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The Effect of Human Placental Lactogen Upon the Metabolism of Rat and Human Adipose Tissue

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The metabolic effects of human placental lactogen (HPL) on rat and human white fat were tested *in vitro*. When tested against rat tissue, HPL resembled insulin in stimulating uptake of glucose and incorporation of [^{14}C] glucose into CO_2, triglyceride and glycogen, but differed from insulin in stimulating glycerol release and in failing to stimulate the incorporation of [^{14}C] acetate into CO_2, glyceride fatty acids and glyceride glycerol. The stimulation of [^{14}C] glucose incorporation and the inhibition of glycerol release by insulin were antagonized by HPL. The effects of HPL on human white fat resembled those on rat white fat, except that glycerol release was not stimulated in human tissue. The possible role of HPL in causing the diabetogenic stress of pregnancy is discussed in the light of these findings.

Human Placental Lactogen (HPL), also known as human chorionic somatomammotrophin, is a peptide which is synthesized in the placenta and which has immunological, structural and somatotrophic properties similar to those of human growth hormone (23). It can also increase the weight of the mammary glands (6) and stimulate lactation (26, 27) in rats. The concentration of HPL in human maternal plasma increases progressively between the 6th and 34th weeks of gestation (23).

However, its precise physiological role is still not clear. The ability of HPL to stimulate insulin release (15) and the mobilization of free fatty acids (FFA) *in vivo* (8), and to antagonize the peripheral actions of insulin (1, 11) suggests that it may be wholly or partly respon-

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sible for those features of pregnancy, such as insulin resistance and an increased concentration of plasma free fatty acids, which are also observed in patients with mild forms of diabetes (18). In this paper we report a simple procedure for the purification of HPL and a study of its effects on the metabolism *in vitro* of adipose tissue from male and female rats and from human subjetcs.

Materials and Methods

TISSUE AND INCUBATIONS. Male and non-pregnant female rats weighing 140-160 g from an inbred Wistar strain, fed *ad libitum*, were used throughout. They were killed by decapitation with a guillotine and the adipose tissue was rapidly removed and placed in 0.9 % NaCl. Abdominal adipose tissue was obtained from human subjects during operations under general anaesthesia for surgical conditions not associated with any endocrine or metabolic disorder.

The tissue was cut into pieces weighing 20-40 mg each and distributed randomly into plastic incubation vessels containing 2 ml of Krebs-Ringer bicarbonate buffer, pH 7.4 (30) with one-half the recommended Ca^{2+} concentration, 2 % defatted albumin, glucose (3 mM) and 0.5 μCi of [U-^{14}C] glucose/ml. Hormones were added as required. The contents of the flasks were gassed with $O_2 + CO_2$ (95:5, v/v) for 2 min, sealed with a rubber cap and incubated at 37° C, with shaking, for 2 hours. At the end of the incubation the reaction was stopped by injecting 0.2 ml of 5 M-H_2SO_4 through the rubber cap. Hyamine hydroxide (0.2 ml of 1.0 M-hyamine hydroxide in methanol) was then injected into a hanging plastic cup inside the incubation vessel and the flasks were gently shaken at 37° C for 2-4 hours to allow complete absorption of the CO_2 released from the medium by the acid.

Isolated fat cells were prepared by the procedure described by RODBELL (22). They were finally resuspended in Krebs-Ringer bicarbonate buffer (as above) containing 2 % defatted albumin and 3 mM-[U-^{14}C] glucose (0.2 μCi/ml). Samples (1 ml) of the suspensions were transferred to plastic flasks. The contents were gassed, after the addition of hormones, and were then incubated at 37° C for 4 hours with shaking. The reactions were stopped and CO_2 was absorbed as described above.

