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Nutrient effects on the hepatic production of somatomedin C (IGF_1) in the milk-fed calf

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The present study was aimed at determining the influence of nutrients supplied by a milk diet (glucose, amino acids, triglycerides) on hepatic somatomedin C (IGF_1) production in vivo in four 30-d-old milk-fed calves fitted with chronically indwelling catheters in hepatic (HV), portal (PV) and mesenteric veins and in the hepatic artery (HA), and with electromagnetic flow-meters on HA and PV. Fasting for 16 h induced a decrease ($P < 0.01$) in hepatic IGF_1 production (nmol/kg body-weight (BW) for 6 h) (1·1 (SE 0·2) *v.* 6·6 (SE 0·7) in control animals). Infusion of glucose (1·8 g/kg BW for 4 h) or a mixture of amino acids (Azonutril; R. Bellon, Neuilly sur Seine; 62·5 mg nitrogen/kg BW for 3 h) in a mesenteric vein led to no significant effect on hepatic IGF_1 production for 6 h (1·2 (SE 0·3) and 0·7 (SE 0·3) nmol/kg BW respectively) compared with fasted calves. Infusion of chylomicrons purified from milk-fed calves (10·5 mg/h per kg BW, i.e. 0·16 mg triglycerides/kg BW per min) enhanced significantly ($P < 0.01$) the hepatic production of IGF_1 (mean value for 6 h: 5·3 (SE 0·8) nmol/kg BW). Infusion of Intralipid (7 mg triglycerides/kg BW per min) induced a slight but significant hepatic IGF_1 production which amounted to 3·5 (SE 0·4) nmol/kg BW ($P < 0.01$ compared with chylomicron treatment) and it began only 5 h after starting the infusion. Neither triglyceride nor chylomicron infusion significantly modified hepatic blood flow. Thus, these results demonstrate for the first time the role of lipids in the regulation of hepatic IGF_1 production in vivo.

Somatomedin C: Liver IGF_1: Calf

Some of the consequences of altered nutrition on growth are direct effects, but nutritional status has an additional impact on endocrine function. Indeed, endocrine adaptations may not only modulate the levels of nutrients circulating in plasma, but also direct the utilization of nutrients for maintenance and growth.

Since both growth hormone (GH) and somatomedin C (IGF_1) play a critical part in promotion of growth, there has been great interest in the effect of nutrition on the somatotrophic axis. Thus nutritional status appears to be one of the most important influences on IGF_1 plasma concentration (Breier *et al.* 1986; Phillips, 1986). However, while a number of studies have attempted to establish what aspects of nutrition are responsible for the modulation of serum IGF_1 (Isley *et al.* 1983; Clemmons *et al.* 1981), the dietary components involved in this regulation have not been determined.

As accumulating evidence indicates that changes in circulating IGF_1 concentrations or activity may reflect regulation by the liver (McConaghey, 1972; Schimpff *et al.* 1976; Coxam *et al.* 1988*b*), the present work was undertaken to assess the influence of plasma metabolite concentrations on the apparent production in vivo of IGF_1 by the liver in preruminant calves fitted with permanent cannulas in the hepatic blood vessels.

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MATERIALS AND METHODS

Animals and diets

Experiments were performed according to a Latin square design on four 30-d-old Holstein × Friesian male calves, weighing 49 ± 2 kg and fitted with chronically indwelling catheters in hepatic (HV), portal (PV) and mesenteric veins and in the hepatic artery (HA), and with electromagnetic flow-meters on PV and HA (Durand *et al.* 1988). At least 6 d were allowed for each calf to recover from surgery before the first experiment (Durand *et al.* 1988). All catheters were patent for the entire study as confirmed at necropsy. No less than 48 h elapsed between two successive experiments on the same animal. Calves were fed twice daily (100 g dry matter (DM) diet/kg body-weight (BW)) on a conventional milk-substitute (230 g protein/kg diet DM and 225 g tallow/kg diet DM), allowing a mean daily weight gain of 1·02 (SE 0·10) kg during the experimental period. Sampling times started at 08.00 hours after an overnight fast (16 h).

Experimental treatments

Nutrients were infused into a mesenteric vein far from the portal vein, allowing a satisfactory mixing with blood. The following treatments were administered in order to reproduce postprandial metabolic events.

Four calves received 1·8 g glucose/kg BW over 4 h given in a 300 g glucose/l saline solution (9 g NaCl/l).

In the same way, according to qualitative and quantitative amino acid hepatic flow measurements (P. Patureau-Mirand, unpublished results), four calves were infused for 3 h with 62·5 mg N/kg BW (Azonutril; R. Bellon, Neuilly sur Seine; total amino acids 148·3 g/l; N 25·0 g/l, amino α-N 14·9 g/l).

