Onderstepoort J. vet. Res., 47, 173–178 (1980)

GLUCOSE TURNOVER, TOLERANCE AND INSULIN RESPONSE IN WETHERS, EWES AND PREGNANT EWES IN THE FED AND FASTED STATE

J. G. VAN DER WALT, J. PROCOS and F. J. LABUSCHAGNE, Veterinary Research Institute, Onderstepoort 0110

ABSTRACT

VAN DER WALT, J. G., PROCOS, J. & LABUSCHAGNE, F. J., 1980. Glucose turnover, tolerance and insulin response in wethers, ewes and pregnant ewes in the fed and fasted state. *Onderstepoort Journal of Veterinary Research*, 47, 173–178 (1980).

Glucose turnover parameters were obtained in fed and fasted wethers, ewes and pregnant ewes in their 2nd and 3rd trimesters, using a jugular bolus injection of D-glucose-2-^{3}H. Fasting significantly ($P<0,05$) reduced glucose turnover (*c.* 40%) in both the wether and the non-pregnant ewe. A somewhat larger difference (*c.* 54%) between the fed and fasted ewes was found in their 3rd trimester of pregnancy due to an increase when fed (*c.* 29% higher turnover than in the non-pregnant ewe) rather than a decrease when fasted, since there was no statistical difference ($P<0,1$) between glucose turnover values of pregnant or non-pregnant fasted ewes. Glucose tolerance was estimated from an intrajugular glucose load (1 g/kg^{0,75} body mass) in these 3 groups of sheep under both fed and fasted conditions, and the resulting insulin response was followed for 4 h after the injection. Fasting reduced the plasma clearance rate of glucose by *c.* 63% in both the wether and the non-pregnant ewe while the reduction was somewhat smaller (*c.* 51%) during the 2nd trimester of pregnancy. Only the pregnant ewe group showed a corresponding reduction in the resulting insulin response of 46% which was similar in magnitude to the diminished clearance, indicating that factors other than insulin are responsible for the reduced glucose clearance associated with fasting in the wether and non-pregnant ewe. Despite similar baseline plasma glucose values the glucose load appeared to distribute in a space that was significantly less than that found in all 3 groups of fed sheep when trace amounts were injected.

Résumé

CONVERSION DU GLUCOSE, TOLÉRANCE ET RÉPONSE À L'INSULINE CHEZ LES BÉLIERS CHÂTRÉS, LES BREBIS ET LES BREBIS EN GESTATION, À L'ÉTAT ALIMENTÉ ET À L'ÉTAT DE JEÛNE

Des paramètres de conversion du glucose ont été obtenus chez des béliers châtrés, des brebis et des brebis en gestation à leur deuxième et troisième trimestres, à l'état alimenté et à l'état de jeûne, en utilisant une injection jugulaire de grosse pilule de glucose-D-2-^{3}H. Le jeûne réduisit de manière significative ($P<0,05$) la conversion de glucose (c 40%) *chez le bélier châtré et la brebis n'étant pas en gestation. Une différence quelque peu plus élevée* (c 54%) *entre les brebis alimentées et les brebis à jeun a été trouvée dans le troisième trimestre de la gestation, due à une augmentation quand elles étaient alimentées* (c 29% *de déplacement plus élevé que chez les brebis n'étant pas en gestation) plutôt qu'à une diminution quand elles étaient à jeun, étant donné qu'il n'y avait aucune différence statistique* ($P<0,1$) *entre les valeurs de conversion du glucose des brebis à jeun étant ou non en gestation. La tolérance au glucose a été estimée avec une charge de glucose intra-jugulaire* (1 g/kg^{0,75} *de poids vif) dans ces trois groupes de moutons étant dans des conditions alimentées et des conditions de jeûne et la réponse à l'insuline résultant de ce traitement fut suivie pendant* 4 *h après l'injection. Le jeûne réduisit le taux de dégagement du glucose du plasma de c* 63% *tant chez les béliers châtrés que chez les brebis n'étant pas en gestation tandis que la réduction fut quelque peu moindre* (c 51%) *pendant le deuxième trimestre de la gestation. Seul, le groupe de brebis en gestation a montré une réduction correspondante de* 46% *dans la résultante réponse d'insuline, ceci étant similaire en magnitude au dégagement diminué, indiquant ainsi que d'autres facteurs que l'insuline sont responsables du dégagement réduit de glucose associé au jeûne chez le bélier châtré ainsi que chez la brebis n'étant pas en gestation. Malgré des valeurs de glucose plasmatique de base similaire, la charge de glucose apparaissait se distribuer dans un espace qui était significativement moindre que celui trouvé chez tous les trois groupes de moutons alimentés quand des quantités de trace furent injectées.*

