Developmental aspects of adipose tissue in GH receptor and prolactin receptor gene disrupted mice: site-specific effects upon proliferation, differentiation and hormone sensitivity

David J Flint[1,2], **Nadine Binart**[2], **Stephanie Boumard**[2], **John J Kopchick**[3] and **Paul Kelly**[2]

[1]Department of Bioscience, University of Strathclyde, Glasgow G1 1XW, UK

[2]Inserm U809, Faculté de Médecine René Descartes- site Necker, Université Paris Descartes, F-75015 Paris, France

[3]College of Osteopathic Medicine, Edison Biotechnology Institute, Ohio University, Athens, Ohio 45701, USA

(Requests for offprints should be addressed to D J Flint; Email: david.flint@strath.ac.uk)

Abstract

Direct metabolic effects of GH on adipose tissue are well established, but effects of prolactin (PRL) have been more controversial. Recent studies have demonstrated PRL receptors on adipocytes and effects of PRL on adipose tissue *in vitro*. The role of GH in adipocyte proliferation and differentiation is also controversial, since GH stimulates adipocyte differentiation in cell lines, whereas it stimulates proliferation but inhibits differentiation of adipocytes in primary cell culture. Using female gene disrupted (ko) mice, we showed that absence of PRL receptors (PRLRko) impaired development of both internal and s.c. adipose tissue, due to reduced numbers of adipocytes, an effect differing from that of reduced food intake, where cell volume is decreased. In contrast, GHRko mice exhibited major decreases in the number of internal adipocytes, whereas s.c. adipocyte numbers were increased, even though body weight was decreased by 40–50%. The changes in adipose tissue in PRLRko mice appeared to be entirely due to extrinsic factors since preadipocytes proliferated and differentiated in similar fashion to wild-type animals *in vitro* and their response to insulin and isoproterenol was similar to wild-type animals. This contrasted with GHRko mice, where s.c. adipocytes proliferated, differentiated, and responded to hormones in identical fashion to controls, whereas parametrial adipocytes exhibited markedly depressed proliferation and differentiation potential and failed to respond to insulin or noradrenaline. Our results provide *in vivo* evidence that both GH and PRL stimulate differentiation of adipocytes but that the effects of GH are site specific and induce intrinsic changes in the precursor population, which are retained *in vitro*.

Journal of Endocrinology (2006) **191,** 101–111

Introduction

Although the effects of growth hormone (GH) upon adipose tissue have been extensively studied and clearly demonstrated, the effects of prolactin (PRL) have not been clearly defined and have generally been considered to be indirect, mainly due to the absence of convincing evidence for the existence of PRL receptors on the mature adipocyte. Whilst PRL and placental lactogen (PL) have been proposed to influence carbohydrate and lipid metabolism (Turtle & Kipnis 1967, Genazzani *et al.* 1969, Strange & Swyer 1974, Oller do Nascimento *et al.* 1989, Bandyopadhyay *et al.* 1995, Freemark *et al.* 2001). Concerns have been expressed about the purity of preparations of PRL and PL used because of potential contamination by either placental or pituitary GH. Negative findings have also been reported in numerous studies examining the lipolytic potential of PL or recombinant bovine (b) PL, bPRL, murine (m) PRL, or mPL in homologous systems (Fielder & Talamantes 1987, Iliou & Demarne 1987, Houseknecht *et al.* 1996).

The PRL receptor (PRLR) belongs to the cytokine receptor superfamily and although it is widely distributed in various tissues, with the exception of birds (Bole-Feysot *et al.* 1998, Ohkubo *et al.* 1998), the receptor had not been detected in white adipose tissue. However, Ling *et al.* (2000) using reverse transcriptase-PCR, identified three PRLR isoforms in mouse adipose tissue, and also detected the protein by immunoblotting and showed it to be upregulated in lactation. They proceeded to show that PRL could induce suppressor of cytokine signaling proteins in adipocytes cultured *in vitro*, or in adipose tissue of PRL-transgenic mice, providing evidence for induction of intracellular signaling in the adipocyte itself, rather than in other cell types present in adipose tissue (Ling & Billig 2001). Furthermore, insulin-induced leptin secretion was inhibited by PRL, suggesting that PRL was acting, like GH, as an insulin-antagonist. PRL receptors have also been reported to be present in human adipose tissue (Ling *et al.* 2003) and PRL was shown to inhibit both basal- and cortisol-induced lipoprotein lipase activity, once again mimicking the effects

0022–0795/06/0191–101 © 2006 Society for Endocrinology *Printed in Great Britain*
DOI: 10.1677/joe.1.06939
Online version via http://www.endocrinology-journals.org

Downloaded from Bioscientifica.com at 02/06/2025 06:58:37PM
via free access

of GH. This series of studies provide compelling evidence for re-examination of the effects of PRL on adipose tissue *in vivo*.

GH has clear-cut effects on growth and nutrient partitioning between muscle and adipose tissue. Following hypophysectomy in rats, body fat increases, lean body mass decreases and such changes can be normalized by GH (Scow 1959). Similarly, transgenic expression of an inactive GH or disruption of the GH receptor (GHR) in mice results in an obese phenotype (Oberbauer *et al.* 1997, Berryman *et al.* 2004). There are clearly direct effects of GH on preadipocyte and adipocyte function, mediated via the GHR (Fagin *et al.* 1980, Vikman *et al.* 1991), although some of its actions have been proposed to be indirect, mediated via insulin-like growth factor-I (IGF-I). This led to the proposal of the dual effector hypothesis by Green *et al.* (1985) which proposed that GH primes mesenchymal cells so that IGF-I can selectively promote cell multiplication, resulting in the clonal expansion of newly differentiated cells. Despite these numerous reports, the effects of GH on adipose tissue proliferation and differentiation remain an area of considerable debate. For example, GH has been demonstrated to exhibit contrasting effects on preadipocyte cell lines when compared with primary preadipocytes. Thus, in clonal cell lines like 3T3-F442A, GH has been demonstrated to decrease proliferation and increase differentiation of preadipocytes (Morikawa *et al.* 1982, Green *et al.* 1985). In contrast, GH stimulates the proliferation of preadipocytes in primary cultures from rat and human adipose tissue and consequently inhibits the adipocyte differentiation process in these cells (Wabitsch *et al.* 1996*a*,*b*). The ability of GH to stimulate the differentiation of clonal preadipocyte cell lines is consistent with the ability of GH to regulate cellular differentiation in a variety of tissues, including osteoblasts (Kassem *et al.* 1993), osteoclasts (Nishiyama *et al.* 1996), and myoblasts (Ewton & Florini 1980). In 3T3-F442A cells, the initial effect of GH is to block cell division and involves decreased expression of cyclin D1 (Corin *et al.* 1990, Tang *et al.* 1995, Wiepz *et al.* 1997, Kim *et al.* 1999). The mechanism by which GH inhibits the differentiation of primary preadipocytes into adipocytes has, however, not been studied as extensively as the differentiation program that occurs in cell lines, and thus, which of these *in vitro* responses most closely represents the situation *in vivo*, is unclear.

