ELSEVIER

Comparative Biochemistry and Physiology Part B 127 (2000) 173–182

CBP

www.elsevier.com/locate/cbpb

Effects of gastric inhibitory polypeptide, somatostatin and epidermal growth factor on lipogenesis in ovine adipose explants

A.S.H. Baba [1], J.M.M. Harper *, P.J. Buttery

Division of Nutritional Biochemistry, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, Leicestershire LE12 5RD, UK

Received 25 January 2000; received in revised form 28 May 2000; accepted 8 June 2000

Abstract

Feeding raises the plasma concentrations of a number of gut-related hormones that may, in turn, influence the metabolism of peripheral tissues. This study investigated the effects of gut-related hormones on lipogenesis in explants from three differing adipose depots in lambs (aged 4–9 months). Incorporation of [^{14}C]-acetate into lipid was measured over a 2-h period, following 24 h pre-incubation in the presence of hormone combinations. In perirenal fat explants, gastric inhibitory polypeptide (GIP) in the concentration range 0.01–10 nM stimulated lipogenesis. Maximal effects were seen at 1 nM (an average increase of 64% over basal values). In contrast, in the presence of insulin (0.1 nM), a dose-dependant decrease in lipogenesis was seen with increasing GIP concentration ($P < 0.001$ for the insulin × GIP interaction). Epidermal growth factor (EGF) and somatostatin in the same concentration range each inhibited lipogenesis, both in the presence and the absence of insulin ($P < 0.001$ in each case). Subcutaneous (back) fat and intermuscular (popliteal) fat responded similarly to each other, but significantly differently from the perirenal depot ($P < 0.001$). Here GIP, somatostatin or EGF (each at 1 nM) all separately stimulated lipogenesis.

Keywords: Sheep; Adipose tissue; Depot fat; Lipid metabolism; Gastrointestinal hormones; Glucose-dependant insulinotropic polypeptide; Insulin; Stimulation; Inhibition; Interactions

1. Introduction

Feeding alters not only nutrient availability, but also increases the plasma concentrations of a number of hormones and bioactive peptides secreted by cells of the gastrointestinal tract. Over recent years, it has been shown that these 'gut hormones' not only increase endocrine secretion from the pancreas, but also directly affect the metabolism of nutrients in other tissues (Guilloteau et al., 1995). To date, there is only limited data as to the action on adipose tissue of polypeptides such as somatostatin and gastric inhibitory polypeptide and epidermal growth factor. Most published studies have concentrated on either isolated fat cells or intact animals, rather than examining effects at the tissue level. Despite the fact that the metabolism of ruminants is adapted to

* Corresponding author. Tel.: +44-115-8516121; fax: +44-15-9516122.

E-mail addressses: jane.harper@nottingam.ac.uk (J.M.M. Harper), ahmad@botany.um.edu.my (A.S.H. Baba).

[1] Present address: Institut Sains Biologi, Universiti Malaya, 50603 Kuala Lumpur, Malaysia.

0305-0491/00/$ - see front matter
PII: S0305-0491(00)00249-2

the use of volatile fatty acids, rather than glucose, as a primary energy source, few studies have examined the action of gut-related hormones on nutrient metabolism in ruminant adipose tissue.

Epidermal growth factor (EGF) is synthesised at multiple sites in the body including, in some species, the salivary glands and pancreas. Its receptors are widespread within the body and it promotes the growth of many cell types. It has well-documented maturation effects in the intestinal tract (Fisher and Lakshmanan, 1990; Vinter-Jensen, 1999) and promotes nutrient utilisation by the intestinal mucosa (Zhang et al., 1997). However its plasma concentrations are very low and do not vary with the physiological status of the animal (Fisher and Lakshmanan, 1990; Vinter-Jensen, 1999). This may be a result of its very rapid clearance from the bloodstream (Tyson et al., 1989). Hence its role as an endocrine mediator is hard to assess. Results of in vitro experiments with isolated adipocytes and preadipocytes are in many cases apparently difficult to reconcile with data obtained from EGF administration to whole animals. To date, there are no published studies of the effect of EGF on adipose explants.

In addition to its role within the pituitary gland, somatostatin is also synthesised by both the gut and the pancreas, but the stimuli to its release differ at the two sites. These are meals of fat, protein or carbohydrate for release from the gut, and increases in plasma concentrations of glucose, fatty acids and amino acids for the endocrine pancreas (Schusdziarra et al., 1978; Wisén et al., 1992; Ipp et al., 1997; Schauder et al., 1997). Somatostatin inhibits the secretion of several gastrointestinal tract hormones including gastrin, GIP, secretin, glucagon and motilin (Bloom et al., 1974; Boden et al., 1975; Sakurai et al., 1975; Martin and Faulkner, 1996). Pharmacological doses of somatostatin were shown to inhibit triglyceride (Schusdziarra et al., 1978) and glucose (Krejs et al., 1980) absorption from the small intestine. It has a wide range of regulatory actions on the growth hormone axis, but has been also reported to influence peripheral tissues (Guilloteau et al., 1995; Patel, 1999). There is no published information about its direct actions on adipose tissue in ruminants.

Gastric inhibitory polypeptide (GIP, also known as glucose-dependant insulinotropic polypeptide) was originally identified as a factor reducing gastric acid secretion, but it also augments insulin release in monogastric animals. Its concentration in plasma is increased by both carbohydrate-rich and fat-rich meals. In humans, absorption and re-esterification of fatty acids is considered to be the major stimulus to its secretion (Knapper et al., 1996; Morgan, 1996), whereas in pigs both carbohydrate and fat are needed for maximal stimulation (Knapper et al., 1995). Studies on pre-ruminant lambs have shown that here too, GIP secretion correlates with the fatty acid content of the meal (Martin and Faulkner, 1994). Elevated GIP concentrations are associated with lower plasma triglycerides in humans (Murphy et al., 1995), and it has been suggested that GIP increases absorption and re-esterification of dietary fat by peripheral tissues (Gama et al., 1997; Knapper et al., 1996). In rats, GIP receptors have been found in liver and adipose tissue (Usdin et al., 1993), and physiological concentrations increase lipogenesis in explants from three different adipose depots (Oben et al., 1991).

