Glucose metabolism in vivo in fed and 48 h starved goats during pregnancy and lactation

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1. Glucose turnover (i.e. glucose entry and utilization rates) in fed and 48 h starved goats during pregnancy and lactation was determined using a continuous infusion of [U-^{14}C]- and [3-^{3}H]glucose.

2. Glucose synthesis and utilization increased during pregnancy and lactation in fed but not in starved goats.

3. Recycling of glucose-C was approximately 10% in fed animals and 15–20% in starved animals and was unaffected by the stage of pregnancy or lactation.

4. Plasma glucose concentrations were maintained during pregnancy and lactation in fed goats but decreased during 48 h starvation in pregnant goats. Little change was seen in the plasma concentration of lipids and their metabolites during pregnancy and lactation in fed goats, but increases were observed after 48 h starvation.

5. The control of glucose metabolism in ruminants during pregnancy and lactation is discussed.

The adult ruminant obtains very little glucose from its diet and its metabolic requirements for glucose are supplied by gluconeogenesis in the liver and kidney (Bergman, 1973). In contrast to the simple-stomached animal, gluconeogenesis is greatest in ruminants in the fed state when gluconeogenic precursors are readily available. Starvation results in a decrease in the rate of glucose synthesis and an increased dependence on lipid as an energy source (Bergman, 1973). During pregnancy and lactation the requirement for glucose increases considerably; in the pregnant animal the foetus and uterus utilize glucose as a major energy source (Lindsay, 1973) and in lactation large quantities of glucose are removed by the mammary glands for lactose synthesis (Annison & Linzell, 1964).

Previous studies on glucose synthesis in pregnant and lactating ruminants in vivo have used glucose labelled with ^{14}C (Bergman, 1973). However, this technique can underestimate the actual rates of glucose turnover since glucose-C metabolized to L-lactate or amino acids can be reincorporated into the glucose skeleton (Katz & Rognstad, 1976). In simple-stomached animals this recycling can be as high as 30–40% (Katz & Rognstad, 1976). However, results from fed non-pregnant, non-lactating sheep suggest that glucose recycling in the ruminant may be lower (Judson & Leng, 1972).

This paper reports changes in the turnover rates of glucose during pregnancy and lactation in fed and 48 h starved goats in vivo using both [U-^{14}C]- and [3-^{3}H]glucose infusions and estimates the recycling of glucose-C in vivo in these animals. Dairy goats were studied at three periods in their normal yearly cycle; in mid-pregnancy when the previous lactation had ceased, in late-pregnancy and in mid-lactation before remating. In addition, plasma concentrations of glucose, insulin and intermediates of lipid metabolism have been determined and related to the changes in the rates of glucose turnover.

MATERIALS AND METHODS

Goats

The experiments were conducted on British-Saanen goats aged 3–10 years and weighing 40–60 kg. The animals were surgically prepared for the collection of mammary-venous and arterial blood (Linzell, 1960) and were accustomed to their surroundings before experimentation. Lactating goats were milked twice daily (08.00 and 16.00 hours). Pregnant and

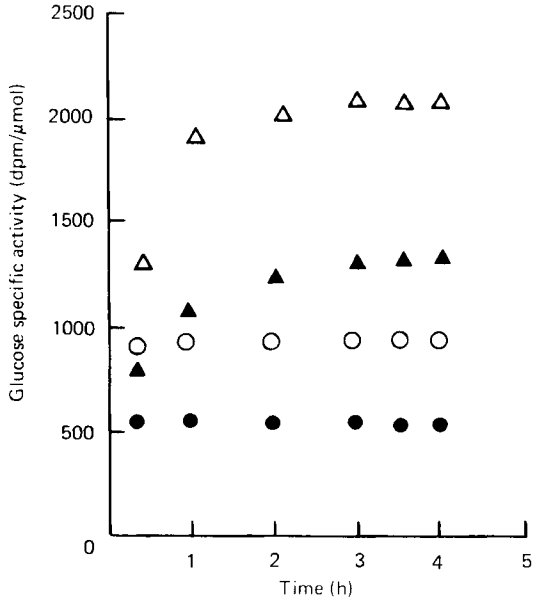

Fig. 1. Time curve of [U-^{14}C]glucose (●, ▲) and [3-^{3}H]glucose (○, △) specific activites in the fed (●, ○) and 48 h starved (▲, △) lactating goat.

lactating animals were fed hay *ad lib.* and concentrates (Rank Hovis McDougal, London) (750 g/d for pregnant animals and 1500 g/d for lactating animals). Starved animals had food withdrawn for 48 h before the experiment. Water was available at all times. Goats were studied in mid-pregnancy (70 ± 3 d); late pregnancy (132 ± 2 d) and lactation (6–12 weeks post partum). Gestation is approximately 150 d in the goat.

