Mannose-binding dietary lectins induce adipogenic differentiation of the marrow-derived mesenchymal cells via an active insulin-like signaling mechanism

Introduction

Dietary components influence the tissue homeostasis in multiple ways, and a direct correlation between health and nutrition is a universally accepted truth. Though the lectins form a very common constituent of vegetarian diet, their effect on the mammalian cells or tissues has not been studied in detail. Lectins are generally regarded as toxic or allergy-causing substances that need to be eliminated from the diet, and yet, one cannot escape an exposure to them, especially through the food items that are consumed in an unprocessed form. Despite varied nutritional habits, foods containing lectins, such as fruits, beans, grains, seeds and nuts, form a major constituent of daily diet across all geographical regions. However, in spite of the daily consumption through dietary sources, relatively few studies examining their in vivo or in vitro biological effects on the cells or tissues have been reported (Freed 1991). Many of the reported studies have focused their attention on gastrointestinal (GI) tract or on immune system with respect to allergic responses as these systems represent the first line of interaction of lectins with the body upon food ingestion. Their effect on tissue homeostasis has, however, remained largely ignored (Pusztaei et al. 1990, 1993; Sasaki et al. 2002; Vasconcelos and Oliveira 2004; Naeem et al. 2007).

We recently examined the in vivo effect of two dietary lectins that are isolated from common food items that are usually consumed in an unprocessed form, namely banana lectin (BL) and garlic lectin (GL), on the hematopoietic compartment of the mice and demonstrated that an oral administration of these lectins resulted in an enhanced hematopoietic stem progenitor cell pool of mice (Hinge et al. 2010).

In this study, we show that consistent with their interaction with insulin receptors on the M210B4 cells and activation of extracellular signal-regulated kinase (ERK) signaling in them (Hinge et al. 2010), both BL and GL exert an adipogenic effect on these mesenchymal cells and this effect is similar to that mediated by insulin, with respect to the gene expression as well as the signaling mechanisms involved.

The data highlight the capacity of dietary lectins to induce adipogenic differentiation of marrow-derived mesenchymal cells. Our study underscores a need to examine the in vivo effects of dietary lectins on tissue homeostasis.

Manmohan Bajaj2,†, Ashwini Hinge2,†, Lalita S Limaye3, Rajesh Kumar Gupta3, Avadhesh Surolia3, and Vaijayanti P Kale1,2

1To whom correspondence should be addressed: Tel: +91-20-2570-8077; Fax: +91-20-2569-2259; e-mail: vpkale@nccs.res.in/vaijayanti.kale@gmail.com
2National Center for Cell Science, NCCS Complex, University of Pune Campus, Pune 411007, India and 3National Institute of Immunology, New Delhi 110067, India

†These authors have contributed equally in the experimental work.

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We have recently demonstrated that the mannose-binding lectins, namely banana lectin (BL) and garlic lectin (GL), interacted with the insulin receptors on M210B4 cells—an established mesenchymal cell line of murine marrow origin—and initiate mitogen-activated protein kinase kinase (MEK)-dependent extracellular signal-regulated kinase (ERK) signaling in them. In this study, we show that this lectin-mediated active ERK signaling culminates into an adipogenic differentiation of these cells. Gene expression studies indicate that the effect takes place at the transcriptional level. Experiments carried out with pharmacological inhibitors show that MEK-dependent ERK and phosphatidylinositol 3-kinase-dependent AKT pathways are positive regulators of the lectin- and insulin-mediated adipogenic differentiation, while stress-activated kinase/c-jun N-terminal kinase pathway acts as a negative one. Since both lectins could efficiently substitute for insulin in the standard adipogenic induction medium, they may perhaps serve as molecular tools to study the mechanistic aspects of the adipogenic process that are independent of cell proliferation. Our study clearly demonstrates the ability of BL and GL to activate insulin-like signaling in the mesenchymal cells in vitro leading to their adipocytic differentiation. The dietary origin of these lectins underscores an urgent need to examine their in vivo effects on tissue homeostasis.

