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At the same hepatic amino acid load, portal infusion of amino acids is more efficient than peripheral infusion in stimulating liver protein synthesis in the dog

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Abstract

Background—Hepatic glucose uptake is enhanced by portal delivery of glucose which creates a negative arterio-portal substrate gradient. Hepatic amino acid (AA) utilization may be regulated by the same phenomenon, but this has not been proven.

Objective—We aimed to assess hepatic AA balance and protein synthesis with or without a negative arterio-portal AA gradient.

Design—Somatostatin was infused IV, and insulin and glucagon were replaced intraportally at 4- and 3-fold basal rates, respectively, in 3 groups (n=9 each) of conscious dogs with catheters for hepatic balance measurement. Arterial glucose concentrations were clamped at 9 mM. An AA mixture was infused IV to maintain basal concentrations (EuAA), intraportally to mimic the post-meal AA increase (PoAA), or IV (PeAA) to match the hepatic AA load in PoAA. Protein synthesis was assessed with a primed, continuous [^{14}C]leucine infusion.

Results—Net hepatic glucose uptake in PoAA was ≤50% of that in EuAA and PeAA ($P<0.05$). The hepatic intracellular leucine concentration was 2- to 2.5-fold greater in PoAA and PeAA than EuAA ($P<0.05$); net hepatic leucine uptake and ^{14}C leucine utilization were ≈2-fold greater ($P<0.05$) and albumin synthesis was 30% greater ($P<0.05$) in PoAA than EuAA and PeAA, Phosphorylation of ribosomal protein S6 (downstream of the mammalian target of Rapamycin complex 1 [mTORC1]) was significantly increased in PoAA, but not PeAA, vs EuAA.

Conclusions—Portal, but not peripheral, AA delivery significantly enhanced hepatic protein synthesis under conditions where AA, glucose, insulin and glucagon did not differ at the liver, an effect apparently mediated by mTORC1 signalling.

Keywords

liver; protein synthesis; portal vein; amino acids

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INTRODUCTION

Protein synthesis in the liver and intestines is considerably higher per gram tissue than in most other tissues, accounting for 25% of whole-body protein synthesis (1). Forty percent of the protein synthesized in the liver is secreted, with albumin alone accounting for almost 40% of secreted proteins. Following a mixed meal, the liver is exposed to high levels of amino acids (AAs) and insulin. In adult humans, increasing amino acid (AA) levels by intravenous infusion results in a stimulation of protein synthesis across the splanchnic bed (2).

Splanchnic metabolism includes both gut and hepatic metabolism, for which regulatory pathways could be different. Studies conducted in mature animals with intravenous infusion of AAs have shown that AAs and insulin have no stimulatory effect on liver protein synthesis (3–5). Conversely, in the neonate, hepatic protein synthesis can be stimulated by the rise in AAs (6–7). Davis et al. (7) have observed a developmental decline in AA-induced stimulation of hepatic protein synthesis in the growing animal. Similarly, aging is associated with a decline in the anabolic response of skeletal muscle to food intake and AAs (8–10). From these studies, one might infer that there is a decreased sensitivity of the liver to AAs during aging. However, this inference is inconsistent with findings in adult human volunteers, in whom dietary AAs have been shown to increase hepatic secretory protein synthesis (11–14).

One difference between the animal and human studies cited above is the route of administration of AAs: peripherally in most of the animal studies versus orally in most of the human investigations. We hypothesized that, in the mature organism, the route of delivery of AAs is critical in the stimulation of hepatic protein synthesis. In support of this hypothesis, human studies have shown that albumin synthesis is stimulated with oral meal feeding whereas it is not responsive to intravenous nutrients (4,12). Furthermore, we have shown that the net hepatic uptake of glutamine and the net fractional extractions of glutamine and serine are significantly increased during the portal vein administration of AAs compared to a peripheral infusion matching hepatic AA loads (15). From the latter study, it is also tempting to hypothesize that the portal delivery of the AAs directed the AAs to hepatic protein synthesis. We therefore designed the present study to compare, using the adult conscious dog, the rate of hepatic protein synthesis observed during portal or peripheral delivery of an AA mixture that reproduces the normal elevation of AAs seen in the portal vein in the post-prandial state. Because protein metabolism is sensitive to AA availability, the concentration and the infusion rate of the AA mixtures were adjusted to maintain the same hepatic AA load between groups under pancreatic clamp conditions. The synthesis rate of resident or exported proteins was then assessed, as was the intracellular signalling pathway leading to protein synthesis.

MATERIALS AND METHODS

Animals and Surgical Procedures

Experiments were performed on twenty seven 42-h fasted conscious adult male mongrel dogs (20–25 kg) that had been fed once daily a standard meat and kibble diet (31% protein, 52% carbohydrate, 11% fat, and 6% fiber based on dry weight, Kal Kan; Vernon, CA; and Purina Lab Canine Diet no. 5006, Purina Mills; St. Louis, MO). The dogs were housed in a facility that met American Association for the Accreditation of Laboratory Animal Care guidelines, and protocols were approved by the Vanderbilt University Medical Center Animal Care Committee. At least 16 days before experimentation, a laparotomy was performed with animals under general anesthesia. Silastic catheters (Dow Corning; Midland, MI) for blood sampling were placed into the portal vein, a hepatic vein, and a femoral artery, and infusion catheters were inserted into a jejunal vein and a splenic vein as previously described (16). Ultrasonic flow probes (Transonic Systems; Ithaca, NY) were placed around the portal vein and hepatic artery. On the day of the experiment, the catheters were exteriorized under local anesthesia,

and intravenous access was established in three peripheral veins. Dogs were used for an experiment only if they met established criteria for good health (17).

Experimental design

In each of the three groups (n = 9 dogs each), the protocol consisted of an equilibration period (0–90 min) and a basal period (90–120 min), followed by an experimental period (120–300 min) (Figure. 1). At time (t) = 0 min, a continuous infusion of indocyanine green dye (0.08 mg/min, Sigma Chemical; St. Louis, MO) was started. At t = 120 min, the timer was stopped briefly. A peripheral venous infusion of somatostatin (0.8 $\mu g \cdot kg^{-1} \cdot min^{-1}$) was begun to inhibit endogenous pancreatic hormone secretion. Two minutes later, an intraportal insulin infusion (1.2 $mU \cdot kg^{-1} \cdot min^{-1}$; to produce 3- to 4-fold basal circulating concentrations) was initiated. Two minutes after the insulin infusion started, an intraportal glucagon infusion (1.65 $ng \cdot kg^{-1} \cdot min^{-1}$; to produce 3-fold basal concentrations) was started. Two minutes later, dextrose and AA infusions were initiated, and the timer was restarted. Over many years, we have demonstrated that this staggered approach to initiating infusions is effective in controlling insulin and glucagon concentrations. A dextrose solution was infused peripherally at variable rates starting at t = 120 min to clamp the arterial plasma glucose level at 9.0 mM. The infusion rate of glucose was adjusted in response to the plasma glucose concentration, which was measured every 5 min. The euaminoacidemia group (EuAA) received a mixture of AAs peripherally to maintain the plasma AAs at their post-absorptive levels since insulin is known to decrease plasma AA levels; the portal group (PoAA) received AAs intraportally to mimic a meal, and the peripheral group (PeAA) received AAs peripherally. Because protein metabolism is sensitive to the AA availability, the concentration of the AA mixtures were adjusted to maintain hepatic AA load equivalence for each amino acid in the PoAA and PeAA groups. AA concentrations of the infusates (Table 1) were determined in pilot experiments, and the mixture was infused at the rate of 0.034 ml $kg^{-1} \cdot min^{-1}$. Protein synthesis was assessed in all groups by a primed (7.6 μCi/kg), continuous infusion (0.76 μCi/kg/min) of [U-^{14}C] leucine (Moravek Biomedicals, Brea, CA) beginning at 120 min. Blood was collected on EDTA. At the end of the 300 min experimental period, animals were sacrificed and samples of each liver lobe were rapidly freeze clamped and stored at −80°C.

