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REGIONAL AND INTERSPECIFIC DIFFERENCES IN THE LIGAND BINDING PROPERTIES OF β-ADRENERGIC RECEPTORS OF INDIVIDUAL WHITE ADIPOSE TISSUE DEPOTS IN THE SHEEP AND RAT

WAYNE P. BOWEN,* DAVID J. FLINT and RICHARD G. VERNON†
Hannah Research Institute, Ayr KA6 5HL, U.K.

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Abstract—The ligand binding of β-adrenergic receptors was characterized in the omental, subcutaneous and popliteal adipose tissue depots in the sheep, and the lumbar and parametrial depots in the rat. Displacement of [^{125}I]iodocyanopindolol ([^{125}I]ICYP) binding to sheep adipose tissue membranes by the β-adrenergic antagonists CGP 20712A and ICI 118,551 (β_1- and β_2-antagonists, respectively) suggested only a single ligand binding site predominantly β_2 in character in all three depots, but revealed differences in affinity for these ligands between depots. Lactation increased β-receptor ligand binding in subcutaneous and omental but not popliteal sheep adipose tissue depots, but had no effect on β-adrenergic receptor affinities for CGP 20712A and ICI 118,551 in the three sheep adipose tissue depots studied. In rat adipocyte membranes, displacement of [^{125}I]ICYP by CGP 20712A and ICI 118,551 was biphasic, indicating high and low affinity sites; displacement profiles differed markedly between lumbar and parametrial depots, the former having a preponderance of β_1-like receptors and the latter a preponderance of β_2-like receptors. The properties of the β-adrenergic receptor binding site thus show species and depot specific differences.

Adipose tissue is found in distinct sites throughout the body located mostly in the abdominal cavity, under the skin or within the musculature. This distribution shows little variation between mammalian species and appears to have occurred early in mammalian evolution [1]. There is an increasing body of evidence suggesting that both anatomic and metabolic characteristics of adipocytes vary between adipose tissue depots. Adipocyte mean cell volume varies between different depots within a species [1] due primarily to different rates of fat accretion during fattening [2]. In general, mean adipocyte cell volumes rank as follows: abdominal > subcutaneous > intermuscular > intramuscular [13]. Several factors probably contribute to such differences. Lipoprotein lipase activity [4–8] and the rate of lipogenesis [5, 9, 10] vary between depots in ruminants, rats, pigs and humans. Blood flow and hence rate of nutrient supply also varies between depots [11, 12].

The rate of net lipid accretion is also dependent on the rate of lipolysis, and this too and its response to catecholamines are reported to vary between adipose depots in rats [13], dogs [14] and humans [7]. The initial step in the stimulation of lipolysis by catecholamines is the interaction with β-adrenergic receptors in the plasma membrane. At least three subtypes of the β-receptor (β_1, β_2 and β_3) have been described in different cells, the subtype present in adipocytes being controversial in rats at least and usually described as atypical [15], although a fairly recent paper has suggested that it is β_1 [16]. When characterizing the β-receptor of sheep adipose tissue we found that it differed markedly from that of the rat. Further studies described here have revealed that the properties of the receptor also vary between depots in both sheep and rats and so may contribute to the differential development and utilization of adipose tissue lipid stores.

MATERIALS AND METHODS

Reagents. CGP 20712A was generously given by Drs Schroter and Schleibli of Ciba-Geigy A.G. (Basel, Switzerland); ICI 118,551 was a gift from ICI Pharmaceuticals (Alderley Park, Macclesfield, U.K.). [^{125}I]Iodocyanopindolol ([^{125}I]ICYP‡) (approximately 2200 Ci/mmol) was purchased from Amersham International (Amersham, U.K.). All other reagents were obtained from either the Sigma Chemical Co. (Poole, U.K.) or BDH (Poole, U.K.).