Keywords: adipocytic differentiation / dietary lectins / insulin receptor-mediated signaling / marrow mesenchymal cells
Results

BL and GL induce adipogenesis in mesenchymal cells of bone marrow origin

In our earlier studies, we have shown that both BL and GL interact with insulin receptors on M210B4 cells and induce a mitogen-activated protein kinase kinase (MEK)-dependent ERK signaling in these cells. This signaling was abrogated when the competing sugar, alpha methyl-D-mannoside, was added to the medium (Hinge et al. 2010), indicating that the effect was indeed mediated via lectin–carbohydrate interactions. In this study, therefore, we examined the in vitro consequences of the longer incubation of the M210B4 cells with these lectins. M210B4 cells were incubated with BL or GL (20 ng/mL) for 14 days and the cells were stained with Oil Red O after fixation. This concentration of lectins was found to be an optimal one for the induction of adipogenesis in the initial dose range study (data not shown). As seen in Figure 1A, a large number of Oil Red O-positive cells formed in BL- and GL-treated M210B4 cells when compared with the control cells, clearly indicating that the lectins induce an adipogenic differentiation of these cells. The effect of BL appeared to be stronger than that of GL and this result is commensurate with the stronger ERK activation induced by BL when compared with that induced by GL (Hinge et al. 2010). Interestingly, unlike insulin, the lectins did not stimulate the proliferation of M210B4 cells even on the face of such strong ERK signaling (data not shown), indicating that the adipogenic differentiation induced by these lectins is perhaps a proliferation-independent event.

Adipogenic effect of lectins is at transcriptional level

Various transcription factors such as peroxisome proliferator-activated receptor gamma (PPARγ) and CCAAT-enhancer binding proteins (C/EBPs) are known to play a key role in the adipocyte differentiation (Spiegelman et al. 1997; Tang and Lane 1999; Grimaldi 2001; Hamm et al. 2001; Tang et al. 2003). Similarly, glyceral-3 phosphate dehydrogenase (GPDH) and adipocyte fatty acid binding protein (aP2) are well-known adipogenic markers (MacDougald and Lane 1995). To determine the transcriptional phenotype of the lectin-treated cells, semi-quantitative polymerase chain reaction (PCR) experiments were carried out with the complementary DNA (cDNA) of M210B4 cells treated with BL or GL using adipocyte-specific markers. The insulin-treated cells were used as a positive control. As seen in Figure 1B, the messenger RNA (mRNA) levels for all the adipocytic markers examined viz. PPARα, PPARγ and GPDH were found to be upregulated when the cells were treated with BL, GL or insulin. The levels of C/EBPβ were marginally upregulated by the lectins. The aP2-specific mRNA was found to be constitutively expressed in these cells and there was no further increase in the expression in response to the lectins or insulin. These results clearly indicated that both BL and GL induced an adipogenic differentiation of M210B4 stromal cells and the effect on most of the genes, except aP2, was taking place at transcriptional level.

In order to examine whether the lectin treatment also resulted in the expression of adipocyte-specific markers at protein level, BL-, GL- or insulin-treated M210B4 cells were

![Fig. 1.](https://academic.oup.com/glycob/article-abstract/21/4/521/1992744)
immunostained with antibodies to the adipogenic markers viz. C/EBPα, C/EBPβ, sterol regulatory element binding protein (SREBP), aP2, adipsin and PPARγ2. The images were analyzed for mean fluorescence intensities (MFIs) using Image J software (NIH). Consistent with the gene expression data, a significant upregulation in PPARγ2 expression was observed in the cells treated with the lectins (Figure 2) and the effect was comparable to that of insulin. Expression of three more adipocyte-specific markers examined, namely C/EBPβ, SREBP and adipsin, was also found to have upregulated significantly. The significant upregulation of C/EBPα and aP2 genes at protein level, but not at transcriptional levels, suggests involvement of translational regulation (Calkhoven et al. 2000). The data confirmed that both BL and GL indeed possessed an adipogenic effect on these cells.

