candidate that links components of the metabolic syndrome such as obesity and insulin resistance.

Visfatin has insulin mimetic properties

Most interesting discoveries are accompanied by some confounding observations. When visfatin was shown by Fukuhara and colleagues to be induced during adipogenesis, secreted from fat cells, and highly and specifically expressed in visceral adipose tissue they also observed an unexpected finding. Contrary to the most intuitive hypothesis, visfatin treatment did not promote insulin resistance, but actually exhibited insulin mimetic properties by causing a glucose lowering effect [2••]. In a manner similar to insulin, visfatin increased glucose transport and lipogenesis when administered to 3T3-L1 adipocytes or L6 myocytes and decreased glucose production by hepatocytes [2••]. When delivered directly to diabetic mice visfatin also improved insulin sensitivity *in vivo* and resulted in decreased glucose and insulin levels. The significance of endogenous visfatin controlling whole body insulin sensitivity was observed by studying heterozygous visfatin mice that have two-thirds the amount of visfatin of wild type mice [2••]. These animals displayed mild but reproducible hyperglycemia [2••]. Subsequent analysis of this insulin mimetic effect revealed two surprising findings; that the effects of visfatin are mediated by the insulin receptor itself with remarkably similar affinities but via a distinct binding site, and that this insulin sensitizing effect of visfatin appears to be additive to the effect of insulin [2••], suggesting that visfatin may activate insulin receptor activated pathways via a novel mechanism.

It is quite a paradox that an insulin mimetic protein is highly produced by visceral fat in a state of insulin resistance. It could be perceived that the adipocyte is employing whatever means necessary to provide an insulin response in obese adipose tissue. Clearly, there are many studies that need to be performed to enhance our understanding of visfatin biology. For example, it will very important to determine whether visfatin, working through the insulin receptor, can modulate all the signaling proteins known to be modulated by insulin. It will also be interesting to determine if visfatin has its own receptor or solely works through the insulin receptor. Also, it will be critical to determine if intracrine signaling is a pathway used by visfatin since PBEF has been shown to be present in the nucleus under some conditions [16]. Finally, it will be important to determine how visfatin is secreted. Perhaps, visfatin/PBEF is secreted when fat cells die. Recent studies have clearly shown a large increase in the amount of dead adipocytes in white adipose tissue of obese mice and these observations suggest that necrotic-like adipocyte death as a pathologic hallmark of obesity [17]. Perhaps, the paradox of visfatin might be more clearly understood if this protein was shown to be released from dying adipocytes? This is just one of many hypotheses that require rigorous testing.

Interestingly, the original paper on the identification of PBEF demonstrated that PBEF itself had no activity but synergized the pre-B-cell colony formation activity of stem cell factor and IL-7 [1]. If visfatin is a legitimate insulin mimetic as has been suggested, then it is sort of surprising that only a few well characterized biological effects of PBEF have been observed in the last 10 years given the broad range of insulin action. The literature on PBEF and visfatin is sparse and although PBEF was discovered over 10 years ago, several studies suggest that this protein does not have expected cytokine like properties. In some ways, this is not surprising since visfatin/PBEF is lacking a signal sequence and can be found in the nucleus of Swiss 3T3 cells following cytokine treatment [16]. Since studies on the regulation of this protein are in its infancy, we think it is premature to speculate on the role of PBEF in metabolic diseases.

Conclusion

The discovery of this curious new adipokine has the potential to enhance our understanding of metabolic diseases such as obesity, type 2 diabetes, and metabolic syndrome. Yet, as with all novel discoveries, these initial observations need to be reproduced and several new related questions need to be addressed before the role of visfatin in metabolic disease can be carefully evaluated. For example, it will be critical to determine the contribution of visceral adipose tissue derived visfatin and it ability to modulate whole body insulin sensitivity.

Although the affinity of visfatin for insulin receptor appears to be similar to insulin, its concentration in plasma is much lower (3–10%) under physiological conditions. Moreover, visfatin/PBEF is not regulated by fasting and feeding. These observations raise some doubts about the physiological importance of the systemic insulin sensitizing effects of visfatin. As suggested above, perhaps the elevation of visfatin in visceral adipose tissue of obese mice is due to its release from dying adipocytes. In this review, we have posed various questions relevant to visfatin/PBEF biology and summarized all the recent papers on visfatin/PBEF. Overall, we were surprised that our survey of the literature revealed minimal progress on the regulation and action of PBEF in the last 10 years and there is no current literature supporting the observation that visfatin has insulin mimetic properties. Clearly, this protein is difficult to study and for this reason the role of visfatin/PBEF remains largely unknown.

References and recommended reading

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

- • of special interest
- •• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 000–000).

1 Samal B, Sun Y, Stearns G, *et al.* Cloning and characterization of the cDNA encoding a novel human pre-B-cell colony-enhancing factor. Mol Cell Biol 1994; 14:1431–1437.

2 •• Fukuhara A, Matsuda M, Nishizawa M, *et al.* Visfatin: a protein secreted by visceral fat that mimics the effects of insulin. Science 2005; 307:426–430.

