HEPATIC BLOOD FLOW RATE, PLASMA TRIGLYCERIDES AND FREE FATTY ACIDS FLOW IN PREGNANT SHEEP DURING FASTED AND FEEDING STATE

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SUMMARY

Hepatic blood flow rates were measured in both pregnant and non-pregnant sheep using the marker dilution technique with creatinine as the marker substance. Hepatic blood flow rates were slightly higher in pregnant than in non pregnant sheep at all times. The effect of fasting/feeding on the flow rates was the same in both pregnant and non-pregnant sheep. Plasma triglycerides (TG) and free fatty acids (FFA) were measured. The concentrations were used to calculate their flow rates from the portal drained tissues and liver uptake. Fasting increased the plasma FFA levels more so in pregnant sheep, while feeding decreased the levels. Similarly TG levels were higher in pregnant sheep than in non-pregnant sheep (as seen in Table 3). It was concluded that fasting is a stress which reduces the energy supply for the animal. The physiological status of the animal such as pregnancy exagerates the effects thereby the energy demand increases.

INTRODUCTION

The portal drained viscera contain significant amounts of adipose tissue in the omenta and mesenteries which, in sheep, can synthesize, store and release lipids.

Lipids are transported in blood largely as plasma lipoprotein - bound triglycerides and albumin - bound free fatty acids. Thus measurements of the hepatic plasma flows coupled with triglyceride and free fatty acid estimation were the basis for determining the TG and FFA transported ont of the portal - drained viscera and the liver.

Roe *et al* (1966) observed that in non-pregnant *ad lib* fed sheep portal venous flow rate (PVF) was 46.3 ± 9.2 ml/min.kg body weight while the pregnant *ad lib* fed sheep had a rate of 44.8 + 8.1 ml/min.kg body weight. In this study (Roe *et al* 1966) observed that fasting decreased the PVF in both non-pregnant and pregnant sheep. Total hepatic blood flow rate (THBF) was observed to be higher in pregnant sheep than in non-pregnant sheep (Katz and Bergman. 1969a) both in fasted state and fed state.

Gestation has a high demand for energy due to the growing foetus (es), hence liver plays an important role in supplying the increased energy requirements. Bowden (1973) observed that levels of FFA, ketone bodies and glucose in the blood of ruminants were related to energy metabolism and may vary between

growing, pregnant and lactating animals. It has been observed that the pregnant sheep shows hypoglycaemia in varying degrees during fasting (Katz and Bergman, 1969c). Plasma FFA in British Saamen goats were observed to increase during the last third of pregnancy, major increase occured just before parturition, (Mabon, Brechancy and Vernon, 1992). Similar resultes were reported in sheep and cows (Vernon, 1980) but the pattern may vary as it is influenced by plane of nutrition and number of foetuses.

In early lactation there is an increased rate of lipid mobilization in ruminants as reported by Jaster and Wegner (1981).

The main objective of this work was to study the effects of feeding and pregnancy on the normal hepatic blood flows and the net fluxes of TG and FFA across the portal-drained viscera and the liver.

MATERIALS AND METHODS

Thirteen female adult sheep with implanted catheters were used. Sheep were kept indoors, fed grass hay and watered *ad libitum*. Additionally were given 1.0kg/sheep/day of commercial sheep nuts. The sheep were cannulated as described by Leek (1976). Portal, hepatic and mesenteric vein catheters (vinyl of 1.5 mm internal diameter) were implanted as described by Katz and Bergman (1969b) with modifications by Kisauzi (1982). Carotid artery loops were surgically done on the right side of each sheep. Five sheep were bred and conceived but only two sheep had their catheters working up to 126 days of pregnancy. Experiments were performed at 110, 117 and 126 days of pregnancy. These animals were catheterized at the 6th-8th week of pregnancy.

