Development 133, 4731-4735 (2006) doi:10.1242/dev.02659

Cytoplasmic activated protein kinase Akt regulates lipid-droplet accumulation in *Drosophila* nurse cells

Natalia Vereshchagina and Clive Wilson*

The insulin/insulin-like growth factor signalling (IIS) cascade performs a broad range of evolutionarily conserved functions, including the regulation of growth, developmental timing and lifespan, and the control of sugar, protein and lipid metabolism. Recently, these functions have been genetically dissected in the fruit fly *Drosophila melanogaster*, revealing a crucial role for cell-surface activation of the downstream effector kinase Akt in many of these processes. However, the mechanisms regulating lipid metabolism and the storage of lipid during development are less well characterized. Here, we use the nutrient-storing nurse cells of the fly ovary to study the cellular effects of intracellular IIS components on lipid accumulation. These cells normally store lipid in a perinuclear pool of small neutral triglyceride-containing droplets. We find that loss of the IIS signalling antagonist PTEN, which stimulates cell growth in most developing tissues, produces a very different phenotype in nurse cells, inducing formation of highly enlarged lipid droplets. Furthermore, we show that the accumulation of activated Akt in the cytoplasm is responsible for this phenotype and leads to a much higher expression of LSD2, the fly homologue of the vertebrate lipid-storage protein perilipin. Our work therefore reveals a signalling mechanism by which the effect of insulin on lipid metabolism could be regulated independently of some of its other functions during development and adulthood. We speculate that this mechanism could be important in explaining the well-established link between obesity and insulin resistance that is observed in Type 2 diabetes.

KEY WORDS: *Drosophila*, Insulin, PTEN, Obesity, Oogenesis

INTRODUCTION

Signalling by insulin and insulin-like molecules plays a central role in the control of sugar, protein and lipid metabolism, both during development and in adults (Dugani and Clip, 2005; Goberdhan and Wilson, 2003a; Holm, 2003). Disruption of this homeostatic mechanism can have significant physiological consequences. For example, reduced insulin signalling underlies the defects in glucose metabolism observed in diabetes, whereas changes in lipid metabolism associated with obesity are an important causative factor in establishing cellular insulin resistance in patients with Type 2 diabetes (Haslam and James, 2005).

Genetic analysis of the insulin/insulin-like growth factor signalling (IIS) cascade in *Drosophila* has highlighted the importance of this pathway in development, revealing a central role in controlling protein synthesis and cell growth (Goberdhan and Wilson, 2003a; Hafen, 2004). This evolutionarily conserved process is mediated by cell-surface activation of the downstream effector kinase Akt (also known as Akt1 – Flybase). Akt is activated by increased levels of phosphatidylinositol 3,4,5-trisphosphate [PtdIns(3,4,5)-P_3], a phospholipid signalling molecule produced by IIS-regulated class I PI3-kinases (Downward, 1998). Hyperactivation of IIS has been implicated in the generation of many different human tumours, which are frequently defective for the tumour-suppressor protein PTEN, a PtdIns(3,4,5)-P_3 phosphatase that directly antagonizes the effects of PI3-kinases (Goberdhan and Wilson, 2003b).

IIS also affects sugar metabolism (Rulifson et al., 2002) and lipid storage during fly development, thus modulating starvation sensitivity (Böhni et al., 1999; Oldham et al., 2002; Teleman et al., 2005). However, the lack of a good in vivo system in which to study cellular changes in lipid accumulation has made it difficult to dissect the genetic mechanisms involved. Interestingly, studies in mice have indicated that, in addition to a global effect on growth, increased IIS can strongly stimulate lipid storage in a narrower range of cell types (Magun et al., 1996; Horie et al., 2004), demonstrating that the effects of IIS on lipid metabolism are highly cell type-specific.

In order to genetically dissect IIS-dependent lipid-storage mechanisms, we analysed the effects of increasing downstream IIS signalling in the nutrient-storing nurse cells of the *Drosophila* ovary. We find that nurse cells lacking *Pten* exhibit a remarkable cell type-specific enlarged lipid-droplet phenotype. Mutant cells have highly elevated levels of activated Akt in their cytoplasm. We show that this pool of Akt is essential for lipid accumulation and controls the expression of an evolutionarily conserved lipid-storage protein, LSD2/perilipin. Our data therefore reveal a novel mechanism by which triglyceride storage can be controlled independently of other IIS-dependent events in specific tissues, potentially explaining how these processes might become uncoupled in certain disease states.

MATERIALS AND METHODS

Genetic crosses

Except where mentioned, all flies were obtained from the Bloomington Stock Centre. The genotype of rescued *Pten* mutant flies was *yw;Pten^{dj189}/Pten^{117};Akt^{3}/Akt^{1}* (Stocker et al., 2002).