Animals. Sheep were 3–4-year-old Finn × Dorset Horn cross-bred ewes. The animals were given hay and water *ad lib*. Non-lactating and lactating ewes also received 400 and 1400 g/day of the cereal mix, respectively. Lactating ewes suckled 2–3 lambs and were used 18 days post partum. Sheep were anesthetized with an intrajugular injection of 20–30 mL of Sagatal (May and Baker, Dagenham, U.K.) and exsanguinated, and adipose tissue was removed and placed in 150 mM saline at 39°. Omental adipose tissue was taken from around the rumen, popliteal (intermuscular) adipose tissue from within the hind limbs and subcutaneous adipose

* Present address: Smith Kline Beecham Pharmaceuticals, Coldharbour Road, The Pinnacles, Harlow, Essex CM19 5AD, U.K.
† Corresponding author.
‡ Abbreviations: ICYP, iodocyanopindolol; BSA, bovine serum albumin.

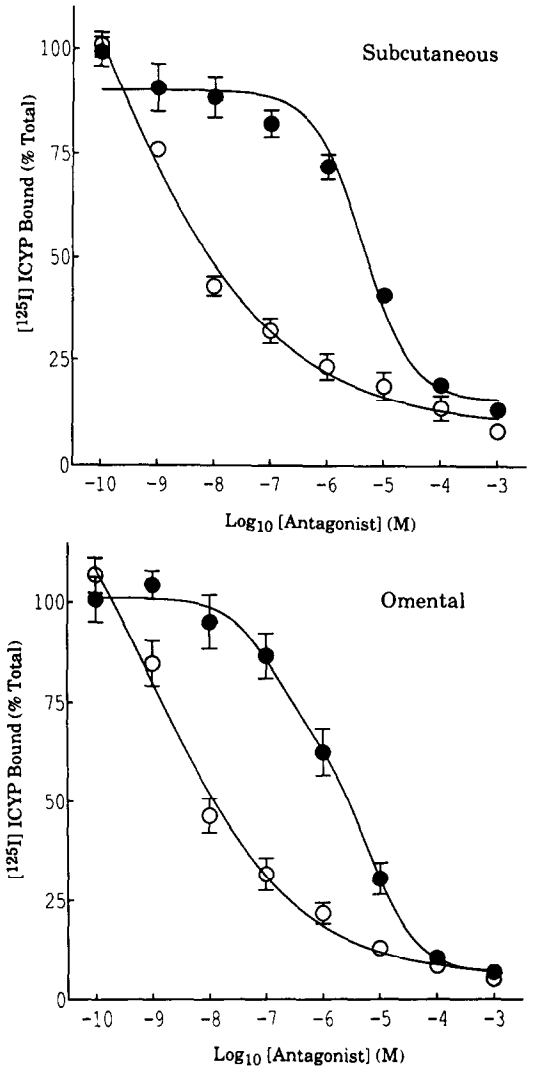

Fig. 1. Competition of [^{125}I]ICYP binding to sheep subcutaneous, popliteal and omental adipose tissue membranes by CGP 20712A and ICI 118,551. Adipose tissue membranes (15 μg protein) from 3–4-year-old non-lactating ewes were incubated with 75 pM [^{125}I]ICYP for 60 min at 22° in the presence of the indicated concentrations of the antagonists CGP 20712A (●) and ICI 118,551 (○). 100% total binding represents [^{125}I]ICYP bound in the absence of any antagonist. Each point is the mean ± SEM of four values each obtained from a different sheep.

tissue from the flank depot immediately anterior to the hind limb.

Wistar female virgin rats (160–180 g) from A. Tuck and Son (Rayleigh, U.K.) were given Labsure irradiated CRM diet (Labsure, Poole, U.K.) and water *ad lib*. Rats were killed by cervical dislocation and adipose tissue was removed and placed in 150 mM saline at 37°. Samples of parametrial adipose tissue were taken from the major lobe of tissue attached to the uterus; lumbar adipose tissue was from the depot along the dorsal wall of the abdomen adjacent to the kidney.