The blood flow measurements were carried out done using the marker dilution principle whereby creatinine was used as the marker substance (Kisauzi, 1982). Continuous infusion, through the mesenteric vein catheter of a creatinine solution of 7.0mg/ml concentration was performed using a perstaltic pump at a rate of 1.0ml/min. A priming dose of a higher concentration, 20.0 mg/ml creatinine was injected during the first 5 minutes to hasten reaching a steady state of the creatinine levels in the body. This means that the quantity given was equal to the amount being excreted out of the body. The blood samples were taken from the portal vein, hepatic vein and carotid loop catheters simultaneously. Prior to experiments the sheep were fasted 48 hours. Refeeding commenced for 40 minutes during the infusion of the creatinine solution. Two blood samples were taken prior to refeeding, at 90 and 110 minutes after the start of infusion. Thereafter sampling took place at 10 minutes interval. After the food was withdrawn sampling continued for every ten minutes for the first thirty minutes then at 2 and 4 hours post-feeding consecutively. Packed cell volume percentage (PCV%), was measured for every sample, the blood was centrifuged and the plasma was stored frozen at -20°C awaiting analysis for plasma creatinine, TG and FFA levels. All these were analysed using commercial kits, for the creatinine a kit from Sigma Chemicals Co.St. Louis Missouri, the TG a kit Peridochron® Triglycerides GPO - PAP, Boehringer Mannhein, GmbH Diagnostica and for the FFA a kit NEFAC,

Wako Chemicals, GmbH, West Germany.

Calculations

The hepatic plasma flows were calculated per unit metabolic size ($BW^{0.73}$) using the following general formula:

$$\text{Plasma flows (ml/min. kg}^{0.73}\text{)} = \frac{\text{infusion rate x } 10^3}{\text{(Creatinine conc. in vein-Creatinine conc. in artery) x BW}^{0.73}}$$

The plasma flow in hepatic artery was estimated by subtraction i.e. total hepatic plasma flow (THPF)-portal vein plasma flow (PVPF).

The TG and FFA flow rates were calculated as a product of plasma flow rate (ml/min.$kg^{0.73}$) and plasma TG or FFA concentrations in the respective blood vessels.

The plasma TG or FFA uptake by the portal drained viscera and liver were calculated as:

(i) Overall viscera uptake (TG or FFA) = (Arterial conc-hepatic vein conc) x THPF

(ii) Splanchnic uptake (TG or FFA)= (Arterial conc-portal vein conc) x PVPF

(iii) Hepatic uptake (TG or FFA) = (i) above - (ii) above.

RESULTS

The hepatic blood flows were higher in the pregnant sheep than in the non-pregnant sheep. The feeding had the same effect on hepatic blood flows in the pregnant and non-pregnant sheep as seen in Figure 1a to 1c. The maximum flow rates were observed at 2 hours post-feeding which were 178±16 and 202±24 ml/min. $kg^{0.73}$ for the THBF in pregnant and non-pregnant sheep respectively. This was the only time when the THBF for the non pregnant sheep was lower than the THBF for the non pregnant sheep PVF increased from 82±6 and 74±3 to 115±16 and 109±14 ml/min. $kg^{0.73}$ for the pregnant and non pregnant sheep respectively, also attained at 2 hours post feeding.

Table 1 shows the blood flows in the pregnant and non-pregnant sheep as described above.

Table 2 and 3 show the plasma TG and FFA concentrations in the pregnant and non-pregnant sheep. The Plasma TG levels were higher in the pregnant sheep than in the non-pregnant sheep. The feeding showed little effect on the TG levels in both groups of animals. The plasma FFA levels were distinctly more elevated during the fasted state in pregnant sheep than in non pregnant sheep. The feeding lowered the levels but for the pregnant sheep the decrease was more moderately than in the non pregnant sheep. In both groups of animals both the FFA and the TG were higher in the portal vein than in the hepatic vein. In Table 4 the TG and the FFA uptake by the liver are shown. Slightly higher values for the pregnant sheep than the non-pregnant sheep were observed but in both groups the effect of feeding was slight.