Males hemizygous for a *hsp70-flp^{122}* X-chromosome insertion, which produces leaky expression of *flp* even in the absence of heat shock (Britton et al., 2002), and heterozygous for *Pten^{1} FRT40A* (Goberdhan et al., 1999) were crossed to females homozygous for *P[w^{+};Ubi-GFP^{nls}S65T] FRT40A* (a gift from Bruce Edgar, Fred Hutchinson Cancer Research Center, Seattle, USA), which preferentially expresses GFP in the nucleus. Newly eclosed *yw^{1118} hsp70-flp^{122}/w^{1118};Pten^{1} FRT40A/ P[w^{+};Ubi-GFP] FRT40A* females were heat shocked in a water bath for 1 hour at 37.5°C and aged for up to 3 days before dissection.

Males hemizygous for the same *hsp70-flp^{122}* chromosome and heterozygous for *UAS-Dp110* were crossed to females homozygous for the actin flp-out stock *Act>CD2>Gal4, UAS-GFP^{nls}S65T* (Neufeld et al., 1998). Newly eclosed F_1 females, heterozygous for *hsp70-flp* and transheterozygous for the flp-out and *UAS-Dp110* chromosomes, were heat

Department of Physiology, Anatomy and Genetics, Le Gros Clark Building, University of Oxford, South Parks Road, Oxford OX1 3QX, UK.

*Author for correspondence (e-mail: clive.wilson@anat.ox.ac.uk)

Accepted 25 September 2006

shocked as described above. In all egg chambers examined, every nurse cell appeared to overexpress the GFP and *Drosophila* (*D*)*p110* transgenes, presumably because of leaky *hsp70-flp*^{122} expression.

Staining of ovaries

Dissected ovaries from 3-day old females were fixed for 30 minutes in 4% paraformaldehyde in PBS. F-actin was stained with 2.5 μg/ml TRITC-phalloidin (Sigma). Rabbit anti-phospho-Ser-505-Akt antibody (Cell Signalling Technologies) was used at 1:500 and rabbit anti-LSD2 (Welte et al., 2005) at 1:500 in PBS, 0.1% Tween-20. The secondary antibody was a Cy5-coupled anti-rabbit antibody raised in donkey (Jackson Labs; 1:800). A 10 mg/ml solution of Nile Red (Sigma) in acetone was diluted 1:2500 or 1:1250 in PBST for staining. To visualize DNA, egg chambers were incubated for 2 hours at room temperature with 0.5 mg/ml RNAase A in PBST, then stained with 1 μg/ml propidium iodide. Images were collected on a Leica TCS SP Confocal System and processed with Adobe Photoshop CS2 version 9.

RESULTS AND DISCUSSION

To understand better the link between IIS and lipid accumulation, we genetically increased downstream IIS signalling in nurse cells of the *Drosophila* ovary, which store triglycerides in small perinuclear droplets during oogenesis for subsequent pumping into the maturing oocyte (Fig. 1A,B; droplets are best visualized at stage 10 of oogenesis with the neutral lipid-soluble dye Nile Red) (Teixeira et al., 2003). Cells in 90% (*n*=50) of clones mutant for the IIS antagonist *Pten* (Goberdhan et al., 1999; Huang et al., 1999) showed cell-autonomous formation of highly enlarged large lipid droplets (Fig. 1D-I) and a corresponding reduction in the number of small perinuclear droplets. This phenotype was never observed in groups of nurse cells in wild-type egg chambers (*n*=50; $P<0.001$ by chi-squared test), and has not previously been reported in *Pten* mutant cells in other tissues. Indeed, analysis of mutant clones in the developing eye imaginal disc revealed no obvious changes in lipid droplet accumulation (see Fig. S1 in the supplementary material).

Cell-surface activation of Akt in response to elevated PtdIns(3,4,5)-P_3 levels involves phosphorylation of both Thr-342 and Ser-505 (equivalent to Ser-473 in mammalian Akt) (Downward, 1998; Pinal et al., 2006). The latter can be assessed using an antibody that cross-reacts specifically with Ser-505-phosphorylated Akt (*P*-Akt; Fig. 2; see Fig. 3J for a schematic of the signalling cascade). Interestingly, unlike *Drosophila* photoreceptor cells in which *P*-Akt is localized at the cell surface (Pinal et al., 2006), we observed modest accumulation of *P*-Akt throughout the cytoplasm of wild-type nurse cells (Fig. 2A,B). Cytoplasmic *P*-Akt levels were greatly increased in *Pten* mutant cells (Fig. 2C-L). Nuclear *P*-Akt was not similarly increased (Fig. 2C,D). A change in cytoplasmic *P*-Akt is observed despite the fact that the germ-line nurse cells and oocyte within an egg chamber form a syncytium via intercellular ring canals, suggesting that IIS signalling complexes (and GFP) are unable to diffuse through these connections. As found in other cell types (Goberdhan et al., 1999; Pinal et al., 2006), there were no gross abnormalities in the actin cytoskeleton of *Pten* mutant cells (Fig. 2G,K) or indications of premature apoptosis (see nuclear staining in Fig. S2 in the supplementary material), indicating a specific defect in lipid storage.