Preparation of rat adipocyte membranes. Adipocytes were prepared by collagenase digestion using the method of Rodbell [17]. Adipose tissue (10 g) was minced in a petri-dish using warm (about 30°) scissors and digested in sealed plastic flasks containing 10 mL of gassed (95% O_2, 5% CO_2) Krebs–Ringer bicarbonate (1.2 mM $CaCl_2$) buffer, containing 25 mM Hepes (pH 7.3), 0.3 mg/mL D-glucose, 1.5 mg/mL collagenase (Type II, Sigma) and 40 mg/mL bovine serum albumin (BSA) (dialysed and fatty acid free), for 1 hr at 37° in a shaking water bath. The digest was passed through a plastic strainer, and the floating adipocytes washed three times with 10 mL of gassed (95% O_2, 5% CO_2) Krebs–Ringer bicarbonate buffer, containing 25 mM Hepes (pH 7.3), 0.3 mg/mL D-glucose, 10 mg/mL BSA at 37°.

Adipocytes were lysed by addition of 10 mL of 10 mM Tris–HCl (pH 7.4), 20 mM EDTA, 0.2 M sucrose containing 0.1 mg/mL bacitracin, 17.5 μg/mL benzamidine HCl, 5 μg/mL soybean trypsin inhibitor, 0.2 mM phenylmethylsulphonyl fluoride at 37°. The suspension was vortexted for 2 min, transferred to a 50-mL plastic centrifuge tube and centrifuged rapidly at 36,900 *g* (SS34 rotor; Sorvall RC-5B Du Pont, U.S.A.) for 20 min at 37°. The fat and infranatant were poured off and the tubes plunged into ice. The pellet was gently resuspended in 5 mL of ice-cold 10 mM Tris–HCl (pH 7.4), 90 mM NaCl using a glass homogenizer and centrifuged at 1000 *g* (Minifuge T, Hereaus) for 10 min at 4°. The supernatant was recovered and

Table 1. Displacement of [^{125}I]ICYP binding to sheep adipose tissue membranes from three depots by CGP 20712A and ICI 118,551

	IC_{50}					
	CGP 20712A (μM)			ICI 118,551 (nM)		
Depot	Non-lactating (N = 4)	Lactating (N = 4)	All sheep (N = 8)	Non-lactating (N = 4)	Lactating (N = 4)	All sheep (N = 8)
Omental	1.47	1.72	1.60	9.37	9.25	9.31
Subcutaneous	3.09	2.90	2.99*	3.76	6.51	5.14*
Popliteal	2.99	4.11	3.55†	7.77	5.88	6.82
SED			0.47			1.52

Results are the means of data from four non-lactating and four lactating sheep. Analysis of variance showed that lactation had no significant effect on IC_{50} value (SED (standard error of the difference) 1.004 and 2.655 for CGP 207112A and ICI 118,511, respectively, for comparing effects of lactation on individual depots). Hence, results were pooled to give values for all sheep; SED values 0.47 and 1.52 are for comparing values in the corresponding column.

*,† Value significantly different from that for omental adipose tissue, $P < 0.05$, $P < 0.01$, respectively.

Table 2. Effect of lactation on [^{125}I]ICYP binding in three sheep adipose tissue depots

	[^{125}I]ICYP bound (fmol/mg protein)			
	Non-lactating		Lactating	
Depot	Mean	Mean of $\log_{10}$ values	Mean	Mean of $\log_{10}$ values
Omental	164	2.19	300	2.47†
Subcutaneous	191	2.26	312	2.48*
Popliteal	171	2.22	231	2.35

Results are means of data from four animals analysed by analysis of variance of log-transformed data.

*, † Value significantly different, $P < 0.02$, $P < 0.01$, respectively, compared with the corresponding value for non-lactating sheep.

Standard error of differences of $\log_{10}$-transformed values is 0.092 for comparing values between lactating and non-lactating sheep, and 0.074 for comparing values between depots from sheep in the same physiological state.

recentrifuged at 36,900 *g* (SS34 rotor; Sorvall RC-5B) for 20 min at 4°. The resulting membrane pellet was resuspended in 10 mM Tris–HCl (pH 7.4), 90 mM NaCl (approximately 2 mg protein/mL) and stored in liquid nitrogen.