Analytical procedures

Plasma glucose, insulin and glucagon levels were measured as previously described (18). Plasma amino acids were analyzed by reverse-phase HPLC of their phenyl isothiocyanate derivatives (PicoTag, Waters, Woburn, MA). Free and bound leucine specific radioactivities were determined as described by Donahue et al (19). Plasma albumin was isolated by ethanol extraction from trichloroacetic acid precipitated plasma proteins as described previously (4).

Methods of calculation

Hepatic load (μmol $kg^{-1} \cdot min^{-1}$)—The load of a substrate or hormone reaching the liver (HL) was calculated as

$$HL = [F_a \times C_a + F_p \times C_p]$$

where F_a and F_p are the arterial and portal flows (ml/kg/min) and C_a and C_p are the concentrations of the variable in question in arterial and portal blood or plasma, as appropriate.

Estimated hepatic plasma sinusoidal hormone concentrations were calculated as HL/total hepatic plasma flow.

Net Hepatic Substrate Balance (nmol $kg^{-1} \cdot min^{-1}$)—Whole blood glucose concentrations and plasma AA concentrations were used, along with blood or plasma flow, as appropriate, to assess net hepatic substrate balance (NHSB). NHSB was determined as

$$NHSB=(F_h \times C_h) - HL$$

where F_h represents blood or plasma flow in the hepatic vein (the sum of F_a and F_p) and C_h represents the substrate concentrations in the hepatic vein. A positive value represents net production and a negative value represents net uptake of the substrate by the liver.

Hepatic leucine uptake (nmol $kg^{-1} \cdot min^{-1}$)—Calculations were based on the differences between the specific radioactivity (Sa) of [^{14}C] leucine entering and exiting the liver (Sa_{in} and Sa_h) and the tracee concentration across the liver (Cin and Ch).

$$Sa_{in}=(Sa_{art} \times F_{art}+Sa_{port} \times F_{port})/F_h$$

where Sa_{art}, Sa_{port} are the hepatic artery and portal vein leucine specific activities and F_{art} and F_{port} are the hepatic and portal vein blood flows, respectively.

$$Cin=(C_{art} \times F_{art}+C_{port} \times F_{port})/F_h$$

where C_{art} and C_{port} are the hepatic artery and portal vein tracee concentrations.

$$\text{Net hepatic leucine balance}=(C_h \times F_h) - (Cin \times F_h)$$

$$\text{Hepatic leucine utilization}=(Sa_{in} \times Cin - Sa_h \times C_h) \times F_h/Sa_{liver}$$

where Sa liver is the liver intracellular specific radioactivity of the tracer.

Protein synthesis rate—Protein synthesis was calculated using the continuous infusion of [^{14}C]leucine as a tracer. Briefly, a primed continuous infusion of [^{14}C]leucine was used to rapidly reach a [^{14}C]leucine Sa plateau in the plasma. A single sample of the product (liver proteins or albumin) was then used and not the incremental increase in specific activity of protein-bound leucine across multiple time points. Liver protein and albumin synthesis rates (Ks in % per day) were calculated using the following equation:

$$(Sa_b \times 100/Sa_{tissue}) \times 1440/t$$

where Sa_b, Sa_{tissue} are the specific radioactivities at time t of the protein-bound leucine and the specific radioactivity of the leucine precursor pool between time 0 and time t. The Sa of the tissue fluid was used as the Sa of the precursor pool since Ahlman et al (5) showed it to be a reliable surrogate measure of leucyl-tRNA specific radioactivity (the real precursor pool for protein synthesis) in a swine model. Protein synthesis was measured in the seven lobes of the liver since it appeared from our preliminary studies that slight differences in the rate of protein synthesis might occur among lobes. Because labelled albumin appears in the blood stream with a lag time of 20 minutes after the beginning of the tracer infusion (secretion time determined in a pilot experiment), the calculation of albumin synthesis was identical to that of protein

synthesis except that it was calculated with a t = t-20, 20 representing the average excretion time in minutes of newly synthesized plasma proteins by the liver.

Measurement of ribosomal protein S6, ribosomal protein S6 kinase 1 (S6K1), eukaryotic initiation factor (eIF)4E binding protein 1 (4E-BP1), and eIF4G Phosphorylation

Phosphorylation of ribosomal protein (rp) S6 on Ser235/236 and Ser240/244 and eIF4G phosphorylation on Ser1108 was measured by Western blot analysis as described previously (20) using anti- phospho-rpS6(Ser235/236) and anti-phospho-rpS6(Ser240/244) antibodies or anti-phospho-eIF4G(Ser1108) antibodies, respectively (Cell Signalling Technology, Beverly, MA). Phosphorylation of S6K1 and 4E-BP1 was measured as changes in migration during gel electrophoresis as described previously (20). Briefly, frozen liver samples were homogenized in 7 volumes of buffer consisting of 20 mM HEPES (pH 7.4), 100 mM KCl, 0.2 mM EDTA, 2 mM EGTA, 50 mM NaF, 50 mM β-glycerophosphate, 0.1 mM phenylmethylsulfonyl fluoride, 1 mM benzamidine, and 0.5 mM sodium vanadate using a Polytron homogenizer. The homogenate was centrifuged at 1,000 × g for 3 min and the supernatant was subjected to Western blot analysis using antibodies that recognize the protein only when it is phosphorylated on specific amino acid residues (rpS6 and eIF4G) or antibodies that recognize the protein regardless of phosphorylation state.

Statistical Analysis

Data are expressed as means ± SE and analyzed by XLStat (Addinsoft NY, USA, version 7.5.2). For net hepatic glucose balance, albumin synthesis, blood flows and sinusoidal hormone concentrations, the statistical evaluation of the data was performed by a 2-way repeated-measures analysis of variance to test the group and time effects and time × group interaction. When significant, the Bonferroni test was employed for post hoc analysis. Differences were considered significant when $P < 0.05$.

For protein metabolism (hepatic leucine balance, hepatic leucine utilization and hepatic protein synthesis rates), statistical evaluation of the data was performed by one-way ANOVA to analyse the effect of the route of delivery of AAs. Within the same animal of each group, values were calculated as the mean of individual values recorded between 210 and 300 min. When a significant overall effect was detected, differences among individual means were assessed with the Bonferroni test to determine significant differences. Differences were considered significant when $P<0.05$.