Four animals were infused with a lipid emulsion (Intralipid, Kabivitrum SA, Noisy le Grand; soya-bean oil 100 g/l, lecithin 12 g/l, glycerol 22·5 g/l) in a saline solution (10 g Intralipid/l) containing heparin (10 IU/ml; Roche, Neuilly sur Seine) at a rate of 0·07 ml/min per kg BW for 60 min, i.e. 7 mg triglycerides and 0·4 IU heparin/kg BW per min.

Two other calves received chylomicrons purified from milk-fed calves (0·1 ml/kg BW per min for 60 min), supplying 0·16 mg triglycerides/kg BW per min.

Four calves fasted for 6 h and four control animals fed on a conventional milk-substitute (50 g/kg BW) were also infused with a saline solution for 4 h.

Methods

Isolation of plasma bovine chylomicrons. Six calves were fed on a conventional milk-substitute (50 g/kg BW) supplemented with 0·5 litres milk cream/kg BW (UHT crème, Toury, Theix) (330 g/l per kg BW). Animals were slaughtered 10 h after feeding and total blood was harvested. Plasma (15 litres) was then isolated by centrifugation at 2500 rev/min for 60 min at 4°. Plasma chylomicrons (Swedberg flotation units > 400) were isolated by ultracentrifugal flotation at 20000 rev/min ($2·25 \times 10^6$ *g*/min) for 45 min at 15° using a Kontron TFT 70–38 rotor, according to Zilversmit (1969).

Assay procedures. Plasma GH concentration was determined by radioimmunoassay (RIA) as previously described (Coxam *et al.* 1987). In the present experiment, all values lower than 0·02 nmol/l were considered undetectable. Within-assay variation was 5·5% and interassay variation 6·2%.

Plasma IGF_1 was measured by RIA after extraction (Underwood *et al.* 1982). In order to dissociate and separate IGF_1 from its carrier protein, calf plasma was mixed with 4 vol. 0·5 M-hydrochloric acid and incubated in stoppered glass tubes for 1 h at room temperature. After incubation, an ODS-silica extraction was performed (Sep Pack C 18 cartridge;

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Table 1. *Effect of fasting or mesenteric infusion of glucose, amino acids, triglycerides or chylomicrons on hepatic somatomedin C (IGF_1) production in calves*

(Mean values with their standard errors)

Treatment	Hepatic IGF_1 production per 6 h (nmol/kg body-weight)	
	Mean	SE
Milk feeding	6·6	0·7
Fasting	1·1*	0·2
Glucose infusion	1·2*	0·3
Amino acid infusion	0·7*	0·3
Triglyceride infusion	3·5*	0·4
Chylomicron infusion	5·3	0·8

Mean values were significantly different from milk-fed controls: $^*P < 0{\cdot}01$.

Waters Associates, Milford, Mass, USA). As IGF_1 is identical in human and bovine species (Honneger & Humbel, 1986), the efficiency of IGF_1 extraction in bovine plasma was examined by adding pure recombinant human IGF_1 (Ciba-Geigy, Basel, Switzerland) to 0·5-ml portions of calf plasma. The RIA dose–response curves of these samples paralleled those of pure IGF_1 alone, but only 88 ± 2% of the added IGF_1 could be detected. Thus the results were corrected according to this extraction efficiency. Under our experimental conditions, intra-assay and interassay variations were 8 and 12%, respectively. The minimum detectable amount was less than 2 nmol/l (Coxam *et al.* 1988*a*).

Plasma insulin concentration was determined by heterologous RIA (Insulin radio-immunoassay kit, SB $INSI^{-1}$; Oris, Gif sur Yvette), the antibody against human insulin cross-reacting with bovine insulin, as shown by the parallelism between human and bovine standard curves. Under our conditions, the sensitivity of the method was 10 nmol/l. The intra- and interassay precisions were 8 and 13% respectively (Richet *et al.* 1985).

Plasma glucose concentration was estimated colorimetrically using the glucose oxidase (*EC* 1.1.3.4) method (Technicon AutoAnalyzer) as previously described (Richet *et al.* 1985), and plasma non-esterified fatty acids (NEFA; test Wako; Biolyon, Lyon) were determined as already reported (Chilliard *et al.* 1984). Triglyceride concentration was estimated by the enzymic method of Fossati & Prencipe (1982) using the Biomérieux reagent kit (PAP 1000, no. 6.123.6; Biomérieux, Charbonnières les Bains) which determined the total glycerol content of the sample. Plasma protein concentration was estimated with the folin phenol reagent (Lowry *et al.* 1951).

Results are expressed as means with their standard errors. Significant differences observed between groups of animals were calculated using the Mann–Whitney *U* test. The influence of treatments was evaluated using one-way analysis of variance. For clarity, only HV plasma concentrations are shown in the figures.