The absorption of long-chain free fatty acids is an important stimulus to GIP secretion in sheep (McCarthy et al., 1992). However factors other than diet per se may also be important in controlling GIP secretion in ruminating animals, since concentrations in plasma rise during lactation (Faulkner and Martin, 1997). Studies in adult sheep show that GIP has effects synergistic with those of insulin, increasing glucose clearance, but it has no effect on insulin-independent clearance (Rose et al., 1998). Two independent studies have shown a negative correlation between plasma insulin and GIP concentrations (Faulkner and Martin, 1997; Dawson et al., 1999), suggesting that GIP may not stimulate insulin production in ruminants. Hence, it is possible that the function of GIP differs between monogastric animals and ruminants, and that its direct actions on adipose tissue may differ from those in rats.

The aim of the study described here was to examine the effects of GIP, EGF and somatostatin on sheep adipose tissue. A number of studies have shown that the in vitro responsiveness of adipose tissue to hormonal stimuli may be dependant on the depot from which the samples are taken (Miller et al., 1991; Oben et al., 1991;

Pond and Mattacks, 1991; Vernon, 1991; Budd et al., 1994). Therefore three contrasting depots were examined.

2. Materials and methods

2.1. Chemicals, radiochemicals, plastics and media

Tissue culture plastics were obtained from Becton Dickinson UK Ltd (Oxford, UK). Except where stated otherwise, laboratory chemicals were obtained from Fisher plc (Loughborough, UK). Sodium [1-^{14}C]acetate (185–222 MBq/mmol) was purchased from Amersham Life Science Ltd (Aylesbury, UK). Dulbecco's phosphate buffered saline solution lacking calcium and magnesium (PBS) and Medium 199 (M199) and antibiotics were obtained in powder form from Imperial Laboratories (Europe) Ltd (Andover, UK). M199 was made up to contain supplementary sodium acetate, to bring the final concentration to 2.6 mM. It was buffered either with 2.2 g/l $NaHCO_3$ or with 25 mM HEPES buffer, adjusted to pH 7.4, as appropriate. Penicillin G (10^5 IU/ml) and streptomycin sulphate (100 mg/l) were added before use. All media were sterilised by filtration through a 0.2 μM membrane.

2.2. Biologically active polypeptides

Natural hormones (porcine GIP and bovine insulin) were obtained from Sigma-Aldrich Chemical Company Ltd (Poole, UK). Synthetic cyclic-somatostatin (somatostatin; 14 amino acids) was obtained from Bachem, UK. All three were dissolved in 154 mM NaCl, 10 mM HCl. Recombinant mouse epidermal growth factor (EGF) was the gift of Coopers Animal Health (Australia) It was dissolved in PBS. Stock solutions of hormones were prepared at 0.1 mM and stored in aliquots at −40°C; repeated freeze–thawing was avoided. All hormones and growth factors were diluted in medium containing 1 g/l crystalline grade bovine serum albumin (BSA; supplied by Sigma).

2.3. Animals and removal of fat tissue samples

Suffolk-cross lambs (aged 4–9 months) were fed a pelleted diet, and had free access to hay and water for at least 1 week prior to use. (Composition of pelleted diet per ton: barley, 225 kg; oats, 450 kg; dried grass, 200 kg; molasses, 100 kg; vitamin and mineral mix, 25 kg). Animals were humanely killed by stunning and exsanguination. Samples of adipose tissue (5–10 g) were aseptically excised within 5–10 min of slaughter. Perirenal fat from immediately around the kidney was used routinely, but as appropriate samples were taken from two other locations in the same animals: (1) the first layer of subcutaneous back fat tissue covering the longissimus dorsi muscle at the 8–12th rib; (2) popliteal fat tissue beneath the semitendinosus muscle. Tissue was transported to the laboratory in warm (39–40°C) HEPES-buffered M199.

2.4. Adipose tissue incubation

Tissue samples were transferred to pre-warmed (39°C) bicarbonate-buffered M199 and aseptically trimmed of excess connective tissues and visible blood vessels. The lymph node and the immediately surrounding tissue from the popliteal depot were discarded. Samples were then chopped into small pieces (20–30 mg). These explants were rinsed at least twice to remove tissue debris, before transfer to 6-well dishes. A total of 500–600 mg of explants were placed in each well, which contained 5 ml bicarbonate-buffered M199 with BSA added to final concentration of 1 g/l. All fat explants were pre-incubated in the presence of their designated concentration of test hormones for 24 h at 39°C under 100% humidity, 6% CO_2 (Vernon, 1982).

Fat explant lipogenesis was determined by measuring the incorporation of [1-^{14}C] acetate into total extractable lipid during a 2 h incubation (Vernon, 1982). Flasks contained 3 ml of HEPES-buffered M199 (with 1 g/l BSA) and 0.7 kBq [1-^{14}C] acetate; appropriately diluted hormones were added in a small volume, to give final concentrations of 0.01–10 nM. Insulin, where present, was used at 0.1 nM. Flask contents were mixed well and pre-warmed to 39–40°C. Approximately 150 mg of appropriately pre-incubated fat explants were quickly blotted to remove excess medium and added to each flask. The flasks were then plugged and were left in shaking waterbath (60–70 shakes/min at 39°C) for 2 h. They were then rapidly cooled on ice. Controls were included in each experiment in which explants were added to labelled media, and the flasks were immediately cooled without incubation.