INTRODUCTION

Gluconeogenesis in the ruminant is of prime importance in providing a continuous supply of glucose to these animals (Bergman, Brockman & Kaufman, 1974) from non-carbohydrate sources such as propionate (Bergman, Roe & Kon, 1966; Van der Walt, 1978) and amino acids (Wolff, Bergman & Williams, 1972) derived from ruminal fermentation. Since the rate of gluconeogenesis may be conveniently estimated from glucose turnover parameters (Bergman, Kats & Kaufman, 1970), valuable insight into the controlling mechanisms may be gained from comparison of such parameters obtained under various physiological conditions such as fasting or pregnancy. Turnover data obtained from either fed or fasted wethers, ewes or pregnant ewes by, among others, Annison & White (1961), Bergman (1963), Bergman (1964), Brockman, Bergman, Pollak & Brondum (1975), Kronfeld & Simesin (1961), Steel & Leng (1968) and Ulya't, Whitelaw & Watson (1970) indicated that fasting depressed the overall level of gluconeogenesis while shifting the emphasis from exogenous precursors to the recycling of endogenous metabolites such as lactate (Roe, Bergman & Kon, 1966), glycerol (Bergman, 1968; Bergman, Starr & Reulein, 1968) and amino acids (Wolff & Bergman, 1972). On the other hand, pregnancy increased the rate of gluconeogenesis (Steel & Leng, 1968; Bergman, 1964) to supply the carbohydrate demand of the foetus.

That differences in the pattern of the gluconeogenic response exist between ruminant and monogastric mammals was further supported by anomalies that have been found in the glucose-insulin axis. Despite the plasma glucose concentration and whole body turnover rate remaining constant during a 12 h feeding cycle (Van der Walt, 1978), the plasma insulin concentration rises, seemingly responding to increased uptake of gluconeogenic precursors (Trenkle, 1978). Nevertheless, insulin response to changes in plasma glucose concentration would appear to be similar to that of other mammals, since a tenfold increase in plasma insulin within 15 min of a glucose load (0,5 g/kg^{0,75} body mass) was reported in wethers by Boda (1964). Furthermore, Boda (1964) found that fasting decreased both the glucose tolerance and the amount of insulin secreted, while Reid (1958) reported an increased tolerance when his wethers were maintained on a high concentrate diet. While plasma glucose concentration does not appear to control insulin secretion to any extent in the ruminant under normal physiological conditions (Trenkle, 1978), plasma insulin concentration appears to play a major role in the control of glucose concentration and turnover

Received 12 May 1980—Editor

(Brockman, Bergman, Joo & Manns, 1975; Van der Walt, 1978).

In view of the scattered nature of the information on glucose turnover, tolerance tests and insulin responses in the wether and ewe under various physiological conditions, we decided to estimate all of these parameters in the same group of sheep, thereby facilitating the comparison of the variously derived parameters under conditions of fasting and pregnancy.

Materials and Methods

Animals

A group of South African Mutton Merino sheep (8 wethers and 16 ewes) were kept in individual pens and fed at 08h00 daily. The wethers received 1 800 and the ewes 1 500 g of lucerne hay which was increased to 1 800 g during the last 6 weeks of pregnancy. The ration was supplemented with 15 g of a 1:1 salt and trace element mixture* daily and 1 g of vitamin A** weekly. The sheep were allowed free access to water. A randomly selected group of 10 ewes were placed in a camp with 2 rams in April for 4 weeks. At the end of that period, 6 of those pregnant, i.e. those not returning to oestrus after 21 days, were returned to their pens and used for the experiment together with the 6 non-pregnant ewes which served as a control group.

