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THE EFFECT OF FEEDING ON: II PLASMA TRIGLYCERIDES FLOW AND UPTAKE BY THE LIVER IN NORMAL ADULT SHEEP.

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SUMMARY

Plasma triglycerides (TG) were measured in portal hepatic veins and arterial blood before, during and after feeding grass hay. In order to determine the liver uptake or output portal blood flows were measured by a continuous creatinine infusion method. Arterial plasma TG concentration in fasted and fed adult sheep was 0.204 ± 0.02 and 0.282 ± 0.02 mmole/l respectively while the venous plasma TG was 0.151 ± 0.01 and 0.184 ± 0.02 mmole/l respectively. No apparent change in plasma TG concentration was noted when sheep were fed. Post feeding TG release from the splanchnic tissues was 0.006 ± 0.001 mmole/min.$kg^{0.73}$ as opposed to 0.004 ± 0.002 mmole/min. $kg^{0.73}$ during the fasted state. During feeding and for a few hours (2-4) post-feeding the hepatic TG uptake was 0.009 ± 0.004 mmole/min. $kg^{0.73}$ in contrast to 0.006 ± 0.002 mmole/min. $kg^{0.73}$ during fasted state.

INTRODUCTION

Lipids are waxy and oily compounds of the body and food. They include neutral fats (i.e. triglycerides), phospholipids, cholesterol and others of less functional importance in the body. These substances are miscible with each other and both triglycerides and phospholipids have the same basic lipid moiety which is fatty acids. The phospholipids are indespensable components of membranes and are also used as detergents to coat fat droplets for transport within the body. Lipids are stored in adipose tissues as triglycerides which are a source of energy and provide heat insulation for the body. Large quantities of triglycerides appear in the liver during starvation, diabetes mellitus and any other condition in which fat is being utilized rapidly for energy. In such conditions triglycerides are mobilized from the adipose tissue, transported as free fatty acids (FFA) and deposited in the liver as triglycerides.

Lipids in the blood arise from intestinal absorption of ingested lipids and the mobilization of triglycerides from storage or synthetic processes (especially in the liver). Blood lipids are mainly present as chylomicrons and lipoproteins (very low density, low density and high density). Also a low proportion occurs as non esterified fatty acids (NEFA = FFA) which are transported bound to plasma albumins.

Adult sheep have a low concentration of plasma lipids compared with man and other ruminants (Leat, 1967) and dietary lipid intake is also low under normal conditions. The portal drained viscera contain significant amount of adipose tissue in the omenta and mesenteries which, in sheep, can synthesize, store and release lipids. The major source of triglycerides in very low density lipoproteins (VLDL) is the circulating plasma FFA. Triglycerides are also found as chylomicrons and VLDL of intestinal source (Bell, 1981).

Prolonged fasting affects the plasma TG concentrations in various species of animals. In the rabbit TG decreases, in the pony TG increases while in the dog and monkey TG varies depending on the state of the animals prior to fasting (Streja, Marliss and Steiner, 1977). In sheep fasting entails mobilization of the fat reserves thereby reducing the plasma TG concentration from 14.1% to 8 - 10% of total lipids but at the same time the plasma FFA concentration is increased (Bouchat, Doize and Paquay, 1980 and 1981).

This work had the objective of assessing the effect of fasting and feeding, in sheep, on plasma TG flow from the portal drained viscera to the liver as well as to see whether increased dietary lipid availability would increase the TG flow and liver TG uptake.

MATERIALS AND METHODS.

Fifteen adult sheep with implanted catheters were used. Sheep were kept indoors, fed grass hay and watered ad libitum. Additionally they were given 1.0 kg/sheep/day of commercial sheep nuts. The sheep were cannulated as described by Leek (1976). The 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 also surgically performed on the right side of each sheep. The loops were made for easy accessibility of arterial blood sampling. Blood flow measurements were measured using a creatinine dilution technique (Kisauzi, 1982). A creatinine solution of 7.0mg/ml was used as an infusion solution while the priming solution contained 20.0mg creatinine/ml. The sheep were fasted for 48 hours prior to the experiment and placed in individual metabolic cages. Infusion was carried out through the mesenteric vein catheter using a peristaltic pump at a rate of 1.0ml/min. Blood sampling was performed simultaneously from the carotid artery, portal and hepatic vein catheters. Sampling took place at 90, 110, 120, 130, 140,150, 160, 170, 180, 270 and 390 minutes after the start of infusion. The feeding commenced from 120 - 150 minutes after the start of infusion (i.e. 40 min). Treatment of blood included PCV% measurement using a hematocrit kit and a plasma harvest which was frozen to be analyzed later for creatinine and TG concentrations. Both creatinine and TG were analyzed using commercial kits (i.e. for creatinine a kit from Sigma Chemicals Co, St. Louis Missouri, and for TG a kit Peridochron(R) Triglycerides, GPO - PAP, Boehringer Mannhein GmbH, Diagnostica).