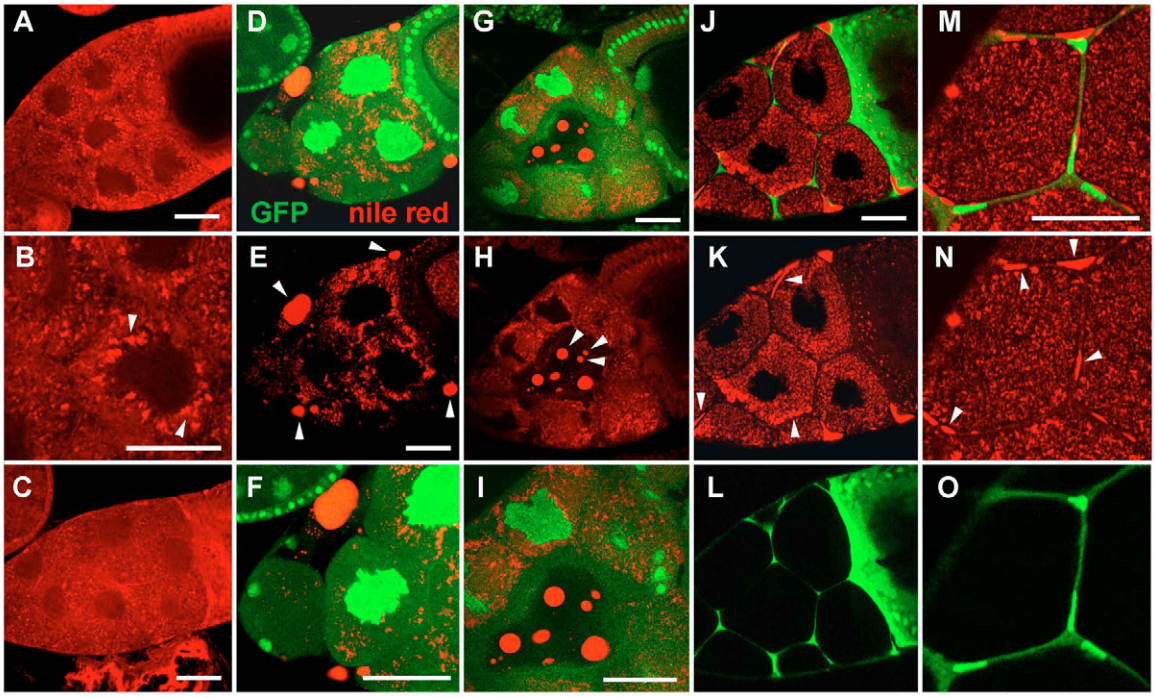

Fig. 1. Increased IIS produces lipid-droplet accumulation defects in nurse cells. (**A**,**B**) Nile Red-positive small neutral lipid droplets (red) accumulate in wild-type nurse cells, particularly in a perinuclear region (arrowheads in B), which appears to co-localize with the endoplasmic reticulum (Teixeira et al., 2003). By contrast, non-GFP-labelled (not green) *Pten* mutant cells (**D-I**) contain large aggregated lipid droplets of up to 15 μm diameter (arrowheads in E,H; shown at high magnification in F,I) and contain far fewer small droplets. (**C**) This phenotype is completely suppressed in *Pten* mutant animals rescued by a specific combination of *Akt* alleles (Stocker et al., 2002). (**J-O**) In some egg chambers, overexpression of *Dp110* in nurse cells induces sporadic formation of superficial, elongated lipid-containing structures close to the plasma membrane (arrowheads in K,N). The profile of these cells is outlined by intense GFP expression in overlying somatic stretched follicular cells (green in L,O; see also deeper section in Fig. 3C). Scale bars: 40 μm in A-C,F,I; 40 μm in E for D,E; 40 μm in G for G,H; 40 μm in J for J,K,L; and 40 μm in M for M,N.O.