Experimental procedures

Preparation for experiment: Polyethylene catheters were inserted into the left jugular vein of each sheep on the afternoon prior to the experiment and filled with a physiological saline-heparin (50 U/mℓ) solution to prevent blockage. This solution was replaced the following morning by physiological saline and the patency of the catheter checked immediately prior to the experiment. Each sheep was transferred to an adjustable pen about an hour prior to the experiment, which began at 08h00. The ration was withheld for the duration of the experiment, although the sheep had free access to water. Sheep were fasted by withholding the ration for 2 days prior to the experiment. A week's recovery period was allowed between glucose turnover and tolerance studies in any one sheep and the ewes were tested at the end of their 2nd and beginning of their 3rd trimesters.

Glucose turnover: The method used to estimate glucose turnover parameters following a single intravenous injection of D-glucose-2-^{3}H at 08h00 has been described by Van der Walt (1975).

Glucose load: A glucose load (1 g/kg^{0,75} body mass, made up in 50 mℓ of sterile physiological saline) was injected over a 30 s period into the catheter at 08h00 and 3 mℓ blood samples were withdrawn at suitable intervals over the next 200 min into heparinized tubes. The plasma fraction of each sample was analysed for glucose and insulin concentrations. Clearance of the glucose load was determined by regression analysis using the method of least squares. The zero time intercept (G), representing the theoretical distribution of the load in the body of the sheep at the time of injection, was corrected for the endogenous glucose concentration (G_E) and divided into the load (G_L) to derive the volume of distribution (V_D) as follows:

$$V_D = \frac{G_L}{G - G_E}\ \ell$$

If the total mass (M) of the sheep was expressed in kg and the metabolic mass (M_M) in kg^{0,75}, then the glucose space (S) was derived from the equation

$$S = \frac{V_D \times 100}{M}\ \%$$ of body mass and the pool size

$$P = \frac{V_D \times G_E}{M_M}\ \text{mg/kg}^{0,75}$$

The slope of the line (K) represents the fractional clearance rate of the pool and is used to calculate the overall metabolic clearance rate (MCR) of the load from the equation $MCR = K.\ V_D.\ 1\,000/\text{m}\ell/\text{min}$.

In some experiments 50 μCi of D-glucose-2-^{3}H was added to the glucose load in order to confirm the distribution volume of the glucose.

Analytical procedures

Plasma glucose: The Glucose-Perid kit provided by Boehringer, Mannheim, was used to determine plasma concentrations of glucose.

Plasma insulin: Plasma insulin concentration was estimated by a radio-immunoassay method using dextran-coated charcoal for the separation of the unbound insulin and the insulin-antigen complex, essentially similar to that described by Vinik, Deppe & Joubert (1970) and adapted in our laboratory.

Results

Glucose turnovers

Regression analysis of the first exponential phase of the decline in glucose specific activity following the single injection of D-glucose-2-^{3}H gave values for fed wethers (Table 1) which agreed with those previously obtained in this laboratory (Van der Walt, 1975).

After 48 h fasting, both the plasma glucose and the entry rate, expressed as a fraction of the pool turned over per minute, declined insignificantly ($P<0,50$, Student's t-test), from $68,4\pm6,6$ to $57,7\pm12,2$ mg/100 mℓ and from $1,04\pm0,26$ to $0,96\pm0,15\%$ of pool/min respectively. However, the decline in the space ($17,5\pm2,3$ to $10,9\pm3,9\%$ of mass) was significant ($P<0,001$), as was the decline in pool size (331 ± 56 to 218 ± 85 mg/kg^{0,75}) and the actual entry rate ($3,43\pm0,46$ to $2,01\pm0,57$ mg/min/kg^{0,75}). The entry rate values calculated for the non-pregnant fed ewes (Table 1) closely resembled those of the fed wethers. However, this decline was due more to a reduction in fractional entry rate ($1,08\pm0,08$ to $0,72\pm0,17\%$ of pool/min) than to a reduction in pool size or space as was the case with the wethers, although none of these group differences were significant.