RESULTS

Effects of fasting

In the three hepatic vessels (HV, PV and HA), mean plasma hormone concentrations (nmol/l) were lower in 16 h fasted than in fed conditions (GH 0·12 (SE 0·01) *v.* 0·89 (SE 0·03),

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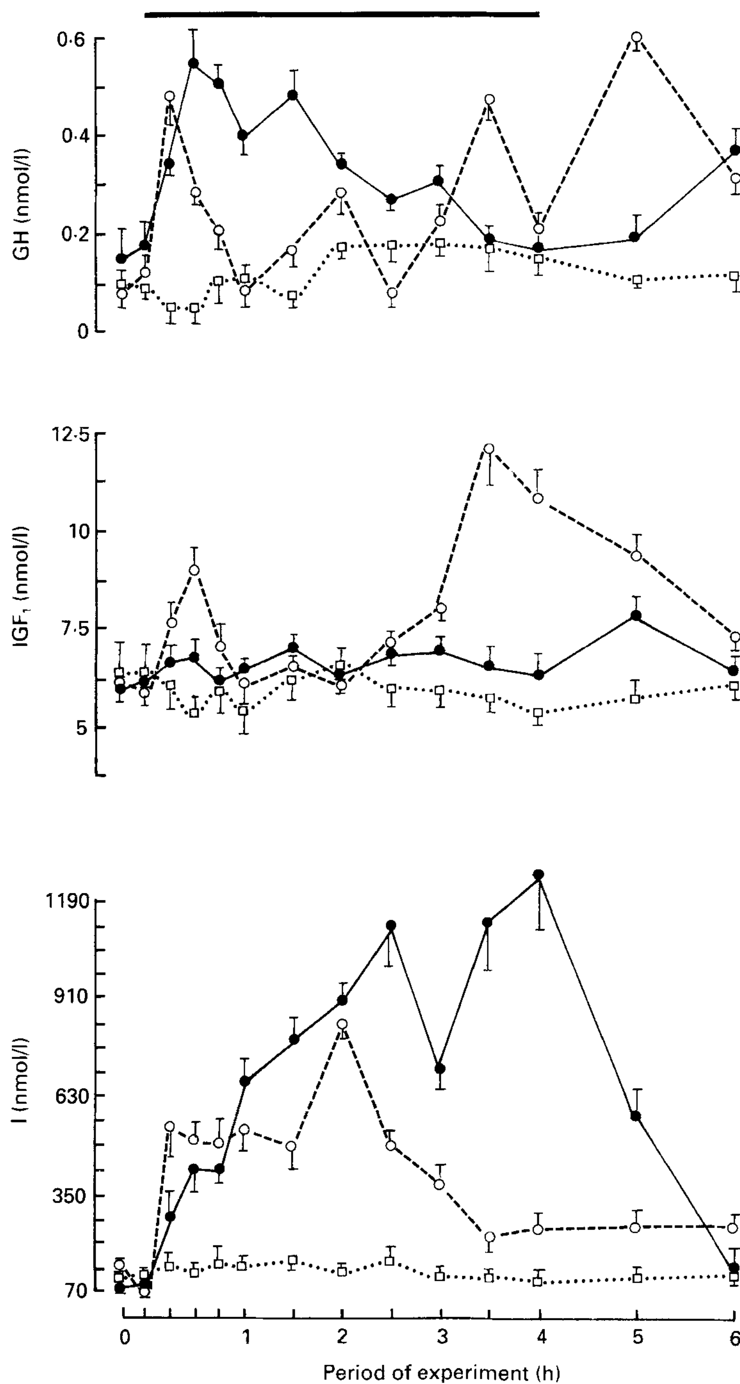

Fig. 1. Plasma growth hormone (GH), somatomedin C (IGF$_1$), insulin (I), proteins (P) and glucose (G) concentrations measured in hepatic veins of young calves infused with glucose (●——●) or vehicle (□······□). The infusion time is indicated by the upper horizontal black bar. Points are mean values with their standard errors represented by vertical bars. Control milk-fed animals (○---○).

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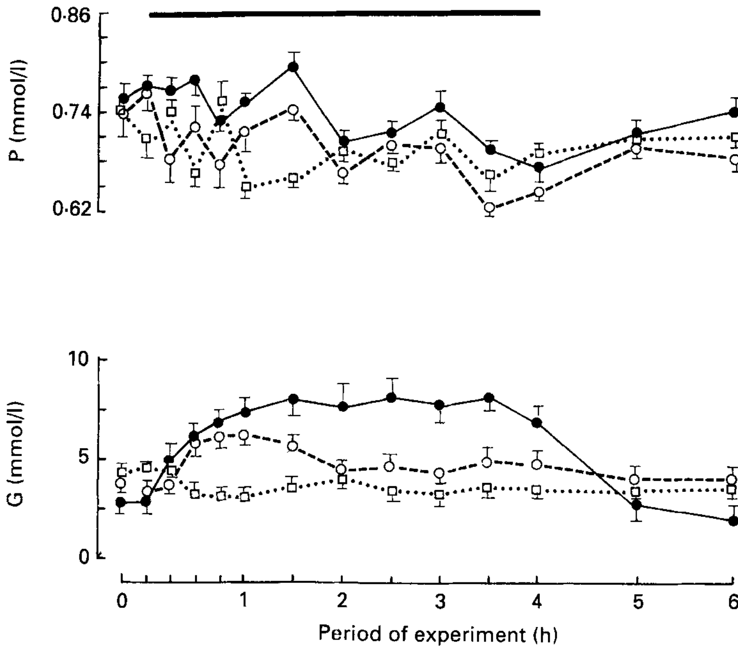

Fig. 1 contd.