In light of these conflicting data, we examined adipocyte development in two mouse models of PRL- and GH-deficiency, the PRLR gene disrupted or knockout (ko) mouse (Ormandy *et al.* 1997) and the GH receptor gene disrupted or ko mouse (Zhou *et al.* 1997). In our initial study of the PRLRko mouse, adipose tissue accretion was reduced, particularly in females and this became progressively more evident with age (Freemark *et al.* 2001). In addition, in female PRLRko mice, there was a significant reduction in serum leptin concentrations. The present study aimed to determine the role of changes in cell number and size in the alterations in adipose tissue mass *in vivo* and to examine the proliferation, differentiation, and hormonal responses of preadipocytes *in vitro* in both PRLRko and GHRko mice.

Materials and Methods

Animals

The production of both GHRko and PRLRko mice has previously been described (Ormandy *et al.* 1997, Zhou *et al.* 1997). All experimental designs and procedures were in agreement with the guidelines of the Animal Ethics Committee of the French Ministère de l'Agriculture. The GHR−/−, PRLR−/− mice, and their wild-type siblings (+/+), were in the inbred 129/Sv background. Female mice were housed on a 12 h light:12 h darkness cycle at 22 °C with food and water available *ad libitum*. Food intakes were monitored at weekly intervals throughout the experiment for PRLRko mice and during the period 2–4 months in GHRko mice. PRLRko mice were killed by cervical dislocation at either 5 or 12 months of age. GHRko mice were killed at 3–4 or 12 months of age.

Preparation of adipocytes and preadipocytes

Adipose tissues from the s.c. (abdominal inguinal) and parametrial depots were dissected, weighed, and a portion chopped with scissors and digested with collagenase. Isolated adipocytes were prepared as previously described, with the exception that the cells were not washed by flotation, in order to avoid the loss of very small adipocytes, which otherwise leads to bias in the estimation of mean cell volume (Doris *et al.* 1994). Adipocyte volumes were determined by capturing images of the isolated adipocytes and determining cell diameters using a Nikon E800 microscope equipped with a video camera and Lucia archive software.

Proliferation studies

A separate portion of tissue was used to prepare precursor adipocytes by centrifugation of the collagenase digest at 100 ***g*** for 5 min. The pellet, containing the precursors, was washed three times and the cells resuspended and counted in a hemocytometer. For proliferation studies, 5000 cells were added to 96-well plates in 100 μl Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum (FCS). Cellular proliferation was determined on a daily basis by adding 10 μl water-soluble tetrazolium salt (WST)-1 into triplicate wells and incubating at 37 °C for 1–2 h. Absorbances were read at 450 nm. A standard curve was used to determine the relationship between absorbance after incubation with WST-1 and known cell numbers, added to culture wells.

Differentiation

For differentiation studies, 50 000 cells were added to six wells of a 24-well plate and cultured in DMEM containing 10% FCS until 2–3 days post-confluence. At this point, three wells were induced to differentiate with a mixture of insulin (1 μg/ml), dexamethasone (100 ng/ml), tri-iodothyronine

Downloaded from Bioscientifica.com at 02/06/2025 06:58:37PM
via free access

(T3) (10 ng/ml), and 3-isobutyl-1-methylxanthine (IBMX) (100 μg/ml) in serum-free DMEM, whilst three wells served as controls for spontaneous differentiation in the presence of DMEM without additions. Medium was replaced every 3–4 days and after 10 days, cells were washed, fixed in 4% paraformaldehyde and stained with oil red O, which is lipid soluble and allows quantification of lipid accumulation in the cells. After 2 h, excess stain was removed with three washes in distilled H_2O and the cells were then photographed before being incubated in 60% isopropanol to solubilize the dye in order to determine the absorbance at 490 nm.

Insulin and isoproterenol sensitivity

Cells that had been cultured and induced to differentiate for 7 days in 96-well plates, as previously described, were washed three times and then cultured for 24 h in various concentrations of either insulin or isoproterenol in serum-free DMEM. The response to insulin was determined by measuring glucose uptake from the medium over 24 h using O-dianisidine, whilst isoproterenol sensitivity was determined by measuring glycerol release into the medium, as a measure of lipolysis, as previously described (Doris *et al.* 1994).

Statistical analyses

Comparisons were made either using Student's paired or unpaired *t*-test or, where multiple comparisons were made by ANOVA, followed by *post hoc* tests involving the Bonferroni correction.

Results

Body weight and food intake

PRLRko mice showed no significant difference in body weight at either 5 or 12 months of age and with no significant changes in food intake (results not shown). In contrast, GHRko mice were significantly lighter than their wild-type (wt) counterparts (wt 39·1±1·7 g; ko 19·3±1·8 g, mean±S.E.M. $P<0·01$, Student's *t*-test). They also ate approximately 75% of the quantity of wt animals (results not shown). Despite the fact that PRLRko mice had similar body weights to controls, adipose tissue mass was reduced. The weight of both the parametrial and s.c. depots were decreased by 45–50% in young animals (Fig. 1a). These changes were almost completely explained by similar decreases in the number of adipocytes at each site (Fig. 1b), with no significant changes in mean adipocyte volume (Fig. 1c). In older animals, the decrease in parametrial adipose tissue weight was still evident (Fig. 1d) and again explained by a decrease in cell numbers (Fig. 1e). In contrast, the effect upon s.c. adipose tissue was almost completely lost in older animals (Fig. 1d), although this was as a result of a compensatory increase in adipocyte volume (Fig. 1f) in the presence of a significant reduction in adipocyte numbers in s.c. tissue (Fig. 1e).

The changes in adipose tissue development in GHRko mice were distinctly different from those in PRLRko mice. In young animals, the parametrial depot was profoundly affected, being decreased by 95% (Fig. 2a). In stark contrast, the effect on s.c. adipose tissue was much less, being decreased by just 35% (Fig. 2a). The decrease in parametrial adipose tissue weight was due to a 75% decrease in both the number and size of adipocytes (Fig. 2b and c). Again, in contrast, whereas the volume of s.c. adipocytes was decreased by about 50%, the number of s.c. adipocytes was almost doubled (Fig. 2b and c). Even when expressed per unit of body weight, the parametrial depot was still significantly reduced in GHRko mice (wt, 7·2±0·6 g/kg body weight, GHRko 3·0±0·7 g/kg, $P<0·01$), whereas the s.c. depot was significantly increased (wt, 2·2±0·2 g/kg body weight, GHRko, 4·1±0·7 g/kg, $P<0·05$, Student's *t*-test). Although the parametrial depot increased in size in older GHRko mice, it still remained decreased by 75% compared with wt animals (Fig. 2d) and this was again due to approximately equivalent decreases in the number (Fig. 2e) and size (Fig. 2f) of adipocytes. The weight of the s.c. depot in wt and GHRko mice was not significantly different at 12 months of age (Fig. 2d). However, this masked the fact that the s.c. depot contained two to three times as many adipocytes in GHRko mice (Fig. 2e), whilst mean adipocyte volume was decreased by a similar magnitude (Fig. 2f).