Despite an increased food intake (1 500–1 800 g of lucerne hay) during the 3rd trimester, the plasma glucose fell significantly ($0,05>P>0,02$) in the fed pregnant ewe to $44,8\pm11,7$ mg/100 mℓ (Table 1). Since glucose space did not change significantly, this lower plasma glucose reflected the significant decrease ($0,05>P>0,02$) in pool size from 310 ± 56 to 192 ± 54 mg/kg^{0,75}. However, the fractional entry rate doubled from $1,08\pm0,08$ to $2,05\pm0,44\%$ of pool/min, which compensated for this smaller pool size, leading to an increase in the actual entry rate ($3,79\pm0,57$ mg/min/kg^{0,75}). Fasting lowered the plasma glucose still further to $25,8\pm6,7$ mg/100 mℓ and the pool

* Kimtrafos 25, Kynoch Feeds, Kimberley, RSA
** Vitamin A, Peter Hand Panvet, Johannesburg, RSA

TABLE 1 Mean values ± SD of glucose turnover parameters calculated from the regression analysis of the decline in specific radioactivity of plasma glucose after an intravenous bolus injection of D-glucose-2-^{3}H into both roughage-fed and fasted wethers, non-pregnant ewes and pregnant ewes in their 3rd trimester

Number of sheep and their treatment	Metabolic mass $kg^{0,75}$	Plasma glucose mg/100 mℓ	Space % of mass	Pool $mg/kg^{0,75}$	Glucose entry rate	
					% of pool/min	$mg/min/kg^{0,75}$
Wethers						
6: fed	27,40±4,75	68,4±6,6	17,6±2,3	331±56	1,04±0,26	3,43±0,46
6: fasted	26,39±2,94	67,7±12,2	10,9±3,9	218±85	0,96±0,15	2,01±0,57
Non-pregnant ewes						
6: fed	21,10±1,70	63,1±9,9	18,2±3,9	310±56	1,08±0,08	3,31±0,44
3: fasted	22,40±1,00	58,5±11,7	16,6±1,3	274±59	0,72±0,17	1,97±0,28
Pregnant ewes						
4: fed	23,80±0,58	44,8±11,7	15,0±2,3	192±54	2,05±0,44	3,79±0,57
4: fasted	23,80±0,58	25,8±6,7	14,7±3,8	112±50	1,70±0,42	1,75±0,31
Pregnant ewes corrected for foetal mass						
4: fed	21,10±1,70	44,8±11,7	17,5±2,7	216±61	2,05±0,44	4,27±0,64
4: fasted	22,40±1,00	25,8±6,7	17,2±4,3	126±56	1,70±0,42	1,97±0,34

TABLE 2 Mean values ± SD of glucose clearance parameters calculated from the regression analysis of the decline in the elevated plasma glucose concentration resulting from an intravenous bolus injection of a glucose load (1 $g/kg^{0,75}$) into both roughage-fed and fasted wethers, non-pregnant ewes and pregnant ewes in their 2nd trimester

Number of sheep and their treatment	Metabolic mass $kg^{0,75}$	Preload plasma glucose mg/100 mℓ	Glucose load clearance			Peak insulin response μU/mℓ
			Space % of mass	Rate % of pool	Plasma clearance mℓ/min	
Wethers						
7: fed	26,46±3,07	67,0±10,0	12,4±1,1	1,26±0,45	123±44	262±61
7: fasted	27,12±2,68	65,0±6,7	14,0±1,5	0,42±0,09	48±10	253±53
Non-pregnant ewes						
6: fed	21,73±1,06	62,4±2,2	14,7±1,9	1,27±0,36	113±32	229±49
6: fasted	21,87±1,33	58,8±6,5	14,1±2,2	0,44±0,12	38±10	251±60
Pregnant ewes						
6: fed	23,74±1,15	55,2±5,7	14,3±1,6	1,21±0,28	118±27	212±58
6: fasted	23,97±1,30	40,5±11,4	15,9±3,8	0,53±0,08	58±9	114±27

size to 112±50 $mg/kg^{0,75}$, while the fractional entry rate decreased slightly, but insignificantly, to 1,70± 0,42% of pool/min. As a result, the glucose entry rate fell dramatically to 1,75±0,31 $mg/min/kg^{0,75}$, lower than the values estimated for the wether or non-pregnant ewe.