$P < 0.01$; insulin 133 (SE 13) *v.* 451 (SE 46), $P < 0.01$; IGF$_1$ 6·45 (SE 0·15) *v.* 8·50 (SE 0·55), $P < 0.01$) (Figs. 1, 2 and 3). In the same way, compared with fasting, milk ingestion induced a significant increase in mean (HV, PV and HA) plasma glucose concentration (mmol/l) (5·5 (SE 0·4) *v.* 3·9 (SE 0·2), $P < 0.01$) and a significant decrease in mean plasma NEFA levels (mmol/l) (0·8 (SE 0·1) *v.* 1·9 (SE 0·1), $P < 0.01$). Feeding did not modify plasma protein nor triglyceride concentrations (mmol/l) (mean values in all three vessels: 0·70 (SE 0·02) and 0·3 (SE 0·02, respectively)) (Figs. 1, 2 and 3). However plasma IGF$_1$ concentrations in the three cannulated hepatic vessels, related to blood flow measurement (ml/min per kg BW) (mean value in fasted calves: PV 39·2 (SE 0·4), HA 6·6 (SE 0·3) *v.* PV 44·7 (SE 1·2), HA 6·3 (SE 0·4) in fed animals), demonstrated a lower IGF$_1$ balance in fasted animals compared with IGF$_1$ production measured in fed calves (Table 1).

Effects of glucose infusion

Compared with fasting calves, glucose infusion led to an increase in mean plasma glucose concentrations for 240 min (8·8 (SE 0·5) *v.* 3·9 (SE 0·2) mmol/l, $P < 0.01$) as well as in plasma insulin levels (683 (SE 83) *v.* 133 (SE 13) nmol/l, $P < 0.01$) but did not modify plasma IGF$_1$ concentrations (6·91 (SE 0·53) *v.* 6·45 (SE 0·15) nmol/l). However, 15 min after starting the glucose infusion, plasma GH concentrations reached a peak value (0·52 (SE 0·04) nmol/l, $P < 0.01$) and persisted at a high level for at least 6 h (mean value 0·32 (SE 0·04) nmol/l, $P < 0.01$) (Fig. 1). Under glucose treatment the mean IGF$_1$ hepatic balance for 6 h was 1·2 (SE 0·3) nmol/kg BW (Table 1).

Effects of amino acid infusion

Compared with values measured in fasting calves, amino acid infusion led to a significant increase in plasma protein concentrations (0·83 (SE 0·04) *v.* 0·69 (SE 0·01) mmol/l, $P < 0.05$)