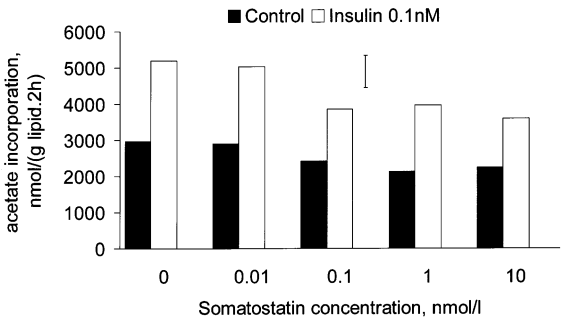

Fig. 1. Effect of somatostatin on lipogenesis in explants of ovine perirenal tissue. Tissue samples were incubated for 24 h with the concentrations of hormones indicated; lipogenesis was then measured over 2 h in the presence of the same hormone concentration. Results shown are the means of three independent experiments, in each of which three measurements of lipogenesis were made per treatment (nine replicates in total). The pooled standard error of the difference is shown.

2.5. Lipid extraction and measurement of radioactivity

Lipid was extracted from the explants by the method of Dole and Meinertz (1960), and the solvent removed by evaporation at room temperature. The lipid residue was dried to constant weight at 60°C. The amount of [^{14}C] acetate incorporated was quantified by liquid scintillation counting (Optiphase scintillator, Fisons).

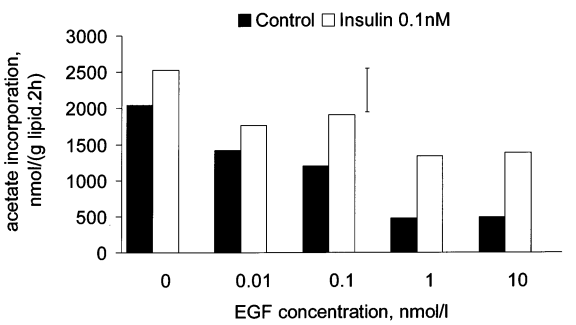

Fig. 2. Effect of epidermal growth factor on lipogenesis in explants of ovine perirenal tissue. Tissue samples were incubated for 24 h with the concentrations of hormones indicated; lipogenesis was then measured over 2 h in the presence of the same hormone concentration. Results shown are the means of five independent experiments: one included all the treatments shown; two examined the effects of EGF only in the absence of insulin, and a further two were carried out only in its presence. In each experiment three measurements of lipogenesis were made per treatment (nine replicates in total). The pooled standard error of the difference is shown.

2.6. Statistical analysis

Measurements in each experiment were performed in triplicate flasks and each experiment was carried out three times. Data were analysed by ANOVA, using the Genstat computer package (Rothamstead Experimental Station, Rothamstead, UK). Replicates within experiments were not meaned separately; experiments were treated as blocks within the analysis. Thus, the pooled standard errors given will include both inter-assay and intra-assay variability.

3. Results

3.1. Effects of epidermal growth factor and somatostatin on perirenal adipose tissue

When ovine perirenal adipose tissue explants were pre-incubated with varying concentrations of either SS or EGF (0.01–10 nM in each case), an inhibition of the incorporation of [1-^{14}C] acetate into lipid was subsequently observed (Figs. 1 and 2). These responses were qualitatively the same, whether or not insulin (0.1 nM) was present during the pre-incubation period.

With somatostatin, a maximal inhibition of incorporation was achieved at between 0.1 and 1 nM; values were of 69–75% of controls lacking somatostatin. Effects of insulin and somatostatin were both highly significant by ANOVA ($P < 0.001$), and there was no significant somatostatin × insulin interaction.

Maximal inhibitory effects of EGF were seen at concentrations of 1 nM or greater. Effects of EGF and insulin were both statistically significant ($P < 0.001$ and 0.008, respectively), and there was no significant insulin x EGF interaction. EGF reduced incorporation to only 25% of control values in the absence of insulin or 55% in its presence.

3.2. Effects of gastric inhibitory polypeptide on perirenal adipose tissue

GIP varied markedly from that of the two factors described above, in that the presence of insulin completely reversed its effects (insulin × GIP interaction statistically significant $P < 0.001$). As can be seen from Fig. 3, a stimulation of lipogenesis was seen with GIP in the absence of

Table 1
Comparative rates of lipogenesis in perirenal fat after pre-incubation with combinations of gut-related hormones[a]

Treatment	[^{14}C]acetate incorporation, nmol/(glipid.2h)		
	Basal	+EGF (1 nM)	+Somatostatin (1 nM)
Basal	2618	1486	–
+GIP 0.1 nM	3214	2460	2500
+Somatostatin 1 nM	2100	1673	–

[a] Results shown are the means of three independent experiments. Each treatment was carried out in triplicate in each experiment. Results were analyzed by ANOVA, using treatments as the source of variation and experiment as the block factor. Incorporation was significantly different between treatments ($P<0.001$; pooled SED = 315.8; 54 df).

insulin (150–160% of control values; $P<0.01$ by t-test at 1 nM), but in its presence, a dose-dependant reduction of up to 50% was seen. Incorporation in the presence of 0.1 nM insulin plus 10 nM GIP was very similar to that in controls lacking both. Effects were close to maximal at 1 nM.

3.3. Interactions between gut-related hormones in the perirenal depot

The effects of combinations of EGF, GIP and SS were examined in the absence of insulin. The effects of the treatments were statistically significant ($P<0.001$). As can be seen from Table 1, the stimulation of lipogenesis seen with 0.1 nM GIP was cancelled out in the presence of either 1 nM EGF or 1 nM SS. However, the combined effects of EGF and SS were less inhibitory than predicted by a model of simple additivity.