DISCUSSION

The eExperiment with the pregnant sheep had to be terminated two weeks before lambing due to failure of drawing blood out of the catheters. In one of the sheep the hepatic vein catheter failed while the portal vein catheter failed in the other sheep. After lambing the catheters were again working perfectly. This suggested that the problem could have been attributed to the displacement and the kinking of the catheters by the growing foetus(es). Katz and Bergman (1969) observed poor results in the twin-pregnant sheep which were catheterized 3 weeks before lambing. Thus in such a situation a possible solution is to have as many animals for the study (i.e. catheterized pregnant sheep) as possible so as to get enough data.

The feeding increased hepatic blood flows in the pregnant sheep in the same way as observed in the non-pregnant. All in all blood flows in the pregnant sheep were higher than in the non-pregnant sheep which could be due to the high demand of energy

Table 1: **Hepatic blood flow rates in pregnant and non-pregnant ewes (ml/min.kg$^{0.73}$) in fasted, during feeding and up to 4 hours post-feeding (mean±SE, n=number of observations shown in brackets).**

PREGNANT SHEEP

STATE OF SHEEP	THBF	PVF	HABF
48 hours fasted	111±6 (12)	82±6 (12)	28±6 (12)
Feeding period	130±30 (6)	97±25 (6)	37±9 (6)
Post-feeding 30 min	162±18 (6)	112±24 (6)	53±18 (6)
Post-feeding 2 hrs	178±16 (6)	115±16 (6)	62±15 (6)
Post feeding 4 hrs	167±11 (6)	102±8 (6)	65±9 (6)

Table 1: continued (NON PREGNANT SHEEP)

STATE OF SHEEP	THBF	PVF	HABF
48 hours fasted	100±3 (52)	74±3 (52)	27±3 (52)
Feeding period	120±9 (20)	78±7 (20)	42±7 (20)
Post feeding 30 min	143±9 (20)	83±7 (20)	61±9 (20)
Post feeding 2 hrs	202±24 (20)	109±14 (20)	94±17 (20)
Post feeding 4 hrs	152±10 (20)	92±9 (20)	60±9 (20)

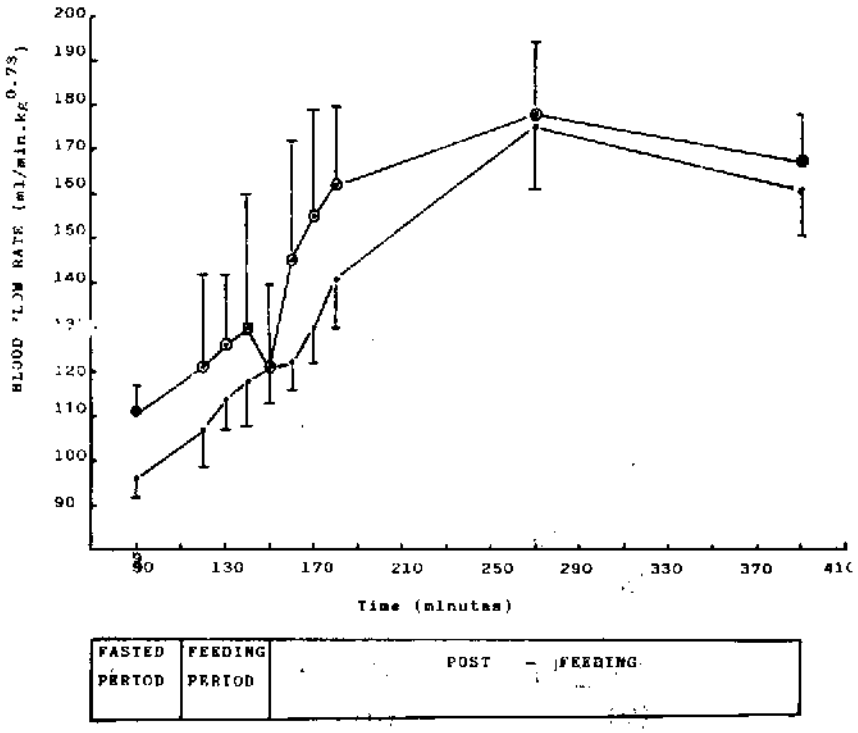

Figure 1a: **Hepatic blood flow rates (ml/min. $kg^{0.73}$) in pregnant and non-pregnant ewes in fasted state, during feeding and up to four hours post-feeding, (Mean±SE).**