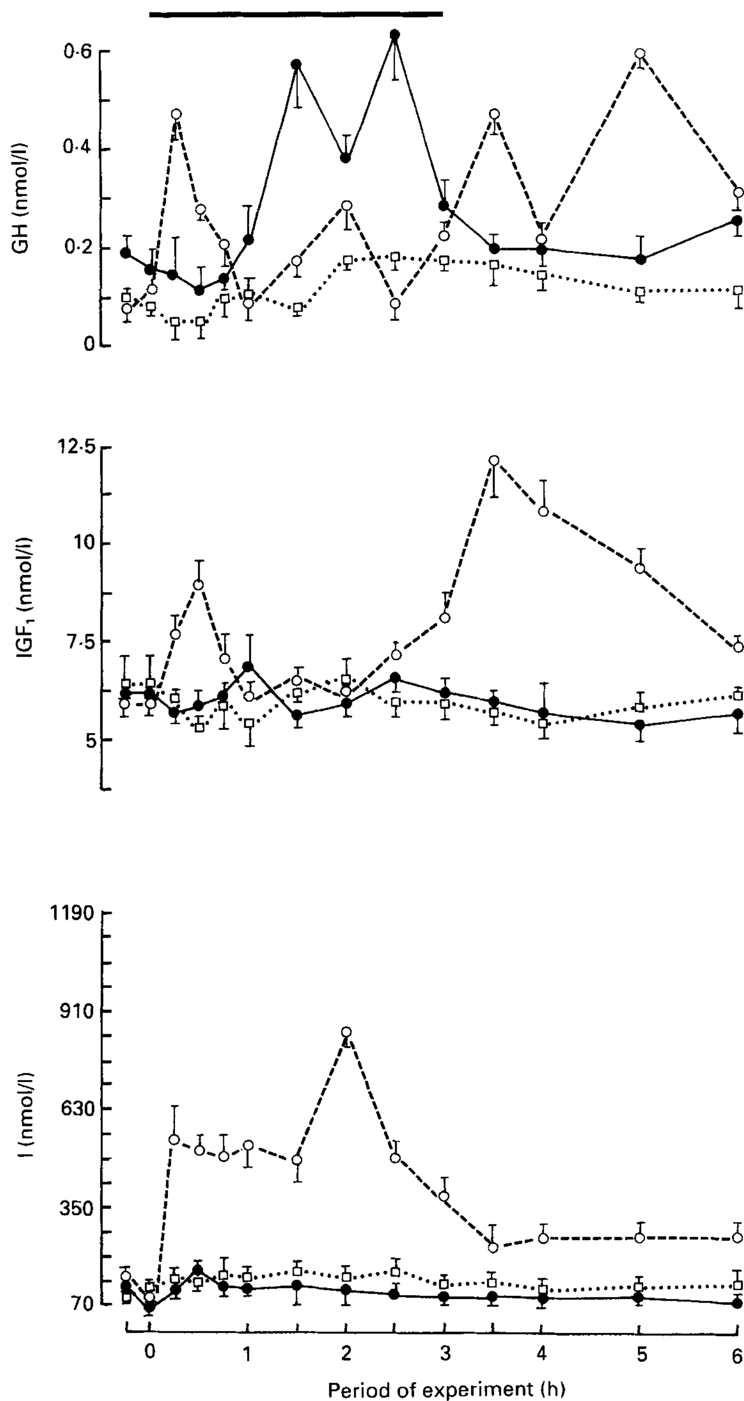

Fig. 2. Plasma growth hormone (GH), somatomedin C (IGF_1), insulin (I), proteins (P) and glucose (G) concentrations measured in hepatic veins of young calves infused with amino acid solution (Azonutril, R. Bellon, Neuilly sur Seine) (●——●) or vehicle (□······□). The infusion time is indicated by the upper horizontal black bar. Points are mean values with their standard errors represented by vertical bars. Control milk-fed animals (○---○).

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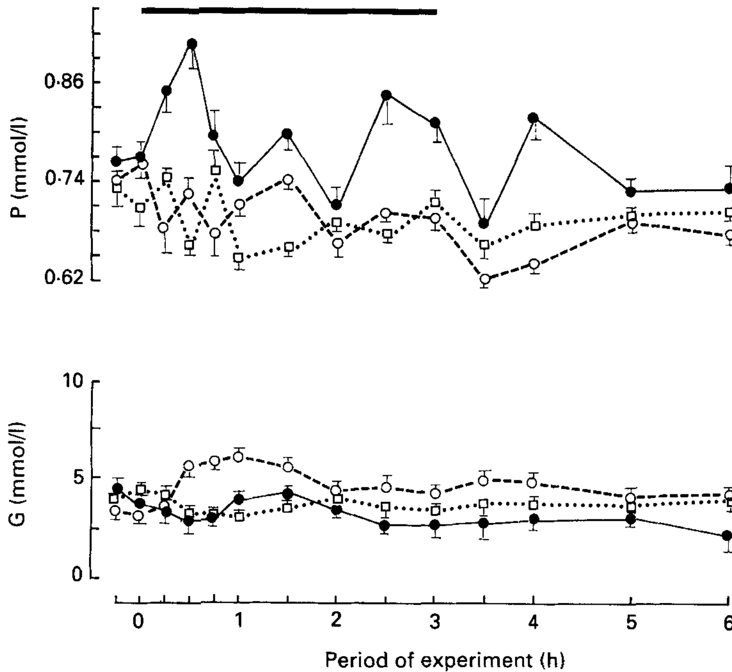

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(Fig. 2). It induced a threefold increase in plasma GH concentrations beginning at 90 min and persisting for the rest of the infusion period (mean value 0·50 (SE 0·07) *v*. 0·16 (SE 0·02) nmol/l at the start of infusion, $P < 0·01$). However, amino acid treatment did not modify plasma IGF_1 concentrations (6·54 (SE 0·40) *v*. 6·50 (SE 0·27) mnol/l at the start of infusion), plasma glucose concentrations (mean value 3·70 (SE 0·30) mmol/l) or plasma insulin concentration (113 (SE 1) nmol/l). Under amino acid treatment, the mean IGF_1 hepatic balance for 6 h was 0·7 (SE 0·3) nmol/kg BW (Table 1).