RESULTS

Hormone concentrations, blood flows and glucose balance data

Arterial blood flows were not different between groups but increased slightly during the experimental period. Portal blood flows were also not different between groups but significantly decreased during the experimental period (Table 2). As a result, total hepatic blood flow changed minimally (<10%) in all groups (not shown). Hepatic sinusoidal insulin concentrations increased by a physiological amount during the experimental period (150–300 min) (Table 2), with no significant differences among the groups. Hepatic sinusoidal glucagon concentrations increased ≈3-fold during the experimental period (150–300 min) compared to the basal period (90–120 min) and were similar in all the groups (Table 2).

The arterial plasma glucose concentration during the basal period and during the AA infusion period did not differ among groups (Figure. 2). The hepatic glucose load increased significantly during glucose infusion and reached 178±10, 175±6 and 172±6 μmol/kg/min in EuAA, PeAA and PoAA, respectively. In the basal period, the animals of the 3 groups exhibited similar net hepatic glucose output at a rate of 10.2±0.8, 8.8±1.4 and 8.2±0.5 μmol/kg/min in EuAA, PeAA

and PoAA, respectively (Figure 2). During the AA infusion period, they shifted to net hepatic glucose uptake (NHGU). With a mean rate of −3.6±1.5 μmol/kg/min (between 210–300 min), NHGU in the PoAA group was significantly lower when compared to the EuAA (−7.7±0.7 μmol/kg/min) and PeAA (−5.7±0.9 μmol/kg/min) groups (Figure 2).

Amino acid concentrations and arterio-portal venous gradient

Hepatic AA loads were similar in the 3 groups (except for arginine) during the basal period (Table 3). Hepatic AA loads in the EuAA group were maintained at the basal values during the experimental period for each amino acid. Hepatic amino acid loads increased in the PeAA and PoAA groups when compared to EuAA (except for Gly, Ala and Cys) but were similar in the PeAA and PoAA groups (except for Trp) (Table 3). During the AA infusion, the total hepatic load of AAs (sum of all AA loads) increased significantly but similarly in PeAA and PoAA compared to EuAA (82.3± 4.3 and 82.5±7.7 compared to 52.6±3.4 μmol/kg/min, respectively). In order to maintain the matching of hepatic AA loads in PeAA and PoAA, the portal infusion of AAs resulted in arterial AA concentrations lower than with the ones generated with the peripheral AA infusion used in the PeAA group (except for Trp and Asp) (Table 3). The PeAA and PoAA groups differed by the generation of a negative arterio-portal venous gradient (A-P gradient) of AAs (i.e., more negative when AAs were infused intraportally; Table 3). The A-P gradient in the PeAA group for most of the AAs was no different from that observed in the post-absorptive state (i.e., the EuAA group).

In the liver, the intracellular leucine concentration increased significantly in the PeAA and PoAA groups compared to EuAA (Figure 3). This increase ranged from 2 to 2.5 times depending of the liver lobe considered, but the same intracellular leucine concentration was observed whatever the route of delivery of the AAs (PeAA or PoAA) (Figure 3).

Hepatic leucine balance and hepatic protein synthesis

During the experimental period, no significant differences in the net hepatic leucine, valine or isoleucine balances was seen with the peripheral AA infusion. By contrast, portal AA infusion to create the same hepatic leucine, valine and isoleucine loads increased net hepatic leucine, valine and isoleucine uptake in comparison with PeAA (Figure 4A). Similar results were observed with other AAs including tryptophan, lysine, glutamine, glutamate and tyrosine (data not shown).

Arterial plasma leucine specific radioactivity was constant and similar among the 3 groups throughout the experimental period (210 to 300 min) and this confirms that a steady state of leucine kinetics was achieved in all groups (Figure 4B). Leucine specific radioactivities were similar in portal plasma, 74.4± 3.9; 71.6± 4.2 and 71.3± 4.3 dpm/nmol in EuAA, PeAA and PoAA, respectively (mean values between 210–300min). Hepatic tracer balance showed similar results to the net hepatic leucine balance. Only the portal infusion of AA was associated with increased leucine uptake into the liver compared to EuAA (Figure 4C).

Liver protein synthesis was measured in the 7 lobes at 300 min (i.e., after 180 min of AA infusion). Hepatic leucine specific radioactivities were 29.4±1.9, 41.6±2.8 and 36.8±3.9 dpm/nmol in EuAA, PeAA and PoAA, respectively. Peripheral delivery of AAs did not increase hepatic protein synthesis significantly, whereas portal delivery stimulated the fractional synthesis in all the liver lobes (Figure 5). The stimulatory effect of portal delivery of AAs was also found on exported hepatic proteins. Indeed, albumin synthesis was significantly increased by 30% whereas peripheral AA infusion had no significant effect compared to EuAA (Figure 5).

Signalling pathways

Because of its central role in mediating increases in protein synthesis, mTORC1 signaling was assessed in the livers of each group. As an index of alterations in mTORC1 signalling, the phosphorylation status of the downstream target, rpS6, on Ser235/236 and Ser240/244 was examined by Western blot analysis. This target was chosen because it was expected to be less subject to transient changes over such a long experimental protocol. As shown in Figure 6, AA infused peripherally had no significant effect on phosphorylation of rpS6 on residues Ser240/244, whereas there was a trend ($P=0.07$) for increased rpS6 phosphorylation on residues Ser235/236. In contrast, AA infused portally led to a significant increase in phosphorylation of rpS6 at both Ser235/236 and Ser240/244 compared to EuAA values. In addition, portal AA delivery produced a significant ($P<0.05$) increase in phosphorylation on Ser235/236 and tended ($P=0.1$) to increase phosphorylation on Ser240/244 compared to peripheral delivery. Activation of mTORC1 was also monitored by assessment of the phosphorylation status of S6K1 as well two other proximal targets of the kinase, i.e. 4E-BP1 and eIF4G. Although all three mTORC1 substrates showed the same pattern of increased phosphorylation as rpS6 in response to portal versus peripheral delivery of AA, the magnitude of the change at the 180 min time point was less than that of rpS6 and did not reach statistical significance. For example, phosphorylation of 4E-BP1, expressed as a percentage of the protein present in the hyperphosphorylated γ-form relative to the total protein, was 59.5±0.8, 61.37±0.7, and 84.2 ±3.8 for control, AA infused peripherally, and AA infused portally. Likewise, phosphorylation of eIF4G on SAer1108, expressed in arbitrary units, was 0.27±0.03, 0.36±0.04, and 0.42±0.1 for control, AA infused peripherally, and AA infused portally, respectively.

DISCUSSION

Peripheral delivery of AAs to produce plasma concentrations seen postprandially did not stimulate hepatic protein synthesis significantly compared with euaminoacidemia, but synthesis of resident liver protein and albumin increased significantly (30%) with portal AA delivery. The stimulation of hepatic protein synthesis with portal delivery of AAs could not be attributed to differences in the hepatic AA load or in insulin, glucagon or glucose concentrations. Thus the route of delivery of AAs is critical for the stimulation of liver protein synthesis in the adult dog.