Chemicals

All enzymes and coenzymes were obtained from Boehringer Corporation (London), Lewes, Sussex and Sigma (London) Chemical Co., Poole, Dorset; [U-^{14}C]- and [3-^{3}H]glucose, ^{125}I-labelled insulin and ^{3}H-labelled cortisol were obtained from The Radiochemical Centre, Amersham, Bucks. All other chemicals were obtained from British Drug Houses, Poole, Dorset.

Methods

On the day before the experiment catheters were placed in a jugular vein, an exteriorized carotid artery and subcutaneous abdominal (milk) vein (Thompson & Thomson, 1977). At approximately 08.00 hours a priming dose of radioactive glucose (3 μCi [U-^{14}C]glucose + 5 μCi[3-^{3}H]glucose) in sterile saline (9 g sodium chloride/l) was injected into the jugular vein, followed by an infusion (0.4 μCi [3-^{3}H]glucose/min + 0·2 μCi [U-^{14}C]glucose/ min) which was maintained for 4 h. This procedure produced a constant specific radioactivity of plasma glucose throughout the final 1 h of infusion (Fig. 1). During the final 1 h blood samples were taken from the carotid artery and the 'milk' vein.

Blood flow through the mammary gland was determined by measuring the dilution of Indocyanine Green dye (Thompson & Thomson, 1977). Plasma glucose concentrations were determined as described by Slein (1963), glycerol as described by Garland & Randle (1962), β-hydroxybutyrate as described by Williamson *et al.* (1962), free fatty acids and triglyceride as described by Thomson *et al.* (1979). Radioactivity in plasma glucose was determined as described by Jones (1965). The bicarbonate content of arterial blood was determined

manometrically (Van Slyke & Folch, 1940). The radioactivity in blood bicarbonate was determined by acidifying a known volume of blood with an equal volume of 1 M-perchloric acid in an air-tight vessel; the $^{14}CO_2$ evolved was trapped in 10 M-sodium hydroxide and the radioactivity determined (Linn & Fritz, 1972). Plasma insulin concentrations were determined as described by Flint *et al.* (1979). Plasma cortisol concentrations were determined using a specific cortisol antiserum (Steranti Research Ltd, St Albans, Herts).

Calculations

Glucose turnover (T) in the whole animal, expressed as μmol/min, was calculated from the equation:

$$T = I/G,$$

where I is the rate of infusion of [U-^{14}C]- or [3-^{3}H]glucose (μCi/min) and G is the specific activity of [U-^{14}C]- or [3-^{3}H]glucose in arterial plasma at equilibrium (μCi/mol).

Recycling of glucose-C (R) in the whole animal, expressed as percentage of the C of newly-formed glucose that came from glucose, was calculated from the equation (Katz *et al.* 1974):

$$R = (T_H - T_C) \times 100/T_H,$$

where T_H is the rate of glucose turnover obtained using [3-^{3}H]glucose and T_C is the rate of glucose turnover obtained using [U-^{14}C]glucose.

Uptake (U) of glucose by the mammary gland, expressed as μmol/min, was calculated from the equation:

$$U = Q_p \times (G_a - G_v),$$

where Q_p is the mammary plasma flow (ml/min), G_a is the concentration of glucose in arterial plasma (μmol/ml) and G_v is the mammary-venous plasma glucose concentration (μmol/ml).

Statistics

Results from 48 h starved animals were compared with those of fed animals using Student's t test for paired obsverations. Stage of pregnancy and lactation effects were compared using an analysis of variance.

RESULTS

Changes in glucose metabolism during pregnancy and lactation in the fed and 48 h starved goat

Glucose turnover, as determined by either [U-^{14}C]- or [3-^{3}H]glucose infusion, increased significantly during pregnancy and lactation. In the lactating animal more than 50% of this glucose was removed by the mammary glands. Utilization of glucose by tissues other than the mammary gland was calculated from the total rate of glucose synthesis and the rate of glucose uptake by the mammary glands to be (mean $\pm$ SE) 326 ± 109 (n 6) and 224 ± 57 (n 6) μmol/min for the fed and starved lactating goat respectively. In the fed lactating animal this rate of non-mammary utilization of glucose was significantly lower ($P < 0.05$) than in the fed goat during mid-pregnancy. The specific radioactivity of arterial blood bicarbonate relative to that of glucose-C decreased significantly in both fed and starved lactating animals when compared with values obtained during pregnancy (Table 1). This may indicate some reduction in the rate of glucose oxidation in the lactating goat. Recycling of glucose-C did not change with advancing pregnancy or into lactation (Table 1).