ANALYTICAL METHODS. HPL was detected during purification by its characteristic reaction of partial identity with antibody to human pituitary growth hormone. Up to 500 μg of preparations of purified HPL were assayed for insulin by radioimmunoassay, following the double-antibody procedure described in the instructions supplied with the anti-insulin serum. The assay could reliably and consistently detect 3 pg (0.07 μunits) of insulin. Polyacrylamide-gel electrophoresis was carried out essentially by the method of DAVIS (5), with the buffer system described by REISFELD and SMALL (21). The methods used for the determination of ^{14}C incorporation into CO_2, and into glycerol and fatty acids of tissue glycerides, and for the assay of glycerol, fatty acids and glucose in the incubation medium, have been described previously (13). Glycogen was extracted from the fat-free tissue residue by the method of WALAAS and WALAAS (31) in the presence of added glycogen as carrier. Further purification (20) did not result in any increase in specific activity.

PREPARATION AND PURIFICATION OF HPL. Human placentas were kept frozen until extracted. After removal of fat and membranes they were homogenized in glacial acetic acid/acetone (400:75, v/v) in a Waring Blendor (2 ml/g tissue). The homogenate was heated to 70° C and allowed to cool. It was then centrifuged at 1,000 × *g* for 30 min. An equal volume

of diethyl-ether was added to the supernatant. The precipitate which formed was dried by successive treatments with acetone, and the dry powder was suspended in 0.2 M acetic acid (14 ml/g powder). After stirring at 4° C overnight, the suspension was centrifuged at 1,000×*g* for 20 min and the precipitate discarded. All subsequent steps were performed at 4° C. The pH of the supernatant was brought to 13.0 by the addition of saturated NaOH while the solution was magnetically stirred, and then immediately reduced to pH 9.0 with glacial acetic acid. The suspension was stirred for 30 minutes, centrifuged at 1,000 × *g* for 30 min and the precipitate discarded. The pH of the supernatant was brought to 7.4 with 0.2 M acetic acid. Proteins which precipitated from this solution at concentration of $(NH_4)_2SO_4$ between 33 and 50 % saturation were collected by centrifugation, dissolved in 10 ml of 0.02 M acetic acid and dialysed against the same solution for 24 hours with two changes of dialysing fluid. The dialysate was brought to pH 6.0 with 0.1 M KOH and centrifuged at 5,000 × *g* for 30 min. Ethanol at —20° C was added to the supernatant to give a final concentration of 15 %. After 3 h at 4° C, the suspension was centrifuged at 5,000 × *g* for 30 min and the precipitate dried by successive treatments with acetone. The powder was dissolved in 0.02 M acetic acid (20 ml/g powder) and applied to a column (0.9 × 80 cm) of Sephadex G-100 equilibrated with the same solution. The column was eluted with 0.02 M acetic acid. The protein in the eluate was assayed in successive 3 ml fractions by measuring UV absorption at 280 nm and HPL was detected immunochemically. The HPL was eluted in approx. 3 times the void volume as the last of four protein peaks. Its elution volume corresponded to a molecular weight of 30,000-35,000 based on a comparison with known proteins (human albumin and human growth hormone). The peak containing the HPL was dried from the frozen state and dissolved in 0.1 M phosphate buffer (pH 7.5). When submitted to polyacrylamide-gel electrophoresis it gave a single dense band on staining, with a faint band of higher mobility. Further purification, with removal of about 10 % of the total protein, was achieved by preparative polyacrylamide-gel electrophoresis. About 50 mg of purified protein was obtained from 1 kg of placenta.

MATERIALS. Sephadex G-100 was obtained from Pharmacia Fine Chemicals, Uppsala. Acrylamide was obtained from BDH. L-arterenol bitartrate and bovine serum albumin (Fraction V powder) were obtained from Sigma. The albumin was defatted by the method described by Chen (3). [U-^{14}C] glucose and sodium [1-^{14}C] acetate were obtained from The Radiochemical Centre, Amersham. Pig insulin (Pro-Actrapid, free from glucagon) was obtained from Novo Industries, London. Samples of HPL were kindly provided by Dr. D. R. Banham, Division of Biological Standards, National Institute for Medical Research, Mill Hill, London, and by Dr. C. H. Li, Laboratory of Hormone Research, University of California, San Francisco. The hormones were dissolved and diluted in Krebs-Ringer bicarbonate buffer, pH 7.4, immediately before use. Antibodies were raised in rabbits to human growth hormone (obtained from Dr. D. H. Banham, as above). Anti-insulin serum was obtained from Wellcome Reagents Ltd., Beckenham, Kent.