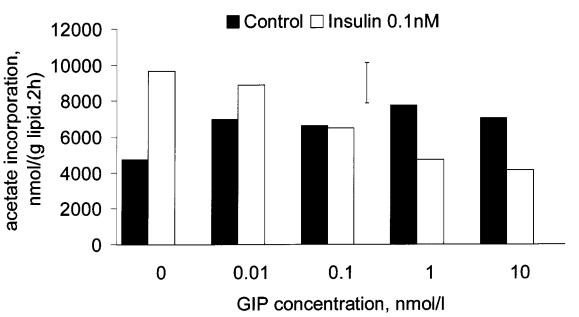

Fig. 3. Effect of gastric inhibitory polypeptide on lipogenesis in explants of ovine perirenal tissue. Tissue samples were incubated for 24 h with the concentrations of hormones indicated; lipogenesis was then measured over 2 h in the presence of the same hormone concentration. Results shown are the means of three independent experiments, in each of which three measurements of lipogenesis were made per treatment. All the treatments shown were carried out in two of the experiments; in the third 0.01 nM GIP, both with and without insulin, was omitted, i.e. nine replicates in the majority of treatments. The pooled standard error of the difference is shown.

3.4. Comparison of the effects of GIP, SS, EGF and insulin in different depots

The responses of the perirenal adipose depot were compared with those of a subcutaneous depot (backfat) and an intermuscular depot (popliteal) taken from the same animals. Overall incorporation by different depots was significantly different ($P=0.008$), with incorporation in the perirenal explants (average 525 nmol/(g lipid·2 h)) being significantly higher than in those from popliteal and backfat (291 and 304 nmol/(g lipid·2 h) respectively; pooled SED = 41.5, 4 df). As

Table 2
Comparison of the response of explants from different adipose depots to treatment with gut-related hormones[a]

Treatment	[^{14}C] acetate incorporation, nmoles/(g lipid.2h)		
	Perirenal	Popliteal	Subcutaneous
Basal	430	209	209
Insulin 0.1 nM	1079	423	438
GIP 1 nM	543	268	255
EGF 1 nM	237	275	284
Somatostatin 1 nM	334	279	330

[a] Results shown are the means of three independent experiments, each with tissue from a single animal. In each experiment treatments were carried out on triplicate samples. Results were analyzed by ANOVA, using treatments and depots as sources of variation, and depots within experiments as the block factor. The treatment × depot interaction was statistically significant ($P<0.001$). For cross-depot comparisons pooled SED = 54.9, 12 df. For within-depot comparisons SED = 40.3, 109 df.

shown in Table 2, the response of the different hormones to the added hormones was also significantly different between depots ($P < 0.001$ for the depot × treatment interaction). When perirenal fat was excluded from the statistical analysis, it was shown that the other two depots behaved similarly. Perirenal fat showed the greatest stimulation with 0.1 nM insulin. In contrast to the results in perirenal fat, EGF and SS both tended to increase lipogenesis in popliteal and subcutaneous fat; in their presence rates for all three depots became indistinguishable.

4. Discussion

The effects of the added hormones on lipogenesis were measured after 24 h pre-incubation of explants, because previous work (Vernon, 1982; Vernon and Finley, 1988) showed that pre-incubation is necessary in order to demonstrate a reproducible effect of insulin in ovine adipose tissue. Thus our results may reflect chronic, as well as acute, regulatory effects. Epidermal growth factor (EGF), somatostatin (SS) and gastric inhibitory polypeptide (GIP) all exerted direct effects on lipogenesis, but the nature and magnitude of these were depot-specific.

Overall rates of lipogenesis were higher in perirenal than popliteal or subcutaneous explants. These results are expressed per unit weight of lipid, so differences may be due to differences in average cell size; this does vary between depots in lambs (Broad et al., 1980; Mendizabal et al., 1997). Relative rates of incorporation between depots are consistent with observations in pigs (Budd et al., 1994), but contrast with results in steers, where incorporation was higher in subcutaneous fat (Dawson et al., 1999). Responses to insulin were smaller than those seen in rats (Oben et al., 1991), but comparable with other studies in ruminants (Miller et al., 1991; Watt et al., 1991).

Receptors for EGF are widely present in mammalian tissues (Fisher and Lakshmanan, 1990; Peng et al., 1997), but its role in adipose tissue development remains unclear. EGF and related ligands promote proliferation in pre-adipocytes from sheep (Broad and Ham, 1983), humans (Hauner et al., 1995) and chickens (Butterwith et al., 1992). In immature fat cells, synthesis of differentiation-specific enzymes and lipid filling may be reduced by EGF (Butterwith et al., 1992; Vassaux et al., 1994; Hauner et al., 1995), or it may be enhanced (Adachi et al., 1994; Bachmeier and Loffler, 1995). In a mixture of isolated mature rat adipocytes from epididymal and perirenal fat, short-term treatment with EGF stimulates the activity of lipogenic enzymes such as acetyl CoA carboxylase and inhibits lipolysis (Haystead and Hardie, 1986; Tebar et al., 1996). In contrast, the chronic systemic administration of EGF in mature male mice decreases perirenal adipose tissue mass, and increases hormone-sensitive lipase in adipose tissue (Kristensen et al., 1998; Vinter-Jensen et al., 1998). Transgenic mice over-expressing the EGF receptor ligand transforming growth factor alpha also have reduced adipose tissue (Luetteke et al., 1993). These may be indirect effects due to alteration of circulating concentrations of hormones and growth factors. Other workers have suggested that the increase in adipose tissue in ageing female mice is also an EGF-mediated phenomenon (Adachi et al., 1995).