In support of our observation, human studies have shown that albumin synthesis is stimulated with oral meal feeding, whereas it is not responsive to intravenous nutrients (4,12). Moreover, 20 to 96% of enterally administered AA are utilized by the splanchnic bed, showing the crucial role of the splanchnic tissues in the distribution and availability of dietary AAs to peripheral tissues (21–24). Interestingly, recent studies conducted in the pig show that the dietary requirement for AAs (lysine, methionine, leucine, valine, isoleucine, and threonine) is lower with total parenteral nutrition than with enteral nutrition (21,25–28), consistent with an increased utilization of the dietary AAs by the gut and liver. Complementary results have shown that dietary AAs are the preferential source of substrate for hepatic protein synthesis (29–31). In the post-prandial state, the AA concentrations are higher in portal vein than in hepatic artery blood (32). However, a difference in AA availability could not explain our results since the hepatic loads of AAs were carefully matched. It is possible that the portal delivery of AA initiates a signal to the liver that stimulates hepatic AA utilization, i.e., protein synthesis. Several studies conducted in our laboratory suggest the existence of an AA-initiated portal signal.

Previously we carried out a series of studies to examine the role of AAs and their route of delivery (peripheral vein vs portal vein infusions) on NHGU in the conscious dog. With a fixed hormonal milieu (4× basal insulin; 1× basal glucagon) and a portal glucose infusion, portal gluconeogenic AA infusion reduced NHGU and net hepatic glycogen synthesis by 50% and

30%, respectively (33). This decrease could have originated from the hepatic AA load itself (i.e, competition between substrates for hepatic uptake) or it could have been specific to the intra-portal route of AA delivery. To answer this question (34), we studied a group of dogs under identical conditions to our previous studies except that the gluconeogenic AAs were delivered peripherally at a rate that would maintain the hepatic AA load equivalent to that existing in the previous studies. Peripheral delivery of AA did not reduce NHGU initiated by portal glucose delivery. This observation is thus consistent with the hypothesis that intra-portal delivery of AAs generates a signal that competes with or modulates the signal that enhances NHGU during portal glucose delivery. In the present experiments, similar conclusions can be drawn, as the portal delivery of all AAs also decreased NHGU when compared to the peripheral AA infusion. In the study of Moore et al. (33), with the peripheral AA infusion, the net hepatic uptake of glucose and AAs (in carbon equivalents) together equalled the net hepatic glycogen deposition and lactate release. With the portal AA infusion, only 70% of their uptake could be accounted for glycogen synthesis and lactate release. Our present study strongly suggests that at least a portion of the remaining 30% of carbon extracted by the liver was then redirected to protein synthesis. Taken together, our data indicate that a portal signal may activate liver protein synthesis. The portal concentrations of almost all of the AAs were statistically indistinguishable in PoAA and PeAA, suggesting that the negative A-P gradient of AAs initiates the signal.

Whether portal AAs modulate the activity of the autonomic nervous system to increase hepatic protein synthesis is unknown. However, the involvement of neuronal input in controlling liver glucose metabolism has already been shown (see 35 for a review)), and Niijima et al. (36–37) have described hepato-portal sensors for 15 different AAs serving as stimulators or inhibitors of the vagal afferent discharge rate. Moreover, Watanabe et al. (38) postulated that plasma protein synthesis is enhanced by vagal-nerve stimulation.

The present study shows that portal, but not peripheral, delivery of AAs leads to a significant increase in phosphorylation of rpS6 on Ser235/236 and Ser240/244. Both sets of sites are phosphorylated by the rpS6 kinase, S6K1 (39). Because mTOR phosphorylates S6K1 (40), an event that is critical for maximal activation of the kinase, this finding suggests that portal AA delivery increases mTORC1 signalling in liver to a greater extent than peripheral delivery. Phosphorylation of S6K1 and 4E-BP1 was maximally increased 60 min after re-feeding fasted rats, and returned toward control values within 180 min (41). In contrast, phosphorylation of rpS6 was maintained through the 180 min time point. Therefore, because the liver samples in the current study were collected following 180 min of amino acid infusion, rpS6 phosphorylation should be a better indicator of mTORC1 activation. Previous studies by our laboratory and others have provided evidence in support of the concept that albumin mRNA is translationally regulated in response to the availability of nutrients. Yap et al. (42) showed that, following a 24 h fast in rats, albumin mRNA shifts out of polysomes and accumulates in 30–50S ribonucleoprotein complexes. Moreover, within 1 h of feeding a complete meal, the albumin mRNA redistributes back into polysomes. Studies in perfused rat liver (43) suggested that the translation of albumin mRNA is repressed by deficiency of essential AAs. Kuwahata et al (44), using acute liver injury in rats as an experimental model, showed that polysome-associated albumin mRNA increases in proportion to the content of branched-chain AAs during total parenteral nutrition. Finally, a cDNA microarray analysis of polysome-associated mRNAs over a time course following meal feeding to fasted rats demonstrated translational control of albumin mRNA (43). Taken together, these data indicate that the mTORC1 signaling pathway is intimately involved in regulating albumin mRNA translation, which helps explain the increase in albumin production observed in PoAA. Leucine modulates the activity of the signalling pathway leading to the initiation of protein translation (45–47). However, intracellular leucine concentrations were identical in the PoAA and PeAA livers, suggesting that the stimulation of the mTOR pathways in the liver is also under the control of signal inputs that are different from the leucine pathways and initiated specifically by the portal AA infusion.

In addition to actions on the mTOR signalling pathway, portal vs peripheral infusion of AAs might impact upon liver protein synthesis via other mechanisms, such as the metabolic sensors AMP-activated protein kinase or general control nonderepressible 2 kinase (GCN2) (48).

Although mTORC1 is a central mediator of the increase in protein synthesis engendered by increased provision of amino acids, other signalling pathways may also be involved in the response. For example, GCN2 is activated in response to deprivation of essential amino acids (49). Upon activation, GCN2 phosphorylates the α-subunit of eIF2 leading to inhibition of the first step in translation initiation. Thus, eIF2α phosphorylation is increased in livers from rats fed a diet lacking either Trp or Leu compared to animals fed a complete meal or a diet lacking the nonessential amino acid Gly (50). However, in the present study there was no difference in eIF2α phosphorylation in livers from dogs infused with amino acids peripherally compared to portally (0.82±0.04 vs 0.79±0.08 arbitrary units, respectively). The basis for the lack of change in eIF2α phosphorylation in the present study is unknown, but is likely related to amino acids being provided in a balanced manner (i.e. one or more essential amino acids were not limiting). In this regard, in previous studies (e.g. 51), no change in eIF2α phosphorylation was observed in liver in response to an overnight fast or after refeeding overnight fasted animals.

In conclusion, portal delivery of AAs stimulated hepatic synthesis of resident and exported proteins whereas peripheral delivery of AAs did not, despite similar AA concentrations within the liver. Our results suggest that a portal signal was generated with the portal infusion of AAs which was necessary to fully stimulate hepatic AA utilization and hepatic protein synthesis. Thus, enteral nutrition may be preferred over parenteral in stimulating or maintaining liver protein synthesis in humans. In contrast, an enhanced splanchnic extraction of AAs may be deleterious in sustaining AA provision to peripheral tissues such as skeletal muscle. This has been suspected, for example, to be responsible for the defect in the post-prandial stimulation of muscle protein synthesis during aging. When muscle protein synthesis is of key importance, our findings suggest that a peripheral infusion of AAs may be advantageous over a portal AA infusion to spare AAs.