Results

IDENTIFICATION OF THE PURIFIED PROTEIN. A protein which gave a reaction of partial identity with antibody to growth hormone was purified from human placenta as described in Methods. The protein had a molecular weight of about 30,000, migrated as a single band when

subjected to polyacrylamide-gel electrophoresis and gave a single antibody when injected into rabbits. Other properties of the protein, in addition to its immunochemical behaviour, were characteristic of HPL. It promoted the growth of female rats whose growth curve had reached a plateau, its activity in this respect being about 1/5 that of growth hormone. It also prolonged dioestrus in intact, immature rats and this luteotrophic effect has been used to establish a bioassay for the hormone (16). The HPL obtained from preparative gel-electrophoresis did not withstand freezing or lyophilization, and its instability in solution at physiological pH rendered it unsuitable for metabolic studies. The material obtained from the penultimate (gel filtration) stage of purification was much more stable. This material contained over 90 % HPL, as indicated by analytical gel-electrophoresis and was shown to contain no hormonal or hormone-like impurity that would interfere with metabolic studies. It was free of growth hormone and chorionic gonadotrophin when tested by the double-diffusion immunochemical technique of OUCHTERLONY (19) and contained no insulin that could be detected by radioimmunoassay (500 μg of protein in an assay that could detect 3 pg of insulin). Thus this material, rather than the unstable protein, was used in the metabolic studies described below.

COMPARISON OF HPL WITH INSULIN. The effects of HPL and insulin on the metabolism of epididymal fat from male rats and of parametrial fat from female rats were compared *in vitro*. Preliminary experiments were performed to determine the concentrations at which insulin and noradrenaline exerted their maximal effects upon the metabolism of glucose by adipose tissues under these conditions. The optimal concentration of each (5 nM for insulin and 10 μM for noradrenaline) was then employed in the experiments with HPL. Under all conditions the rates of incorporation or uptake were constant throughout the 2-hr incubations. Table I shows the effect of HPL and of insulin on glycerol release and the metabolism of [^{14}C] glucose by parametrial and epididymal adipose tissues *in vitro*. Glucose uptake, glycerol release and incorporation of ^{14}C into CO_2, glyceride and glycogen were all higher in the presence of HPL than in control samples incubated without HPL. Some of these effects could be detected with 50 μg of HPL/ml, but higher concentrations (100 μg/ml for female tissue and 500 μg/ml for male tissue) were required before the differences achieved statistical significance. Assuming a molecular weight of 30,000 for HPL, 100 μg/ml corresponds approximately to 3 μM.

Insulin stimulated glucose uptake and incorporation of ^{14}C into CO_2, glycerides and glycogen. In contrast to the action of HPL, insulin significantly depressed the release of glycerol from rat adipose tissue from both sources. Moreover, a dose of HPL whose effect on glucose uptake was comparable with that of insulin had a smaller effect than that of insulin on the incorporation of ^{14}C into glycogen and glyceride fatty acids, and a greater effect on incorporation of ^{14}C into glyceride glycerol. The effects of HPL and insulin on glucose uptake and ^{14}C incorporation were essentially the same in the absence and in the presence of noradrenaline. However, in the presence of noradrenaline HPL did not stimulate glycerol release, whereas insulin caused the increase in glycerol release which is normally observed with these concentrations of hormones in the presence of glucose (10, 12).