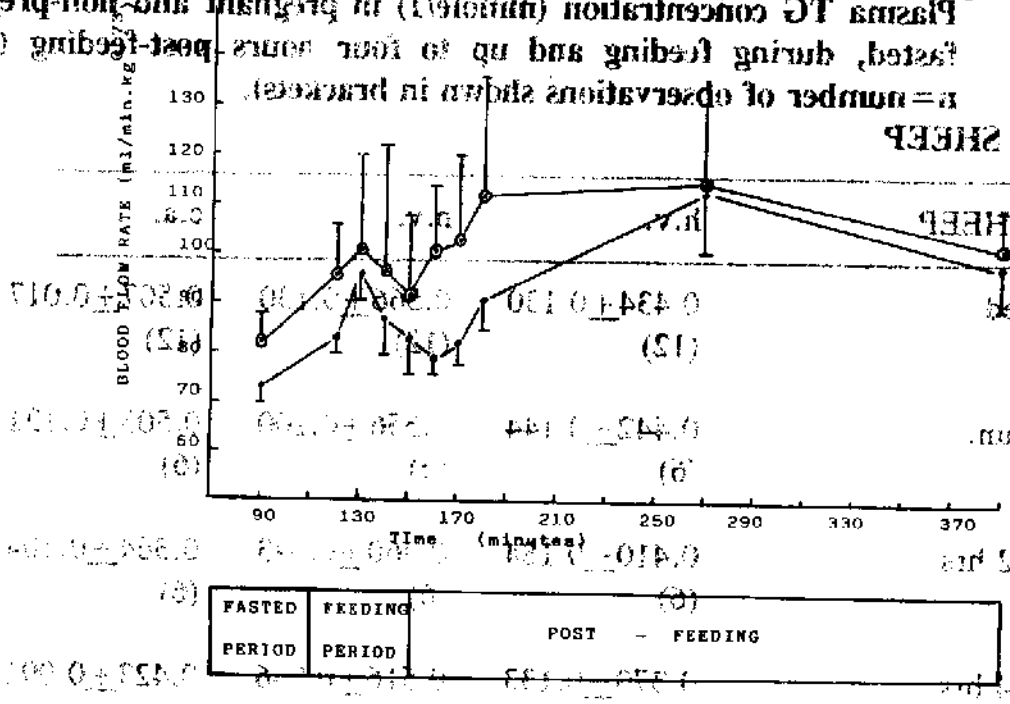

Figure 1b: **Hepatic blood flow rates (ml/min. $kg^{0.73}$) in pregnant and non-pregnant ewes in fasted state, during feeding and up to four hours post-feeding, (Mean±SE).**