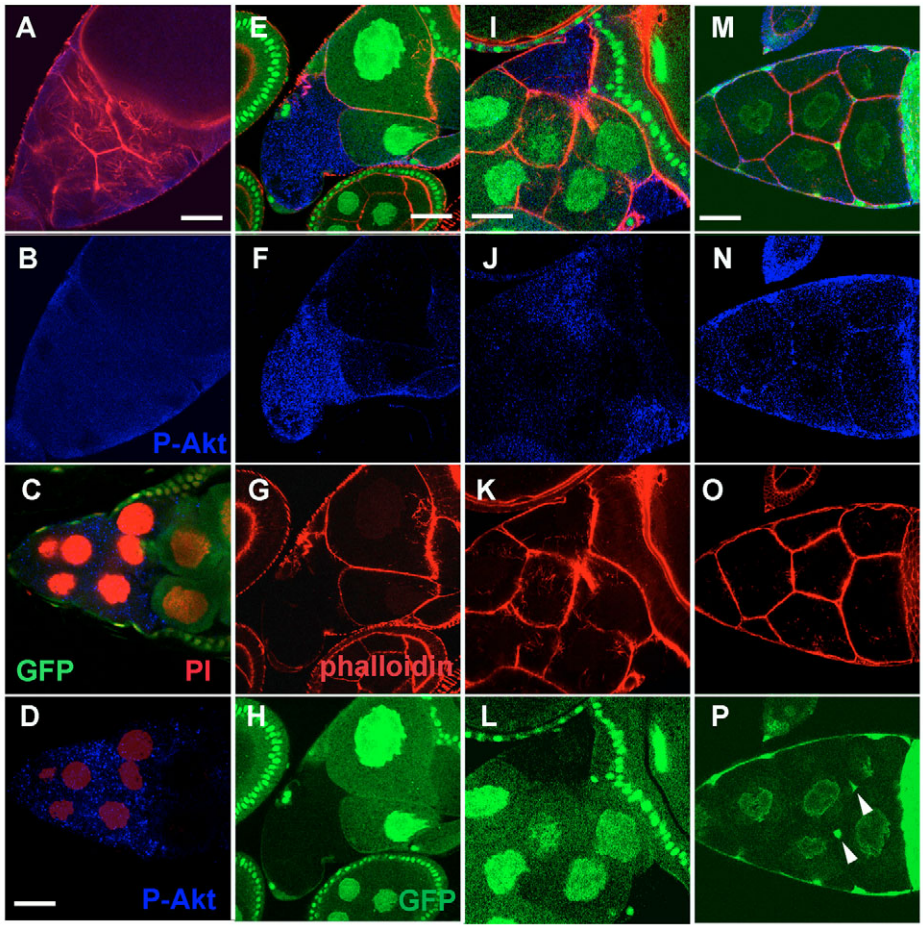

Fig. 2. Cytoplasmic activated Akt is greatly increased in *Pten*-mutant nurse cells. (**A**,**B**) Nurse cells from wild-type egg chambers show a modest accumulation of activated *P*-Akt (blue) in the cytoplasm. The actin cytoskeleton of these cells is labelled with TRITC-phalloidin (red). (**E-L**) Non-GFP-labelled (not green in H,L) *Pten* mutant cells have no obvious cortical cytoskeletal abnormalities (G,K), but contain much higher levels of cytoplasmic *P*-Akt (F,J). (**C**,**D**) Elevated levels of *P*-Akt are observed in the cytoplasm of mutant cells but not in nuclei, which are stained with propidium iodide (red) in this egg chamber. The positions of *Pten* mutant nuclei are marked in red in D. (**M-P**) Nurse cells overexpressing *Dp110* produce increased cell-surface *P*-Akt (N), co-localizing with cortical actin (O). Intensely GFP-positive nuclei of peripheral *Dp110*-overexpressing stretched follicle cells overlying the nurse cells are also observed in this image (P, arrowheads). Scale bar: 40 μm in A for A,B; 40 μm in D for C,D; 40 μm in E for E-H; 40 μm in I for I-L; and 40 μm in M for M-P.

Stocker et al. have identified a combination of *Akt* mutant alleles that entirely suppresses the lethality, growth and polarity defects observed in strong hypomorphic *Pten* mutants (Stocker et al., 2002). Viable females of this genotype showed normal storage of lipids in nurse cells (Fig. 1C), demonstrating that Akt is an essential mediator of the effects of PTENs on lipid storage.

To test the hypothesis that cytoplasmic *P*-Akt is crucial in promoting large lipid-droplet accumulation, we overexpressed *Drosophila* PI3-kinase (Dp110, also known as Pi3K92E – Flybase) (Leevers et al., 1996) in nurse cells. Overexpression of Dp110 does not fully phenocopy the effects of *Pten* mutation in other tissues (Gao et al., 2000), in part, perhaps, because these molecules can control different subcellular pools of activated Akt. Indeed, levels of cell-surface *P*-Akt were selectively increased by Dp110 overexpression (Fig. 2M-P), but there was no obvious change in bulk cytoplasmic *P*-Akt. Many of these overexpressing cells appeared to contain a normal distribution of small lipid droplets. However, in 16% of overexpressing egg chambers (n=100), elongated cell-surface Nile Red-positive structures, which are not observed in normal egg chambers (n=100, P<0.001), were seen in nurse cells at the periphery of the egg chamber (Fig. 1J-O).