Effects of lipid administration

At 15 min after starting chylomicron administration at a rate of 10·5 mg/h per kg BW, an increase in mean plasma GH was observed (0·58 (SE 0·05) nmol/l, $P < 0·01$). A simultaneous increase in mean plasma IGF_1 concentrations was also observed (HV 11·8 (SE 0·8), HA 6·2 (SE 0·9), PV 6·4 (SE 1·1) nmol/l, $P < 0·01$), and both persisted for at least 300 min (Fig. 3). However, when triglycerides alone (Intralipid) were infused, plasma GH or IGF_1 concentrations began to increase only 300 min after starting the infusion. In both cases, plasma NEFA levels were not different from those measured in fed animals (mean values 0·7 (SE 0·1) and 0·8 (SE 0·2) mmol/l respectively). Chylomicron administration induced a slow and progressive increase in plasma triglyceride concentration at 240 min (0·55 (SE 0·15) mmol/l, $P < 0·01$), while Intralipid treatment was immediately associated with high levels of triglyceride (mean values 0·6 (SE 0·1) *v*. 0·2 (SE 0·01) mmol/l in fasted calves, $P < 0·01$) (Fig. 3). Under chylomicron or Intralipid treatments the mean IGF_1 hepatic balances (nmol/kg BW) were 5·3 (SE 0·8) and 3·5 (SE 0·4) respectively *v*. 1·1 (SE 0·02) in fasted calves (Table 1).

Neither glucose, nor amino acid, nor lipid infusion significantly modified HA or PV blood flows.

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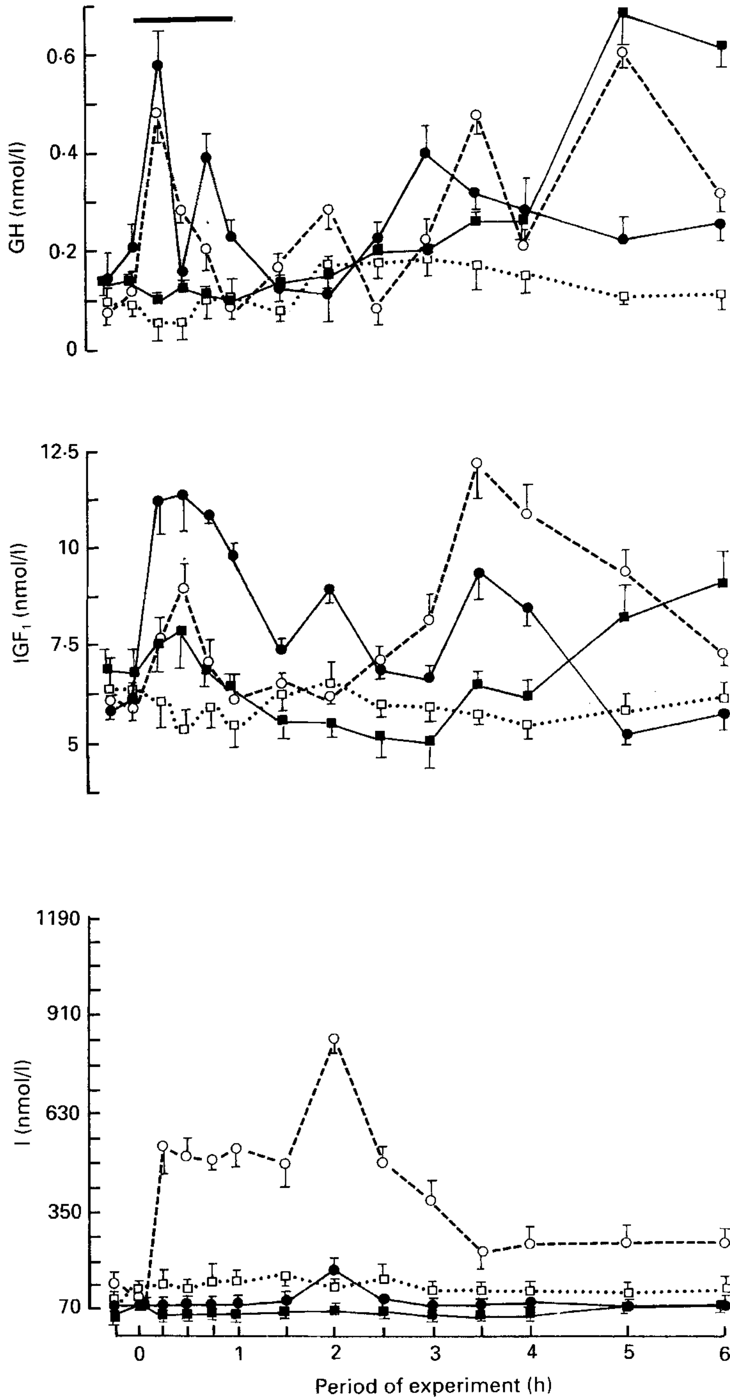

Fig. 3. Plasma growth hormone (GH), somatomedin C (IGF_1), insulin (I), triglycerides (TR), non-esterified fatty acids (NEFA), proteins (P) and glucose (G) concentrations measured in hepatic veins of young calves infused with chylomicrons (●——●), Intralipid (Kabivitrum SA, Noisy le Grand) (■——■) or vehicle (□······□). The infusion time is indicated by the upper horizontal black bar. Points are mean values with their standard errors represented by vertical bars. Control milk-fed animals (○---○).