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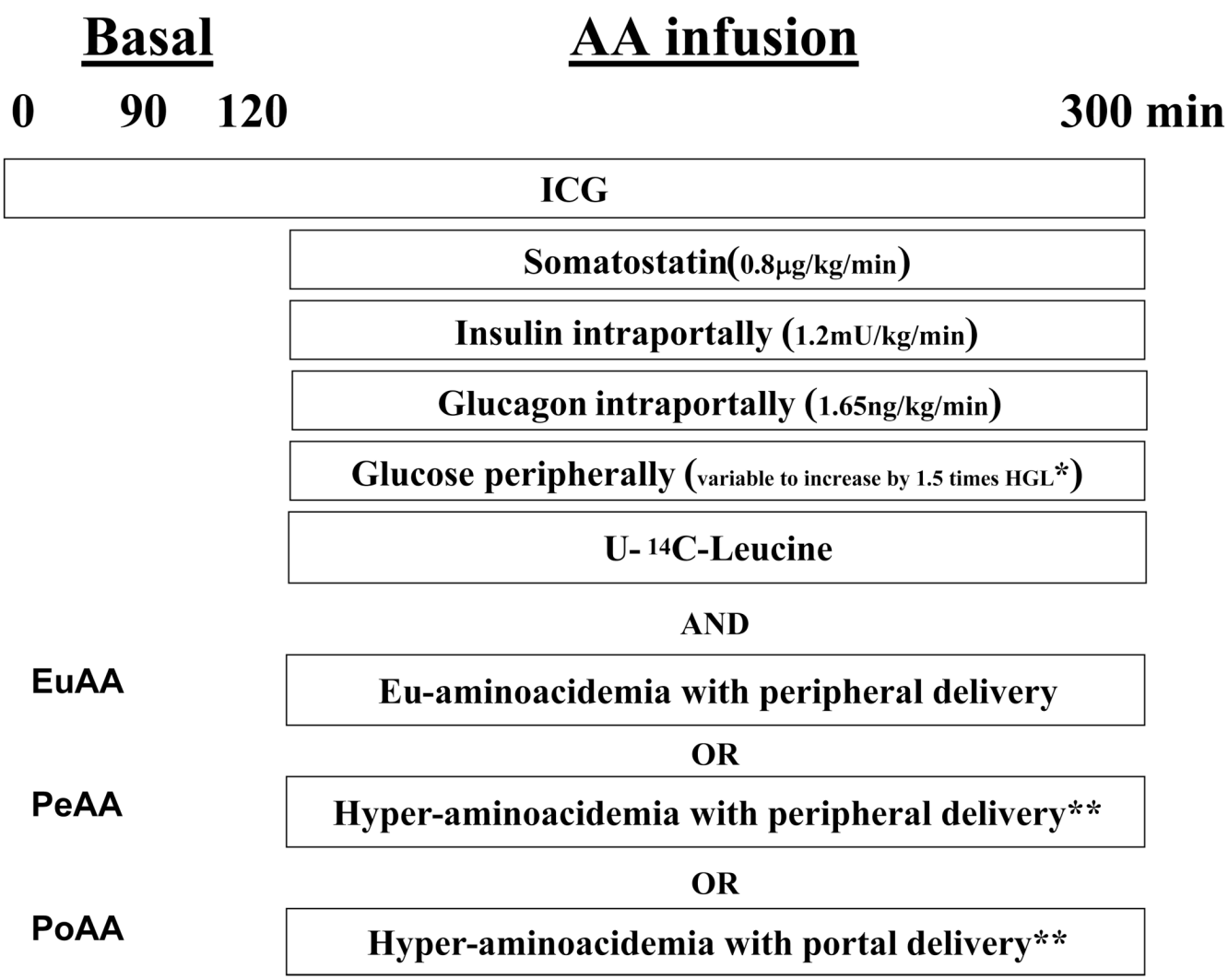

Figure 1.
Experimental design. * HGL= Hepatic glucose load; ** same amino acid hepatic load.

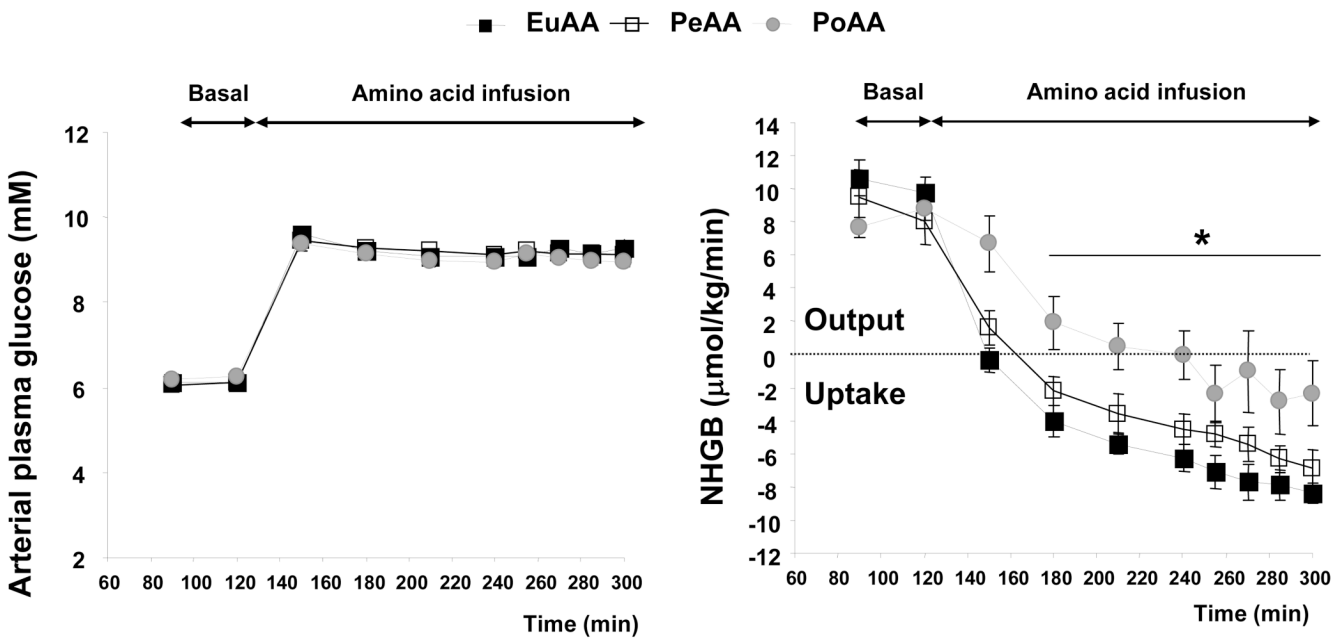

Figure 2.

Portal amino acid infusion reduced net hepatic glucose uptake (NHGU). Amino acids were infused as described in Figure 1 and Table 1. Arterial, portal and hepatic blood samples were drawn at time indicated in the figure. Glucose concentration was determined and net hepatic glucose balance (NHGB) was calculated as described in “Materials and Methods”. Results represent the mean ± SE of 9 animals for each group. The statistical evaluation of the data was performed by a 2-way repeated-measures analysis of variance to test the group and time effects and time × group interaction. When significant, the Bonferroni test was employed for post hoc analysis. Differences were considered significant when $P < 0.05$.