In vertebrates. triglyceride storage is regulated by a number of evolutionarily conserved lipases and droplet-binding proteins, including perilipin. Perilipin can promote lipid-droplet formation, and its expression and activity are upregulated by insulin (Holm, 2003; Prusty et al., 2002; Akimoto et al., 2005). In wild-type *Drosophila* nurse cells the perilipin homologue LSD2, which normally modulates lipid storage, is distributed throughout the cytoplasm, but excluded from lipid droplets (Teixera et al., 2003) (Fig. 3A,B). Levels of LSD2 were greatly increased in *Pten* mutant cells (Fig. 3E-I). As expected, the protein was excluded from all large lipid droplets and, in some cases, there was increased accumulation at the periphery of these structures. PI3-kinase-overexpressing nurse cells do not express LSD2 at elevated levels (Fig. 3C,D), but this protein is excluded from the surface-localized Nile Red-stained structures formed in these cells.

Subcellular localization of IIS components facilitates independent regulation of multiple processes

We have shown that the accumulation of large lipid droplets in bulk cytoplasm requires activation of Akt throughout this compartment, as is observed in *Pten*-mutant nurse cells. Previous functional studies of *Pten* in *Drosophila* have primarily highlighted a crucial role for this molecule in controlling cell growth in the wing, eye and other developing adult tissues (Goberdhan et al., 1999; Huang et al., 1999; Oldham et al., 2002). The effects on lipid-droplet formation appear more cell type-specific and, indeed, our analysis of the developing eye imaginal disc indicates that the large droplet phenotype observed in nurse cells is not reproduced in photoreceptors, in which, at least at later stages of development, activated Akt is localized to the cell surface (Pinal et al., 2006).

Plasma membrane-associated *P*-Akt mediates many normal functions of IIS, including growth (Goberdhan and Wilson, 2003a). Furthermore, surface activation in restricted plasma-membrane domains potentially permits independent regulation of other localized cellular properties such as polarity and migration (Sulis and Parsons, 2003; Pinal et al., 2006). However, significant

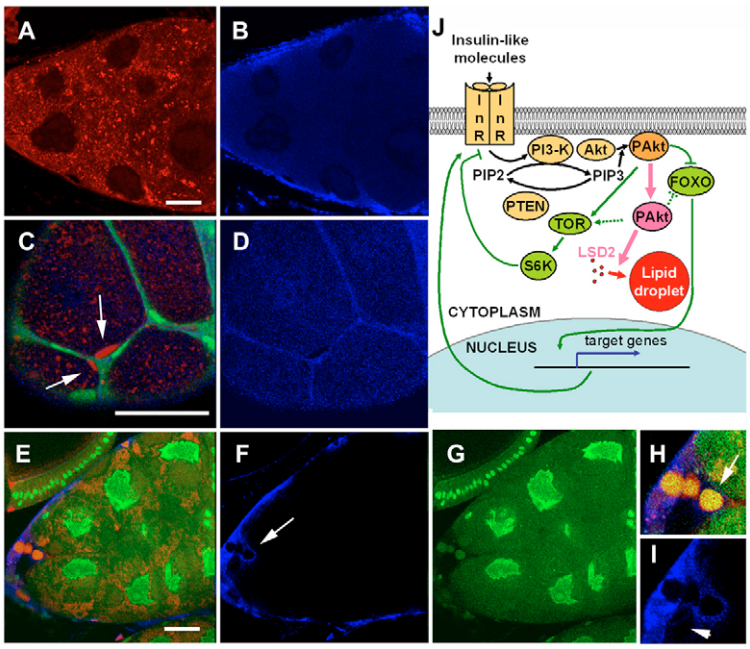

Fig. 3. Cytoplasmic activated Akt controls *Lsd2* expression and specific IIS-dependent functions. (**A**,**B**) Perilipin homologue LSD2 (B, blue) is expressed relatively homogeneously in the nurse cell cytoplasm, which is stained for lipid with Nile Red (A, red). (**C-I**) Nile Red-positive droplets (red; arrows) in *Pten*-mutant (GFP-negative cell in E-I; I,H are magnified views of anterior nurse cell in E) and *Dp110*-overexpressing (C,D) nurse cells exclude the perilipin homologue LSD2. In G,H, Nile Red is also detected in the green channel in intensely stained large lipid droplets. LSD2 levels are highly elevated in *Pten* mutant cells (F,I); normal perilipin levels are not visible at this low imaging sensitivity. There is evidence for elevated LSD2 accumulation around some lipid droplets (arrow in H). Arrowhead in I points to a region in the mutant cell that does not contain LSD2 – which is presumably the nucleus. (**J**) Schematic showing the established InR/PI3-kinase/Akt signalling cassette, involving InR-dependent activation of cell-surface Akt (orange), a process antagonized by PTEN. Our data reveal a role for cytoplasmic activated Akt (pink) in regulating the accumulation of large lipid droplets (red) in *Drosophila* nurse cells via a mechanism that, in part, involves LSD2. Note potential negative-feedback mechanisms (green), which might be controlled by cytoplasmic as well as surface *P*-Akt, and could therefore induce differential sensitivity to insulin in different subcellular domains. Scale bars: 40 μm.