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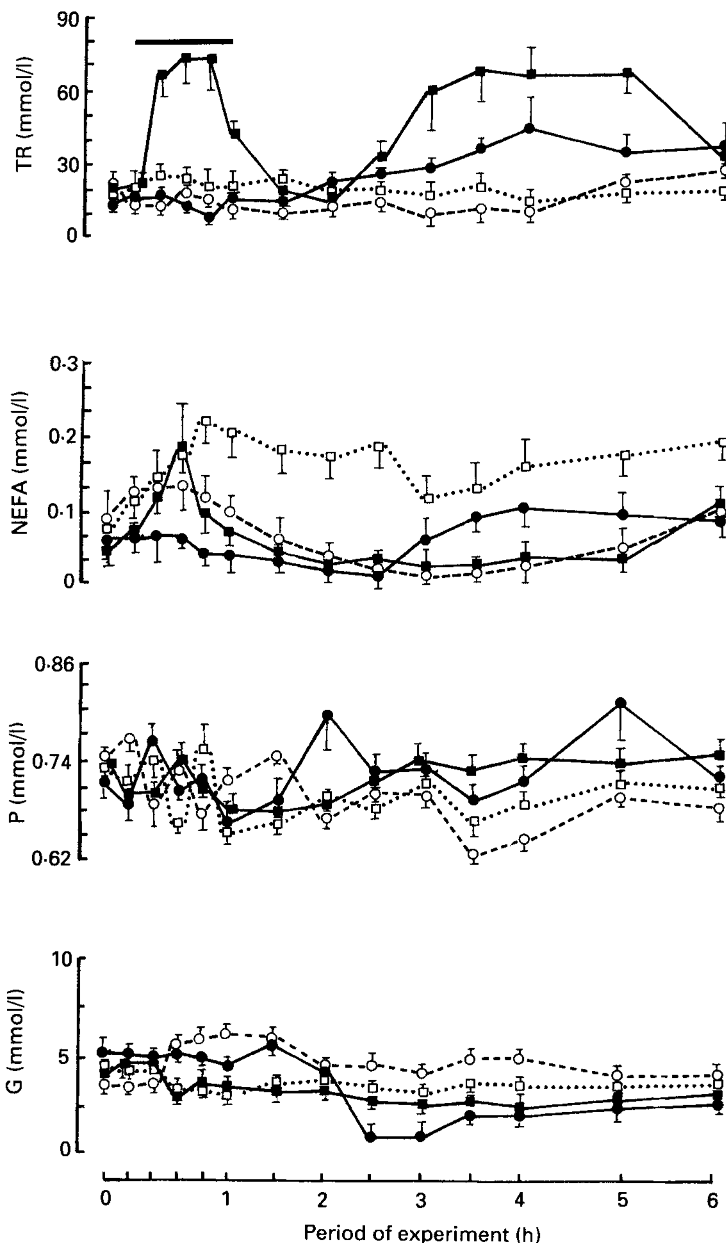

Fig. 3 contd.

DISCUSSION

A number of tissues are able to generate IGF_1, which would have an endocrine (McConaghey, 1972) or autocrine-paracrine (Canalis *et al.* 1989) role. However, an important regulation of IGF_1 activity occurs in the liver (Schimpff *et al.* 1976; Coxam *et al.* 1988*b*). Thus the present study in rapidly growing young calves reflects nutritional regulation of hepatic IGF_1 production. However, before commenting on the possible physiological significance of the observed events, some of the methodological aspects of the

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study have to be discussed. First, the experimental conditions were free from any evidence of stress, as shown by the mean daily weight gain of the animals (1·02 (SE 0·10) kg). Second, experiments were performed on fasted animals with stable metabolite concentrations. Third, all the treatments were administered at physiological doses in order to mimic postprandial metabolic shifts. Finally, most studies of GH secretion are of limited value in that the assessment of GH release is based either on infrequent sampling or sampling over very short periods; this was the reason for taking blood samples for at least 6 h in the present study.

As previously demonstrated in man (Clemmons *et al.* 1981), rats (Maes *et al.* 1983) and dogs (Eigenman *et al.* 1985), plasma IGF_1 concentrations are reduced during fasting and increased with refeeding. Starvation of humans (Roth *et al.* 1963) results in sustained increased plasma GH concentrations, whereas starvation of pigs (Machlin *et al.* 1968) gives only a transient increase in plasma GH. On the other hand, Machlin *et al.* (1968) have shown that sheep starved for 7 d do not increase their blood GH concentrations and, as previously described (Coxam *et al.* 1987), our calves decreased their plasma GH concentration during fasting.

The correlation between plasma glucose concentration and GH concentration in the present study corresponds well with the assumption of Hunter (1968) that acute variations in the availability of energy substrates, such as glucose, seem to be primarily regulated by the acute GH secretion in humans, contrary to what has been suggested in adult cattle (McAtee & Trenkle, 1971). Nevertheless, glucose infusion has been shown to depress plasma GH concentrations in men (Glick *et al.* 1965). However, feeding decreases plasma GH concentrations in humans (Hintz *et al.* 1978) while it increases them in calves (Coxam *et al.* 1987). On the other hand, our results indicate that plasma glucose concentration did not seem to be an important regulator of IGF_1 secretion in calves (Fig. 1). No other information concerning glucose influence on plasma IGF_1 levels is now available.