We observed that EGF reproducibly stimulated lipogenesis in subcutaneous and intermuscular fat, but inhibited it in perirenal fat. Depot-specific differences in adipose tissue response to various stimuli have been reported in guinea pigs (Pond and Mattacks, 1991), pigs (Budd et al., 1994), rats (Oben et al., 1991), and cattle (Smith et al., 1998). None of these studies show qualitative differences in hormone action between depots, unlike the results reported here. However, adipose tissue itself produces hormones and cytokines, some of which show depot-specific variation, e.g. leptin (GottschlingZeller et al., 1999) and interleukin-6 (Fried et al., 1998). Hence EGF interaction with varying amounts of endogenous hormones might account for the contrast in response observed when subcutaneous and popliteal depots are compared with perirenal adipose tissue. EGF or related growth factors could modify the partitioning of de novo lipid synthesis between depots.

Somatostatin appears to have a species-specific role in glucose clearance. In man, it usually has little effect (Meneilly et al., 1987), but in dairy cattle its infusion enhances insulin-mediated uptake of radio-labelled glucose (Rose et al., 1997). This is a net effect on many tissues including muscle and liver, and may not reflect its action on adipose tissue. The importance of SS to the regulation of lipid metabolism is highlighted by a number of recent studies involving immuno-neutralisation of the endogenous hormone. An in-

crease in fat deposition has been reported for cattle (Ingvartsen and Sejrsen, 1995; Dawson et al., 1997) and sheep (Westbrook and McDowell, 1994). However this effect may be indirect since SS-immunisation also results in increases in plasma concentrations of GH, IGF-1, and thyroid hormone (Vankessel et al., 1993; Farmer et al., 1994; Dawson et al., 1997).

There are five subtypes of somatostatin receptor, which are expressed in a tissue-specific manner (Patel and Skrikant, 1997), allowing a diversity of response to the hormone. Adipocytes are known to express SS receptors (Simon et al., 1988). The binding of SS to them reduces hormone-stimulated lipolysis in adipocytes from the abdominal fat-pad of chickens (Suniga and Oscar, 1994; Oscar, 1996). In contrast, SS has been reported to inhibit insulin-stimulated glucose uptake in mature rat adipocytes (Pullen et al., 1985). Our studies indicate that, depending on the site of adipose tissues from which the samples were taken, SS directly affects fat metabolism in vitro by stimulating or inhibiting lipogenesis independently of insulin action. As discussed above for EGF, the reason for this difference in response is not clear. However, its implications are important, since circulating SS may differentially affect lipogenesis in adipose depots.

In man, GIP stimulates pancreatic insulin release (Dupre et al., 1973), for which reason it was re-named glucose-dependant insulinotropic polypeptide. In contrast, GIP concentrations in the plasma are negatively correlated with insulin concentrations both in growing steers fed different diets (Dawson et al., 1999) and in lactating sheep (Faulkner and Martin, 1997). These results suggest that the function of GIP in ruminants is not the same as in monogastric animals. In vitro studies have shown that GIP receptors are present on both normal rat adipocytes and the 3T3-L1 mouse pre-adipocyte cell line, and that they are up-regulated during differentiation (Yip et al., 1998). In both adipose explants and isolated adipocytes from the rat GIP has insulin-like effects, increasing lipogenesis and enhancing the effects of insulin (Hauner et al., 1988; Oben et al., 1991). GIP had little effect on basal or insulin-stimulated lipogenesis in adipose explants from steers (Dawson et al., 1999). At supraphysiological concentrations, GIP increases lipolysis in the mouse 3T3-L1 adipocyte cell line; this action is consistent with its elevation of intracellular cyclic AMP concentrations and is counteracted by the addition of insulin (McIntosh et al., 1999).

Our studies show that treatment with GIP alone can increase lipogenesis in ovine adipose tissue, but that it acts as an antagonist, rather than a synergist of insulin action. The insulin concentration used (0.1 nM) was close to the physiological concentration in the plasma of ruminating animals (Hart et al., 1985; Francis et al., 1999). The concentrations of GIP in the plasma of adult sheep have been measured in several independent studies; these give values of 0.04–0.12 nM for non-lactating animals (McCarthy et al., 1992; Faulkner and Martin, 1997; Rose et al., 1998), rising to a maximum of 0.3 nM in fed, lactating ewes (Faulkner and Martin, 1997). The data presented here show that at a concentration of 0.1 nM GIP there is no difference in lipogenic rates between explants with and without insulin, whereas 0.01 nM GIP has little effect on insulin-stimulated lipogenesis. Since there is a negative correlation between insulin and GIP concentrations in ruminant plasma under normal, non-fasting conditions, the net effect of these interactions could be to stabilise rates of lipogenesis in adipose tissue.

In conclusion, this study is apparently the first to show that the action of hormones and growth factors on adipose tissue may vary qualitatively, rather than just quantitatively, between depots. In particular, popliteal and subcutaneous tissues respond similarly to somatostatin and EGF, but this response is different to that seen in perirenal tissue. It is therefore not possible to make reliable predictions about changes in adiposity in response to a hormone, based on observations from one depot alone. Our data also show that, in ovine perirenal adipose tissue, GIP is not an insulin synergist but an antagonist. Clearly, more studies are needed to elucidate the role of gut-related hormones in ruminant lipid metabolism, since data obtained from monogastric species are not always applicable.

Acknowledgements

The authors are grateful to J. Craigon for statistical advice. A.S.H. Baba was supported during the course of this work by a scholarship from the Malaysian government.

References

Adachi, H., Kurachi, H., Homma, H., Adachi, K., Imai, T., Morishige, K.I., Matsuzawa, Y., Miyake, A., 1994. Epidermal growth factor promotes adipogenesis of 3T3-L1 cell in vitro. Endocrinology 135, 1824–1830.