Preparation of sheep adipose tissue membranes. Sheep adipose tissue (100 g) was mixed with 200 mL of 10 mM Tris–HCl (pH 7.4), 20 mM EDTA, 0.2 M sucrose containing 0.1 mg/mL bacitracin, 17.5 μg/mL benzamidine HCl, 5 μg/mL soybean trypsin inhibitor, 0.2 mM phenylmethylsulphonyl fluoride at 40°, and homogenized in a warm (about 30°) blender (Waring, U.S.A.) at high speed for 35 sec. The homogenate was quickly centrifuged at 2800 *g* (Minifuge T, Hereaus for 5 min at 40°. The sample was cooled on ice until the fat solidified and the infranatant was then removed and recentrifuged at 40,000 *g* (6 × 100 mL rotor; Prepsin 50, MSE) for 20 min at 4°. The pellet was resuspended in ice-cold 10 mM Tris–HCl (pH 7.4), 90 mM NaCl using six strokes of a large-bore glass homogenizer fitted with a Teflon pestle. The suspension was centrifuged at 1000 *g* (Minifuge T, Hereaus) for 10 min at 4°. The supernatant was recovered and recentrifuged at 36,900 *g* (SS34 rotor; Sorvall RC-5B) for 20 min at 4°. The resulting membrane pellet was resuspended in 10 mM Tris–HCl (pH 7.4), 90 mM NaCl (approximately 2 mg protein/mL) and stored in liquid nitrogen.

Radioligand binding assays. β-Adrenergic receptors in membranes were assayed using the method of Malbon *et al.* [18] with some modification. Membranes (15 μg protein) were incubated with 75 pM [^{125}I]ICYP, 50 mM Tris–HCl (pH 7.4), 10 mM $MgCl_2$, 0.1 mM ascorbate in a total volume of 200 μL for 1 hr at 22°. The reaction was quenched by addition of 5 mL of ice-cold 50 mM Tris–HCl (pH 7.4), $MgCl_2$ (10 mM) and the incubation mixture was filtered rapidly through a single 2.5-cm Whatman GF/C filter disc. The filter was washed twice with 5 mL of ice-cold 50 mM Tris–HCl (pH 7.4), 10 mM $MgCl_2$. The radioactivity trapped on the filters was quantified using a LKB γ counter. When assessing the number of β-adrenergic receptors of sheep adipocyte membranes, non-specific binding was determined by adding 100 μM (−)-isoproterenol.

Protein determinations. Protein was measured by the method of Bradford [19] using the Bio-Rad protein assay (Cat. No. 500–0006) using BSA as standard.

Analysis of data. Radioligand competition curves were analysed using a four-parameter logistic equation (ALLFIT) [20]. Biphasic competition curves were analysed using non-linear iterative analysis (GraphPad Inplot; GraphPad Software, 10855 Sorrento Valley Road, 9, San Diego, CA 92121, U.S.A.). β-Adrenergic receptor affinities (IC_{50} values) from competition curves obtained for lactating and non-lactating sheep were interpreted using analysis of variance utilizing the GENSTAT

