Level of nutrition and visceral organ size and metabolic activity in sheep*

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(*Received 4 July 1989 – Accepted 7 March 1990*)

Thirty-two crossbred wether lambs (initial live-weight 31 kg) were fed on a diet (metabolizable energy (ME) 12·8 MJ/kg) *ad lib.* (ADLIB) or restricted to maintain body-weight (MAINT) for a 21 d period. On days 0, 7, 14 and 21, four lambs per treatment were slaughtered, visceral organs weighed and tissues sampled. During the 21 d period, ME intake in ADLIB lambs increased quadratically with an average rate of live-weight gain of 425 g/d. In MAINT lambs, live weight (30 kg) was maintained, and daily ME intake (kJ/kg empty body-weight (EBW)$^{0.75}$) declined ($P < 0.01$) quadratically with time. Weights of liver, stomach and small intestines as a percentage of EBW were increased in ADLIB lambs and decreased by 10–33% in MAINT lambs (treatment × day, $P < 0.01$). In vitro liver oxygen consumption was not affected by level of feed intake. Estimates of whole-liver O_2 consumption (mmol O_2/d per kg EBW) increased in ADLIB lambs and were relatively constant in MAINT lambs. These findings suggest that level of feed intake changes the relative proportion of visceral organs to body mass. In addition, the effect of level of feed intake on changes in the relative contribution of visceral organs to whole-body metabolic rate appears to be primarily a result of differences in organ size rather than tissue-specific metabolic activity.

Nutrient restriction: Organ size and metabolism: Sheep

Several studies have indicated that a decreased plane of nutrition results in a decrease in metabolic rate (Marston, 1948; Ledger & Sayers, 1977; Gray & McCracken, 1979). In a series of experiments with pigs, rats and sheep, a decreased plane of nutrition consistently resulted in decreased relative sizes of visceral organs, such as liver, kidney, stomach and intestines (Koong *et al.* 1982; Ferrell & Koong, 1986; Ferrell *et al.* 1986). Regression analysis of data from these studies indicates that a good relationship exists between weights of liver and gut tissues and estimates of maintenance energy requirements. Furthermore, the observed differences in maintenance energy requirements between breeds of animal and stage or level of production has also been attributed to the relative weights of visceral organs (Smith & Baldwin, 1974; Jenkins & Ferrell, 1983; Jenkins *et al.* 1986).

Although the visceral organs represent approximately 6–10% of body-weight, estimates indicate that visceral tissues account for 40–50% of whole-body cardiac output, protein synthesis and heat production (Davis *et al.* 1981; Webster, 1981). Metabolic activity of an organ is the product of organ size and metabolic activity per unit of tissue. Conceivably, changes in organ size alone can account for observed differences in whole-body metabolism (Smith & Baldwin, 1974; Canas *et al.* 1982); however, changes in total organ metabolic

* Published as paper no. 8581, Journal Service, Nebraska Agriculture Experiment Station.

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activity could result from differences in metabolic activity per unit tissue as well. The objective of our study was to measure the effect of level of nutrition on visceral organ size and metabolic activity in young sheep.

MATERIALS AND METHODS

Experimental diet and design

Thirty-two crossbred wether lambs (approximately 4 months old and weighing approximately 31 kg) were assigned to a 2×4 factorial arrangement of treatments in a completely random design with either an *ad lib.* (ADLIB) or maintenance (MAINT) treatment and slaughter times of 0, 7, 14 or 21 d. In the 14 d period before initiation of the experiment, all animals were individually fed on the experimental diet (Table 1) once daily at a level of 30 g/kg live weight. During the 21 d experimental period, the MAINT lambs were fed to maintain live weight by restricting feed intake rather than nutrient density. Initially, feed intake required to maintain live weight in the MAINT lambs was estimated based on initial live weight; thereafter, wethers were weighed twice weekly and feed allowances were adjusted to achieve maintenance of live weight. Intakes of lambs in the ADLIB group were recorded daily. Both the intake and live weight values at 0, 7, 14 and 21 d, represent measurements from the animals slaughtered on those respective days.