In order to examine whether these changes represented intrinsic defects in the adipocytes of PRLRko and GHRko mice, we first examined proliferation of preadipocytes *in vitro*. There was no evidence that proliferation rates were impaired in PRLRko mice in either parametrial or s.c. sites. In fact, proliferation tended to be higher in PRLRko cells at both sites although neither achieved statistical significance (Fig. 3a). Similarly, in GHRko mice, rates of proliferation of preadipocytes derived from the s.c. site were unaffected (Fig. 3b). However, this was not the case for cells derived from the parametrial site of GHRko mice, since these failed to proliferate during the 7-day culture period in all six animals tested (Fig. 3b).

The ability of preadipocytes to differentiate *in vitro* was also examined using a differentiation mixture, including insulin, dexamethasone, T3, and IBMX. The differentiation of the stromal vascular fraction involved a high proportion of the total number of cells, typically in excess of 75% (Fig. 4). However, the individual response of cells varied markedly in stimulated cells, with lipid accumulation initially involving many small lipid droplets. Ultimately, these droplets coalesced to form smaller numbers of larger lipid droplets becoming highly characteristic of the fully differentiated adipocyte. The degree of differentiation was quantified by solubilization of the oil red O staining (Fig. 5). In wt animals, the magnitude of lipid accumulation was considerably greater in the s.c. depot compared with the parametrial (note the difference in axis scales) and was also considerably greater in young animals compared with older animals (this was particularly evident in

Downloaded from Bioscientifica.com at 02/06/2025 06:58:37PM
via free access

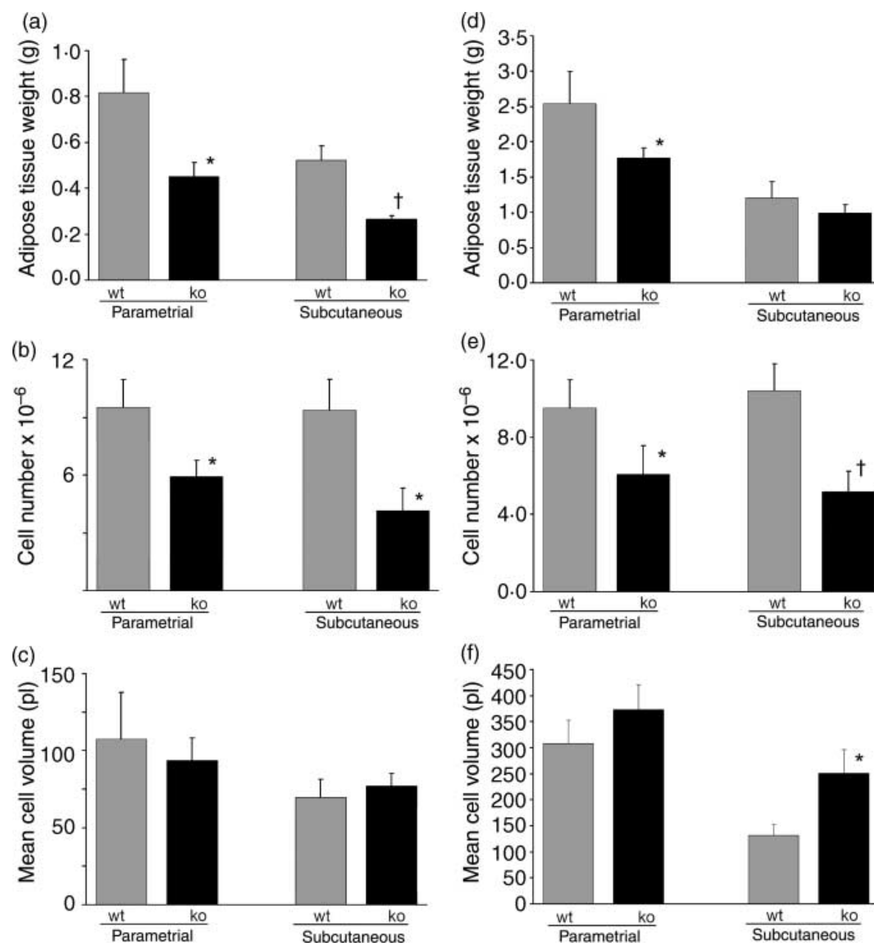

Figure 1 (a) Adipose tissue weight, (b) adipocyte number, and (c) mean adipocyte volume in parametrial and s.c. sites in wild-type (wt) and PRLR knockout (ko) mice at 5 months of age. Values are mean ± S.E.M. of four animals. (d) Adipose tissue weight, (e) adipocyte number, (f) and mean adipocyte volume in parametrial and s.c. sites in wt and PRLRko mice at 12 months of age. Values are means ± S.E.M. of six wt and seven ko animals. $^*P<0·05$; $^†P<0·01$ compared with wild-type animals. Note the difference in axis scales between young and old animals.

the s.c. depot). Differentiation potential was unaffected in PRLRko animals, when compared with wt animals, independently of adipose tissue site or age, under either basal- or hormone-stimulated conditions (Fig. 5).

In GHRko mice, differentiation potential was also much greater in s.c. adipose tissue of young wt mice when compared with parametrial adipose tissue (Fig. 6). Differentiation in s.c. adipose tissue of GHRko mice was identical to that of wt animals (Fig. 6, lower panel). Once again, however, preadipocytes from the parametrial depot of GHRko mice behaved anomalously *in vitro*, showing no differentiation response (Fig. 6, upper panel).

Hormone responsiveness of these tissues was also examined during the post-differentiation period. Insulin sensitivity, as determined by glucose disappearance from the culture medium was similar in wt and PRLRko mice, independent of the site of adipose tissue (Fig. 7a). Insulin sensitivity in GHRko mice was also similar to wt mice in adipocytes derived from the s.c. depot, but parametrial adipocytes of GHRko mice were completely unresponsive to insulin (Fig. 7b).

Sensitivity to isoproterenol, determined by glycerol release into the medium, was similar in wt and PRLRko mice, independent of the site of adipose tissue (Fig. 8a). Sensitivity to isoproterenol in GHRko mice was also similar to wt mice in the s.c. depot but, as in the case for insulin, parametrial adipocytes of GHRko mice were completely unresponsive to this lipolytic agent (Fig. 8b).

Discussion

In this study, we have examined the roles of PRL and GH in the development of adipose tissue *in vivo* and their subsequent influence on preadipocyte proliferation and differentiation