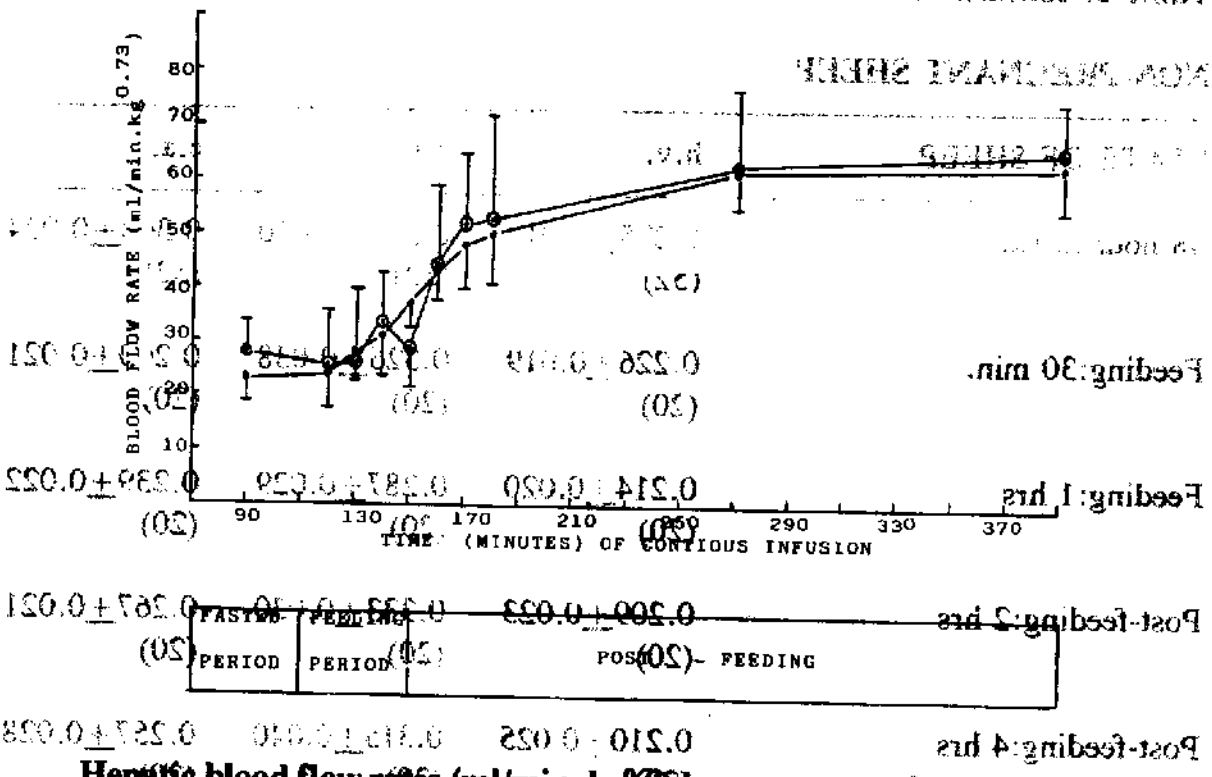

Figure 1c: **Hepatic blood flow rates (ml/min. $kg^{0.73}$) in pregnant and non-pregnant ewes in fasted state, during feeding and up to four hours post-feeding, (Mean±SE).**

Table 2: Plasma TG concentration (mmole/1) in pregnant and non-pregnant ewes fasted, during feeding and up to four hours post-feeding (mean±SE, n=number of observations shown in brackets).

PREGNANT SHEEP

STATE OF SHEEP	h.v.	p.v.	c.a.
48 hours fasted	0.434±0.130 (12)	0.566±0.130 (12)	0.507±0.017 (12)
Feeding:30 min.	0.442±0.144 (6)	0.556±0.100 (6)	0.503±0.131 (6)
Post feeding:2 hrs	0.410±0.184 (6)	0.460±0.043 (6)	0.364±0.104 (6)
Post feeding:4 hrs	0.370±0.133 (6)	0.516±0.046 (6)	0.423±0.091 (6)

Table 2: continued.