levels of *P*-Akt also dissociate into the cytoplasm and nucleus in some cell types (Downward, 1998; Ayala et al., 2004; Kumar and Hung, 2005). Nuclear *P*-Akt has been proposed to have several cell-biological roles (Déléris et al., 2006), but the specific functions of the cytoplasmic pool have not previously been genetically analysed. Although we have focused our study on the effects of downstream IIS signalling components, insulin receptor signalling normally modulates Akt activity throughout the entire fly (Goberdhan et al., 2003a), so we anticipate that upstream IIS components will also play a modulatory role in the lipid-storage process. Indeed, these latter components do affect ovarian development (Drummond-Barbosa and Spradling, 2001; Brogiolo et al., 2001). In fact, we believe our study has uncovered a conserved function of IIS in lipid-storage cells, as insulin can also increase perilipin expression in mammalian adipocytes and sebocytes (Prusty et al., 2002; Akimoto et al., 2005).

Our data suggest that the effect of cytoplasmic *P*-Akt on lipid storage may be cell type-specific. Increasing IIS throughout the whole organism in viable *Pten* mutants surprisingly reduces total lipid content (Oldham et al., 2002), whereas decreasing IIS elevates lipid levels both in flies and mice (Böhni et al., 1999; Burks et al., 2000). We have not been able to show biochemically whether triglycerides are increased rather than merely redistributed in *Pten* mutant ovaries, because only a small minority of the nurse cells is mutant. However, in some late-stage clones, in which cytoplasmic volumes are decreasing, lipid levels often appear elevated in mutant cells (e.g. Fig. S2 in the supplementary material). Insulin-stimulated increases in lipid-droplet number and size have been observed in mammals (Schwertfeger et al., 2003; Magun et al., 1996), and, interestingly, when tumours are induced in mouse liver by raising IIS, lipid-storage mechanisms are also activated (Horie et al., 2004). We propose that these cell type-specific responses are due to selective accumulation of an IIS-modulated, cytoplasmic activated Akt pool that must, to some extent, be controlled independently of cell-surface *P*-Akt. Indeed, we have recently identified a *Drosophila* phosphatase that regulates levels of cytoplasmic *P*-Akt in nurse cells and have shown that mutations in this gene produce a very similar enlarged lipid-droplet phenotype, confirming this hypothesis (our unpublished observations).

How could elevated cytoplasmic *P*-Akt induce such a dramatic lipid-droplet phenotype in nurse cells? In mammals, Akt can promote the transcription of genes involved in lipid biosynthesis and storage pathways (Eberle et al., 2004). Our data indicate LSD2/perilipin is one of these targets. IIS also post-translationally upregulates the activity of mammalian perilipin (Holm, 2003). Interestingly, ovaries mutant for *Lsd2* show altered lipid accumulation, but droplets are still formed (Teixeira et al., 2003). Therefore, LSD2 is almost certainly one, but not the only, target for IIS in the control of lipid-droplet accumulation in nurse cells. In this context, it is interesting to note that, in addition to its proposed role in coating lipid droplets, LSD2 has recently been shown to regulate microtubule-dependent trafficking of these organelles (Welte et al., 2005). As cytoplasmic *P*-Akt could still be associated with intracellular membranes or the droplet surface, it may be well positioned to modulate this transport process.

Obesity is a well-established predisposing factor in the acquisition of cellular insulin resistance and Type 2 diabetes (Haslam and James, 2005). Increased levels of circulating free fatty acids (FFAs) associated with obesity appear to be important in this link (Kovacs and Stumvoll, 2005). However, it is unclear whether other mechanisms are also involved or how reduced insulin sensitivity ultimately impacts on lipid storage. Molecules downstream of Akt are known to regulate cell-surface IIS through at least two negative-feedback loops (Fig. 3J) involving downstream S6 kinase and the transcription factor FOXO (Harrington et al., 2005; Goberdhan et al., 2005; Puig and Tjian, 2005). Our work therefore raises the possibility that any predisposition towards increased cytoplasmic *P*-Akt could specifically promote lipid storage and also selectively suppress insulin-dependent events at the cell surface (Fig. 3J). It will be interesting to investigate further the molecules involved in controlling this *P*-Akt pool and whether the feedback mechanisms have any role to play in linking obesity and insulin resistance in Type 2 diabetes.