Downloaded from Bioscientifica.com at 02/06/2025 06:58:37PM
via free access

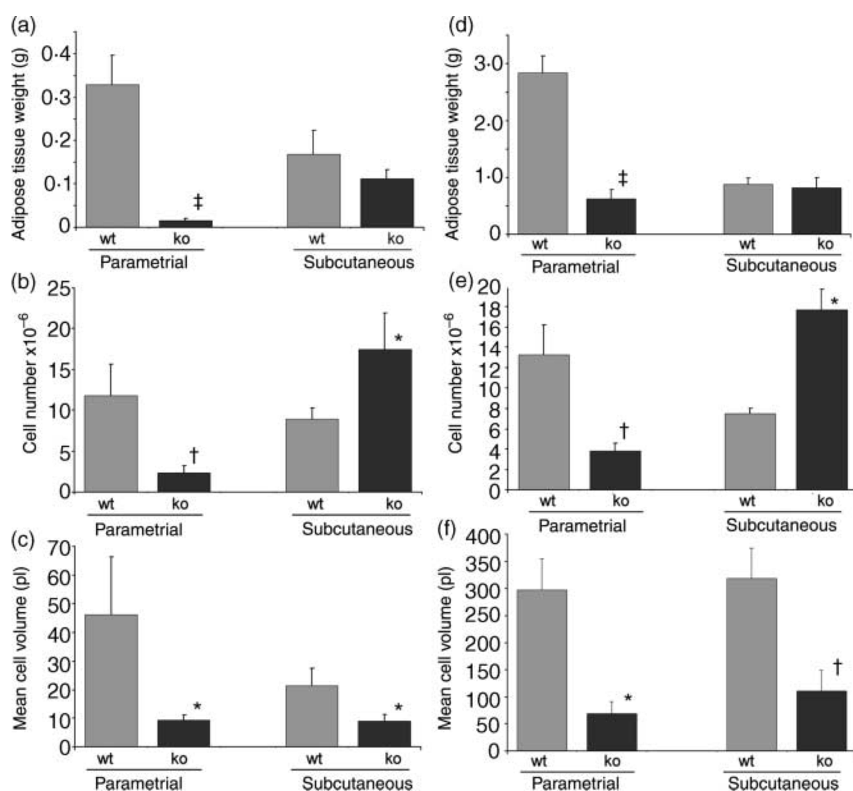

Figure 2 (a) Adipose tissue weight, (b) adipocyte number, and (c) mean adipocyte volume in parametrial and s.c. sites in wild-type (wt) and GHR knockout (ko) mice at 3–4 months of age. Values are means±S.E.M. of five wt and six ko animals. (d) Adipose tissue weight, (e) adipocyte number, and (f) mean adipocyte volume in parametrial and s.c. sites in wt and GHRko mice at 12 months of age. Values are means±S.E.M. of seven wt and eight ko animals. $^{*}P<0{\cdot}05$; $^{\dagger}P<0{\cdot}01$; $^{\ddagger}P<0{\cdot}001$ compared with wild-type animals. Note the difference in axis scales between young and old animals.

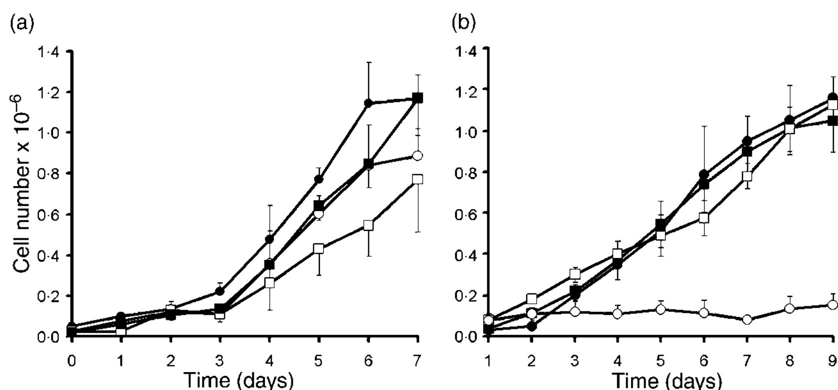

Figure 3 Proliferation of stromal-vascular cells derived from (a) PRLRko and (b) GHRko mice at 5 months of age. Wild-type (wt) parametrial (□), ko parametrial (○), wt s.c. (■), and ko s.c. (●) cell numbers were determined, in triplicate, on a daily basis by addition of 10 µl water-soluble tetrazolium salt (WST)-1 followed by incubation for 1–2 h. Cell numbers were calculated from a standard curve in which WST-1 values were calculated from serial dilutions of a known number of cells added to tissue culture wells under identical conditions. Values are means±S.E.M. of four to six animals.