3T3L1, a pre-adipocytic cell line, is a commonly used model system to study adipogenesis. We, therefore, used this well-characterized cell line to further confirm the adipogenic potential of BL and GL. In order to do so, we carried out semi-quantitative reverse transcription–polymerase chain reaction (RT–PCR) experiments with the cDNA prepared from 3T3L1 cells incubated with BL, GL or insulin. As seen in Supplementary data, Figure S1, except for aP2, the mRNAs for most of the adipocyte-specific genes examined were upregulated in response to BL and GL, confirming their adipogenic potential. The effect on the expression of C/EBPα, GPDH and PPARγ mRNAs was even better than that of insulin. The expression of PPARα was upregulated by the lectins, but was downregulated by the insulin treatment, at least at this selected time point. A constitutive expression of aP2 was seen in these cells as well and was only marginally upregulated by GL, while BL and insulin did not have any further enhancing effect. The reason behind the observed differences in the upregulation of the genes in response to lectins versus insulin is not clear at present. It is possible that the kinetics of gene expression in response to the lectins or insulin may vary as a function of time, and the expression levels of various genes may peak at different time points. However, this possibility needs to be examined formally. Nonetheless, the data indicate that the lectins have an adipogenic effect on 3T3L1 cells.

**Fig. 2.** Enhanced adipocyte-specific proteome of M210B4 cells after BL or GL treatment: M210B4 cells seeded on cover slips were treated for 4 days with either BL or GL. The cells were immunostained with antibodies against adipocyte-specific markers. A significant upregulation in the adipocyte-specific proteome was observed in the cells treated with the lectins or insulin (positive control) when compared with the untreated cells. The images were analyzed for MFIs using Image J software and these data have been depicted in the graphical form on the right-hand side of the respective images.

BL and GL mimic insulin action

In the earlier experiments, it was observed that though the M210B4 cells treated with insulin or lectins showed the presence of Oil Red O-positive cells, the lipid granules formed were very small giving an overall grainy appearance making it difficult to score individual adipocytes. It was possible that the formation of typical adipocytes having large intracellular lipid droplets may require the presence of other components of adipocyte induction medium such as dexamethasone, 3-isobutyl-1-methylxanthine (IBMX) and indomethacin (Yu et al. 2008). In this set of experiments, therefore, we incubated the M210B4 cells in the standard adipocyte induction medium [insulin induction medium (IIM)] for 10 days. As seen in Figure 3Ab, the M210B4 cells incubated in IIM showed the formation of cells possessing large lipid droplets which stained brightly with Oil Red O (Figure 3Bb). These cells could easily be scored under an inverted microscope. In the parallel sets, we substituted insulin with either BL [BL induction medium (BIM)] or GL [GL induction medium (GIM)] and scored the number of adipocytes formed in them (Figure 3Ac and d) that stained brightly with Oil Red O (Figure 3Bc and d). Induction medium without insulin, BL or GL was used as control (C-IM, Figure 3Aa and Ba). As seen in Figure 3C, a significantly higher number of adipocytes were observed in the cells incubated in BIM as well as in GIM when compared with the C-IM. In BIM and IIM sets, the number of adipocytes was more than that in the IIM, but the adipocytes in the GIM and IIM sets had larger lipid droplets in them (see the insets in Figure 3A) when compared with those in BIM set, indicating subtle differences in the lectin actions.

**Lectin-induced adipogenesis involves active signaling mechanisms**

Several studies have clearly demonstrated that mitogen activated protein kinase (MAPK) activation controls the gene expression by phosphorylating a variety of transcription factors and altering their transcriptional efficiency in a variety of cell types (Yang et al. 2003). We have previously shown that both BL and GL induced an MEK-dependent ERK activation in the M210B4 cells (Hinge et al. 2010). We, therefore, examined whether incorporation of U0126, an inhibitor of MEK-dependent ERK activation, in the induction media had any effect on the adipogenic differentiation of these cells. It
was observed that the insulin- and lectin-mediated adipogenesis was indeed susceptible to the presence of U0126 in the medium (Figure 4A and B). The adipocyte formation in IIM, BIM and GIM was significantly more than that seen in C-IM as observed before. Since AKT pathway is also known to be involved in the adipogenic process (Xia and Serrerao 1999; Yu et al. 2008), we added LY294002, an inhibitor of phosphatidylinositol 3-kinase (PI3K)-dependent AKT pathway, in the induction media. It was observed that there was a significant decrease in the adipocyte formation in the presence of this inhibitor as well, indicating that both MEK-dependent ERK signaling as well as PI3K-dependent AKT signaling are the positive mediators of insulin- and lectin-mediated adipogenesis of M210B4 cells (Figure 4C). Stress-activated kinase/c-jun N-terminal kinase (SAPK/JNK) is known to phosphorylate PPARγ1 and downregulate its transcriptional activity (Camp et al. 1999) and, thus, acts as a negative regulator of adipogenesis (Hong et al. 2007). In keeping with these reports, inclusion of SP600125, an SAPK/JNK inhibitor, in the induction medium, a clear upregulation of adipogenesis was seen in all sets (Figure 4D), indicating that SAPK/JNK pathway negatively regulates the lectin- and insulin-mediated adipogenesis in the mesenchymal cells. In all these experiments, the adipocytes formed in IIM, BIM and GIM sets were significantly more than those formed in C-IM as seen in the earlier experiments.