Time effect $P<0.0001$, Group effect $P<0.0001$ and Time × Group interaction $P<0.011$.* PoAA significantly different from both EuAA and PeAA. PeAA not significantly different from EuAA

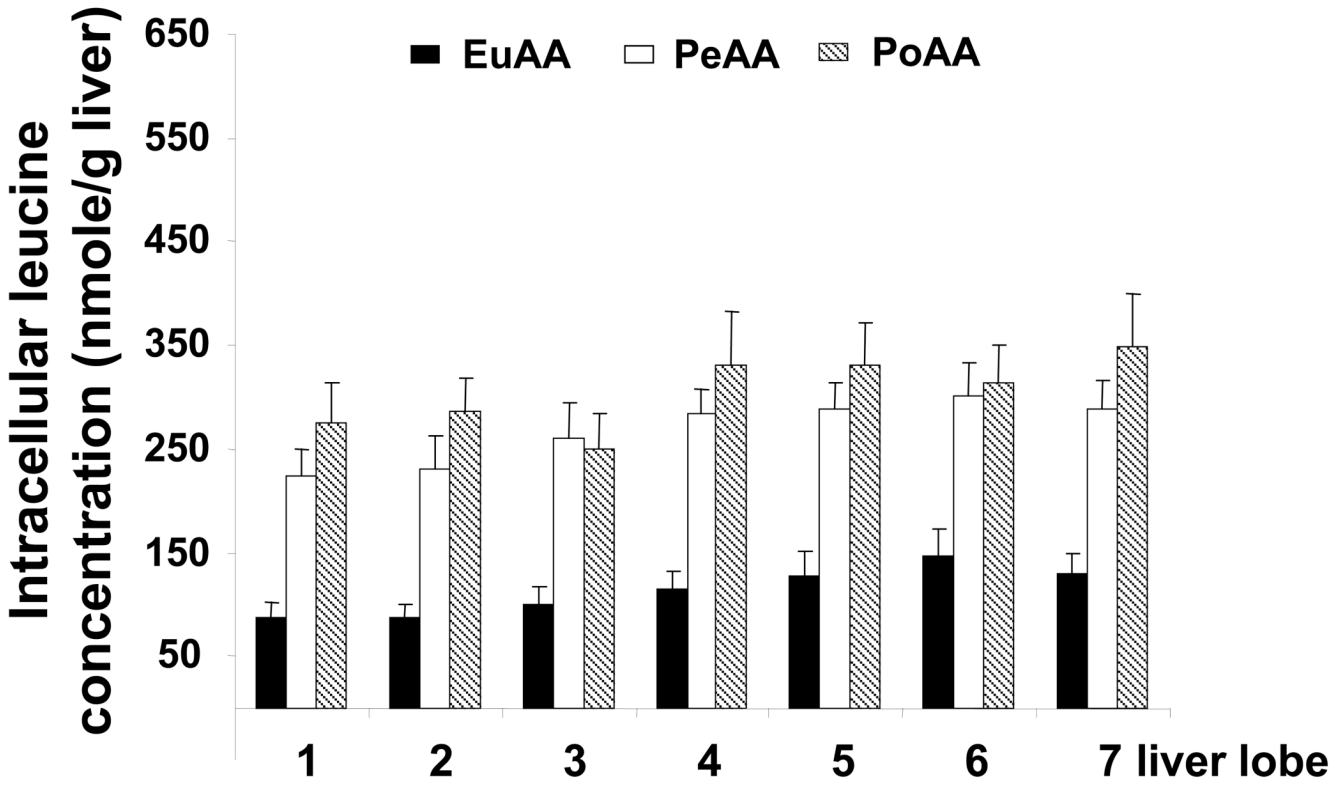

Figure 3.

Intracellular leucine concentration in the liver lobes of the dogs within the three experimental groups. At the end of the infusion period, liver samples were quickly removed and frozen in liquid nitrogen and stored at −80°C until analyzed. Liver samples were prepared as described in “Materials and Methods” and leucine was determined as described in Donahue et al. (19). The results represent the mean ± SE of 9 animals for each group. The statistical evaluation of the data was performed by a 2-way repeated-measures analysis of variance to test the group and lobe effects and lobe × group interaction. When significant, the Bonferroni test was employed for post hoc analysis. Differences were considered significant when $P < 0.05$. Group effect $P<0.0001$, Lobe effect $P<0.036$ and no significant Lobe × Group interaction. EuAA significantly different from PoAA and PeAA.; PeAA not significantly different from PoAA.