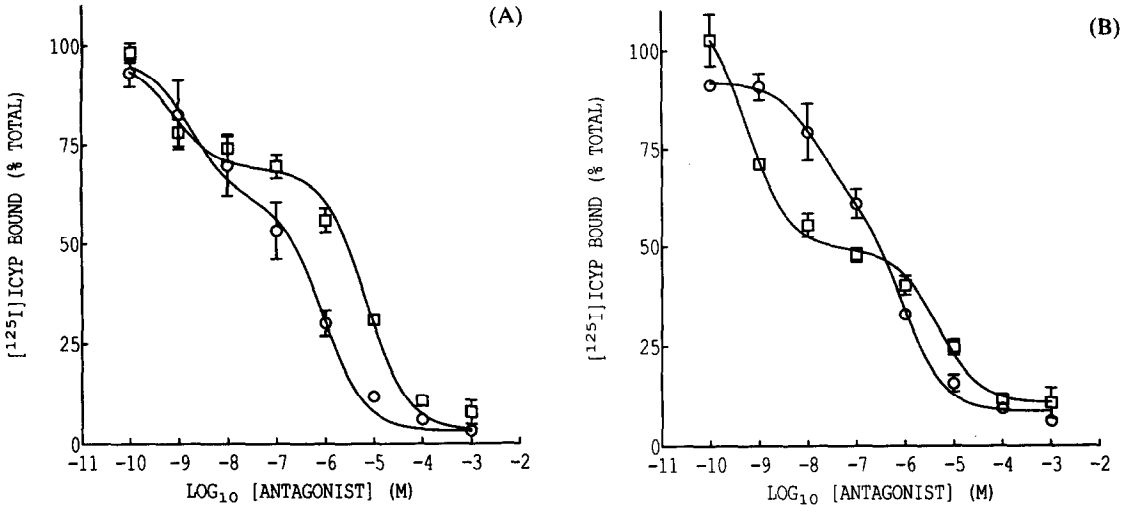

Fig. 2. Displacement of [^{125}I]ICYP binding to rat parametrial and lumbar adipocyte membranes by CGP 20712A and ICI 118,551. Rat adipocyte membranes (20 μg protein) were incubated with 93 pM [^{125}I]ICYP for 60 min at 22° in the presence of various concentrations of the antagonists of β_1 and β_2 CGP 20712A (□) and ICI 118,551 (○), respectively. Total binding represents [^{125}I]ICYP bound in the absence of antagonist. The data were analysed using non-linear iterative analysis. Each point is the mean ± SEM, calculated from three separate experiments performed in duplicate. (A) Parametrial adipocytes; (B) lumbar adipocytes.

Table 3. Displacement of [^{125}I]ICYP binding to rat adipocyte membranes by CGP 20712A and ICI 118,551: assessment of proportion and IC_{50} of high and low affinity binding sites

		Adipose tissue			
		Parametrial binding site		Lumbar binding site	
Ligand	Variable	R_H	R_L	R_H	R_L
CGP 20712A	IC_{50}	0.27 ± 0.01 nM	4.80 ± 0.10 μM	0.55 ± 0.01 nM‡	4.66 ± 0.13 μM
	Percentage of sites	38.0 ± 3.8	62.0 ± 6.2	61.9 ± 4.0†	38.1 ± 2.5*
ICI 118,551	IC_{50}	3.52 ± 0.12 nM	0.86 ± 0.02 μM	14.29 ± 0.49 nM‡	0.87 ± 0.02 μM
	Percentage of total sites	61.7 ± 5.2	38.3 ± 3.2	35.8 ± 3.8†	64.2 ± 6.8*

Results for [^{125}I]ICYP (93 pM) binding to rat parametrial and lumbar adipocyte membranes in the presence and absence of various concentrations of CGP 20712A (β_1-antagonist) and ICI 118,551 (β_2-antagonist) ranging from 10^{-10} to 10^{-3} M (Fig. 2) were analysed using non-linear iterative analysis. The quality of curve fitting for one and two site models was assessed using an *F*-test; this suggested a two site model. The analysis provided estimates of the IC_{50} for high (R_H) and low (R_L) affinity binding sites and provided an estimate of the number of each sites as a percentage of the total number of sites.

Each value is mean ± SEM of three values, each obtained from a separate experiment.

*, †, ‡ Value differs from corresponding value for parametrial adipocytes, $P < 0.05$, 0.01, 0.001, respectively.

computer program. Statistical analysis was either analysis of variance or Student's *t*-test of either untransformed or log-transformed data, where the latter reduced the coefficient of variation.