The data described above indicated that the insulin- and lectin-mediated adipogenesis is a net result of interplay between at least three important signaling pathways, namely ERK, AKT and SAPK/JNK. In order to specifically detect the activation status of the signaling molecules involved in the process, western blot experiments were carried out with the lysates of insulin- or lectin-treated cells in the presence or absence of specific inhibitors. The blots were sequentially probed with the antibodies to phospho-specific or native forms of signaling molecules, viz. p44/42 (ERK1/2), AKT and SAPK/JNK, and the data were normalized after densitometric analyses. As seen in Figure 4E and F, treatment of M210B4 cells with insulin, BL or GL induced phosphorylation of ERK1/2 (Figure 4Ea and Fa) and AKT (Figure 4Eb and Fb) in a U0126- and LY294002-sensitive manner, respectively. A low-level constitutive activation of ERK2 and AKT was seen in these cells. The effect of insulin on ERK1 was much stronger than that of the lectins. On the other hand, M210B4 cells also possessed a constitutively active SAPK/JNK that was downregulated by the treatment with insulin or lectin. As expected, SP600125 further downregulated the phosphorylation of SAPK/JNK (Figure 4Ec and Fc). These data are commensurate with the data obtained in the earlier experiments and clearly show that the lectin-mediated adipogenesis involves active signaling mechanisms that are very similar to that evoked by insulin.

Discussion
Lectins form an unavoidable component of vegetarian diet and yet, their effects on various tissues have not been extensively studied. GI tract and immune system have been the two principal target tissues studied with respect to lectin intake and most of the times the lectins under study are the toxic ones (Pusztai et al. 1990, 1993; Sasaki et al. 2002; Vasconcelos and Oliveira 2004; Naeem et al. 2007). However, it is imperative not only to differentiate between the lectins having salutary effects versus the ones having obvious toxic effects, but also to examine the effects of apparently nontoxic dietary lectins on human and animal tissues, both in vitro as well as in vivo. This becomes...
more relevant in the light of the efforts that are being made to develop insect-resistant crop varieties having a higher expression of certain lectins that are not tolerated by the insects (Baintner et al. 2000; Vasconcelos and Oliveira 2004). These lectins may evoke an unforeseen response in human or animal bodies after their prolonged ingestion.

Lectins can be transported through the gut wall into the blood circulation, where they directly influence peripheral...
tissues and body metabolism through the binding to glycosylated structures, such as the insulin receptor, the epidermal growth factor receptor, the interleukin 2 receptor and also the leptin receptors (Hedo et al. 1981; Livingston and Purvis 1981; Ponzio et al. 1990; Freed 1991; Zeng et al. 1995; Jönsson et al. 2005). Since lectins can reach various tissues after ingestion, it may be a worthy endeavor to examine in vivo effects of various dietary lectins on tissue homeostasis (Jönsson et al. 2005). We ourselves have demonstrated the systemic effects of BL and GL on the bone marrow tissue of the mice (Hinge et al. 2010). Koshte et al. (1992) have demonstrated that BL induced a strong IgG4 immune response in healthy blood donors having banana fruit in the diet and their study highlights the fact that in addition to the actual content of the lectins in the food, the amount of biologically active lectin that can escape antibody binding in the human body is an important issue in lectin biology and should be carefully examined.