Sampling procedure

At day 0 and 7, 14 and 21 d thereafter, four lambs previously assigned to each treatment were removed from their pens following an overnight fast, weighed and jugular blood samples were drawn into heparinized tubes. The lambs were then stunned with a captive bolt and exsanguinated. Lambs were eviscerated and the livers, kidneys, and full and digesta-free components of the gastrointestinal tract were weighed. All visible mesenteric fat was dissected from the gastrointestinal tissue before recording its weight. In addition, a portion of ventral sac from the reticulo-rumen (approximately 100 cm^2) and a segment (approximately 100 mm) of the proximal jejunum was removed, rinsed free of feed particles with cold tap water and placed in ice-cold oxygenated Krebs–Henseleit buffered saline (9 g sodium chloride/l; KHS) containing 10 mM-glucose. A section of the right lobe of the liver was also placed in ice-cold oxygenated KHS plus glucose (10 mM). Typically, the elapsed time between the exsanguination of the lamb and the beginning of in vitro tissue incubations was 15–20 min. Plasma was separated from whole blood by centrifugation at 5000 ***g*** for 15 min. Plasma glucose was measured using a glucose oxidase (*EC* 1.1.3.4) method. Plasma triiodothyronine (T_3) and thyroxine (T_4) concentrations were measured using a radioimmunoassay kit (Cambridge Medical Diagnostics, Billerica, MA). Inter- and intra-assay coefficients of variation for both T_3 and T_4 were within 5–10%.

In vitro oxygen consumption

Liver slices weighing approximately 100 mg were prepared using a hand microtome. Rumen papillas were sliced from the epithelial layer of the rumen mucosa using a microtome blade. Jejunal tissue was prepared by cutting transverse sections of the gut into 100 mg pieces. O_2 consumption was measured polarigraphically using a system that employed a Clark electrode positioned within a thermostatically-controlled (37°) cell chamber (Gilson Medical Electronics, Middletown, WI). Approximately 50 mg wet tissue was placed in the chamber containing air-saturated KHS plus glucose (10 mM) and O_2

Table 1. *Diet composition (g/kg on a dry matter basis)*

Ingredient	
Ground maize	788·6
Lucerne (*Medicago sativa*)	100·0
Soya-bean meal	50·0
Molasses	50·0
Limestone	7·8
Sodium chloride	3·0
Trace mineral*	0·5
Vitamin premix†	0·1
Metabolizable energy‡	12·80
Crude protein§	120·0

* Contained (g/kg): 100 magnesium, 60 zinc, 45 iron, 20 manganese, 5 copper, 3 iodine and 0·5 cobalt.
† 4500 μg retinol, 75 μg cholecalciferol and 4·13 μg DL-α-tocopherol/g premix.
‡ MJ/kg dry matter based on National Research Council (1984).
§ Percent crude protein based on Kjeldahl nitrogen.

consumption was recorded for a 3 min period and repeated three or four times to estimate an average O_2 consumption rate. Preliminary observations indicated linearity of O_2 consumption rates during the 3 min period measured.

Statistics

Results were analysed according to the general linear models procedure of the Statistical Analysis System (SAS, 1982) with a 2×4 factorial arrangement of treatments in a completely random design. Independent variables were dietary treatment, day of sampling and the interaction between treatment and day partitioned into linear, quadratic and cubic effects. Results are presented as least-squares means with their respective standard errors.

RESULTS

During the 21 d period, feed intake, live weight, empty body-weight (EBW) and carcass weight increased in ADLIB lambs and decreased or remained constant in the MAINT lambs (treatment × day, $P < 0{\cdot}01$, linear; Table 2). Throughout the 21 d period, mean EBW of the MAINT groups was maintained within 6% of that in the initial control group. The calculated daily metabolizable energy (ME) intakes of MAINT lambs were 618, 523 and 494 (kJ/kg $EBW^{0{\cdot}75}$) after 7, 14 and 21 d respectively; thus ME intake declined about 20% during this interval. The average rate of empty body gain in the ADLIB lambs was 395 g/d for the 21 d period.