In order to test whether the effects shown in table I were due to a direct action of HPL on fat cells or were mediated by an action on other cells within the tissue, the effect of the hormone on isolated fat cells was investigated. HPL at concentrations greater than 50 μg/ml in-

Table I. *Effect of HPL on the metabolism of adipose tissue from male and female rats.* Insulin (5 mM) and noradrenaline (10 μM) were present where indicated. Basal values (with no additions) are given as μmoles per gram of tissue in two hours of incubation. Where hormones were present results are given as percentages of their corresponding basal values. Each result is expressed as the mean ± S.E.M. of four separate incubations (epididymal fat) and eight separate incubations (parametrial fat).

Additions	Glucose uptake	Incorporation of [U-^{14}C] glucose into				Glycerol release	FFA release
		CO_2	Glyceride FA	Glyceride glycerol	Glycogen		
Parametrial fat							
None	3.26±0.20	0.43±0.05	0.75±0.15	0.07±0.01	0.11±0.01	1.71±0.14	
50 μg/ml HPL	102 ±11	119 ±20	116 ±24	111 ±17	97 ±10	121 ±10	
100 μg/ml HPL	124 ±8 *	139 ±17 *	153 ±30	127 ±13	117 ±4 *	130 ±15 *	
500 μg/ml HPL	232 ±25 *	303 ±62 *	313 ±73 *	253 ±27 *	133 ±17	198 ±20 *	
Insulin	274 ±10 *	391 ±36 *	387 ±59 *	185 ±23 *†	375 ±29 *†	58 ±9 *†	
Noradrenaline	193 ±9 *	279 ±11 *	152 ±16	310 ±18 *	72 ±2	460 ±15 *	374 ±35 *
Noradrenaline+ 500 μg/ml HPL	226 ±13 **	329 ±29	362 ±133 **	293 ±34	148 ±55 **	395 ±87	263 ±29
Noradrenaline+ Insulin	223 ±5 **	351 ±8 **	202 ±40	370 ±19	82 ±3	572 ±24 **	452 ±56
Epididymal fat							
None	2.96±0.63	0.78±0.04	0.79±0.28	0.12±0.03		1.45±0.13	
50 μg/ml HPL	125 ±5	102 ±15	91 ±8	68 ±15		101 ±13	
100 μg/ml HPL	147 ±17	117 ±9	93 ±13	86 ±15		106 ±6	
500 μg/ml HPL	318 ±12 *	237 ±24 *	262 ±29 *	286 ±31 *		244 ±72 *	
Insulin	384 ±30 *	299 ±33 *	361 ±46 *†	130 ±36 †		78 ±14 †	

* Significant effect ($P < 0.05$) of single hormone. † Significant effect ($P < 0.05$) between insulin and HPL (500 μg/ml). ** Significant effect ($P < 0.05$) between noradrenaline and its combination with HPL or insulin.

Table II. *Effect of HPL and insulin on the metabolism of [^{14}C] acetate by epididymal fat pads.*
Incubations contained 3 mM non-radioactive glucose and 1 mM Na [1-^{14}C] acetate (1 μCi/ml). Results are given as the mean ± S.E.M. of four sets of incubations.

Additions	Incorporation of [1-^{14}C] acetate into		
	CO_2	Glyceride fatty acids	Glyceride glycerol
	μmoles/g tissue/2 h		
None	6.79 ± 0.19	4.05 ± 0.16	0.099 ± 0.007
100 μg/ml HPL	6.68 ± 0.30	3.31 ± 0.36	0.137 ± 0.012
500 μg/ml HPL	6.23 ± 0.40	3.62 ± 0.22	0.120 ± 0.008
Insulin (5 nM)	5.70 ± 0.15 *	17.90 ± 0.32 *	0.044 ± 0.004 *

* Significantly ($P < 0.05$) different from basal.

creased the incorporation of ^{14}C from [U-^{14}C] glucose into CO_2 and into glycerol and fatty acids of glyceride. This stimulatory effect showed no evidence of a lag period.