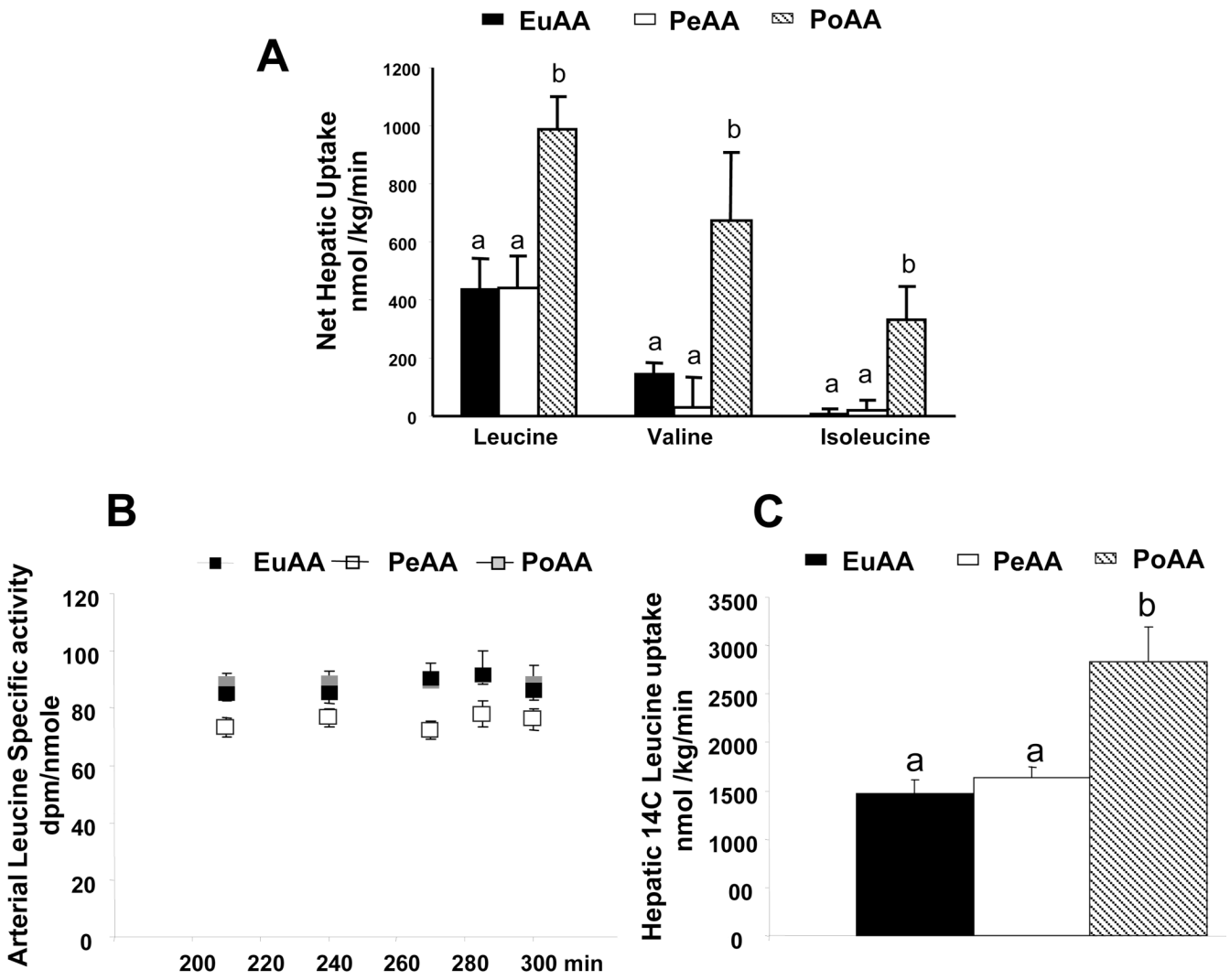

Figure 4.

Arterial specific radioactivity of ^{14}C leucine, net hepatic branched chain amino acid balance and net ^{14}C leucine utilization. Amino acids were infused as described in the legend to Figure 1 and Table 1. Arterial, portal and hepatic blood samples were drawn at the time indicated in the figure. Leucine and leucine SA were determined as described in "Materials and Methods". The results represent the mean ± SE of 9 animals for each group. The statistical evaluation of the data was performed by a one-way measures analysis of variance to test the group effect. The Bonferroni test was employed for post hoc analysis. Values with different letters are significantly different, $P < 0.05$.

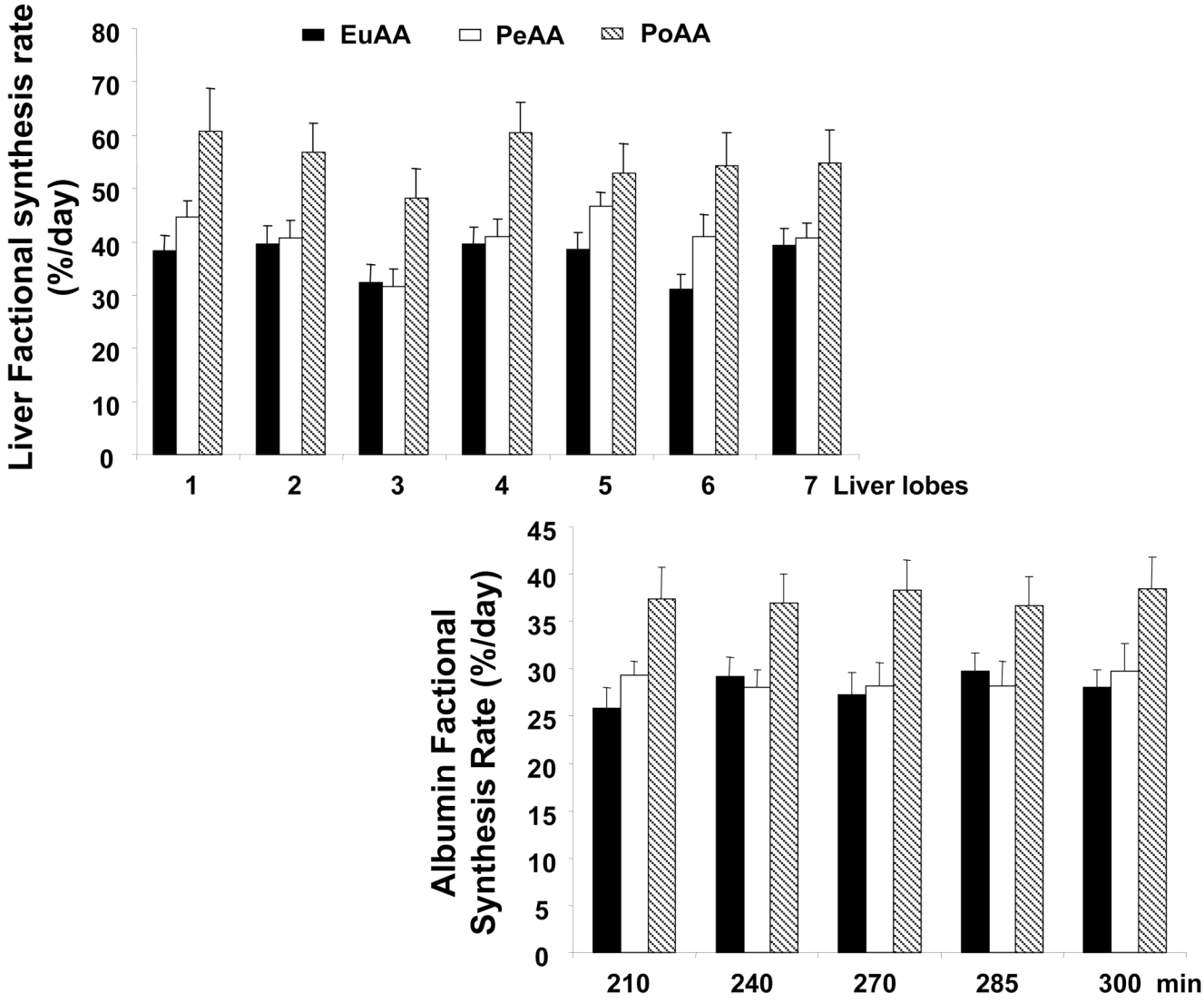

Figure 5.

Hepatic and albumin fractional protein synthesis rates. Amino acids were infused as described in the legend to Figure 1 and Table 1. Arterial, portal and hepatic blood samples were drawn at times indicated in the figure. At the end of the infusion period, liver samples were quickly removed and frozen in liquid nitrogen and stored at −80°C until analyzed. Liver samples were prepared as described in "Materials and Methods" and leucine and leucine SA were determined as described in Donahue et al. (19). Liver protein synthesis was analyzed on the seven hepatic lobes in each dog. Albumin synthesis was determined kinetically from 210 to 300 min. The results represent the mean ± SE of 9 animals for each group.

For liver synthesis, the statistical evaluation of the data was performed by a 2-way measures analysis of variance to test the group and lobe effects and lobe × group interaction. When significant, the Bonferroni test was employed for post hoc analysis. Differences were considered significant when $P < 0.05$. Group effect $P<0.0001$; Lobe effect $P<0.025$ and no significant interaction interaction. PoAA significantly different from EuAA and PeAA. PeAA not significantly different to EuAA

For albumin synthesis, the statistical evaluation of the data was performed by a 2-way repeated-measures analysis of variance to test the group and time effects and time × group interaction.