The absolute weights of all visceral organs (treatment × day, $P < 0{\cdot}01$, linear) and the relative weights (% EBW) of liver (treatment × day, $P < 0{\cdot}01$, linear), stomach (treatment × day, $P < 0{\cdot}05$, linear) and small intestines (treatment × day, $P < 0{\cdot}10$, linear and quadratic) were increased in ADLIB lambs and decreased in MAINT lambs (Table 3). Mean relative large intestines weight averaged across the 21 d period was not different between ADLIB and MAINT lambs. After 21 d, the absolute weights of liver, stomach, small intestines and large intestines in MAINT lambs were approximately 52, 72, 63, 63, and 63%, respectively of that in the ADLIB lambs. In comparison, EBW in MAINT lambs was 78% of that in ADLIB lambs after 21 d. More than half the total decrease in liver weight in the MAINT lambs occurred in the initial 7 d period. The changes in liver weight in response to level of nutrition were of greater magnitude than that of any other organ

Table 2. *Feed intake and components of live weight in sheep fed* ad lib. *or at maintenance levels*‡

	Day of treatment				
	0	7	14	21	SEM
ME intake (kJ/kg $EBW^{0.75}$ per d)§					
Ad lib.	1097*	1249	1335	1309	198
Maintenance	1087	618	523	494	—
Live wt (kg)					
Ad lib.	30·4*	32·7	36·9	39·3	2·0
Maintenance	32·3	30·7	29·3	30·3	—
EBW (kg)					
Ad lib.	26·4*	28·3	31·9	34·7	1·9
Maintenance	28·1	28·0	26·5	27·2	—
Digesta (kg)					
Ad lib.	3·9†	4·4	5·0	4·6	0·4
Maintenance	4·2	2·7	2·8	3·2	—
Carcass wt (kg)					
Ad lib.	16·8*	17·8	19·9	22·0	1·1
Maintenance	17·5	17·6	17·2	16·8	—

ME, metabolizable energy; EBW, empty body-weight.
* Treatment × day ($P < 0.01$), linear.
† Treatment × day ($P < 0.05$), quadratic.
‡ For details, see p. 440.
§ Represents average of previous 7 d period.

measured. The relationships between liver weight and body weight in each treatment were determined using allometric regression. Based on the model $Y = aW^b$, where Y is liver weight (g) and W is body weight (kg), the following equations were generated

$$Y = 10{\cdot}5\ (\text{SE}\ 1{\cdot}95)\ W^{1{\cdot}15}\ (\text{SE}\ 0{\cdot}187)\ (\text{ADLIB}, n\ 16),$$
$$Y = 21{\cdot}6\ (\text{SE}\ 2{\cdot}87)\ W^{0{\cdot}87}\ (\text{SE}\ 0{\cdot}308)\ (\text{MAINT}, n\ 16).$$

In vitro tissue O_2 consumption rates expressed per g tissue were not affected by level of nutrition (Table 4). In general, rates of O_2 consumption of rumen epithelial tissue were higher than those of liver tissue. Whole-liver O_2 consumption relative to EBW was calculated as a product of in vitro estimates and liver weight of each lamb (Fig. 1). During the 21 d period, whole-liver O_2 consumption increased in ADLIB lambs and remained unchanged in MAINT lambs (treatment × day, $P < 0.01$, linear). After 21 d, whole-liver O_2 consumption in the ADLIB lambs increased by 77% above that of the initial control values.

Averaged across all sampling days, mean plasma glucose concentrations in ADLIB lambs were higher ($P < 0.05$) than those in MAINT lambs (Table 5). Plasma glucose concentrations in the MAINT lambs decreased (31%) in the initial 7 d period and remained relatively constant from day 7 to day 21. Plasma concentrations of both T_3 and T_4 were not affected ($P > 0.10$) by level of nutrition (Table 5). However, T_3 concentrations in ADLIB lambs were increased by 34% and 47% at 7 and 14 d respectively, but returned to initial control concentrations by day 21. Inter- and intra-assay coefficients of variation for both T_3 and T_4 were 5–10%; however, coefficients of variation for both T_3 and T_4 were 44–46%, indicating large between-animal variation in concentrations of these hormones.