The preparation of HPL used for the present work had a greater effect upon glucose metabolism in adipose tissue than the preparation of Dr. C. H. Li and was considerably more active than the Mill Hill preparation. For instance, when tested at a concentration of 125 μg/ml the present preparation produced a 55 % increase in the incorporation of [^{14}C] glucose into CO_2 and a 150 % increase in incorporation into glyceride fatty acids. In identical experiments performed with the same pool of tissue and the same concentrations of HPL, the increases were 26 and 64 % respectively for the preparation from Dr. Li and 15 and 49 % for the preparation from Mill Hill.

Our preparation of PHL differed from insulin in its effect on the metabolism of acetate in adipose tissue (table II). Whereas insulin stimulated the incorporation of ^{14}C from [1-^{14}C] acetate into fatty acids and inhibited ^{14}C incorporation into CO_2 and glyceride glycerol, HPL had no significant effect on any of these aspects of adipose tissue metabolism.

ANTAGONISM BETWEEN HPL AND INSULIN. Although several of the actions of HPL on glucose metabolism in adipose tissue were similar to those of insulin, some of the effects of insulin were modified by HPL. This was most marked in the case of those effects of insulin which differed significantly from those of HPL (table I). Thus, HPL reversed the inhibitory effect of insulin upon glycerol release and significantly diminished the stimulatory effect of insulin upon the incorporation of ^{14}C from [U-^{14}C] glucose into glycogen and into the fatty acids of glyceride (table III).

EFFECT OF HPL ON HUMAN ADIPOSE TISSUE. HPL did not stimulate glycerol release from human adipose tissue, but in other respects its actions upon human fat, in the presence and in the absence of insulin, were similar to those observed in rat adipose tissue (table III). In particular, glucose uptake and the incorporation of ^{14}C from [U^{14}C] glucose into CO_2 and glycogen were stimulated in the absence of insulin.

Discussion

The immunochemical properties of the HPL used for the present work were similar to those preparations described by others (2, 4, 9, 28). However, our

Table III. *The effect of HPL on the metabolism of rat parametrial fat in the presence of insulin, and of human adipose tissue in the presence and absence of insulin.*
Tissue was incubated with [U-^{14}C] glucose in the presence of insulin (5 nM) and HPL (500 μg/ml) as indicated. Basal values are given as μmoles per gram of tissue in two hours of incubation (rat parametrial fat) and four hours (human fat). Where hormons were present, results are given as percentages of their corresponding basal values. Each result is expressed as the mean ± S.E.M. of four separate incubations.

		Incorporation of [U-^{14}C] glucose into					
Additions	Glucose uptake	CO_2	Glyceride FA	Glyceride glycerol	Glycogen	Glycerol release	FFA release
Rat parametrial fat							
None	5.48±0.98	0.84±0.07	0.42±0.04	0.83±0.09	0.03±0.01	2.20±0.12	1.20±0.27
Insulin	209 ±7	374 ±20	740 ±57	131 ±6	734 ±72	74 ±6	114 ±20
Insulin+HPL	211 ±7	319 ±29	526 ±69 *	143 ±9	455 ±62 *	128 ±8 *	112 ±19
Human fat							
None	2.98±0.26	0.16±0.03	0.003±0.001	0.20±0.01	0.015±0.005	0.69±0.17	
HPL	164 ±19 *	196 ±30 *	479 ±247	127 ±18	242 ±37 *	112 ±16	
Insulin	146 ±10 *	175 ±40	991 ±657	113 ±14	491 ±128	88 ±29	
Insulin+HPL	152 ±9	193 ±33	459 ±192	115 ±11	430 ±104	115 ±11	

* Significant effect ($P < 0.05$) of HPL.

preparation appears to be biologically more active than preparations used by other workers (C. Osorio and C. Ferrer, unpublished observations). Thus, compared with equal weights of human pituitary growth hormone, its somatotrophic activity was more than twice that of the preparations used by MURAKAWA and RABEN (17). Moreover, the effect of our preparation upon the metabolism of adipose tissue was considerably greater than that of the sample of purified HPL provided by Dr. C. H. Li. Even so, the concentration of the hormone preparation required to elicit a significant response upon adipose tissue *in vitro* was about 10 times higher than that found in blood during pregnancy. Similarly high or higher concentrations have been required by other workers to show any effect of HPL on glucose metabolism (7) or lipolysis (25, 29). This could be explained by unphysiological constraints imposed by using an *in vitro* system and by loss of biological activity during the purification procedure. However, until a more active preparation is isolated, any interpretations of the physiological significance of effects of HPL observed *in vitro* should be treated with caution.