Adachi, H., Kurachi, H., Homma, H., Adachi, K., Imai, T., Sakata, M., Matsuzawa, Y., Miyake, A., 1995. Involvement of epidermal growth factor in inducing adiposity of aged female mice. J. Endocrinol. 146, 381–393.

Bachmeier, M., Loffler, G., 1995. Influence of growth-factors on growth and differentiation of 3T3-L1 preadipocytes in serum-free conditions. Eur. J. Cell Biol. 68, 323–329.

Bloom, S.R., Mortimer, C.H., Thorner, M.O., Basser, G.M., Hall, R., Gomez-Pan, A., Roy, V.M., Russell, R.L.G., Coy, D.H., Kastin, A.J., Schally, A.V., 1974. Inhibition of gastrin and gastric acid secretion by growth hormone release inhibiting hormone. Lancet 2, 1106–1109.

Boden, G., Sivitz, M.C., Owen, O.E., 1975. Somatostatin suppresses secretin and pancreatic exocrine secretion. Science 190, 163–165.

Broad, T.E., Davies, A.S., Tan, G.Y., 1980. Pre- and postnatal study of the carcass growth of sheep, 2. The cellular growth of adipose tissues. Anim. Prod. 31, 73–79.

Broad, T.E., Ham, R.G., 1983. Growth and adipose differentiation of sheep preadipocyte fibroblasts in serum-free medium. Eur. J. Biochem. 135, 33–39.

Budd, T.J., Atkinson, J.L., Buttery, P.J., Salter, A.M., Wiseman, J., 1994. Effect of insulin and isoproterenol on lipid metabolism in porcine adipose tissue from different depots. Comp. Biochem. Physiol. 108C, 137–143.

Butterwith, S.C., Peddie, C.D., Goddard, C., 1992. Effects of transforming growth factor-α on chicken adipocyte precursor cells in vitro. J. Endocrinol. 134, 163–168.

Dawson, J.M., Greathead, H.M.R., Sessions, V.A., Tye, F.M., Buttery, P.J., 1999. Effect of gastric inhibitory polypeptide on bovine fat metabolism. Comp. Biochem. Physiol. 123B, 79–88.

Dawson, J.M., Soar, J.B., Buttery, P.J., Craigon, J., Gill, M., Beever, D.E., 1997. The effect of immunization against somatostatin and beta-agonist administration, alone and in combination, on growth and carcass composition in young steers. Anim. Sci. 64, 37–51.

Dole, V.P., Meinertz, H., 1960. Microdetermination of long chain fatty acids in plasma and tissue. J. Biol. Chem. 235, 2595–2599.

Dupre, J., Ross, S.A., Watson, D., Brown, J.C., 1973. Stimulation of insulin secretion by gastric inhibitory polypeptide in man. J. Clin. Endocrinol. Metab. 37, 826–828.

Farmer, C., Rushen, J., Brazeau, P., 1994. Hormonal concentrations during parturition in sows immunized against somatostatin. Can. J. Anim. Sci. 74, 711–713.

Faulkner, A., Martin, P.A., 1997. The concentrations of some gut polypeptides are elevated during lactation in ruminants. Comp. Biochem. Physiol. 118B, 563–568.

Fisher, D.A., Lakshmanan, J., 1990. Metabolism and effects of epidermal growth factor and related growth factors in mammals. Endocr. Rev. 11, 418–442.

Francis, S.M., Veevliet, B.A., Littlejohn, R.P., Suttie, J.M., 1999. Plasma glucose and insulin levels in genetically lean and fat sheep. Gen. Comp. Endocrinol. 116, 104–113.

Fried, S.K., Bunkin, D.A., Greenberg, A.S., 1998. Omental and subcutaneous adipose tissue of obese subjects release interleukin-6: depot difference and regulation by glucocorticoid. J. Clin. Endocrinol. Metab. 83, 847–850.

Gama, R., Norris, F., Morgan, L., Hampton, S., Wright, J., Marks, V., 1997. Elevated post-prandial gastric inhibitory polypeptide concentrations in hypertriglyceridaemic subjects. Clin. Sci. 93, 343–347.

GottschlingZeller, H., Birgel, M., Scriba, D., Blum, W.F., Hauner, H., 1999. Depot-specific release of leptin from subcutaneous and omental adipocytes in suspension culture: effect of tumor necrosis factor-alpha and transforming growth factor-beta 1. Eur. J. Endocrinol. 141, 436–442.

Guilloteau, P., LeHuërou-Luron, I., Toullec, R., Chayvialle, J.A., Blum, J.W., 1995. Regulatory peptides in young ruminants. In: Englehart, W.V., Leonhard-Marek, S., Breves, G., Giesecke, D. (Eds.), Ruminant Physiology: Digestion, Metabolism, Growth and Reproduction, Proceedings of the Eighth International Symposium on Ruminant Physiology. Ferdinand Enke Verlag, Stuttgart, pp. 519–537.

Hart, I.C., Chadwick, P.M.E., Coert, A., James, S., Simmonds, A.D., 1985. Effect of different growth hormone releasing factors on the concentrations of growth hormone, insulin and metabolites in the plasma of sheep maintained in positive and negative energy balance. J. Endocrinol. 105, 113–119.

Hauner, H., Glatting, G., Kaminska, D., Pfeiffer, E.F., 1988. Effects of gastric inhibitory polypeptide on glucose and lipid metabolism of isolated rat adipocytes. Ann. Nutr. Metab. 32, 282–288.