NON-PREGNANT SHEEP

STATE OF SHEEP	h.v.	p.v.	c.a.
48 hour fasted	0.275±0.017 (52)	0.374±0.020 (52)	0.3[illegible]±0.024 (52)
Feeding:30 min.	0.226±0.019 (20)	0.326±0.038 (20)	0.2[illegible]±0.021 (20)
Feeding:1 hrs	0.214±0.020 (20)	0.287±0.029 (20)	0.239±0.022 (20)
Post-feeding:2 hrs	0.209±0.023 (20)	0.333±0.030 (20)	0.267±0.021 (20)
Post-feeding:4 hrs	0.210±0.025 (20)	0.315±0.040 (20)	0.257±0.028 (20)

Table 3. **Plasma FFA concentrations (umole/1) in pregnant and non-pregnant ewes fasted, during feeding and up to four hours post-feeding (mean+SE, n=number of observation in brackets).**

PREGNANT SHEEP

STATE OF SHEEP	h.v.	p.v.	c.a.
48 hours fasted	1485±163(12)	1644±201(12)	854±50(12)
Feeding: 30 min	1397±269(6)	1648±341(6)	1591±401(6)
Post-feeding:30 min	1220±211(6)	1358±338(6)	1427
Post-feeding 2 hrs	1276±420(6)	1243±356(6)	1280±400(6)
Post-feeding 4 hrs	1228±238(6)	1381±226(6)	1279±259(6)

Table 3: continued.

NON-PREGNANT SHEEP

STATE OF SHEEP	h.v.	p.v.	c.a.
48 hours fasted	854±50(40)	980±56(40)	936±52(40)
Feeding:30 min	541±67(16)	594±72(16)	557±74(16)
Post-feeding 30 min	566±61(16)	621±63(16)	623±57(16)
Post-feeding 2 hrs	621±62(16)	693±58(16)	656±56(16)
Post-feeding 4 hrs	631±46(16)	689±46(16)	668±59(16)

Table 4: Liver uptake of plasma TG and FFA in pregnant and non-pregnant ewes fasted, during feeding, up to four hours post feeding, (mean±SE, n=number of observations shown in brackets).

	TG mmole/min.kg$^{0.73}$		FFA umole/min.kg$^{0.73}$	
STATE OF SHEEP	PREG.	NON-PREG.	PREG.	NON-PREG
48 hrs fasted	0.011±0.003 (12)	0.006±0.002 (52)	10.0±2.6 (12)	6.6±1.7 (40)
Feeding:30 min	0.012±0.012 (6)	0.007±0.003 (20)	16.5±9.5 (6)	5.0±2.2 (16)
Post-feeding 30 min	0.006±0.007 (6)	0.004±0.003 (20)	24.8±36.4 (6)	5.8±2.2 (16)
Post-feeding 2hrs	0.004±0.017 (6)	0.013±0.004 (20)	(-)0.7±10.7 (6)	7.5±4.0 (16)
Post-feeding 4hrs	0.015±0.013 (6)	0.009±0.002 (20)	9.0±3.4 (6)	5.4±4.4 (16)

PREG. = Pregnant NON-PREG. = Non-pregnant

required by the growing foetus(es). In the pregnant sheep THBF increased from 111±8 to 121±13ml/min. kg$^{0.73}$ an increase of 9% within ten minutes of feeding. The maximum THBF rates were reached at 2 hours post-feeding corresponding to a 51% increase over the pre-feeding values. These values did not differ from that of 49% obtained by Katz and Bergman, (1969a) but deviated markedly from that of 18% reported by Bergman *et al.* (1970). The feeding caused a biphasic increase in PVF in both the pregnant and in the non-pregnant ewes. The temporary decrease in between the two peaks could be due to a general decreased plasma volume as a result of saliva production. PVF increased by 36% over pre-feeding values which was inconsistent with those of 9% (Roe *et al.* 1966), 52% (Katz and Bergman, 1969a) and 17% (Bergman *et al.* 1970) reported in the literature. The difference could be due to the difference in sampling time, the type of feed and the perceutage calculation.