There is a slight but insignificant decrease in space in both the fed and the fasted pregnant ewe due to the sharing of available glucose between maternal and foetal metabolism. If the mass of the foetus is subtracted from the maternal mass, an estimate of 17,5± 2,7% is obtained for the glucose space in fed sheep and 17,2±4,3% for fasted sheep, which is similar to comparable values obtained for the non-pregnant animal. When the pool size is similarly corrected, it is still significantly lower than that in the non-pregnant animal both fed and fasted. As a result, the pregnant ewes displayed a much higher glucose turnover rate (4,27±0,64 $mg/min/kg^{0,75}$) when fed, and a similar rate (1,97±0,34 $mg/min/kg^{0,75}$) when fasted, than the non-pregnant ewes.

Glucose clearance

Table 2 shows that the average plasma glucose concentrations prior to the glucose load were similar to those found in the sheep used for the turnover experiments (Table 1), except for the pregnant ewes that were in the 2nd trimester of pregnancy. Although the plasma glucose values in both the fed and fasted pregnant ewe (55,2±5,7 and 40,5±11,4 mg/100 mℓ respectively) had not yet declined to the lower concentrations found later in the pregnancy, they were still significantly lower ($0,02>P>0,01$ and $P<0,001$ respectively) than those found in the non-pregnant ewe (62,4±2,2 and 58,8±6,5 mg/100 mℓ respectively).

There was no statistical difference between the glucose clearance rates of fed wethers, ewes or pregnant ewes. Fasting affected all 3 groups equally and reduced the rate significantly ($P<0,001$) to about 30% of the fed value. The average peak insulin response to the load was generally unaffected by sex or nutritional status. However, the significant decrease ($P<0,01$) of the response in fasted pregant ewes (212±58 to 114±27 μU/mℓ) indicates a reduced secretory capacity of the endocrine pancreas under these conditions. The reduction in glucose load clearance rate in fasted wethers and non-pregnant ewes despite normal insulin response implies that another factor is responsible for the control of glucose uptake under these circumstances.

The glucose load was distributed in a remarkably constant space (*c.* 14% of body mass) that remained essentially unaffected by either pregnancy or fasting. Correction of the glucose space in pregnant ewes for the foetal mass results in values that are higher (16,2±1,8% and 18,3±4,7% for fed and fasted ewes respectively) than those in the non-pregnant

ewes. Since Boyd, Moriss, Meschia, Makowski & Battaglia (1973) have shown that glucose uptake by the ovine foetus is directly proportional to the arterial glucose concentration, it is reasonable to propose that a portion of the load will distribute in the foetus. Therefore, the uncorrected values are probably closer to the actual distribution in both fed pregnant ewes ($P<0,001$) and fasted pregnant ewes ($0,10>P>0,05$). That these values were significantly lower than those calculated from the tracer data given in Table 1 suggests a block in glucose uptake. Fasting caused a slight increase in the glucose space which, in the case of wethers, was significantly higher ($0,10>P>0,05$) than that calculated from the tracer data.

Inclusion of D-glucose-2-^{3}H in the load in order to confirm these apparent differences between the turnover and load data confirmed the effect of load, nutritional status and pregnancy on the glucose space in sheep. The results summarized in Fig. 2 show clearly that the load significantly reduces the space in fed wethers and non-pregnant ewes but not pregnant ewes in which a reduction in space in the fed animal has already occurred. Fasting causes a similar reduction in space in the wether and non-pregnant ewe but does not seem to affect the pregnant animal.