RESULTS

β-Adrenergic receptors were radiolabelled with the radioligand [^{125}I]ICYP, which binds to β-adrenergic receptors with high affinity, in a non-subtype-selective manner. Competition curves for the displacement of [^{125}I]ICYP binding to sheep adipose tissue membranes by ICI 118,551 (β_2 selective) and CGP 20712A (β_1 selective) were steep and monophasic in all three adipose depots (subcutaneous adipose tissue, Fig. 1; other results not shown). However, the affinity of the β-adrenergic receptor for each of these antagonists varied between depots. Data from eight sheep (four non-lactating, four lactating) were analysed using the ALLFIT computer program. The rank order of IC_{50} values for CGP 20712A displacement of [^{125}I]ICYP binding was popliteal = subcutaneous > omental (Table 1). Lactation had no effect on the IC_{50} values for either

CGP 20712A or ICI 118,551 in any of the three depots studies (Table 1). The pooled IC_{50} values for all sheep for popliteal ($P < 0.01$) and subcutaneous ($P < 0.02$) adipose tissue were significantly different to that for omental adipose. The rank order of IC_{50} values for IC 118,551 competition was omental > popliteal = subcutaneous (Table 1). The pooled IC_{50} for subcutaneous adipose tissue for all sheep was 45% lower than that for omental adipose ($P < 0.02$).

β-Adrenergic receptor ligand binding of 75 pM [^{125}I]ICYP was very similar in membranes prepared from omental, subcutaneous and popliteal adipose depots of non-lactating sheep (Table 2). Analysis of $\log_{10}$-transformed results showed that lactation increased β-adrenergic receptor ligand binding in omental ($P < 0.01$) and subcutaneous ($P < 0.02$) depots (Table 2) by about 40%.

In view of the findings in sheep adipose tissue, the study of regional variation of β-adrenergic receptor properties was extended to rat adipose tissue. Adipocyte membranes were prepared separately from parametrial and lumbar adipose depots. [^{125}I]-ICYP binding (93 pM) to β-adrenergic receptors in both depots was displaced with increasing concentrations of ICI 118,551 and CGP 20712A. Competition curves of ICI 118,551 and CGP 20712A were biphasic in both depots, indicating the presence of high and low affinity binding sites (Fig. 2). Simultaneous analysis of pooled data from three independent experiments with non-linear iterative analysis (GraphPad InPlot) revealed that the lumbar depot possessed a higher percentage of high affinity CGP 20712A binding sites (62%; $P < 0.02$) and a lower percentage of low affinity CGP 20712A sites (38%; $P < 0.05$) as compared with the parametrial depot (Table 3). In addition, the IC_{50} of the high affinity CGP 20712A site (0.53 nM) in the lumbar depot was greater ($P < 0.001$) than the corresponding value for the parametrial depot 0.27 nM) (Table 3). In contrast, the parametrial adipocytes possessed the highest percentage of high affinity ICI 118,551 binding sites (62%; $P < 0.02$) and the lowest percentage of low affinity sites (38%; $P < 0.05$). Also, these adipocytes contained a high affinity ICI 118,551 site with a lower IC_{50} (i.e. higher affinity) than the same site in lumbar adipocytes ($P < 0.001$). However, the IC_{50} values for the low affinity binding sites for CGP 20712A and ICI 118,551 in parametrial adipocytes were the same as the corresponding values for lumbar adipocytes.

DISCUSSION

The study shows that not only are there major species differences in the properties of β-adrenergic receptors of white adipose tissue, but there are also marked differences in properties in different depots within a species, even in depots such as lumbar and parametrial which are in close proximity in the abdominal cavity.

The sub-type of the rat adipocyte β-adrenergic receptor has been a subject of controversy for a long time. Lands *et al.* [21] originally classified it as β_1, a conclusion also reached more recently by Bahouth and Malbon [16]. Most recent studies, however, have proposed that the rat adipocyte β-receptor is atypical [15]. Our findings support the view that the receptor is atypical or that there may be two populations of receptors, as the displacement curves shown in Fig. 2 differ markedly from those for sheep and are not consistent with a single β_1 or β_2 component. Results for parametrical adipocytes are very similar to those reported by Valet *et al.* [22] for dog omental adipocytes (in both studies there appears to be about 65% β_2 and 35% β_1 subtypes). Curiously this ratio of β_2:β_1 subtypes was reversed in canine adipocytes by long-term exposure to increased endogenous catecholamine levels [22]. In contrast, our findings for lumbar adipose tissue, which appears to have a substantial proportion of a β_1-like component, are similar to those of Bahouth and Malbon [16] for parametrial adipocytes. In the present study great care was taken to ensure that the lumbar and parametrial preparations were not cross-contaminated; the substantial amount of adipose tissue lying between the ovary and kidney was not included in either preparation. Previous studies by others have mostly used either epididymal adipose tissue from male rats or parametrial adipose tissue from female rats. Because of their close proximity, preparations of the latter may have contained varying amounts of adipose tissue from other sites, especially when older, fatter rats were used. The present study shows that even adjacent adipose tissue depots can differ markedly in the properties of their β-adrenergic receptors; hence, some of the differences between the various studies and even species reported previously may well arise from the source of adipose tissue within the body.