Calculations

Hepatic plasma flows were calculated per unit metabolic body size ($BW^{0.73}$) as following:

(i) Total hepatic plasma flow (THPF)ml/min. $kg^{0.73}$

$$= \frac{i \times 10^3}{[(CRT)h.v. - (CRT)c.a \times (BW)^{0.73}]}$$

(ii) Portal venous plasma flow (PVPF) ml/min. $kg^{0.73}$

$$= \frac{i \times 10^3}{[(CRT)\ p.v. - (CRT)\ c.a. \times (BW)^{0.73}]}$$

(iii) Hepatic arterial plasma flow (HAPF) ml/min. $kg^{0.73}$ = THPH - PVPF;

Where:
i = infusion rate, 1.0 ml/min of 7.0 mg/ml conc.

(CRT) h.v., p.v. and c.a. are plasma creatinine concentrations in hepatic vein, portal vein and carotid artey respectively.

$(BW)^{0.73}$ = metabolic body weight.

Hepatic plasma TG flows were calculated as plasma flows x plasma TG concentrations in the respective blood vessels.

Plasma TG uptake was calculated as the product of the TG flows x respective plasma flow
i.e.

(i) Overall visceral uptake (mmole/min. $kg^{0.73}$) = [(arterial plasma TG) - (hepatic vein plasma TG) x THPF]

(ii) Splanchnic TG uptake (mmole/min.$kg^{0.73}$) = [(Arterial plasma TG) - portal vein plasma TG) x PVPF]

(iii) Hepatic TG uptake (mmole/min. $kg^{0.73}$) = Overall Visceral Uptake - Splanchnic Uptake

RESULTS

In the normal sheep the plasma TG concentration was 0.204±0.02 and 0.151±0.01 mmole/l for the arterial and venous blood respectively in the fasted state while in fed state the concentrations were 0.282±0.02 and 0.184±0.02 mmole/l (mean±SE, n=29) arterial and venous blood respectively.

Table 1: The effect of feeding on hepatic plasma flow rate, mean±SE, ml /min.$kg^{0.73}$ in adult sheep. $BW^{0.73}$ = 78.3±3.2kg (n=8).

STATE OF SHEEP	THPF	PVPF	HAPF
Fasted: 48 hours (n=16)	67.8±2.7	52.0±2.1	15.8±2.2
Feeding: 10 min (n=8)	74.1±5.4	56.4±2.7	17.7±3.8
Feeding: 20 min (n=8)	78.8±4.8	58.7±3.8	20.2±3.1
Feeding: 30 min (n=8)	81.1±6.8	59.4±5.0	21.7±4.3
Feeding: 40 min (n=8)	83.7±5.1	57.6±4.7	26.1±2.9
Post feeding: 10 min (n=8)	85.3±4.6	55.3±2.7	30.0±3.3
Post feeding: 20 min (n=8)	91.0±5.5	58.3±3.5	32.7±5.3
Post feeding: 30 min (n=8)	96.2±7.1	63.9±3.8	32.3±6.3
Post feeding: 2 hours (n=8)	120.1±9.6	77.7±8.3	42.4±5.5
Post feeding: 4 hours (n=8)	112.9±9.2	69.4±6.5	43.6±5.9

Table 2: **Effect of feeding on plasma TG concentration, (Mean SE) mmole/1 in adult sheep.**

State of Sheep	Hepatic vein	Portal vein	Carotid artery
Fasted: 48 hours (n=16)	0.281±0.021	0.377±0.025	0.030±0.032
Feeding: 10 min (n=8)	0.238±0.025	0.288±0.037	0.254±0.024
Feeding: 20 min (n=8)	0.210±0.018	0.289±0.051	0.206±0.021
Feeding: 30 min (n=8)	0.238±0.022	0.357±0.061	0.279±0.022
Feeding: 40 min (n=8)	0.236±0.023	0.350±0.032	0.279±0.022
Post feeding: 10 min (n=8)	0.252±0.022	0.350±0.036	0.286±0.035
Post feeding: 20 min (n=8)	0.222±0.31	0.279±0.034	0.232±0.020
Post feeding: 30 min (n=8)	0.233±0.022	0.278±0.038	0.227±0.025
Post feeding: 2 hours (n=8)	0.227±0.026	0.337±0.031	0.251±0.018
Post feeding: 4 hours (n=8)	0.207±0.023	0.321±0.55	0.237±0.32