Starvation resulted in a significant decrease in glucose turnover in both pregnant and lactating goats, as measured by either [U-^{14}C]- or [3-^{3}H]glucose infusion (Table 1). The percentage of glucose-C which was recycled increased significantly in the starved pregnant animal from approximately 10 to 15; the increase in the percentage of glucose-C recycled in the lactating animal was not statistically significant (Table 1) but four of five lactating

Table 1. *Aspects of glucose metabolism in the fed and 48 h starved goat during pregnancy and lactation*

(Mean values with their standard errors; no. of animals in parentheses)

	Mid-pregnancy				Late pregnancy				Lactation				Significance of pregnancy and lactation effects	
	Fed (9)		Starved (9)		Fed (9)		Starved (8)		Fed (6)		Starved (6)			
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Fed	Starved
Glucose turnover (μmol/min) using:														
[U-^{14}C]glucose infusion	493	28	353	38***	639	51	412	49***	819	80	333	36**	††	NS
[3-^{3}H]glucose infusion	551	47	405	51**	720	61	484	51***	929	118	423	56*	††	NS
Recycling of glucose-C (%)	9·7	3·3	14·4	2·6*	10·7	2·7	15·4	2·2*	10·2	3·4	21·0	3·8	NS	NS
Arterial plasma glucose (μmol/ml)	3·06	0·28	2·04	0·16**	2·74	0·22	2·11	0·18*	3·06	0·29	2·92	0·18	NS	NS
Arterial blood $^{14}CO_2$/ arterial blood [^{14}C] glucose (%)	2·34	0·26	2·74	0·61	3·33	0·52	3·43	0·53	1·31	0·26	0·94	0·12	†	†

Values were statistically significantly different from those for the fed goat at the same stage of pregnancy or lactation: * $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.
Significance of stage of pregnancy and lactational effects: † $P < 0·05$, †† $P < 0·01$.

Table 2. *Arterial concentrations of lipid metabolism in fed and 48 h starved goats during pregnancy and lactation*

(Mean values with their standard errors for six goats)

	Mid-pregnancy				Late pregnancy				Lactation				Significance of pregnancy and lactation effects	
	Fed (9)		Starved (9)		Fed (9)		Starved (8)		Fed (6)		Starved (6)			
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Fed	Starved
Free fatty acids (μmol/ml)	0·38	0·14	1·10	0·17***	0·63	0·11	1·57	0·16**	0·37	0·10	2·28	0·61*	NS	NS
Triglyceride (μmol/ml)	0·13	0·03	0·06	0·01*	0·12	0·03	0·17	0·03	0·08	0·01	0·33	0·01*	NS	††
Gycerol (μmol/ml)	0·022	0·006	0·049	0·011*	0·020	0·004	0·037	0·008*	0·022	0·006	0·044	0·009*	NS	NS
β-hydroxybutyrate (μmol/ml)	0·29	0·03	0·48	0·12	0·27	0·04	0·56	0·10*	0·71	0·17	0·77	0·07	†	NS

Values were statistically significantly different from those for the fed goat at the same stage of pregnancy or lactation: * $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.
Significance of pregnancy and lactational effects: † $P < 0·05$, †† $P < 0·01$.

Table 3. *Arterial concentrations of insulin and cortisol in fed and 48 h starved goats during pregnancy and lactation*

(Mean values with their standard errors; no. of animals in parentheses)

	Mid-pregnancy (4)				Late pregnancy (5)				Lactation (6)			
	Fed		Starved		Fed		Starved		Fed		Starved	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Insulin (ng/ml)	1·12	0·08	0·58	0·05**	0·85	0·09	0·45	0·04**	0·89	0·20	0·62	0·10
Cortsiol (ng/ml)	18·4	5·5	43·5	11·6*	25·3	11·6	49·8	13·5*	13·0	4·6	10·0	5·7

Values were statistically significantly different from those for the fed goat at the same stage of pregnancy or lactation: * $P < 0·05$, ** $P < 0·01$.

goats showed an increased recycling of glucose-C on starvation. Arterial plasma glucose concentrations decreased significantly after 48 h starvation in the pregnant but not in the lactating animal (Table 1). The specific radioactivity of arterial blood bicarbonate relative to that of glucose-C showed no apparent change as a result of starvation (Table 1).