In contrast to the rat, it would appear that for sheep each of the three depots studied contains a single population of β-receptors of the β_2-subtype. This is suggested by the displacement curves which are consistent with a single component. In addition, while the number of β-receptors appears to be increased by lactation in two of the depots this increase did not result in any apparent change in the properties of the receptors. Nevertheless, the properties of the receptors of omental adipose tissue do differ from those of subcutaneous and popliteal in their affinity for β_2- and β_1-specific ligands. The structural reason for this is not known but there are several possibilities, for example, different patterns of glycosylation or phosphorylation; a different lipid and/or protein structure surrounding the receptor; and possibly even different amino acid sequences in the receptors.

In the present study, the concentration of ligand used (75 pM) was not completely saturating hence differences in binding with lactation could have been due to changes in affinity rather than B_{max}. However, lactation does not appear to change affinity (at least for the two antagonists used). Furthermore, other studies using a range of concentrations of [^{3}H]-dihydroalprenalol as ligand also show that lactation increases binding by omental but not popliteal adipocyte membranes [23]. For subcutaneous adipose tissue there are conflicting reports with an increase noted by Watt *et al.* [24] but not by Kennedy and Vernon [23].

The physiological consequences and advantages of having β-adrenergic receptors with different

affinity for ligands in individual adipose tissue depots is unclear. Lactation for example results in a proportionately greater loss of fat from omental and subcutaneous adipocytes than from popliteal adipocytes in sheep (de la Hoz and Vernon, unpublished observation) but this parallels differences in receptor number rather than properties of the receptors in the three depots found in the present study. Under-nutrition usually results in a greater loss of subcutaneous than abdominal fat in sheep (see Ref. 25), but while this might be due to differences in β-receptor properties, other factors such as rate of lipid synthesis and blood flow, both of which vary between depots [3], will also influence the net rate of lipid loss. Whatever the reasons, differences in properties of β-receptors in different adipose tissue depots in commercially important animals like sheep does raise the possibility of devising means for selective manipulation of adiposity with production of animals with leaner carcases but with adequate reserves of abdominal fat.