HPL had direct effects upon the metabolism of adipose tissue, some of which were qualitatively similar to those of insulin, both in the absence and in the presence of noradrenaline. Stimulation of the uptake and metabolism of glucose by epididymal fat pads was also observed with the preparation of HPL used by FRIESEN (7) but has not been demonstrated previously in adipose tissue from female rats or human subjects. In contrast to our preparation of HPL, that used by TURTLE and KIPNIS (29) did not stimulate glucose metabolism in adipose tissue. We therefore considered the possibility that some of the metabolic effects of our preparations of HPL were due to contamination with insulin. However, insulin could not be detected by radioimmunoassay in any preparation. From the limit of sensitivity of the assay, it can be calculated that the maximun concentration of insulin in a solution of 500 μg of the HPL preparation/ml was 0.5 pM (0.07 μunit/ml). This is far below the lowest concentration of insulin (1-5 μunits/ml) that can elicit a response from adipose tissue *in vitro* (22, 24). Contamination with insulin or insulin-like hormones is also difficult to reconcile with the finding that some effects of insulin were antagonized by HPL and that, for a given degree of stimulation of glucose uptake, HPL had a greater effect than insulin on the incorporation of [^{14}C] glucose into glyceride glycerol and a smaller effect on incorporation into fatty acids and glycogen. Finally, HPL differed from insulin in having no effect on the incorporation of [1-^{14}C] acetate into fatty acids. Since there was no consistent lipolytic effect of our preparation of HPL, it is unlikely to be contaminated with lipolytic hormones.

Adipose tissue from pregnant rats has a high basal uptake of glucose (14). There is also a resistance to the actions of insulin in human peripheral tissues during pregnancy (18). The stimulatory effects of HPL on glucose metabolism and its inhibitory effect on some of the actions of insulin suggest that HPL could be responsible for some of these changes. However, the present results do not indicate how far HPL is responsible for the raised plasma FFA concentration that occurs in human pregnancy. TURTLE and KIPNIS (29) and STRANGE and SWYER (25) found that a lag period of 2 hours was required before any stimulation of lipolysis in adipose tissue could be observed after addition of HPL *in vitro*. Our preparations of HPL increased the rate of glycerol output by rat adipose tissue during a 2 hours incubation, but the magnitude of the effect was variable and no effect was obtained with human adipose tissue. It is unlikely, therefore, that the increase in serum FFA concentration

observed in pregnant women is due to an activation by HPL of adipose-tissue triglyceride lipase.

Resumen

Se han estudiado los efectos metabólicos de HPL sobre el tejido adiposo humano y el de rata *in vitro*. En tejido de rata, HPL se asemejó a insulina, estimulando la captación de glucosa y la incorporación de [C^{14}] glucosa a CO_2, triglicéridos y glucógeno, pero se comportó de manera diferente a insulina, estimulando la liberación de glicerol y no estimulando la incorporación de [C^{14}] acetato a CO_2, ácidos grasos y glicerol de glicéridos. HPL antagonizó la estimulación de la incorporación de [C^{14}] glucosa y la inhibición de la liberación de glicerol producidas por insulina. Los efectos de HPL sobre el tejido adiposo humano fueron similares a los producidos en tejido adiposo de rata, excepto que no estimuló la liberación de glicerol en tejido adiposo humano. A la vista de estos resultados se discute el posible papel que ejerza HPL en la sobrecarga diabetogénica del embarazo.

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