Downloaded from Bioscientifica.com at 02/06/2025 06:58:37PM
via free access

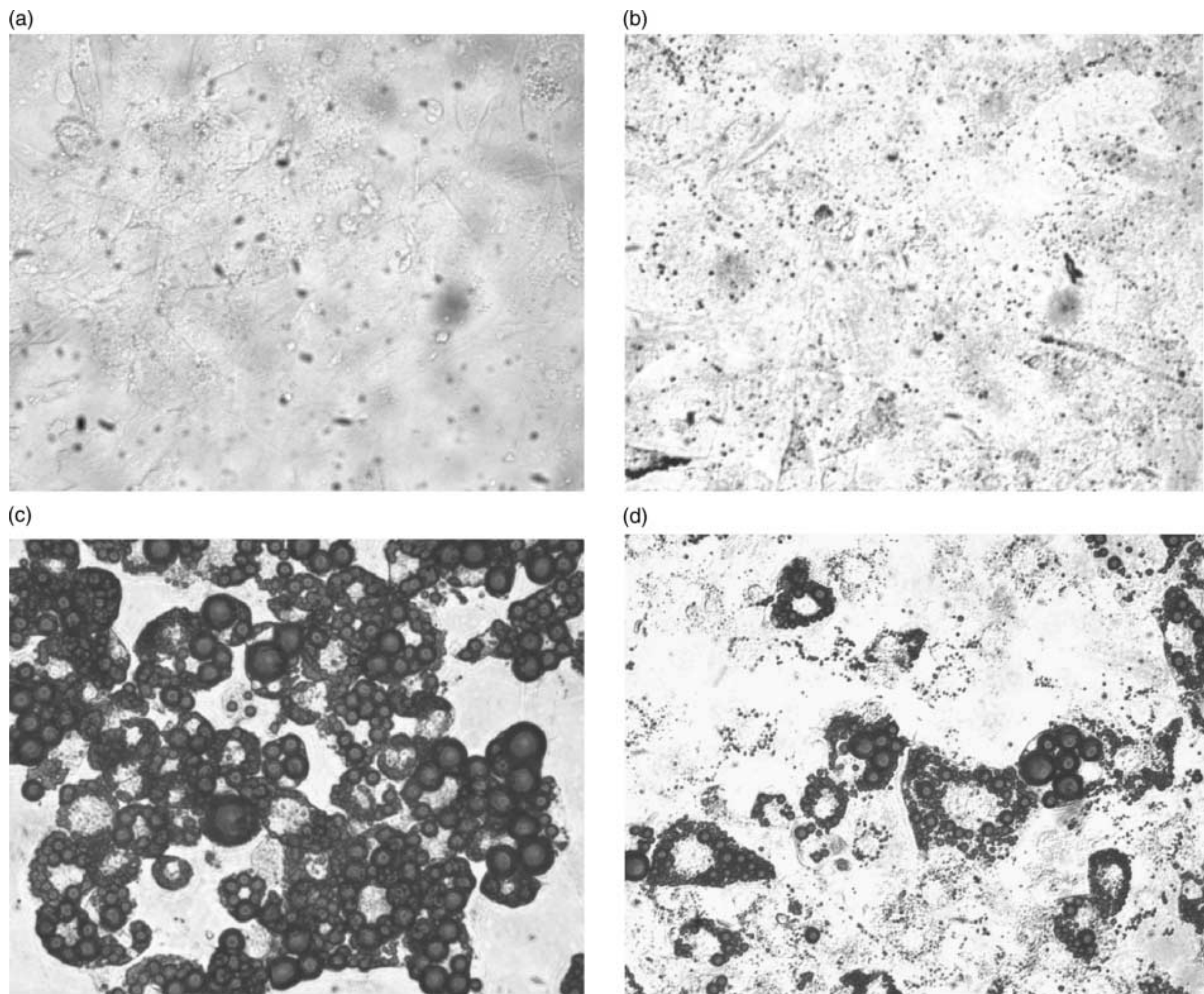

Figure 4 Differentiation of adipocytes *in vitro*. Stromal-vascular cells were cultured to confluence. Cells stained with oil red O at confluence (a) showed no oil red O staining irrespective of animal status or tissue site. Cells cultured for a further 10 days in serum-free DMEM showed little accumulation of lipid although this was increased compared with immediate post-confluent cells, again irrespective of animal status or tissue site. (b) Representative cells from a wild-type animal. (c) Lipid accumulation in s.c. tissue from a GHRko mouse, illustrating a high degree of differentiation, with some cells exhibiting a small number of large lipid droplets. The differentiation response in parametrial tissue (d) was less than that of s.c. although note that, even in this case, most cells are accumulating lipid in a perinuclear position. (d) Adipocytes from parametrial tissue of a GHRko mouse.

in vitro using two knockout models, the PRLRko and GHRko mouse. These studies confirm and extend our previous findings in the PRLRko mouse, which revealed decreases in adipose tissue mass. In this study, we describe significant reductions in adipose tissue mass at two sites in female PRLRko mice and are able to extend our initial findings by illustrating that the decrease in adipose mass was explicable exclusively in terms of a reduced number of adipocytes, with no evidence of a reduction in cell volume. Thus, we have identified for the first time, an important effect of PRL in determining adipocyte numbers. When precursor populations of cells from two anatomical sites were cultured *in vitro*, we found no evidence of any intrinsic defect due to the *in vivo* absence of PRL, since they demonstrated normal proliferative and differentiative responses *in vitro*, as well as normal responses to insulin and isoproterenol. These findings could be a result of *in vivo* changes of circulating, or locally produced, modulators of adipocyte function in PRLRko mice. However, there is evidence that PRL influences differentiation of adipocytes *in vitro*, although this involved a cell line, 3T3-L1 (Stewart *et al*. 2004). Others, using primary cultures of adipocytes, have shown that PRLR exist on adipocytes and that they respond metabolically in terms of lipoprotein lipase expression although proliferation and differentiation were not examined (Ling *et al*. 2000, 2003, Ling & Billig 2001). Such findings suggest that the effects on

Downloaded from Bioscientifica.com at 02/06/2025 06:58:37PM
via free access

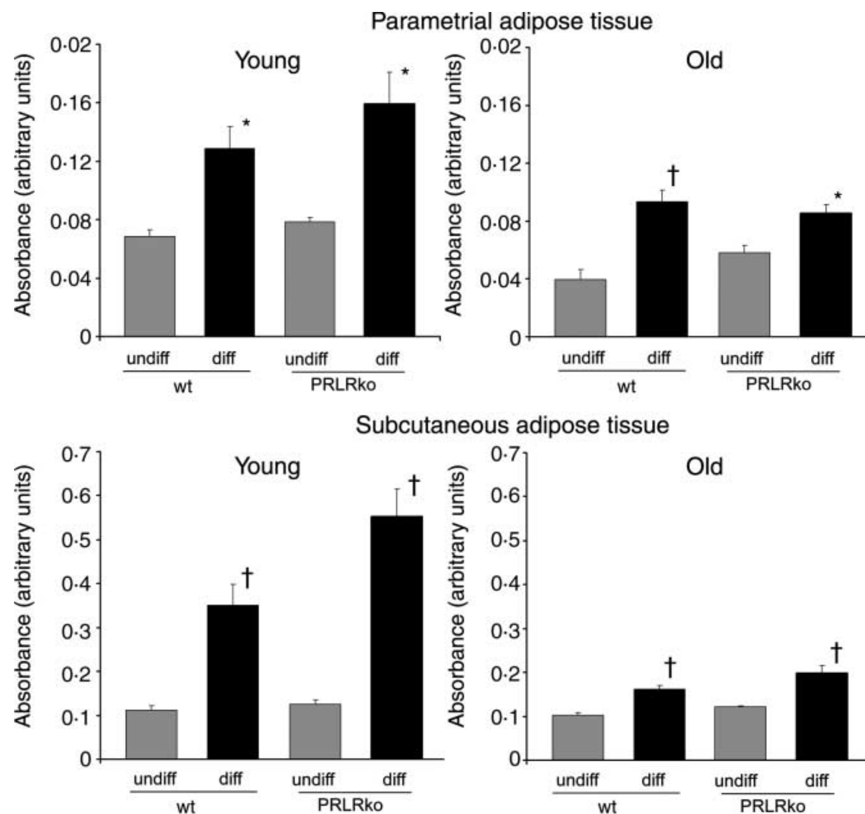

Figure 5 Differentiation of adipocytes from wild-type (wt) and PRLRko mice at 5 (young) and 12 (old) months of age. Stromal-vascular cells were cultured until confluent. Two to three days later, cells in triplicate wells were cultured in serum-free DMEM to provide undifferentiated (undiff) cells and triplicate wells were cultured in DMEM containing insulin, T3, dexamethasone, and IBMX for 10 days to provide differentiated (diff) cells. Lipid accumulation was determined using oil red O. Values are mean ± S.E.M. of four animals per group. $^{*}P<0{\cdot}01$; $^{\dagger}P<0{\cdot}001$ compared with undifferentiated cells.

adipocyte development described in our study could be the result of direct effects of PRL. Our model does not, however, permit us to explore this possibility further, since PRLRko mice possess no PRLR and thus cannot respond to exogenous PRL *in vitro* or *in vivo*.

The effect of PRLR deficiency on adipocyte numbers was unexpected, since one possible explanation for such a decrease in adipose tissue mass would be a decrease in food intake. However, we noted no change in food intake in our animals. Furthermore, the effect of food restriction is to decrease adipocyte volume, not number. In addition, we noted that the effects of PRLR-deficiency on adipocyte numbers persisted until 1 year of age, although the effect on adipose tissue mass was partially abrogated by an increase in adipocyte volume in the s.c. but not the parametrial site. This is the first report of site-specific regulation of adipose tissue metabolism by PRL.