The THBF increased mainly due to an increased PVF especially during feeding up to 2 hours post feeding. This could be the result of an increased blood supply to the rumen as

reported by Dobson *et al.* (1981 and Barnes *et al.* (1982, 1983) but also hepatic arterial flow rate (HAF) may have contributed significantly especially during the 2 - 4 hour period post feeding. In sheep Thompson *et al.* (1975) observed, an increase in the venous oxygen in the first 1 - 2 hours post feeding but the portal - drained viscera apparently did not contribute to this increase as reported by Webster and White (1973). Thus due to the apparent increased hepatic metabolism at 2 - 4 hours post-feeding the apparent increase in HAF supplied oxygen to top up the limited oxygen supplied by the portal vein.

The plasma TG and FFA were assayed in pregnant ewes between 15 - 18 weeks of pregnancy. The Temporary blockage of the catheters as described above prevented further assay during the last weeks of pregnancy. The plasma TG concentrations were higher in pregnant sheep than in the non-pregnant sheep. Noble *et al.* (1971) reported increased plasma TG concentration in sheep while Mabon *et al.* (1982) found no change during the first weeks of pregnancy which was followed by an increase up to 20 weeks of pregnancy. The increased TG could probably serve as a reserve for an increased substrate demand especially towards the end of the gestation period.

The TG transported into the splanchnic tissues (arterial plasma TG) was lower than that drained out of the tissues at all times i.e. in fasted state and during feeding. As there was little or no absorption from the gastro-intestinal tract during fasting the TG probably was a result of lipolysis in the omental and mesenteric adipose tissue. Furthermore the uptake of TG by the liver was higher in pregnant sheep though not so much noticeable especially during feeding. The plasma FFA concentrations like those of plasma TG were higher in the pregnant than in the non-pregnant ewes. The FFA levels were higher in the portal vein and arterial plasma than in the hepatic vein though not significant. Annison (1960) also found that arterial plasma FFA increased more rapidly on fasting in the pregnant sheep than in the non-pregnant sheep. In the present study plasma FFA were 1542 ± 190 and 923 ± 53 umole/1 in the fasted pregnant and non-pregnant ewes respectively (mean $\pm$ SE for the three vessels). These values were in accordance with those of 1281 ± 48 umole/1 in the fasted pregnant ewes (Leat and Ford, 1966); 1724 umole/1 for the hypoglycemic fasted pregnant sheep (Bergman *et al.* 1968) and 860 umole/1 in fasted pregnant ewes recorded by Katz and Bergman (1969a). There was less effect on plasma the FFA with feeding similarly as was observed for the non pregnant ewes. This could be due to the inadequate supply of nutrients through absorption as feeding was restricted for only 30 - 40 minutes during the experiment. After 48 hours of fasting the feed uptake was probably far too little to depress the lipolysis which in normal case or with adequate refeeding would be reversed to lipogenesis as there would be adequate substrate for that from absorbed dietary lipids. Mabon *et al.* (1982) observed a significant increase of plasma FFA between weeks 14-20 of pregnancy with a higher and more rapid increase closer to parturition time.

The increment in the FFA concentration was probably due to an increased mobilization of fatty acids from the adipose tissues in response to the increased requirement for endogenous

substrate for energy production during pregnancy.

In this study the hepatic FFA uptake was 10.0 ± 1.7 umole/min.$kg^{0.73}$ in fasted state, and 12.0 ± 1.9 umole/min.$kg^{0.73}$ during the feeding in the pregnant ewes. The difference was insignificant. The tissues were releasing FFA into the portal circulation at all times. Katz and Bergman (1969c) observed no significant difference in hepatic FFA uptake between the fed and fasted sheep which were 6.3 ± 1.2 and 9.3 ± 11.7 umole/min. $kg^{0.73}$ respectively.

To conclude, plasma TG and FFA concentration were higher in the pregnant ewes probably due to the increased demand by the foetal growth coupled by an inadequate carbohydrate supply (fasting). This led to increased lipid mobilization reflected by a iincreased hepatic TG and FFA uptake. The Restricted feeding as used in the study did not provide adequate dietary substrates for energy neither in the pregnant nor in the non pregnant ewes as the decreased plasma FFA already started to raise within four hours post-feeding.

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