A relationship between the induced insulin response and the uptake of the injected load was found by correlating the insulin response parameters, such as total insulin secreted over the period 0–100 min after the load (area under the response curve), average peak (average of 10, 20 and 30 min values) and highest peak against either the load clearance rate or the labelled glucose disappearance rate, using a least squares regression analysis (see example given in Fig. 1). All combinations tested proved to be highly significant (26 data pairs; $0,001>P>0,0001$) in fed wethers, ewes and pregnant ewes as well as in fasted pregnant ewes, although the best correlation was obtained with the peak of insulin response and the labelled glucose disappearance rate. Since the disappearance of the D-glucose-2-^{3}H (or for that matter, the load) is directly related to the uptake of glucose by peripheral tissues, this high correlation with the peak values of insulin response suggests that insulin control of glucose uptake is exerted by momentary releases of hormone rather than by average plasma concentration. Despite high insulin response values, the fasted wethers and non-pregnant ewes showed no increase in glucose uptake, pointing to an anti-insulin effect of some other factor induced in these sheep by fasting.

Discussion

The apparent lack of a response in the glucose turnover rate following the post-prandial increase in fermentation end products, which are gluconeogenic, may be due to the modulating effect of the concurrent increase in insulin levels. West & Passey (1967) found that a long-term infusion of glucose, which would elevate the plasma insulin concentration, depressed the endogenous entry rate of glucose. This conclusion was further supported by obtaining a similar depression from the infusion of insulin. Other factors, such as glucagon (Bassett, 1972), probably also play a role in regulating the supply of glucose to the peripheral tissues by ensuring a nearly constant plasma concentration (Newsholme & Start, 1973). That insulin is not the sole controlling factor is confirmed by our findings with the fasted wethers and non-pregnant ewes, in which the glucose clearance rate declined despite a normal insulin response to the glucose load.

When deprived of food, the wethers, the ewes and the pregnant ewes all displayed a "starvation diabetes" of the same order as that found in man, i.e. a 40% decrease in the glucose tolerance. Of all the sheep only the pregnant ewe group showed a corresponding reduction (46% from 212 ± 58 to 114 ± 27 μU/mℓ) in insulin response to the glucose load similar to that reported in humans. Thus the reduced uptake during fasting in the wethers and non-pregnant ewes must have been due to some factor other than insulin. Factors that have previously been proposed include adrenalin (Shikama & Ui, 1975), corticosteroids (Bassett, 1963), impaired hepatic uptake (Mahler & Szabo, 1970), impaired insulin coupling to tissue receptor sites and a lowered intracellular metabolism (Kasuga, Akanuma, Iwamoto & Kosaka, 1977). Since there is little net hepatic uptake of glucose normally in sheep (Ballard, Hanson & Kronfeld, 1969) despite rapid equilibration between portal blood and liver (Hooper & Short, 1975), the impaired tolerance must be associated with peripheral uptake. The same factor may also be linked to the decreased glucose entry rate associated with fasting. Some of the decreased production may be ascribed to a diminished supply of absorbed gluconeogenic precursors during fasting (Lindsay, 1971), since an increase in daily ration (20% over basal) in the pregnant ewe group corresponded closely to an increase in glucose turnover rate (23% when corrected for foetal mass). However, a concurrent decline in the plasma glucagon level has been reported (Bassett, 1972) which would further depress the hepatic gluconeogenic rate. The concomitant decrease in peripheral uptake may be due simply to the restricted availability of glucose or, more likely, to an insulin antagonist. In addition, the parallel decrease in insulin response in the pregnant ewe further restricts the maternal peripheral glucose uptake, thus ensuring an adequate supply both for foetus and maternal central nervous system.

A study of the glucose space results revealed several striking anomalies. The glucose distribution was changed by the addition of the load (17,6–12,4% and 18,2–14,7% for fed wethers and ewes respectively), fasting (17,6–10,9% and 18,2–16,6% for wethers and ewes respectively) and by pregnancy *per se* (18,2–15,0%). This reduction in distribution volume may be explained by postulating 2 compartments or pools of glucose, the one extracellular, i.e. the plasma plus the extracellular fluid and the other, intracellular. The increase in adrenalin accompanying the glucose tolerance test (Robertson & Porte, 1974) could therefore depress the insulin mediated glucose uptake and so diminish the apparent volume of distribution or glucose space. The space reduction found in fasting is probably caused by a similar mechanism linked to the increased plasma cortisol concentrations (Bassett & Madill, 1974) which would also inhibit the action of insulin on glucose uptake.