Hauner, H., Rohrig, K., Petruschke, T., 1995. Effects of epidermal growth factor (EGF), platelet-derived growth-factor (PDGF) and fibroblast growth-factor (FGF) on human adipocyte development and function. Eur. J. Clin. Invest. 25, 90–96.

Haystead, T.A.J., Hardie, D.G., 1986. Both insulin and epidermal growth factor stimulate lipogenesis and acetyl-CoA carboxylase activity in isolated adipocytes. Biochem. J. 234, 279–284.

Ingvartsen, K.L., Sejrsen, K., 1995. Effect of immunization against somatostatin (SS) in cattle-a review of performance, carcass composition and possible mode of action. Acta Agri. Scand. 45A, 124–131.

Ipp, E., Dobbs, E., Arimura, A., Vale, W., Harris, V., Unger, R.H., 1997. Release of immunoreactive somatostatin from pancreas in response to glucose, amino acids, pancreozymin-cholecystokinin and tolbutamide. J. Clin. Invest. 60, 760–765.

Knapper, J.M.E., Heath, A., Fletcher, J.M., Morgan, L.M., Marks, V., 1995. GIP and GLP-1(7-36)amide secretion in response to intraduodenal infusions of nutrients in pigs. Comp. Biochem. Physiol. 111C, 445–450.

Knapper, J.M.E., Morgan, L.M., Fletcher, J.M., 1996. Nutrient-induced secretion and metabolic effects of glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1. Proc. Nutr. Soc. 55, 291–305.

Krejs, G.J., Browne, R., Raskin, P., 1980. Effect of intravenous somatostatin on jejunal absorption of glucose, amino acids, water and electrolytes. Gastroenterol. 78, 26–31.

Kristensen, K., Pedersen, S.B., Vinter-Jensen, L., Flyvbjerg, A., Richelsen, B., 1998. Systemic administration of epidermal growth factor reduces fat mass in rats: effects on the hormone-sensitive-lipase, lipoprotein lipase and leptin. Horm. Res. 50, 292–296.

Luetteke, N.C., Lee, D.C., Palmiter, R.D., Brinster, R.L., Sandgren, E.P., 1993. Regulation of fat and muscle development by transforming growth factor α in transgenic mice and cultured cells. Cell Growth Diff. 4, 203–213.

Martin, P.A., Faulkner, A., 1994. Gastric inhibitory polypeptide concentrations in lambs fed milk or milk constituents. Comp. Biochem. Physiol. 108A, 371–375.

Martin, P.A., Faulkner, A., 1996. Effects of somatostatin-28 on circulating concentrations of insulin and gut hormones in sheep. J. Endocrinol. 151, 107–112.

McCarthy, J.P., Faulkner, A., Martin, P.A., Flint, D.J., 1992. Changes in the plasma concentration of gastric-inhibitory polypeptide and other metabolites in response to feeding in sheep. J. Endocrinol. 134, 235–240.

McIntosh, C.H.S., Bremsak, I., Lynn, F.C., Gill, R., Hinke, S.A., Gelling, R., Nian, C., McKnight, G., Jaspers, S., Pederson, R.A., 1999. Glucose-dependent insulinotropic polypeptide stimulation of lipolysis in differentiated 3T3-L1 cells: wortmannin-sensitive inhibition by insulin. Endocrinology 140, 398–404.

Mendizabal, J.A., Soret, B., Purroy, A., Arana, A., Horcada, A., 1997. Influence of sex on cellularity and lipogenic enzymes of Spanish lamb breeds (Lacha and Rasa Aragonesa). Anim. Sci. 64, 283–289.

Meneilly, G.S., Elahi, D., Minaker, K.L., Rowe, J.W., 1987. Somatostatin does not alter insulin-mediated glucose disposal. J. Clin. Endocr. Metab. 65, 364–367.

Miller, M.F., Cross, H.R., Lunt, D.K., Smith, S.B., 1991. Lipogenesis in acute and 48-h cultures of bovine intramuscular and subcutaneous adipose tissue explants. J. Anim. Sci. 69, 162–170.

Morgan, L.M., 1996. The metabolic role of GIP: physiology and pathology. Biochem. Soc. Trans. 24, 585–591.

Murphy, M.C., Isherwood, S.G., Sethi, S., Gould, B.J., Wright, J.W., Knapper, J.A., Williams, C.M., 1995. Postprandial lipid and hormone responses to meals of varying fat contents: modulatory role of lipoprotein lipase? Eur. J. Clin. Nutr. 49, 579–588.

Oben, J., Morgan, L., Fletcher, J., Marks, V., 1991. Effect of the entero-pancreatic hormones, gastric inhibitory polypeptide and glucagon-like polypeptide(7-36)amide, on fatty acid synthesis in explants of rat adipose tissue. J. Endocrinol. 130, 267–272.

Oscar, T.P., 1996. Prolonged in vitro exposure of broiler adipocytes to somatostatin enhances lipolysis and induces desensitization of antilipolysis. Poultry Sci. 75, 393–401.

Patel, Y.C., 1999. Somatostatin and its receptor family. Front. Neuroendocrinol. 20, 157–198.

Patel, Y.C., Skrikant, C.B., 1997. Somatostatin receptors. Trends Endocrinol. Metab. 8, 398–405.

Peng, M., Palin, M.-F., Véronneau, S., LeBel, D., Pelletier, G., 1997. Ontogeny of epidermal growth factor (EGF), epidermal growth factor receptor (EGFR) and basic fibroblast growth factor (bFGF) mRNA levels in pancreas, liver, kidney and skeletal muscle of pig. Dom. Anim. Endocrinol. 14, 286–294.

Pond, C.M., Mattacks, C.A., 1991. The effects of noradrenaline and insulin on lipolysis in adipocytes isolated from nine different depots of guinea-pigs. Int. J. Obes. 15, 609–618.