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REFERENCES

1. Pond CM and Mattacks CA, Anatomical organisation of mammalian adipose tissue. In: *Functional Morphology of Vertebrates* (Eds. Dunker HR and Fleischer G), pp. 485–489. Springer, Stuttgart, 1985.
2. Vernon RG, The growth and metabolism of adipocytes. In: *Control and Manipulation of Animal Growth* (Eds. Buttery PJ, Haynes NB and Lindsay DB), pp. 67–83. Butterworths, London, 1986.
3. Vernon RG, Control of lipogenesis and lipolysis. In: *The Control of Fat and Lean Deposition* (Eds. Buttery PJ, Boorman KN and Lindsay DB). Butterworths, London, in press.
4. Haugebak CD, Hedrick HB and Asplund JM, Relationship between extramuscular adipose tissue lipoprotein lipase activity and intramuscular lipid deposition in fattening lambs. *J Anim Sci* **39**: 1026–1031, 1974.
5. Lee YB and Kauffman RG, Cellular and enzymatic changes with animal growth in porcine intramuscular adipose tissue. *J Anim Sci* **38**: 532–537, 1974.
6. Savard R and Greenwood MRC, Site-specific adipose tissue LPL responses to endurance training in female lean Zucker rats. *J Appl Physiol* **65**: 693–699, 1988.
7. Rebuffe-Scrive M, Andersson B, Olbe L and Bjorntorp P, Metabolism of adipose tissue in intraabdominal depots of nonobese men and women. *Metabolism* **38**: 453–458, 1989.
8. Bjorntorp P, Ottosson M, Rebuffe-Scrive M and Xu X, Regional obesity and steroid hormone interactions in human adipose tissue. In: *Obesity: Towards a Molecular Approach* (Eds. Bray GA, Ricquier D and Spiegelman BM), pp. 147–157. Alan R. Liss, New York, 1990.
9. Whitehurst GB, Beitz DC, Cianzio D and Topel DG, Fatty acid synthesis from lactate in growing cattle. *J Nutr* **111**: 1454–1461, 1981.
10. Fried SK, Lavau M and Pi-Sunyer FX, Variations in glucose metabolism by fat cells from three adipose depots of the rat. *Metabolism* **31**: 876–883, 1982.
11. Gregory NG, Christopherson RJ and Lister D, Adipose tissue capillary blood flow in relation to fatness in sheep. *Res Vet Sci* **40**: 352–356, 1986.
12. West DB, Prinz WA and Greenwood MRC, Regional changes in adipose tissue blood flow and metabolism in rats after a meal. *Am J Physiol* **257**: R711–R716, 1989.
13. Hartman AD and Christ DW, Effect of cell size, age and anatomical location on the lipolytic response of adipocytes. *Life Sci* **22**: 1087–1096, 1978.
14. Berlan M, Carpene C, Lafontan M and Dang-Tran L, Alpha-2 adrenergic antilipolytic effect in dog fat cells: incidence of obesity and adipose tissue localisation. *Norm Metab Res* **14**: 257–260, 1982.
15. Zaagsma J and Nahorski SR, Is the adipocyte β-adrenoceptor a prototype for the recently cloned atypical "β_3-adrenoceptor"? *Trends Pharmacol Sci* **11**: 3–7, 1990.
16. Bahouth SW and Malbon CC, Subclassification of β-adrenergic receptors of rat fat cells: a re-evaluation. *Mol Pharmacol* **34**: 318–326, 1988.
17. Rodbell M, Metabolism of isolated fat cells. *J Biol Chem* **239**: 375–380, 1964.
18. Malbon CC, Moreno FJ, Cabelli RJ and Fain JN, Fat cell adenylate cyclase and β-adrenergic receptors in altered thyroid states. *J Biol Chem* **253**; 671–678, 1978.
19. Bradford MM, A rapid and sensitive method for the quantitation of microgram quantities of protein utilising the principle of protein-dye binding. *Anal Biochem* **72**: 248–254, 1976.
20. De Lean A, Munson PJ and Rodbard D, Simultaneous analysis of families of sigmoidal curves: application to bioassay, radioligand assay, and physiological dose-response curves. *Am J Physiol* **235**: E97–E102, 1978.
21. Lands AM, Arnold A, McAuliff JP, Ludena FP and Brown TG Jr, Differentiation of receptor systems activated by sympathomimetic amines. *Nature* **214**: 597–598, 1967.
22. Valet P, Montastruc J-L, Berlan M, Tran M-A, Lafontan M and Montastruc P, Differential regulation of fat cell Beta-2 and Beta-1 adrenoceptors by endogenous catecholamines in dog. *J Pharmacol Exp Ther* **249**: 271–277, 1988.
23. Kennedy AD and Vernon RG, Beta and alpha receptor binding in subcutaneous, omental and popliteal adipocyte membranes from lactating and non-lactating ewes. *J Anim Sci* **68**(Suppl 1): 283–284, 1990.
24. Watt PW, Clegg RA, Flint DJ and Vernon RG, Increases in sheep adipocyte β-receptor number on exposure to growth hormone *in vitro*. In: *Biotechnology in Growth Regulation* (Eds. Heap RB, Prosser CG and Lamming GE), p. 230. Butterworths, London, 1989.
25. Vernon RG, Lipid metabolism in the adipose tissue of ruminant animals. *Prog Lipid Res* **19**: 23–106, 1980.