We thank Bruce Edgar, Ernst Hafen and Hugo Stocker for fly strains; Steve Gross for antiserum; Simon North for technical support; and Marie-Christine Ramel for help with manuscript preparation. This work was funded by Diabetes UK (ref. BDA:RD02/0002540), and the Biotechnology and Biological Sciences Research Council, UK.

Supplementary material

Supplementary material for this article is available at http://dev.biologists.org/cgi/content/full/133/23/4731/DC1

References

Akimoto, N., Sato, T., Iwata, C., Koshizuka, M., Shibata, F., Nagai, A., Sumida, M. and Ito, A. (2005). Expression of perilipin A on the surface of lipid droplets increases along with the differentiation of hamster sebocytes in vivo and in vitro. *J. Invest. Dermatol.* **124**, 1127-1133.

Ayala, G., Thompson, T., Yang, G., Frolov, A., Li, R., Scardino, P., Ohori, M., Wheeler, T. and Harper, W. (2004). High levels of phosphorylated form of Akt-1 in prostate cancer and non-neoplastic prostate tissues are strong predictors of biochemical recurrence. *Clin. Cancer Res.* **10**, 6572-6578.

Böhni, R., Riesgo-Escovar, J., Oldham, S., Brogiolo, W., Stocker, H., Andruss, B. F., Beckingham, K. and Hafen, E. (1999). Autonomous control of cell, and organ size by CHICO, a *Drosophila* homolog of vertebrate IRS1-4. *Cell* **97**, 865-875.

Britton, J. S., Lockwood, W. K., Li, L., Cohen, S. M. and Edgar, B. A. (2002). *Drosophila*'s insulin/PI3-kinase pathway coordinates cellular metabolism with nutritional conditions. *Dev. Cell* **2**, 239-249.

Brogiolo, W., Stocker, H., Ikeya, T., Rintelen, F., Fernandez, R. and Hafen, E. (2001). An evolutionarily conserved function of the *Drosophila* insulin receptor and insulin-like peptides in growth control. *Curr. Biol.* **11**, 213-221.

Burks, D. J., Font de Mora, J., Schubert, M., Withers, D. J., Myers, M. G., Towery, H. H., Altamuro, S. L., Flint, C. L. and White, M. F. (2000). IRS-2 pathways integrate female reproduction and energy homeostasis. *Nature* **407**, 377-382.

Déléris, P., Gayral, S. and Breton-Douillon, M. (2006). Nuclear PtdIns(3,4,5)P(3) signaling: an ongoing story. *J. Cell. Biochem.* **98**, 469-485.

Downward, J. (1998). Mechanisms and consequences of activation of protein kinase B/Akt. *Curr. Opin. Cell Biol.* **10**, 262-267.

Drummond-Barbosa, D. and Spradling, A. C. (2001). Stem cells and their progeny respond to nutritional changes during *Drosophila* oogenesis. *Dev. Biol.* **231**, 265-278.

Dugani, C. B. and Klip, A. (2005). Glucose transporter 4, cycling, compartments and controversies. *EMBO Rep.* **6**, 1137-1142.

Eberle, D., Hegarty, B., Bossard, P., Ferre, P. and Foufelle, F. (2004). SREBP transcription factors: master regulators of lipid homeostasis. *Biochimie* **86**, 839-848.

Gao, X., Neufeld, T. P. and Pan, D. (2000). *Drosophila* PTEN regulates cell growth and proliferation through PI3K-dependent and -independent pathways. *Dev. Biol.* **221**, 404-418.

Goberdhan, D. C. I. and Wilson, C. (2003a). The functions of insulin signalling: size isn't everything, even in *Drosophila*. *Differentiation* **71**, 375-397.

Goberdhan, D. C. I. and Wilson, C. (2003b). PTEN: multifunctional growth regulator and more. *Hum. Mol. Genet.* **12**, R239-R248.

Goberdhan, D. C., Paricio, N., Goodman, E. C., Mlodzik, M. and Wilson, C. (1999). *Drosophila* tumour suppressor *PTEN* controls cell size and number by antagonising the PI3-kinase/Chico signaling pathway. *Genes Dev.* **13**, 3244-3258.

Goberdhan, D. C. I., Meredith, D., Boyd, C. A. R. and Wilson, C. (2005). PAT-related amino acid transporters regulate growth via a novel mechanism that does not require bulk transport of amino acids. *Development* **132**, 2365-2375.

Hafen, E. (2004). Cancer, type 2 diabetes, and ageing: news from flies and worms. *Swiss Med. Wkly* **134**, 711-719.

Harrington, L. S., Findlay, G. M. and Lamb, R. F. (2005). Restraining PI3K: mTOR signalling goes back to the membrane. *Trends Biochem. Sci.* **30**, 35-42.

Haslam, D. W. and James, W. P. (2005). Obesity. *Lancet* **366**, 1197-1209.