This paper demonstrates that PBEF is produced in fat cells, regulated during adipogenesis and highly expressed in mouse and human visceral adipose tissue in obesity. In addition, it was shown that visfatin has insulin mimetic properties in various cell types and administration to animal could increase insulin sensitivity.

3 •• Chen MP, Chung FM, Chang DM, *et al.* Elevated plasma level of visfatin/pre-B cell colony-enhancing factor in patients with type 2 diabetes mellitus. J Clin Endocrinol Metab 2005; Oct 18 [Epub ahead of print].

To date, this is the only study to confirm the novel observations by Fukuhara *et al.* that demonstrate elevated levels of visfatin in type II diabetes.

4 •• Berndt J, Kloting N, Kralisch S, *et al.* Plasma visfatin concentrations and fat depot-specific mRNA expression in humans. Diabetes 2005; 54:2911–2916.

This was the first major study to cast some doubt on the modulation of visfatin expression in subcutaneous versus visceral adipose tissue and indicated that there was no significant correlation between visfatin plasma concentrations and parameters of insulin sensitivity.

5 •• Jia SH, Li Y, Parodo J, *et al.* Pre-B cell colony-enhancing factor inhibits neutrophil apoptosis in experimental inflammation and clinical sepsis. J Clin Invest 2004; 113:1318–1327.

These data identify PBEF as a novel inflammatory cytokine that plays a requisite role in the delayed neutrophil apoptosis of clinical and experimental sepsis.

6 • Ye SQ, Simon BA, Maloney JP, *et al.* Pre-B-cell colony-enhancing factor as a potential novel biomarker in acute lung injury. Am J Respir Crit Care Med 2005; 171:361–370.

This paper shows that PBEF is highly expressed in mouse, canine, and human acute lung injury and demonstrates that PBEF is strong candidate as a biomarker for lung pathophysiology.

7 • Ye SQ, Zhang LQ, Adyshev D, *et al.* Pre-B-cell-colony-enhancing factor is critically involved in thrombin-induced lung endothelial cell barrier dysregulation. Microvasc Res. 2005; Sep 23 [Epub ahead of print].

This study followed up previous work indicating that PBEF was a novel candidate gene and biomarker in acute lung injury and demonstrated that PBEF play a role in thrombin-induced lung endothelial cell barrier dysregulation.

8 Ognjanovic S, Bryant-Greenwood GD. Pre-B-cell colony-enhancing factor, a novel cytokine of human fetal membranes. Am J Obstet Gynecol 2002; 187:1051–1058.

9 Ognjanovic S, Bao S, Yamamoto SY, *et al.* Genomic organization of the gene coding for human pre-B-cell colony enhancing factor and expression in human fetal membranes. J Mol Endocrinol 2001; 26:107–117.

10 • van der Veer E, Nong Z, O'Neil C, *et al.* Pre-B-cell colony-enhancing factor regulates NAD+-dependent protein deacetylase activity and promotes vascular smooth muscle cell maturation. Circ Res 2005; 1:25–34.

These findings identify PBEF as a regulator of NAD+ dependent reactions in smooth muscle cells and indicate that this protein is important in the acquisition of a mature smooth muscle cell phenotype.

11 Rongvaux A, Shea RJ, Mulks MH, *et al.* Pre-B-cell colony-enhancing factor, whose expression is upregulated in activated lymphocytes, is a nicotinamide phosphoribosyltransferase, a cytosolic enzyme involved in NAD biosynthesis. Eur J Immunol 2002; 11:3225–3234.

12 • Kloting N, Kloting I. Visfatin: gene expression in isolated adipocytes and sequence analysis in obese WOKW rats compared with lean control rats. Biochem Biophys Res Commun 2005; 332:1070–1072.

This paper demonstrated that visfatin expression was not modulated in a rodent model of metabolic syndrome.

13 •• Choi KC, Ryu OH, Lee KW, *et al.* Effect of PPAR-alpha and -gamma agonist on the expression of visfatin, adiponectin, and TNF-alpha in visceral fat of OLETF rats. Biochem Biophys Res Commun 2005; 336:747–753.

This was the first study to demonstrate that visfatin levels were increased in diabetic rats following rosiglitazone treatment.

14 • Kralisch S, Klein J, Lossner U, *et al.* Isoproterenol, TNFalpha, and insulin downregulate adipose triglyceride lipase in 3T3-L1 adipocytes. Mol Cell Endocrinol 2005; 240:43–49.

This study examined the modulation of visfatin by various cytokines in cultured adipocytes. Although, these observations are interesting, only the levels of visfatin mRNA were examined. This group had previously shown that IL-6 also modulates visfatin.

15 Kralisch S, Klein J, Lossner U, *et al.* Interleukin-6 is a negative regulator of visfatin gene expression in 3T3-L1 adipocytes. Am J Physiol Endocrinol Metab 2005; 289:E586–E590.

16 Kitani T, Okuno S, Fujisawa H. Growth phase-dependent changes in the subcellular localization of pre-B-cell colony-enhancing factor. FEBS Lett 2003; 544:74–78.

17 Cinti S, Mitchell G, Barbatelli G, *et al.* Adipocyte death defines macrophage localization and function in adipose tissue of obese mice and humans. J Lipid Res 2005; 46:2347–2355.

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