Table 3. *Visceral organ weights of sheep fed* ad lib. *or at maintenance levels*§

(Values expressed in g wet tissue and, in parentheses, g/kg empty body-weight)

	Day of treatment				
	0	7	14	21	SEM
Liver					
Ad lib.	495*	558	674	773	29
	(18·8)	(19·8)	(21·2)	(22·4)	(0·8)
Maintenance	520	432	385	369	—
	(18·6)	(15·5)	(14·6)	(13·6)	
Kidney					
Ad lib.	80*	90	103	98	5
	(3·05)	(3·20)	(3·23)	(2·83)	(0·14)
Maintenance	84	86	71	70	—
	(3·03)	(3·05)	(2·70)	(2·60)	
Stomach					
Ad lib.	878*	1039	1137	1245	54
	(33·3)†	(36·7)	(35·8)	(36·0)	(1·2)
Maintenance	904	872	769	784	—
	(32·5)	(31·0)	(29·1)	(29·1)	
Small intestines					
Ad lib.	608*	707	734	680	34
	(23·3)‡	(25·0)	(23·0)	(19·7)	(1·4)
Maintenance	654	563	421	425	—
	(23·5)	(20·4)	(15·9)	(15·8)	
Large intestines					
Ad lib.	436	504	529	601	26
	(16·5)	(17·8)	(16·7)	(17·6)	(1·0)
Maintenance	433	485	414	379	—
	(15·6)	(17·4)	(15·7)	(14·1)	

* Treatment × day ($P < 0·01$), linear.
† Treatment × day ($P < 0·05$), linear.
‡ Treatment × day ($P < 0·01$), linear and quadratic.
§ For details, see p. 440.

Table 4. *In vitro tissue oxygen consumption rates (μmol/min per g wet tissue) in sheep fed* ad lib. *or at maintenance levels*‡

	Day of treatment				
	0	7	14	21	SEM
Liver					
Ad lib.	0·498*	0·716	0·733	0·733	0·041
Maintenance	0·552	0·738	0·654	0·717	—
Rumen epithelium					
Ad lib.	0·95†	1·09	1·14	1·02	0·05
Maintenance	0·98	1·23	1·25	1·18	—
Jejunum§					
Ad lib.	—	0·57	0·56	0·58	0·08
Maintenance	—	0·58	0·51	0·60	—

* Day ($P < 0·01$), linear.
† Day ($P < 0·01$), linear and quadratic.
‡ For details, see p. 440.
§ Missing values were a result of time constraints during laboratory measurements.

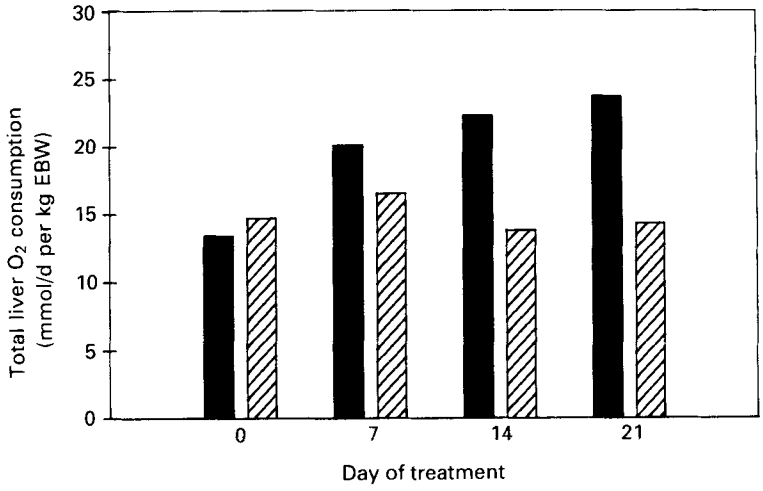

Fig. 1. Estimated whole-liver oxygen consumption expressed per unit empty body-weight (EBW) of sheep fed *ad lib.* (■) or at maintenance levels (▨). Values represent means, with the pooled standard error of the mean of 2·2. Treatment × day effect ($P < 0·01$) linear. For details of dietary treatments, see p. 440.