Marrow-derived mesenchymal cells have the capacity to differentiate along various pathways to form bone, cartilage, tendon, muscle and adipose tissue (Pittenger et al. 1999). Though the role of adipocytes in the regulation of hematopoietic stem cells (HSCs) is a debated issue (DiMascio et al. 2007; Naveiras et al. 2009; Sera et al. 2009), the adipose tissue is known to be a reservoir of stem cells (De Ugarte et al. 2003). In this study, we have shown that M210B4—a mouse marrow-derived mesenchymal cell line—undergoes an adipogenic differentiation in response to the lectins. It is thus possible that the enhanced stem-cell pool that was observed by us in the lectin-fed mice could partly be attributed to the adipogenic nature of these lectins. Though the mice fed for 28 weeks with these lectins did not show any significant increase in weight (Hinge et al. 2010), the adherent cells present in the cultures of the marrow cells isolated from the lectin-fed mice spontaneously underwent adipogenic differentiation (our unpublished data). Though preliminary in nature, these results suggest that the lectins perhaps induce an adipogenic commitment in the marrow mesenchymal cells, but they need some other factor(s) to undergo a frank adipogenic differentiation in vivo.

The course of adipogenic conversion of uncommitted stem cells and pre-adipocytes in vitro is achieved by the addition of specific growth factors that are selected depending on the cell system to be used. For instance, adipogenesis is induced in the rodent cell line OB17 by incubation with fatty acids, whereas 3T3-L1 and 3T3-F442A cells require incubation with cyclic AMP, dexamethasone and insulin (Grimaldi 2001). Likewise, human pre-adipocytes isolated from subcutaneous fat can readily be induced to undergo adipose conversion upon stimulation with thiazolidinediones, while pre-adipocytes from omental fat cannot (Adams et al. 1997). It has also been established that incubating human mesenchymal stem cells (MSCs) with a mixture of insulin and dexamethasone supplemented with either rabbit serum or with the synthetic PPAR-γ2 ligand, rosiglitazone, will induce the conversion of uncommitted precursors cells to adipocytes (Janderová et al. 2003). In addition to the chemical induction, adipogenic pathway in mesenchymal stem cells (MSCs) has also been shown to be controlled by hypoxia (Fink et al. 2004).

Using standard adipogenic induction medium, we have clearly shown that insulin in the induction medium could be replaced by BL and GL, suggesting that they mimic insulin action. This is commensurate with our earlier report that both these lectins interact with insulin receptor on this cell line and activate MEK-dependent ERK pathway (Hinge et al. 2010). The gene expression studies using semi-quantitative RT–PCR or immunofluorescence approaches show that the adipogenic induction by the lectins is congruent to that induced by insulin. Though in case of BL and GL the ERK activation was found to be transient in nature (Hinge et al. 2010), diet may contain certain as-yet-uncharacterized lectins having a capacity to initiate a constitutive signaling, and such unwaranted constitutive signaling may have consequences such as insulin resistance, leptin resistance (Jönsson et al. 2005) or even neoplastic growth (Evans et al. 2002; Rhodes et al. 2008) via feed-forward mechanisms, and therefore, this aspect warrants investigation.

A detailed knowledge about the signaling pathways involved in a process leads to the development of specific pharmacological study reagents or mimetic compounds. Since the signaling pathways activated by both BL and GL mimic the insulin-mediated one, these lectins can be used as specific molecular tools to study the mechanistic aspects of the adipogenic process that are independent of cell proliferation. It may also be possible to use specific subfragments or peptide sequences derived from these lectins to develop agonists or antagonists of insulin receptor-mediated signaling.

In summary, our data clearly show that two mannose-specific dietary lectins, BL and GL, induce adipogenic differentiation of mesenchymal cells via active signaling mechanisms and also underscore the need to investigate the possible involvement of the dietary constituents in the tissue differentiation processes. The present data, taken together with our earlier published results (Hinge et al. 2010), indicate that the lectin-mediated changes in the mesenchymal cells that participate in the formation of stroma for various tissues and organs may indirectly affect the function of the stem cells. Thus, a detailed study on the effect of dietary lectins on the tissue-specific stem cells may yield valuable information about the diet-mediated regulation of tissue homeostasis.