Pullen, L., Spencer, G.S.G., Hallett, K.G., Williams, J.P.G., Carssen, G.J., Hill, D.J., 1985. Somatostatin inhibits the insulin-stimulated glucose uptake into adipocytes in vitro. ICRS Med. Sci. 13, 1094.

Rose, M.T., Obara, Y., Itoh, F., Hashimoto, H., Takahashi, Y., 1997. Non-insulin- and insulin-mediated glucose uptake in dairy cows. J. Dairy. Res. 64, 341–353.

Rose, M.T., Itoh, F., Takahashi, Y., 1998. Effect of glucose-dependent insulinotropic polypeptide on whole-body glucose utilization in sheep. Exp. Physiol. 83, 783–792.

Sakurai, H., Dobbs, R.E., Unger, R.H., 1975. The effect of somatostatin on the response of GLI to the intraduodenal administration of glucose, protein and fat. Diabetologia 11, 427–436.

Schauder, P., McIntosh, C., Arends, J., Arnold, R., Freichs, H., Creutzfeld, W., 1997. Somatostatin and insulin release from pancreatic islets in response to D-glucose, L-leucine, α-ketoisocaproic acid or D-glyceraldehyde; evidence for a regulatory role of adenosine-3′5′-cyclic monophosphate. Biochem. Biophys. Res. Commun. 75, 630–635.

Schusdziarra, V., Harris, J.M., Conlon, J.M., Arimura, A., Unger, R.H., 1978. Pancreatic and gastric somatostatin release in response to intragastric and intraduodenal nutrients and HCl in the dog. J. Clin. Invest. 62, 509–518.

Simon, M.A., Romero, B., Calle, C., 1988. Characterization of somatostatin binding-sites in isolated rat adipocytes. Regulat. Pept. 23, 261–270.

Smith, S.B., Lin, K.C., Wilson, J.J., Lunt, D.K., Cross, H.R., 1998. Starvation depresses acylglycerol biosynthesis in bovine subcutaneous but not intramuscular adipose tissue homogenates. Comp. Biochem. Physiol. 120B, 165–174.

Suniga, R.G., Oscar, T.P., 1994. Triiodothyronine attenuates somatostatin inhibition of broiler adipocyte lipolysis. Poultry Sci. 73, 564–570.

Tebar, F., Soley, M., Ramirez, I., 1996. The antilipolytic effects of insulin and epidermal growth factor in rat adipocytes are mediated by different mechanisms. Endocrinology 137, 4181–4188.

Tyson, L.M., Browne, C.A., Jenkin, G., Thorburn, G.D., 1989. The fate and uptake of murine epidermal growth factor in sheep. J. Endocrinol. 123, 121–130.

Usdin, T., Mezey, É., Button, D.C., Brownstein, M.J., Bonner, T.I., 1993. Gastric inhibitory polypeptide receptor, a member of the secretin-vasoactive intestinal polypeptide receptor family, is widely distributed in peripheral organs and the brain. Endocrinology 133, 2861–2870.

Vankessel, A.G., Korchinski, R.S., Laarveld, B., 1993. Growth and serum thyroid-hormone and insulin-like growth factor-I (IGF-I) responses to passive-immunization against somatostatin in the lamb. Can. J. Anim. Sci. 73, 509–516.

Vassaux, G., Negrel, R., Ailhaud, G., Gaillard, D., 1994. Proliferation and differentiation of rat adipose precursor cells in chemically defined medium- differential action of anti-adipogenic agents. J. Cell. Physiol. 161, 249–256.

Vernon, R.G., 1982. Effects of growth hormone on fatty acid synthesis in sheep adipose tissue. Int. J. Biochem. 14, 255–258.

Vernon, R.G., 1991. Depot specific endocrine control of fatty acid synthesis in adipose tissues of foetal lambs. Dom. Anim. Endocrinol. 8, 161–164.

Vernon, R.G., Finley, E., 1988. Roles of insulin and growth hormone in the adaptations of fatty acid synthesis in white adipose tissue during the lactation cycle in sheep. Biochem. J. 256, 873–878.

Vinter-Jensen, L., 1999. Pharmacological effects of epidermal growth factor (EGF), with focus on the urinary and gastrointestinal tracts. APMIS (Suppl. 93) 107, 4–42.

Vinter-Jensen, L., Flyvbjerg, A., Nexo, E., 1998. Systemic treatment with epidermal growth factor causes organ growth concomitant with reduced circulating levels of IGF-I and IGF-BP3: time-dependent changes in female rats. Growth Horm. IGF Res. 8, 411–419.

Watt, P.W., Finley, E., Cork, S., Clegg, R.A., Vernon, R.G., 1991. Chronic control of the β- and α_2-adrenergic systems of sheep adipose tissue by growth hormone and insulin. Biochem. J. 273, 39–42.

Westbrook, S.L., McDowell, G.H., 1994. Immunization of lambs against somatotropin release inhibiting factor to improve productivity — comparison of adjuvants. Austr. J. Agric. Res. 45, 1693–1700.

Wisén, O., Björvell, H., Cantor, P., Johansson, C., Theodorsson, E., 1992. Plasma concentrations of regulatory peptides in obesity following modified sham feeding (MSF) and a liquid test meal. Regulat. Pept. 39, 43–54.

Yip, R.G.C., Boylan, M.O., Kieffer, T.J., Wolfe, M.M., 1998. Functional GIP receptors are present on adipocytes. Endocrinology 139, 4004–4007.

Zhang, G.X., Lai, J.H., Jia, T.W., Wang, W.Z., Wang, J.Y., 1997. Effects of epidermal growth factor on glutamine metabolic enzymes in small intestine and skeletal muscle of parenterally fed rats. Nutrition 13, 652–655.