Holm, C. (2003). Molecular mechanisms regulating hormone-sensitive lipase and lipolysis. *Biochem. Soc. Trans.* **31**, 1120-1124.

Horie, Y., Suzuki, A., Kataoka, E., Sasaki, T., Hamada, K., Sasaki, J., Mizuno, K., Hasegawa, G., Kishimoto, H., Iizuka, M. et al. (2004). Hepatocyte-specific Pten deficiency results in steatohepatitis and hepatocellular carcinomas. *J. Clin. Invest.* **113**, 1774-1783.

Huang, H., Potter, C. J., Tao, W., Li, D. M., Brogiolo, W., Hafen, E., Sun, H. and Xu, T. (1999). *PTEN* affects cell size, cell proliferation and apoptosis during *Drosophila* eye development. *Development* **126**, 5365-5372.

Kovacs, P. and Stumvoll, M. (2005). Fatty acids and insulin resistance in muscle and liver. *Best Pract. Res. Clin. Endocrinol. Metab.* **19**, 625-635.

Kumar, R. and Hung, M. C. (2005). Signaling intricacies take center stage in cancer cells. *Cancer Res.* **65**, 2511-2515.

Leevers, S. J., Weinkove, D., MacDougall, L. K., Hafen, E. and Waterfield, M. D. (1996). The *Drosophila* phosphoinositide 3-kinase Dp110 promotes cell growth. *EMBO J.* **15**, 6584-6594.

Magun, R., Burgering, B. M., Coffer, P. J., Pardasani, D., Lin, Y., Chabot, J. and Sorisky, A. (1996). Expression of a constitutively activated form of protein kinase B (c-Akt) in 3T3-L1 preadipose cells causes spontaneous differentiation. *Endocrinology* **137**, 3590-3593.

Neufeld, T. P., de la Cruz, A. D. A., Johnston, L. A. and Edgar, B. A. (1998). Coordination of growth and cell division in the *Drosophila* wing. *Cell* **93**, 1183-1193.

Oldham, S., Stocker, H., Laffargue, M., Wittwer, F., Wymann, M. and Hafen, E. (2002). The *Drosophila* insulin/IGF receptor controls growth and size by modulating PtdInsP(3) levels. *Development* **129**, 4103-4109.

Pinal, N., Goberdhan, D. C., Collinson, L., Fujita, Y., Cox, I. M., Wilson, C. and Pichaud, F. (2006). Regulated and polarized accumulation of PtdIns(3,4,5)P3 is essential for morphogenesis of the apical membrane in photoreceptor epithelial cells. *Curr. Biol.* **16**, 140-149.

Prusty, D., Park, B. H., Davis, K. E. and Farmer, S. R. (2002). Activation of MEK/ERK signaling promotes adipogenesis by enhancing peroxisome proliferator-activated receptor gamma (PPARgamma) and C/EBPalpha gene expression during the differentiation of 3T3-L1 preadipocytes. *J. Biol. Chem.* **277**, 46226-46232.

Puig, O. and Tjian, R. (2005). Transcriptional feedback control of insulin receptor by dFOXO/FOXO1. *Genes Dev.* **19**, 2435-2446.

Rulifson, E. J., Kim, S. K. and Nusse, R. (2002). Ablation of insulin-producing neurons in flies: growth and diabetic phenotypes. *Science* **296**, 1118-1120.

Schwertfeger, K. L., McManaman, J. L., Palmer, C. A., Neville, M. C. and Anderson, S. M. (2003). Expression of constitutively activated Akt in the mammary gland leads to excess lipid synthesis during pregnancy and lactation. *J. Lipid Res.* **44**, 1100-1112.

Stocker, H., Andjelkovic, M., Oldham, S., Laffargue, M., Wymann, M. P., Hemmings, B. A. and Hafen, E. (2002). Living with lethal PIP3 levels: viability of flies lacking PTEN restored by a PH domain mutation in Akt/PKB. *Science* **295**, 2088-2091.

Sulis, M. L. and Parsons, R. (2003). PTEN: from pathology to biology. *Trends Cell Biol.* **13**, 478-483.

Teixeira, L., Rabouille, C., Rorth, P., Ephrussi, A. and Vanzo, N. F. (2003). *Drosophila* Perilipin/ADRP homologue Lsd2 regulates lipid metabolism. *Mech. Dev.* **120**, 1071-1081.

Teleman, A. A., Chen, Y. W. and Cohen, S. M. (2005). *Drosophila* Melted modulates FOXO and TOR activity. *Dev. Cell* **9**, 271-281.

Welte, M. A., Cermelli, S., Griner, J., Viera, A., Guo, Y., Kim, D. H., Gindhart, J. G. and Gross, S. P. (2005). Regulation of lipid-droplet transport by the perilipin homolog LSD2. *Curr. Biol.* **15**, 1266-1275.