**Materials and methods**

**Lectins**

Purification isolation and characterization of BL and GL has been described before (Chandra et al. 1997, 1999; Dam et al. 1998; Bachhawat et al. 2001; Singh et al. 2004, 2005; Gupta et al. 2008; Hinge et al. 2010). The lectin solutions were stored at 4°C and the protein content was estimated by Micro BCA Kit (Pierce, Rockford, IL) before use.

**Cell culture**

1 × 10^5 M210B4 cells or 1 × 10^3 3T3L1 cells were seeded in their respective growth medium [Roswell Park Memorial Institute (RPMI) 1640 supplemented with 10% fetal bovine serum (FBS) and Dulbecco’s modified Eagle’s medium supplemented with 10% new born calf serum] and were allowed...
to adhere overnight. They were then treated with 20 ng/mL of either BL or GL and were fed after every 72 h with fresh medium supplemented with the respective lectin.

The cells were either stained with Oil Red O or were processed for gene expression studies. In another set of experiments, 2 × 10^4 M210B4 cells were seeded in the wells of a 24-well plate and 48 h after confluence, they were treated with the standard adipogenic induction medium containing IBMX (0.5 mM; Sigma, St. Louis, MO, USA), dexamethasone (1 µM; Sigma), indomethacin (0.2 mM; Sigma), along with insulin (10 µg/mL; hereafter referred to as IIM; Invitrogen, Carlsbad, CA), or with the medium where insulin was replaced with either BL (20 ng/mL; hereafter referred to as BIM) or GL (20 ng/mL; hereafter referred to as GIM). The control cells received the medium containing IBMX, dexamethasone and indomethacin only (hereafter referred to as C-IM). The cells were treated with specific inhibitors 1 h before feeding with the respective adipogenic differentiation medium, and the respective inhibitors were also continued in the medium. The different inhibitors used were as follows: U0126 (10 µM, an MEK-dependent ERK phosphorylation inhibitor; Calbiochem, Darmstadt, Germany), SP600125 (10 µM; a SAPK/JNK inhibitor; Calbiochem) and LY294002 (10 µM; a PI3K inhibitor; Calbiochem). After 48 h of treatment with the induction medium, the cells were fed with the growth medium containing 10 µg/mL of insulin in IIM set, 20 ng/mL of BL in BIM set and 20 ng/mL of GL in GIM set. The cultures were set in triplicate and a mean ± SEM was calculated.

**Oil Red O staining**

Accumulation of triglycerides was visualized by staining the cells with Oil Red O (Sigma) as per Lillie and Ashburin’s method (Culling 1974). In brief, the cells were fixed in freshly prepared 10% buffered formalin for 10 min and were washed with distilled water before staining with Oil Red O for 35 min.

**Semi-quantitative RT–PCR**

M210B4 and 3T3L1 cells were treated with BL, GL or insulin, and mRNA was isolated using mRNA Micro Kit (Dynal, AS, Oslo, Norway) according to the manufacturer’s instructions. The isolated mRNA was reverse transcribed by using Superscript II (Invitrogen, Carlsbad, CA) and the cDNAs were subjected to semi-quantitative RT–PCR using gene-specific primers, namely PPARα, PPARγ, C/EBPα, aP2 (fatty acid binding protein) and GPDH. The levels of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were used for normalization of the signal.

**Primer sequences and the accession numbers of the genes that were used as templates**

C/EBPα (NM_007678.3)
F: 5′-GTTGACCAAAGACAGACAGA-3′
R: 5′-AGACCTGACCTCGGCTCAG-3′

PPARα (NM_011144.6)
F: 5′-CGAGAGAAAAGGCGAGAGG-3′
R: 5′-CTTAAGGACTTCGCTTGA-3′

PPARγ (NM_011146.3)
F: 5′-AGACACAGGACAAATCACA-3′
R: 5′-GAAAATGACCCCTCTTGTA-3′

GPDH (NM_010271.2)
F: 5′-AGAGATGCTGCGGACAGAA-3′
R: 5′-AAAGGGTGCTCTGGGGTCTGT-3′

αP2 (NM_024406.2)
F: 5′-CAATGCAGCTTAAATGGGGAT-3′
R: 5′-CGACTTTCCATGCCACTTC-3′

GAPDH (NM_000884.2)
F: 5′-ACTGCCACCCAGAGACTGT-3′
R: 5′-CCATGCCAGTGAGCTTCC-3′

The PCR products were separated on a 2% agarose gel and the gel was imaged on gel documentation system (Bio Imaging System from Syngene, Cambridge, UK). The densitometric analysis of the bands was carried out using GeneTools analysis software, Syngene.