Table 3. **The effect of feeding on hepatic plasma TG flow rate (mean±SE), mmole/min. $kg^{0.73}$ in adult sheep. Values obtained as product of plasma fow rates (Table 1) X plasma concentration (Table 2).**

State of Sheep	Hepatic vein	Portal vein	Carotid artery
Fasted: 48 hours (n=16)	0.019±0.001	0.020±0.003	0.004±0.001
Feeding: 10 min (n=8)	0.017±0.002	0.016±0.002	0.004±0.001
Feeding: 20 min (n=8)	0.016±0.002	0.017±0.004	0.004±0.001
Feeding: 30 min (n=8)	0.019±0.002	0.021±0.004	0.006±0.002
Feeding: 40 min (n=8)	0.019±0.002	0.020±0.002	0.007±0.001
Post feeding: 10 min (n=8)	0.021±0.002	0.019±0.001	0.009±0.001
Post feeding: 20 min (n=8)	0.020±0.002	0.016±0.002	0.007±0.001
Post feeding: 30 min (n=8)	0.022±0.003	0.017±0.002	0.007±0.001
Post feeding: 2 hours (n=8)	0.026±0.003	0.025±0.002	0.011±0.002
Post feeding: 4 hours (n=8)	0.023±0.002	0.022±0.004	0.010±0.002

Table 4: The effect of feeding on splanchnic and hepatic plasma TG uptake, (mean ± SE); mmole/min. $kg^{0.73}$ (Negative values indicate release of TG from the tissue/organ).

State of sheep	Splachnic vein	Hepatic vein
Fasted: 48 hrs (n=16)	(-)0.004 ± 0.002	0.006 ± 0.001
Feeding: 10 min (n=8)	(-)0.002 ± 0.002	0.003 ± 0.001
Feeding: 20 min (n=8)	(-)0.005 ± 0.002	0.005 ± 0.003
Feeding: 30 min (n=8)	(-)0.006 ± 0.003	0.008 ± 0.004
Feeding: 40 min (n=8)	(-)0.004 ± 0.002	0.008 ± 0.002
Post feeding: 10 min (n=8)	(-)0.004 ± 0.002	0.006 ± 0.002
Post feeding: 20 min (n=8)	(-)0.003 ± 0.001	0.004 ± 0.003
Post feeding: 30 min (n=8)	(-)0.003 ± 0.001	0.002 ± 0.004
Post feeding: 2 hours (n=8)	(-)0.006 ± 0.001	0.009 ± 0.004
Post feeding: 4 hours (n=8)	(-)0.005 ± 0.001	0.009 ± 0.003

In the catheterized sheep the plasma TG concentrations at all times i.e. in the fasted state, during feeding and a few hours (2-4) post-feeding, were higher in the portal vein than in the hepatic vein and carotid artery. The trend of plasma TG response, due to feeding can be seen in Table 1. In Table 2, plasma TG flow rates can be noted, while the plasma TG uptake by the splanchnic tissue and liver is shown in Table 3. During feeding the plasma TG concentrations in all the vessels decreased. This was noted up to 4 hours post feeding. The decrease was not significantly different from the pre-fed values.

DISCUSSION AND CONCLUSION

The arterial plasma TG in both the fasted and the fed state was higher than in the venous blood, indicating that plasma TG has been extracted from the arterial blood and if any TG had been secreted into the venous blood it was less than was extracted. The arterial plasma Tg was 0.204 ± 0.02 and 0.280 ± 0.02 in fasted and fed sheep respectively while the venous plasma TG was 0.151 ± 0.01 and 0.184 ± 0.02 mmole/1 (Mean ± SE, n = 29) in fasted and fed state respectively. These values in both fasted and fed states were consistent with those of 0.076 and 0.194 mmole/1 for venous blood in *ad lib* fed sheep recorded by Bergman *et*

al., (1971) and Noble *et al.*, (1971). They were also in accordance with those of 0.205 - 0.285 mmole/1 in mature fed sheep (Christie, *et al.*, 1978).