In GHRko mice, the situation was distinctly different, not least, perhaps, because absence of the GHR leads to severe dwarfism. The development of the parametrial adipose tissue depot was dramatically impaired and this impairment was also due to a decrease in the number of adipocytes, although there was, in addition, a significant reduction in adipocyte volume. Such a finding provides strong support for a stimulatory effect of GH on both adipocyte proliferation (cell numbers) and differentiation (adipocyte lipid content or mean cell volume). In complete contrast, however, adipose tissue development in s.c. depots of GHRko mice was not significantly impaired even though these mice weighed only 40–50% of the weight of wt mice. In fact, adipocyte numbers were doubled in s.c. adipose tissue of GHR-deficient animals, whereas mean adipocyte volume was significantly decreased. The fact that, *in vitro*, cells from the s.c. depot also proliferated, differentiated, and responded to hormones in identical fashion to those of wt animals, suggests that the effects of GH on development and function of the s.c. depot were not due to intrinsic changes but, as for PRLRko mice, reflected events pertaining *in vivo*. In contrast to the s.c. site, both proliferation and differentiation *in vivo* of adipocytes from the parametrial depot were critically dependent upon the presence of the GHR. The distinction between the apparent fundamental requirement for GH in the parametrial depot and the total independence of the s.c. depot is clearly worthy of further

Downloaded from Bioscientifica.com at 02/06/2025 06:58:37PM
via free access

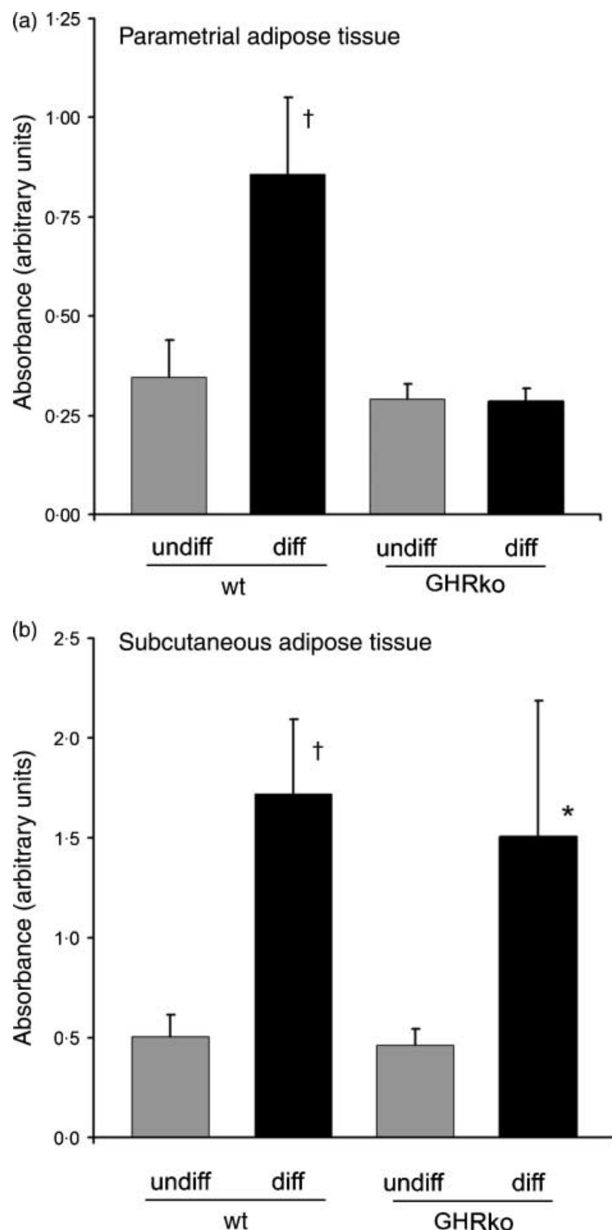

Figure 6 Differentiation of adipocytes from wild-type (wt) and GHRko mice at 3–4 months of age. Stromal-vascular cells were cultured until confluent. Two to three days later, cells in triplicate wells were cultured in serum-free Dulbecco's modified Eagle's medium (DMEM) to provide undifferentiated (undiff) cells and triplicate wells were cultured in DMEM containing insulin, T3, dexamethasone, and IBMX for 10 days to provide differentiated (diff) cells. Lipid accumulation was determined using oil red O. Values are means ± S.E.M. of five animals per group. *$P<0·05$; †$P<0·01$; compared with undifferentiated cells.

investigation. In addition to identifying these site-specific effects, we also demonstrated, for the first time, that absence of the GHR creates intrinsic changes in adipocytes from this depot, which persist for at least 10 days *in vitro*. These findings confirm and extend a previous study where we examined the effects of an antiserum to GH which, when administered to neonatal rats, also produced major decreases in parametrial but not s.c. fat (Flint & Gardner 1993). Whilst the effects upon the parametrial depot described in our study clearly demonstrate that GH is required for adipocyte proliferation and differentiation *in vivo*, in similar fashion to that seen in 3T3-F442A cells *in vitro*, the site-specific effects of GH may in part explain the contradictory results obtained with different cell lines, or in comparisons of primary cell culture, where cells have originated from different anatomical sites.

How might these site-specific differences be explained? There is differential expression of the GHR depending on the location of the adipose tissue depot with, for example, high level expression of the GHR in the epididymal fat pad compared with the retroperitoneal fat pad in male rats (LaFranchi *et al.* 1985). However, no such studies have compared GHR numbers in s.c. and parametrial depots of female mice. Expression of the receptor is regulated during the differentiation process with increased levels of receptor expression during adipocyte differentiation (Landron *et al.* 1987, Zou *et al.* 1997) and these different levels of receptor expression could explain the differential sensitivity to GH of individual adipose sites. However, the differences in GHR expression reported have generally not been large and the differences in adipose tissue development in the respective sites have, similarly, not been dramatically different. We thus believe that the dramatic changes in development of the parametrial depot in GHRko mice described in this study make this site an excellent model to examine the nature of this effect of GH.

In our study, the depot most protected from GH-deficiency, the s.c., possesses a large lymph node in the inguinal region, whereas the parametrial depot does not. Pond (1999) has proposed an intriguing hypothesis, which relates to one of the latest proposed therapeutic uses of GH. This involves the lipodystrophic condition in HIV patients where adipose tissue accumulates specifically in the abdominal cavity. Pond noted that, even in the face of starvation, some depots which are rich in lymphoid tissue are conserved. She suggests that this is a process whereby lymphoid tissues are provided with a local energy source so that immune responses are not compromised (Pond & Mattacks 2002, Mattacks *et al.* 2003, Pond 2003). Thus, part of the GH insensitivity of the s.c. depot may be due to the influence of lymphoid cytokines.

In summary, our studies provide a number of novel observations. We demonstrate that, although the parametrial depot becomes the major depot as female mice age, the s.c. depot differentiates to a much greater extent *in vitro*. We also noted a considerable decrease in the ability of preadipocytes from both sites to accumulate lipid *in vitro* as the animals aged. For the first time, we describe effects of PRL on adipocyte development. These are not explicable in terms of effects on appetite, since the reduction in mass was attained by decreased numbers of adipocytes, rather than a decrease in their size. We also demonstrate that the actions of GH are site specific,