Direct evidence of another factor inhibiting insulin mediated glucose uptake is provided by the load clearance-insulin response data. In fed animals the rate of uptake of glucose (i.e. labelled glucose disappearance rate) was clearly linked to the magnitude of the induced insulin response (peak insulin values). On the other hand, there was no such correlation when the wethers and ewes were fasted, indicating the presence of another factor inhibiting the effect of the normal insulin response.

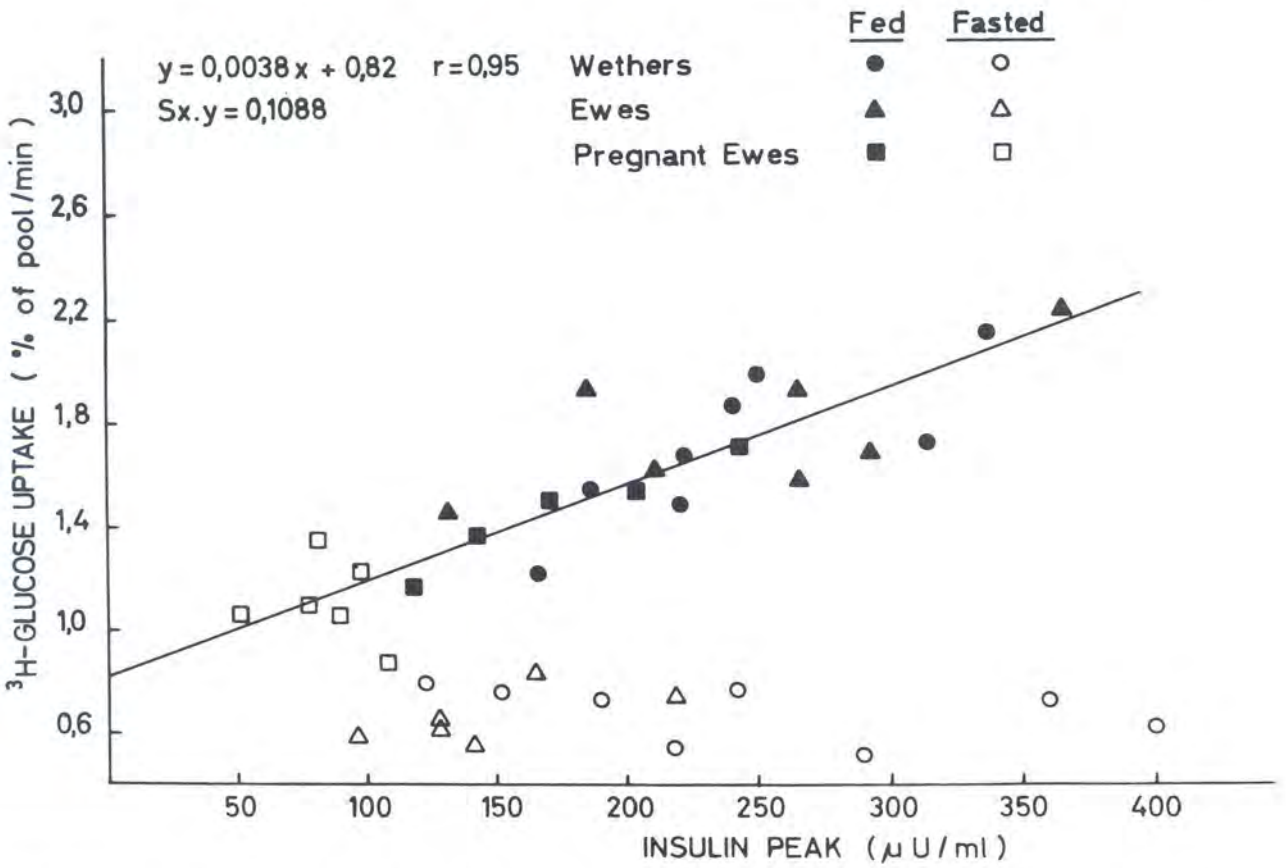

FIG. 1 Correlation between the height of the initial insulin peak and the D-glucose -2-^{3}H disappearance rate is shown for the fed wethers, ewes, pregnant ewes and fasted pregnant ewes. The fasted wethers and ewes were not included in the regression analysis