In the fasted state, during feeding and 2 - 4 hours post - feeding the splanchnic tissues were releasing TG in the portal vein hence the insignificant difference in the plasma levels. The only difference could be the source of TG during the different times. In the fasted state as observed by Bouchat *et al.* (1980, 1981) there is mobilization from the omenta and mesenteric adipose tissue. This could be the main source for TG as in the intestinal tract, due to fasting, no dietary lipids can be obtained. At 2 - 4 hours post feeding TG release increased probably due to increased gastro-intestinal mucosa VLDL - TG synthesis caused by increased substrates from fermentation and digestion of lipids. Also the flow of TG into the liver at this period was higher than the outflow (TG in the hepatic vein). This suggests that the liver takes up TG from the plasma at all times but more so when there is an increased supply. At 2 - 4 hours post-feeding the splanchnic tissues released TG in the portal circulation at a rate of 0.006 ± 0.001 in contrast to 0.004 ± 0.002 mmole/min. $kg^{0.73}$ during fasted state. The hepatic TG uptake was 0.009 ± 0.004 mmole/min $kg^{0.73}$ at 2-4 hours post feeding in contrast to 0.006 ± 0.002 mmole/min. $kg^{0.73}$ in fasted state. The difference is insignificant. This is in accordance to what Morris (1963) observed in man, dog and rat where in vivo and in vitro experiment showed that perfused liver extracted 8% of chylomicron - TG with no significant difference in the efficiency with which starved or fed livers took up chylomicron - TG or FFA. Morris (1963) observed that isolated perfused rat liver showed simultaneous uptake and retransport of TG. Bergman *et al.* (1971) observed 10% TG uptake by the liver in unanaesthetized sheep. In the present study hepatic TG uptake was 21% of the total TG presented to the liver i.e. TG in portal vein and TG in hepatic artery. This seems to be higher than the values of Bergman *et al.* (1971) and Morris (1963) probably due to the method by which the percentage was derived.

Bouchat *et al.* (1980, 1981) showed that fasting entails mobilization of lipids through lipolysis thus releasing lipids in plasma in form of FFA and glycerol. As observed in this study there was no difference in TG plasma concentration during fasted or fed state in the sheep, thus giving rise to a question as to the origin of TG during the fasted state. Is it possible that, besides FFA and glycerol, TG might be a by product of lipolysis released directly into plasma (unlikely) or could it be that FFA and glycerol are used as substrates to resynthesize VLDL-TG when they are passing through the blood vessels in the gut mucosa?

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REFERENCES

Barnes, R.J., Comline, R.S. and Dobson, A. (1983) Changes in the blood flow to the digestive organs of sheep induced by feeding. **Quarterly Journal of Experimental Physiology** 68:77 - 78.

Bell, A.W. (1981) Lipid metabolism in liver and selected tissues and in the whole body of ruminant animals. In: Lipid Metabolism in Ruminant Animals. Edited by Christie, W.W. Pergamon Press pp 363.

Bergman, E.N.; Havel, R.J.; Wolfe, B.M. and Bohmer, T. (1971) Quantitative studies of the metabolism of chylomicron triglycerides and cholesterol by liver and extra-hepatic tissues of sheep and dogs. **Journal of Clinical Investigation** 50:1831 - 1839.

Bouchat, J.Cl.; Doize, F and Paquay, R. (1980) Effects of fasting on blood composition and nitrogen losses in the adult sheep depending on previous diet and body weight. **Reproduction, Nutrition, Development** 20: 77-92.

Bouchat, J.C.L.; Doize F. and Paquay, R. (1981) Influence of diet and prolonged fasting on blood lipids, ketone bodies, glucose and insulin in adult sheep. **Reprod. Nutr. Develop.** 21 : 69-89.

Christie, W.W. (1978) The composition, structure and function of lipids in the tissues of ruminant animals. **Progressive Lipids Research** 17:111 - 205.

Katz, M.L. and Bergman, E.N. (1969) A method for simultaneous cannulation of the major splanchnic blood vessels of the sheep. **Am. J. Vet. Res.** 30:655-661.

Kisauzi, D.N. (1982) Liver blood flow and metabolite fluxes in normal sheep and sheep with liver dysfunction. PhD Thesis UCD Ireland.

Leat, W.W.F. (1967) Plasma lipids of newborn and adult ruminants and of lambs from birth to weaning. **Journal of Agriculture Science** 69:241-246.

Leek, B.F. (1976) A simple and effective rumen cannulation technique for sheep. **Journal of Physiology** 263: 233 - 234.

Morris, B. (1963) The metabolism of free fatty acids and chylomicrons triglycerides by the isolated perfused liver of the rat. **Journal of Physiology** 168:564.

Noble, R.C.; Steele, W. and Moore, J.H. (1971) Fatty acid composition of liver lipids of young lambs. **British Journal of Nutrition** 26:97 - 105.

Streja, D. A.; Marliss, E.B. and Steiner, G. (1977) The effects of prolonged fasting on plasma triglyceride kinetics in man. **Metabolism** 26: 505 - 516.