Downloaded from Bioscientifica.com at 02/06/2025 06:58:37PM
via free access

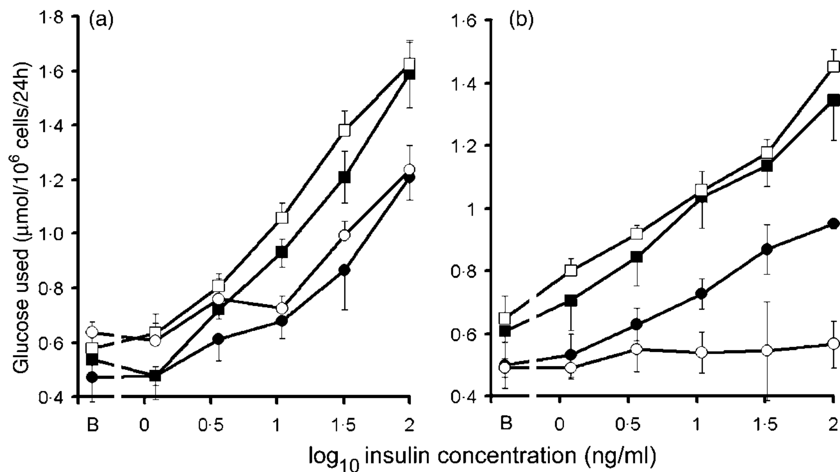

Figure 7 *In vitro* responses to insulin of preadipocytes from (a) PRLRko (5 months old) and (b) GHRko mice (4 months old) differentiated *in vitro* with insulin, T3, dexamethasone, and IBMX for 7 days. After differentiation, cells were washed three times and then cultured in serum-free DMEM containing varying concentrations of insulin as indicated. Glucose concentrations in the medium were determined 24 h later. Cells were derived from wild-type (wt) parametrial (●), ko parametrial (○), wt s.c. (■), and ko s.c. (□) adipose tissue. B indicates basal glucose uptake in the absence of insulin. Values are means ± S.E.M. of four animals per group.

a finding which may explain the conflicting results from *in vitro* studies involving both primary tissue culture and preadipocyte cell lines, which may be due to derivation of precursor cells from different anatomical sites *in vivo*. Finally, we describe intrinsic defects in parametrial adipocytes from GHRko mice and believe that this provides an excellent model to identify novel factors involved in this long-lived effect of GH.

Acknowledgements

We thank Miss M Gardner for skilled technical assistance. D J F was in receipt of a Poste Orange from Inserm. These studies were funded, in part, by the Scottish Executive Environmental and Rural Affairs Department. J J K was supported, in part, by the State of Ohio's Eminent Scholar Program that

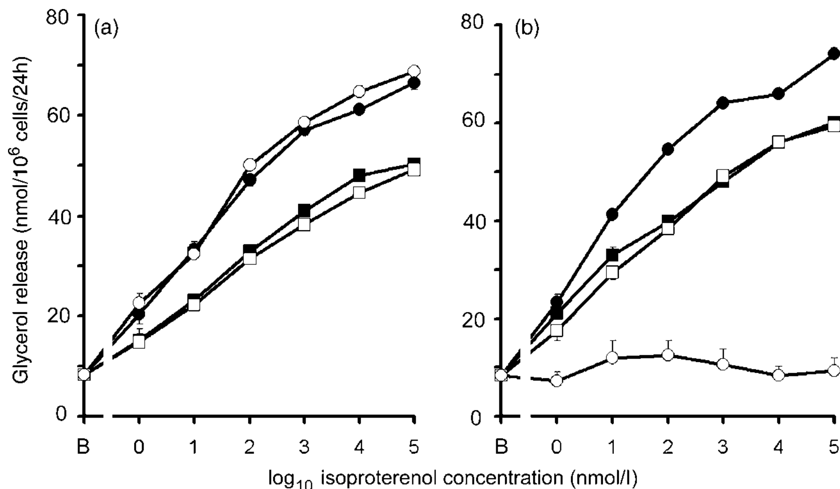

Figure 8 *In vitro* responses to isoproterenol of preadipocytes from (a) PRLRko (5 months old) and (b) GHRko mice (4 months old) differentiated *in vitro* with insulin, T3, dexamethasone, and IBMX for 7 days. After differentiation, cells were washed three times and then cultured in serum-free DMEM containing varying concentrations of isoproterenol as indicated. Glycerol concentrations in the medium were determined 24 h later. Cells were derived from wild-type (wt) parametrial (●), ko parametrial (○), wt s.c. (■), and ko subcutaneous (□) adipose tissue. B indicates basal glycerol release in the absence of isoproterenol. Values are means ± S.E.M. of four animals per group.

Downloaded from Bioscientifica.com at 02/06/2025 06:58:37PM
via free access

includes a gift by Milton and Lawrence Goll and NIH RO1 AG19899-05. The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

References

Bandyopadhyay GK, Lee LY, Guzman RC & Nandi S 1995 Effect of reproductive states on lipid mobilization and linoleic acid metabolism in mammary glands. *Lipids* **30** 155–162.

Berryman DE, List EO, Coschigano KT, Behar K, Kim JK & Kopchick JJ 2004 Comparing adiposity profiles in three mouse models with altered GH signaling. *Growth Hormone and IGF Research* **14** 309–318.

Bole-Feysot C, Goffin V, Edery M, Binart N & Kelly PA 1998 Prolactin (PRL) and its receptor: actions, signal transduction pathways and phenotypes observed in PRL receptor knockout mice. *Endocrine Reviews* **19** 225–268.

Corin RE, Guller S, Wu KY & Sonenberg M 1990 Growth hormone and adipose differentiation: growth hormone-induced antimitogenic state in 3T3-F442A preadipose cells. *PNAS* **87** 7507–7511.

Doris R, Vernon RG, Houslay MD & Kilgour E 1994 Growth hormone decreases the response to anti-lipolytic agonists and decreases the levels of Gi2 in rat adipocytes. *Biochemical Journal* **297** 41–45.

Ewton DZ & Florini JR 1980 Relative effects of the somatomedins, multiplication-stimulating activity, and growth hormone on myoblasts and myotubes in culture. *Endocrinology* **106** 577–583.

Fagin KD, Lackey SL, Reagan CR & DiGirolamo M 1980 Specific binding of growth hormone by rat adipocytes. *Endocrinology* **107** 608–615.

Fielder PJ & Talamantes F 1987 The lipolytic effects of mouse placental lactogen II, mouse prolactin, and mouse growth hormone on adipose tissue from virgin and pregnant mice. *Endocrinology* **121** 493–497.

Flint DJ & Gardner MJ 1993 Influence of growth hormone deficiency on growth and body composition in rats: site-specific effects upon adipose tissue development. *Journal of Endocrinology* **137** 203–211.

Freemark M, Fleenor D, Driscoll P, Binart N & Kelly P 2001 Body weight and fat deposition in prolactin receptor-deficient mice. *Endocrinology* **142** 532–537.

Genazzani AR, Benuzzi-Badoni M & Felber JP 1969 Human chorionic somato-mammotropine (HCSM): lipolytic action of a pure preparation on isolated fat cells. *Metabolism* **18** 593–598.

Green H, Morikawa M & Nixon T 1985 A dual effector theory of growth-hormone action. *Differentiation* **29** 195–198.

Houseknecht KL, Bauman DE, Vernon RG, Byatt JC & Collier RJ 1996 Insulin-like growth factors-I and -II, somatotropin, prolactin, and placental lactogen are not acute effectors of lipolysis in ruminants. *Domestic Animal Endocrinology* **13** 239–249.

Iliou JP & Demarne Y 1987 Evolution of the sensitivity of isolated adipocytes of ewes to the lipolytic effects of different stimuli during pregnancy and lactation. *International Journal of Biochemistry* **19** 253–258.