Circulating concentrations of intermediates of lipid metabolism during pregnancy and lactation in fed and starved goats

The concentration of free fatty acids and glycerol in arterial plasma increased significantly after 48 h starvation in both pregnant and lactating goats (Table 2). Circulating concentrations of triglyceride fell during starvation in mid-pregnancy but increased after starvation in lactation (Table 2). The concentration of β-hydroxybutyrate increased significantly in late pregnancy (Table 2).

In the fed animal there was a significant increase in the circulating concentration of free fatty acids as pregnancy progressed (mean $\pm$ SE difference $0{\cdot}25 \pm 0{\cdot}06$ (n 6) μmol/ml; $P < 0{\cdot}02$) but a fall occurred during lactation (Table 2). There were no significant changes in the concentrations of triglyceride or free glycerol during advancing pregnancy and lactation in the fed or starved goat, but β-hydroxybutyrate concentrations were significantly higher during lactation in the fed animal (Table 2).

Circulating concentrations of insulin and cortisol in fed and starved goats during pregnancy and lactation

There was a significant decrease in the arterial plasma concentration of insulin and a significant increase in cortisol concentration during starvation in pregnant animals (Table 3). Advancing pregnancy and lactation had no significant effect on plasma insulin concentrations in fed or starved goats (Table 3). Plasma cortisol concentrations were also unchanged by advancing pregnancy, but the significant increase in concentration seen during starvation in the pregnant goat did not occur during starvation of the lactating animal (Table 3).

DISCUSSION

We have followed the changes in glucose turnover using [3-^{3}H]- and [U-^{14}C]glucose in fed and 48 h starved goats from mid-pregnancy to mid-lactation. This procedure has enabled us to determine both the actual rate of glucose turnover as opposed to the irreversible rate determined previously (Annison & Linzell, 1964; Bergman, 1973) and also the proportion of newly-synthesized glucose which was derived from glucose-C during the infusion period (i.e. glucose recycling). We have observed recycling of glucose-C of approximately 10% of the total rate of glucose turnover in fed goats. These values are similar to the values of approximately 13% obtained with fed non-pregnant sheep (Judson & Leng, 1972). This low rate of glucose recycling in the ruminant is surprising in view of the animals' dependence on gluconeogenesis, but suggests that the input of dietary C3 and C4 compounds is adequate to maintain the required rate of glucose production in the fed animal. After 48 h starvation recycling of glucose-C increased to 15–20% indicating a slightly greater dependence on recently-produced glucose metabolites for glucose resynthesis. This is consistent with the increase in the extraction of L-lactate and pyruvate by livers of lactating and non-lactating cows during starvation (Baird *et al.* 1980). Simple-stomached animals appear to be more efficient in recycling glucose-C during starvation, and in the starved rabbit and rat recycling of glucose-C has been estimated to be 25–40% (Katz & Rognstad, 1976). However, in the goat the major precursors of glucose during starvation would appear to be storage products such as amino acids from protein and glycerol from triglyceride depots.

In fed goats the rates of glucose turnover in mid-pregnancy were similar to those seen in non-pregnant sheep of similar body-weight (Bergman, 1973) and it is generally believed

that during early and mid-pregnancy the demands of the foetus are minimal resulting in little additional glucose utilization. The rate of glucose turnover increased in the fed goat in late pregnancy as is seen in the ewe (Bergman, 1973). This increase in glucose production may be inadequate to meet the increasing requirements of the growing foetus as well as the normal demands of the extra-uterine tissues as some mobilization of lipid reserves appeared to occur which resulted in a doubling of the plasma concentrations of free fatty acids although this increase was not statistically significant in late pregnancy. In fed lactating goats glucose synthesis and utilization increased almost 2-fold compared with the values obtained in mid-pregnancy and approximately 65% of this glucose was removed by the mammary glands. These values are comparable to those obtained previously in goats (Annison & Linzell, 1964) and sheep (Bergman, 1973). The existence of such high rates of glucose uptake by the mammary glands means that other tissues of the lactating animal exist on a reduced rate of glucose utilization even in the fed animal. The rate of glucose utilization by the non-mammary tissues of the fed lactating goat was calculated to be comparable with that of the 48 h starved animal in mid-pregnancy. An indication of a decreased rate of glucose utilization by the non-mammary tissues was the decrease in the specific radioactivity of blood bicarbonate relative to that of blood glucose, suggesting decreased rates of glucose oxidation in lactating as compared with mid-pregnant goats. Decreased rates of glucose oxidation have been observed in lactating as compared with non-lactating cows (Bartley & Black, 1966). Hormonal changes are probably involved in this metabolic change although plasma insulin and cortisol showed no significant changes during pregnancy and lactation.