The infusion of amino acids resulted in an increase in plasma GH concentrations (Fig. 2). This confirms previous findings. Effectively, arginine infusion into fasted heifers increases plasma GH levels (McAtee & Trenkle, 1971). Leucine also significantly increases plasma GH levels while phenylalanine treatment does not modify plasma GH concentration in sheep (Davis, 1972). In our study, amino acid infusion did not modify plasma IGF_1 concentrations. However, plasma IGF_1 levels have been shown to reflect changes in N balance induced by manipulation of nutrient intake (Clemmons *et al.* 1985). Effectively, Takano *et al.* (1978) have demonstrated an increase in plasma IGF_1 concentration following refeeding with a high-protein diet, but not after high-carbohydrate–low-protein intake (Phillips & Unterman, 1984). Furthermore, plasma IGF_1 concentrations measured in protein-restricted Leghorn and broiler chicks were only 50 and 34% of those of controls respectively (Lauterio & Scanes, 1987). Thus, further studies using different amino acids in physiological amounts in our experimental model are necessary before the nutritional and metabolic significance of these observations can be accurately assessed.

Previous studies reported an influence of NEFA on plasma GH concentrations. Thus, Hertelendy & Kipnis (1973) have shown that a rapid decrease in plasma NEFA concentrations can stimulate GH release in sheep. In the same way, a drop in serum NEFA could provoke a rise in GH plasma concentrations in calves (Reynaert & Franchimont, 1974). No such studies on the influence of lipids on plasma IGF_1 concentrations are available. However, Isley *et al.* (1983) demonstrated that both protein and energy intakes are regulators of serum IGF_1 concentrations in adult humans, and that the energy intake may be of greater importance.

Our findings emphasized the important physiological role of lipids in the regulation of the somatotrophic axis. Effectively, our experiment was performed under physiological

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conditions because we administered 10·5 mg chylomicrons/h per kg BW, while the lipoprotein flux in the portal vein at peak absorption of lipids (10 h after a milk meal) is 415 mg/h per kg BW (Laplaud *et al.* 1989). Chylomicron infusion was associated with a simultaneous elevation in plasma GH and IGF_1 concentrations, while such an increase was observed only 5 h after starting intralipid administration. In the same way, plasma IGF_1 concentrations in the three hepatic vessels related to blood flow measurement indicated that IGF_1 hepatic production was more important with chylomicron treatment than with Intralipid. On the other hand, chylomicron infusion induced a slight increase in plasma triglyceride and NEFA levels only 4 h after starting treatment, while this increase was immediate in Intralipid-infused calves. The difference between chylomicrons and triglycerides would be related to the presence of apoproteins (B/E) that are recognized by specific receptors (Brown & Goldstein, 1983). Effectively, the liver of the bovine appears able to recognize, to incorporate and to metabolize chylomicrons and very-low-density lipoproteins (VLDL) of density $< 1·018$ g/ml, as shown by Bauchart *et al.* (1989). These authors found that the hepatic balance for VLDL was markedly negative (−83%) and corresponded to a net capture of 6·4 (SE 1·2) mg/h per kg BW by the liver. Thus plasma triglycerides have to be associated with proteins (apo B/E) to be absorbed efficiently by hepatocytes (Brown & Goldstein, 1983). However, the uptake of chylomicrons or VLDL is modulated by the nature of the apoprotein (Windler *et al.* 1980). Effectively, native lipoproteins have to be metabolized into remnants, i.e. chylomicrons have to lose apo C to be recognized by the liver, the half-life of native chylomicrons being very short. This also supposes that the lipoprotein lipase acts in the neighbourhood of hepatocytes. Under standard conditions it acts in extrahepatic tissues, but a fraction can be found in systemic circulation (T. Olivecrona, unpublished results). Thus apoproteins, and more especially apo B/E, appear to be important. This would explain the better hepatic utilization of chylomicrons compared with Intralipid.

Thus, although further investigation of the probable role of lipid metabolism on the GH–IGF_1 axis is clearly warranted, our studies suggest the important role of lipids in the regulation of hepatic IGF_1 production. However, our results do not indicate whether the apoprotein or the lipid moiety of chylomicrons (or VLDL) is able to stimulate the hepatic release of IGF_1. The infusion of apo VLDL or apo B would be of great interest.

The observations reported here provide evidence on the roles of end-products of digestion in the regulation of the somatotrophic axis. Effectively, under the various dietary conditions studied, plasma GH concentrations are correlated with the intake of digestible nutrients, and confirm that in young milk-fed calves, as in adult sheep (Bassett, 1974), the metabolic disposition of absorbed nutrients is hormonally regulated.

Our results are the first which demonstrate that lipids appear to play a major role in the regulation of hepatic IGF_1 production.

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