Table 5. *Plasma glucose and thyroid hormone concentrations in sheep fed* ad lib. *or at maintenance levels*†

	Day of treatment				
	0	7	14	21	SEM
Glucose (mg/l)					
Ad lib.	930*	890	880	800	90
Maintenance	940	650	670	650	—
Triiodothyroxine (ng/l)					
Ad lib.	947*	1265	1390	998	250
Maintenance	1062	850	951	744	—
Thyroxine (μg/l)					
Ad lib.	87	137	92	87	38
Maintenance	103	114	133	73	—

* Treatment ($P < 0·05$).
† For details, see p. 440.

The variation in hormone concentrations may have been a consequence of untimely or insufficient sampling.

DISCUSSION

The observed changes in live weight, EBW and visceral organ weight are consistent with several previous reports (Marston, 1948; Meyer & Clawson, 1964; Ledger & Sayers, 1977; Ferrell *et al.* 1986). These findings clearly demonstrate that restriction of food intake results in a decrease in the relative proportion of visceral tissues to body EBW and that weights of these organs change more rapidly than does body weight in response to nutrition. Koong *et al.* (1982) and Ferrell *et al.* (1986) observed significant decreases in weights of liver, stomach and small intestines of sheep and pigs following periods of nutrient restriction for

6–10 weeks. In this study, liver and gut tissue weights decreased during a 21 d feed restriction. In addition, much of the decrease observed in visceral organ weights occurred within 7–14 d and illustrates the ability of these tissues to adapt rapidly to changing nutrient supply.

Ledger & Sayers (1977) demonstrated the effect of prolonged maintenance of live weight on energy requirements of steers. During a 24 week period, the daily feed intake required to maintain live weight of steers at three different weights (185, 275 and 450 kg) decreased by 18–52% depending on live weight. In a similar study (Meyer & Clawson, 1964), the average daily feed intake of sheep maintained at 35 kg for 42 d was approximately 375 kJ ME/kg live weight$^{0.75}$ which is 20% lower than estimates reported for similar sized sheep fed *ad lib.* (Ferrell *et al.* 1986). Live weight of the MAINT lambs was maintained at approximately 30 kg throughout the 21 d period. The daily calculated ME intakes (kJ/kg EBW$^{0.75}$) of the MAINT lambs gradually decreased from 618 at day 7 to 494 at day 21. Also in the period between 7 and 21 d, digesta weight or gut-fill was relatively unchanged or increased. Therefore, the reduced estimates of ME intake suggest a true decrease in dietary energy required to maintain live weight. A decrease in feed intake required to maintain live weight could result from an increased utilization of the diet at the lower intake level, a reduction in maintenance energy requirements or a combination of both effects (Meyer & Clawson, 1964; Ledger & Sayers, 1977).

One of the primary objectives of the present study was to measure the effect of level of nutrition on visceral organ metabolic activity per unit tissue. Rates of O_2 consumption by liver and rumen epithelial tissue were similar to those reported previously (Ferrell & Koong, 1985; McBride & Milligan, 1985*a*). Rates of O_2 consumption by jejunal tissue were lower than the estimates reported by McBride & Milligan (1985*b*). This was probably because the jejunal tissue preparation used in the present study included both epithelial tissue and the less metabolically active smooth muscle layers (D. G. Burrin, unpublished results), which may have lowered the relative metabolic rate of the tissue.

Measurements of O_2 consumption per unit tissue for the various organs were unaffected by nutritional level. This is consistent with previous results in hepatocytes from fed and fasted rats (Burrin *et al.* 1988) and sheep liver slices (Ferrell & Koong, 1985). In the latter study, liver O_2 consumption per unit tissue was unchanged during a 3-week period in which feed was restricted to maintain live weight. However, prolonged periods of nutrient restriction (6 weeks) did appear to decrease liver tissue O_2 consumption (Ferrell & Koong, 1985). Contrary to our results, in vitro hepatocyte O_2 consumption was reduced by 30% in sheep starved for 5 d compared with fed controls (McBride & Milligan, 1985*a*) and duodenal mucosal O_2 consumption was decreased when sheep were starved for 48 h or feed intake was reduced from 14·8 to 7·6 MJ/d (McBride & Milligan, 1985*b*).