**Immunofluorescence staining**

M210B4 cells seeded on cover slips were treated for 4 days with either BL or GL. The cells were immunostained with antibodies against adipocyte-specific markers, namely C/EBPα, C/EBPβ, PPARγ2, SREBP, Adipsin and aP2 (Santa Cruz Biotech, Santa Cruz, CA). Cy3-labeled anti-rabbit antibody raised in Donkey (Chemicon, Billerica, MA) was used as the secondary antibody. Images were acquired on a confocal laser scanning microscope (Carl Zeiss, North America) and the images were analyzed for MFI using Image J software (NIH).

**Western blot**

M210B4 cells were serum-starved overnight in RPMI 1640 medium containing 1% FBS. Cells were then treated with respective inhibitors of the signaling pathways for 1 h. Cells treated with equivalent amount of dimethyl sulfoxide (DMSO) were used as controls. The inhibitors used in the study were U0126, LY294002 and SP600125 (all at 10 µM) for inhibiting MEK-dependent ERK, PI3K-dependent Akt and SAPK/JNK pathways, respectively. The cells were incubated with C-IM, IIM, BIM and GIM and were lysed at different time intervals after the treatment viz. 5 min for ERK, 30 min for Akt and 15 h for SAPK/JNK. The selection of the time points was based on the peak level signaling observed in our time kinetic experiments. The protein estimation was done by Micro BCA Kit (Pierce), and the protein was determined using specific antibodies to the native and phosphorylated forms of p44/42, Akt and SAPK/JNK (Cell Signaling Tech, Danvers, MA).

**Statistical analysis**

Statistical analysis was carried out by using one-way repeated-measures analysis of variance using Sigma Stat Software (Jendal Scientific) and the graphs were created using Sigma Plot software (Jendal Scientific).
Supplementary data

**Supplementary data for this article is available online at [http://glycob.oxfordjournals.org/](http://glycob.oxfordjournals.org/).**

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**Abbreviations**

- aP2, adipocyte protein 2;
- BIM, BL induction medium;
- BL, banana lectin;
- C/EBPs, CCAAT-enhancer binding proteins;
- cDNA, complementary DNA;
- C-IM, control induction medium;
- ERK, extracellular signal-regulated kinase;
- FBS, fetal bovine serum;
- GAPDH, glyceraldehyde 3-phosphate dehydrogenase;
- GIM, GL induction medium;
- GL, garlic lectin;
- GPDH, glycerol-3 phosphate dehydrogenase;
- IBMX, 3-isobutyl-1-methylxanthine;
- IIM, insulin induction medium;
- MAPK, mitogen-activated protein kinase;
- MEK, mitogen-activated protein kinase kinase;
- MFI, mean fluorescence intensity;
- mRNA, messenger RNA;
- PCR, polymerase chain reaction;
- P3K, phosphatidylinositol 3-kinase;
- PPAR, peroxisome proliferator-activated receptor;
- PI3K, phosphatidylinositol 3-kinase;
- PPAR, peroxisome proliferator-activated receptor;
- RT–PCR, reverse transcription–polymerase chain reaction;
- SAPK/JNK, stress-activated kinase/c-jun N-terminal kinase;
- SREBP, sterol regulatory element binding protein.

**References**


Camp HS, Tafuri SR, Leff T. 1999. c-Jun N-terminal kinase phosphorylates PI3K, phosphatidylinositol 3-kinase; PPAR, peroxisome proliferator-activated receptor; RT–PCR, reverse transcription–polymerase chain reaction; SAPK/JNK, stress-activated kinase/c-jun N-terminal kinase; SREBP, sterol regulatory element binding protein.