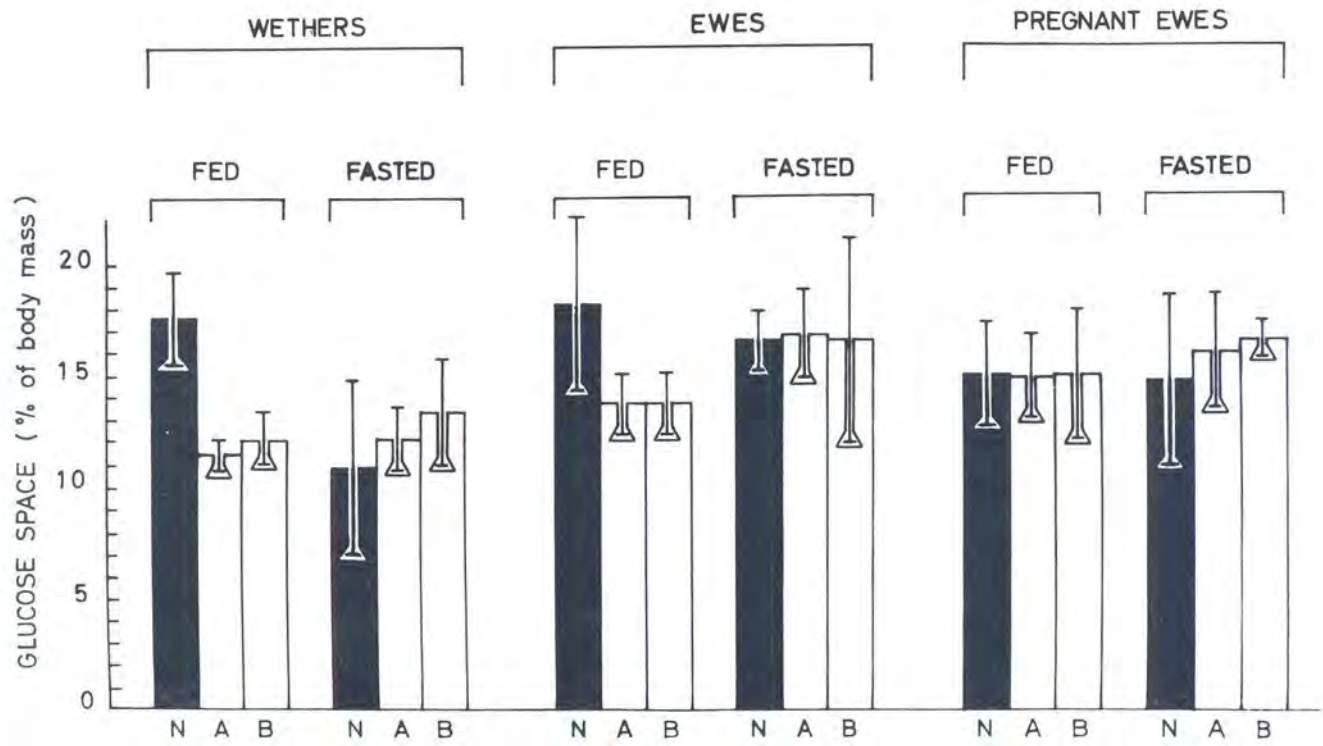

FIG. 2 The variation in glucose space in wethers, ewes and pregnant ewes both fed and fasted when injected intravenously with a labelled glucose load (1 g/kg^{0,75}). Baseline values (solid black columns) were derived from the load-free turnover data in Table 1, while the open columns (derived from A = label disappearance and B = load clearance data) denote the glucose spaces obtained after administration of the load

Acknowledgements

The technical assistance of Miss L. Grei and Miss S. de Villiers is gratefully acknowledged, as is the help received from Mr B. Briel in supervising the animal management.

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