Failure to detect a change in the in vitro O_2 consumption of liver slices in response to level of nutrition should be viewed with some caution. As is the case with many in vitro estimates of tissue metabolism, the extrapolated rates of liver O_2 consumption from in vitro values in the present study are substantially lower than measurements we have obtained in vivo (Burrin *et al.* 1989). Similarly, the inability accurately to predict absolute rates of de novo fatty acid synthesis in vivo from estimates made in vitro has been critically reviewed (Mersmann, 1986). The fact that estimates of in vitro tissue metabolism typically do not accurately represent in vivo measurements is probably related to the incomplete perfusion of nutrients and hormones and the catabolic nature of in vitro tissue preparations.

Nevertheless, the calculated estimates of whole-liver O_2 consumption suggest that level of nutrition affects organ metabolic activity. Although liver O_2 consumption in MAINT lambs did not change during the 21 d period, ADLIB lambs exhibited a large increase in liver O_2 consumption. A decrease in liver O_2 consumption was not observed in MAINT

lambs even though liver weight decreased and EBW was maintained. This was because in vitro O_2 consumption in MAINT lambs increased from day 0 to day 7. Literature estimates of in vivo O_2 consumption by gut and liver in response to level of intake are scarce. However, available measurements suggest that O_2 consumption and blood flow by the tissues of the portal-drained viscera increases with increasing ME intake (Webster *et al.* 1975; Huntington, 1984; Huntington *et al.* 1985; Wieghart *et al.* 1986). A similar effect of feed or energy intake on in vivo O_2 consumption and blood flow of the liver of sheep and cattle has been reported (Burrin *et al.* 1989; Reynolds & Tyrrell, 1987). Our findings suggest that the observed increase in liver O_2 consumption was primarily a result of increased weight of the liver and not a change in tissue specific metabolic activity.

The decrease in jugular blood glucose concentration in MAINT lambs is in agreement with previous reports in ruminants (Basset, 1974; DeJong, 1981). In sheep, the rate of glucose turnover decreases during development of hypoglycaemia, and is positively correlated with plasma glucose concentration (Bergman, 1974). Interpreting the significance of plasma thyroid hormone concentrations was complicated by the large animal-to-animal variation. Thyroid hormones are involved in control of cellular metabolism (Tata *et al.* 1963) and appear to be influenced by level of nutrition (Blum *et al.* 1979, 1980, 1985; Ingram & Ramsden, 1981). In the present study, the concentration of T_3 in MAINT lambs at 7, 14 and 21 d was lower than that in ADLIB lambs. The lower plasma T_3 levels combined with the decrease in ME required for maintenance in MAINT compared with ADLIB lambs is consistent with a decreased rate of metabolism in response to nutrient restriction.

In summary, results from the present study provide further understanding of the metabolic changes that occur in visceral tissues during nutrient restriction. Nutrient restriction decreased metabolic activity of the liver primarily by affecting liver size, and this response appears to occur within a short time period. Previously, we (Burrin *et al.* 1989) and others (Reynolds & Tyrrell, 1987) have shown that nutrient restriction decreases the relative contribution of the liver to whole-body O_2 consumption. Thus, taken together, these results suggest that a decrease in the proportion of metabolically active visceral organs is involved in reduced total body energy expenditures during adaptation to nutrient restriction. In addition, other biochemical processes, such as protein synthesis, make an important contribution to cellular energy expenditures (Millward *et al.* 1976; Milligan & Summers, 1986). Protein synthesis in liver and gut tissue of rats appears to be susceptible to modulation by nutrition (McNurlan *et al.* 1979). Additional studies that define the effects of nutrient intake on visceral organ metabolism will provide a better understanding of the components of whole-body energy expenditures.

The authors wish to thank Roger Mandigo, Jim Lamkey and Randy Kemp for assistance in slaughtering animals and collecting tissue. They also thank Laurie Dubrovin for laboratory assistance.

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Printed in Great Britain