Kassem M, Blum W, Ristelli J, Mosekilde L & Eriksen EF 1993 Growth hormone stimulates proliferation and differentiation of normal human osteoblast-like cells *in vitro*. *Calcified Tissue International* **52** 222–226.

Kim SO, Houtman JC, Jiang J, Ruppert JM, Bertics PJ & Frank SJ 1999 Growth hormone-induced alteration in ErbB-2 phosphorylation status in 3T3-F442A fibroblasts. *Journal of Biological Chemistry* **274** 36015–36024.

LaFranchi S, Hanna CE, Torresani T, Schoenle E & Illig R 1985 Comparison of growth hormone binding and metabolic response in rat adipocytes of epididymal, subcutaneous, and retroperitoneal origin. *Acta Endocrinologica* **110** 50–55.

Landron D, Dugail I, Ardouin B, Quignard-Boulange A & Postel-Vinay MC 1987 Growth hormone binding to cultured preadipocytes from obese fa/fa rats increases during cell differentiation. *Hormone and Metabolic Research* **19** 403–406.

Ling C & Billig H 2001 PRL receptor-mediated effects in female mouse adipocytes: PRL induces suppressors of cytokine signaling expression and suppresses insulin-induced leptin production in adipocytes *in vitro*. *Endocrinology* **142** 4880–4890.

Ling C, Hellgren G, Gebre-Medhin M, Dillner K, Wennbo H, Carlsson B & Billig H 2000 Prolactin (PRL) receptor gene expression in mouse adipose tissue: increases during lactation and in PRL-transgenic mice. *Endocrinology* **141** 3564–3572.

Ling C, Svensson L, Oden B, Weijdegard B, Eden B, Eden S & Billig H 2003 Identification of functional prolactin (PRL) receptor gene expression: PRL inhibits lipoprotein lipase activity in human white adipose tissue. *Journal of Clinical Endocrinology and Metabolism* **88** 1804–1808.

Mattacks CA, Sadler D & Pond CM 2003 The cellular structure and lipid/protein composition of adipose tissue surrounding chronically stimulated lymph nodes in rats. *Journal of Anatomy* **202** 551–561.

Morikawa M, Nixon T & Green H 1982 Growth hormone and the adipose conversion of 3T3 cells. *Cell* **29** 783–789.

Nishiyama K, Sugimoto T, Kaji H, Kanatani M, Kobayashi T & Chihara K 1996 Stimulatory effect of growth hormone on bone resorption and osteoclast differentiation. *Endocrinology* **137** 35–41.

Oberbauer AM, Stern JS, Johnson PR, Horwitz BA, German JB, Phinney SD, Beermann DH, Pomp D & Murray JD 1997 Body composition of inactivated growth hormone (oMt1a-oGH) transgenic mice: generation of an obese phenotype. *Growth, Development and Aging* **61** 169–179.

Ohkubo T, Tanaka M, Nakashima K, Talbot RT & Sharp PJ 1998 Prolactin receptor gene expression in the brain and peripheral tissues in broody and nonbroody breeds of domestic hen. *General and Comparative Endocrinology* **109** 60–68.

Oller do Nascimento CM, Ilic V & Williamson DH 1989 Re-examination of the putative roles of insulin and prolactin in the regulation of lipid deposition and lipogenesis *in vivo* in mammary gland and white and brown adipose tissue of lactating rats and litter-removed rats. *Biochemical Journal* **258** 273–278.

Ormandy CJ, Camus A, Barra J, Damotte D, Lucas B, Buteau H, Edery M, Brousse N, Babinet C, Binart N *et al*. 1997 Null mutation of the prolactin receptor gene produces multiple reproductive defects in the mouse. *Genes and Development* **11** 167–178.

Pond CM 1999 Physiological specialisation of adipose tissue. *Progress in Lipid Research* **38** 225–248.

Pond CM 2003 Paracrine interactions of mammalian adipose tissue. *Journal of Experimental Zoology. Part A, Comparative Experimental Biology* **295** 99–110.

Pond CM & Mattacks CA 2002 The activation of the adipose tissue associated with lymph nodes during the early stages of an immune response. *Cytokine* **17** 131–139.

Scow RO 1959 Effect of growth hormone and thyroxine on growth and chemical composition of muscle, bone and other tissues in thyroidecto-mized-hypophysectomized rats. *American Journal of Physiology* **196** 859–865.

Stewart WC, Baugh JE Jr, Floyd ZE & Stephens JM 2004 STAT 5 activators can replace the requirement of FBS in the adipogenesis of 3T3-L1 cells. *Biochemical and Biophysical Research Communications* **324** 355–359.

Strange RC & Swyer GI 1974 The effect of human placental lactogen on adipose tissue lipolysis. *Journal of Endocrinology* **61** 147–152.

Tang B, Jeoung DI & Sonenberg M 1995 Effect of human growth hormone and insulin on [^{3}H]thymidine incorporation, cell cycle progression, and cyclin D expression in 3T3-F442A preadipose cells. *Endocrinology* **136** 3062–3069.

Turtle JR & Kipnis DM 1967 The lipolytic action of human placental lactogen on isolated fat cells. *Biochimica et Biophysica Acta* **144** 583–593.

Vikman K, Carlsson B, Billig H & Eden S 1991 Expression and regulation of growth hormone (GH) receptor messenger ribonucleic acid (mRNA) in rat adipose tissue, adipocytes, and adipocyte precursor cells: GH regulation of GH receptor mRNA. *Endocrinology* **129** 1155–1161.

Wabitsch M, Heinze E, Hauner H, Shymko RM, Teller WM, De Meyts P & Ilondo MM 1996*a* Biological effects of human growth hormone in rat adipocyte precursor cells and newly differentiated adipocytes in primary culture. *Metabolism* **45** 34–42.

Downloaded from Bioscientifica.com at 02/06/2025 06:58:37PM
via free access

Wabitsch M, Braun S, Hauner H, Heinze E, Ilondo MM, Shymko R, De Meyts P & Teller WM 1996*b* Mitogenic and antiadipogenic properties of human growth hormone in differentiating human adipocyte precursor cells in primary culture. *Pediatric Research* **40** 450–456.

Wiepz GJ, Houtman JC, Cha D & Bertics PJ 1997 Growth hormone attenuation of epidermal growth factor-induced mitogenesis. *Journal of Cellular Physiology* **173** 44–53.

Zhou Y, Xu BC, Maheshwari HG, He L, Reed M, Lozykowski M, Okada S, Cataldo L, Coschigamo K, Wagner TE *et al*. 1997 A mammalian model for Laron syndrome produced by targeted disruption of the mouse growth hormone receptor/binding protein gene (the Laron mouse). *PNAS* **94** 13215–13220.

Zou L, Menon RK & Sperling MA 1997 Induction of mRNAs for the growth hormone receptor gene during mouse 3T3-L1 preadipocyte differentiation. *Metabolism* **46** 114–118.

Received 28 April 2006
Accepted 6 July 2006
Made available online as an Accepted Preprint
4 August 2006

Downloaded from Bioscientifica.com at 02/06/2025 06:58:37PM
via free access