Starvation of pregnant and lactating goats resulted in decreases in plasma insulin concentrations and increases in the rate of lipid mobilization as indicated by the elevation in the arterial concentrations of free fatty acids, glycerol and β-hydroxybutyrate. Plasma cortisol concentrations were also increased in starved pregnant goats. Rates of glucose synthesis decreased and were similar in starved animals irrespective of stage of pregnancy or lactation. Thus, when glucose precursors are no longer available from the diet, rates of gluconeogenesis fall to a low level which is independent of physiological state. In pregnancy this rate appeared to be below that required for the maintenance of glucose concentrations. The lactating animals reduced glucose utilization during starvation by reducing milk production by approximately 70% and were more able to maintain plasma glucose concentrations. These observations are consistent with the suggestion that glucose turnover is related to food intake rather than to pregnancy or lactation *per se* (Steel & Leng, 1968; Lindsay, 1971).

In conclusion, the use of [3-^{3}H]- and [U-^{14}C]glucose to determine glucose turnover has enabled us to calculate actual (rather than irreversible) rates of glucose turnover and recycling of glucose-C in goats during pregnancy and lactation. The low values of glucose recycling in the goat suggest that previous determinations using only [U-^{14}C]glucose underestimate rates of glucose turnover to only a small extent. The recycling of glucose-C may be considered low when compared with values obtained in non-ruminants, especially when it is considered that ruminants depend almost entirely on gluconeogenesis for their glucose supply. However, this may merely reflect a more efficient utilization of glucose in the ruminant.

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REFERENCES

Annison, E. F. & Linzell, J. L. (1964). *J. Physiol., Lond.* **175**, 372.
Baird, G. D., Lomax, M. A., Symonds, H. W. & Shaw, S. R. (1980). *Biochem. J.* **186**, 47.
Bartley, J. C. & Black, A. L. (1966). *J. Nutr.* **89**, 317.
Bergman, E. N. (1973). *Cornell Vet.* **63**, 341.
Flint, D. J., Sinnett-Smith, P. A., Clegg, R. A. & Vernon, R. G. (1979). *Biochem. J.* **182**, 421.
Garland, P. B. & Randle, P. J. (1962). *Nature, Lond.* **196**, 987.
Jones, G. B. (1965). *Analyt. Biochem.* **12**, 249.
Judson, G. J. & Leng, R. A. (1972). *Aust. J. biol. Sci.* **25**, 1313.
Katz, J. & Rognstad, R. (1976). *Curr. Topics Cell Regul.* **10**, 237.
Katz, J., Rostami, H. & Dunn, A. (1974). *Biochem. J.* **142**, 161.
Lindsay, D. B. (1971). *Proc. Nutr. Soc.* **30**, 272.
Lindsay, D. B. (1973). In *Production Diseases in Farm Animals*, p. 107 [J. M. Payne, K. G. Hibbitt and B. F. Sansom, editors]. London: Bailliere Tindall.
Linn, C. H. & Fritz, I. B. (1972). *Can. J. Biochem.* **50**, 963.
Linzell, J. L. (1960). *J. Physiol., Lond.* **153**, 481.
Slein, M. W. (1963). In *Methods of Enzymatic Analysis*, p. 117 [H. U. Bergmeyer, editor]. New York: Academic Press.
Steel, J. W. & Leng, R. A. (1968). *Proc. Aust. Soc. Anim. Prod.* **7**, 343.
Thompson, G. E. & Thomson, E. M. (1977). *J. Physiol., Lond.* **272**, 187.
Thomson, E. M., Snoswell, A. M., Clarke, P. L. & Thompson, G. E. (1979). *Q. Jl exp. Physiol.* **64**, 7.
Van Slyke, D. D. & Folch, J. (1940). *J. biol. Chem.* **136**, 509.
Williamson, D. H., Mellanby, J. & Krebs, H. A. (1962). *Biochem. J.* **82**, 90.

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