When significant, the Bonferroni test was employed for post hoc analysis. Differences were considered significant when $P < 0.05$. No significant time effect; Group effect $P<0.0001$ and no significant interaction. PoAA significantly different from EuAA and PeAA. PeAA not significantly different from EuAA

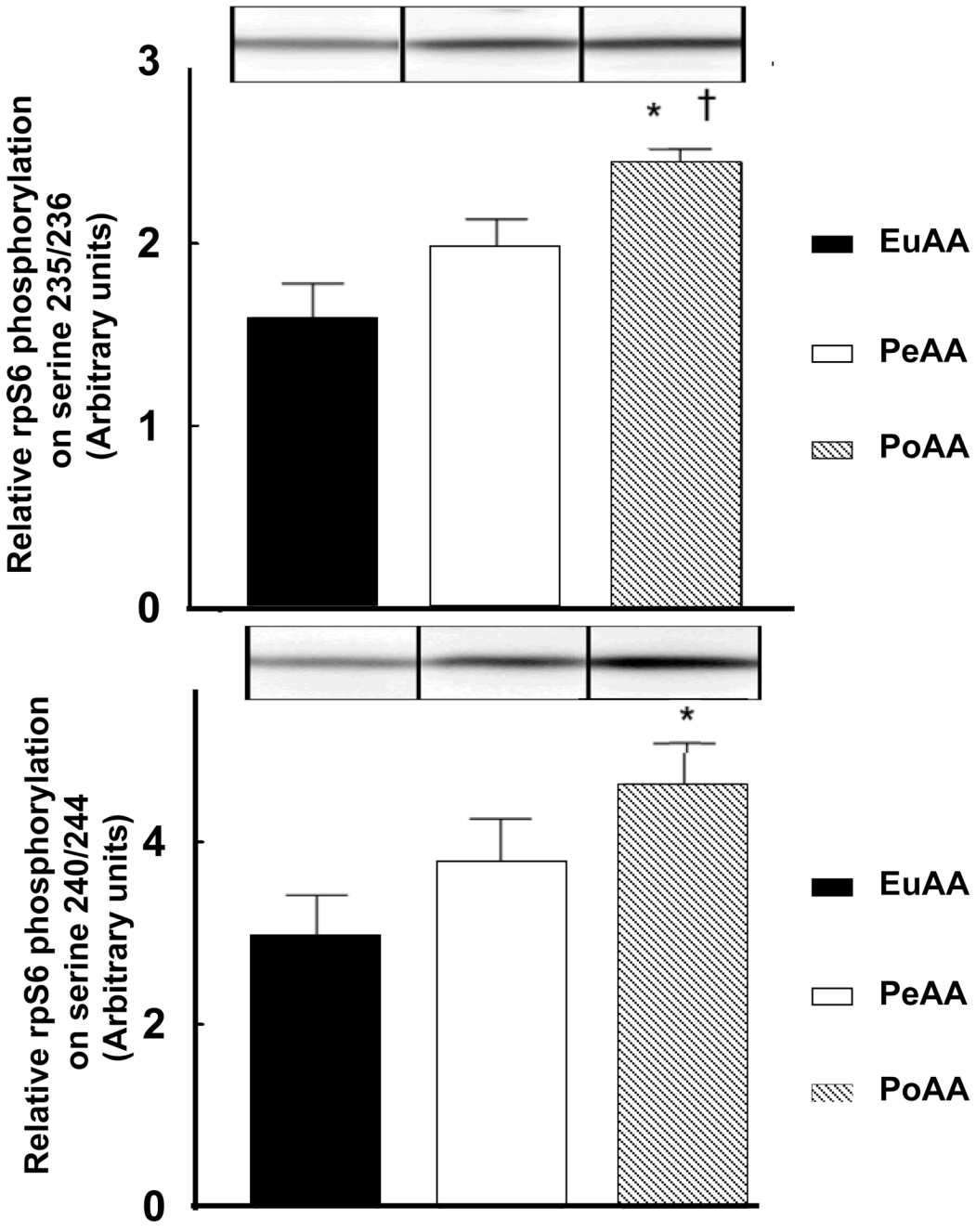

Figure 6.

Amino acids infused portally, but not peripherally, activate mTORC1 signaling as indicated by increased phosphorylation of rpS6. Amino acids were infused as described in Figure 1 and Table 1. At the end of the infusion period, liver samples were quickly removed and frozen in liquid nitrogen and stored at −80°C until analyzed. Phosphorylation of rpS6 on (A) Ser235/236 and (B) Ser240/244 was measured by Western blot analysis as described under “Materials and Methods”. In addition, samples were analyzed for eEF2 content to demonstrate equal loading of protein onto the gel (see insert to panel A). eEF2 was used as a loading control because its expression did not change under the experimental conditions used in this study. The results represent the mean ± SE of 9 animals for each group. Representative Western blots are shown

in the insets to each panel. The statistical evaluation of the data was performed by a one-way measures analysis of variance to test the group effect. The Bonferroni test was employed for post hoc analysis. * $P<0.002$ vs. EuAA; † $P<0.05$ vs. peripheral amino acid condition.

Table 1

Concentration of the aminoacid mixture infused in the three experimental groups

	Amino acid infusate concentration μM		
	EuAA	**PeAA**	**PoAA**
Arginine	32.1	62.0	40.2
Histidine	6.7	11.6	11.4
Isoleucine	21.7	74.6	32.8
Leucine	45.7	137.2	70.1
Lysine	54.7	102.6	64.3
Methionine	11.6	31.5	14.4
Threonine	35.9	84.0	60.8
Tryptophane	8.2	11.8	13.7
Valine	27.3	102.5	56.0
Phenylalanine	9.7	36.3	21.8
Alanine	35.9	107.8	88.0
Asparagine	15.5	30.6	16.7
Aspartate	2.4	8.0	3.3
Glutamate	16.3	54.4	21.8
Glutamine	153.3	178.0	150.6
Glycine	51.4	45.3	31.0
Serine	26.1	66.6	39.8
Tyrosine	13.2	17.7	11.9
Cysteine	6.6	17.1	21.5
Proline	27.8	93.8	52.1

Table 2

Hepatic plasma flows, hepatic sinusoidal insulin and glucagon concentrations

	Basal	Amino acid infusion					
Time (min)	**90**	**120**	**150**	**210**	**240**	**270**	**300**	**Variance analysis**
Arterial flows (ml/kg/min)								
EuAA	3.1±0.2	3.0±0.2	4.0±0.3	4.3±0.4	4.1±0.4	4.5±0.4	4.5±0.4	Group effect = NS
PeAA	3.1±0.2	3.1±0.2	3.9±0.3	4.2±0.2	3.8±0.3	4.1±0.2	4.3±0.3	Time effect P<0.001
PoAA	3.0±0.3	3.2±0.3	3.7±0.5	4.5±0.4	4.1±0.4	4.0±0.4	4.1±0.3	Time×Group =NS
Portal flows (ml/kg/min)								
EuAA	14.6±1.0	14.9±1.0	13.0±0.7	12.5±0.6	12.6±0.8	13.1±1.0	13.1±0.9	Group effect = NS
PeAA	13.6±0.8	14.0±0.9	12.4±0.9	12.4±0.8	12.6±0.8	12.9±0.8	12.9±0.9	Time effect P<0.006
PoAA	13.7±0.6	14.2±0.6	12.7±0.8	12.4±0.7	12.7±0.6	12.8±0.6	12.9±0.7	Time×Group =NS
Hepatic sinusoidal insulin (mU/ml)								
EuAA	17.1±1.6	15.4±2.4	75.8±9.6	74.7±5.7	60.1±8.8	63.9±9.2	74.9±10.7	Group effect = NS
PeAA	16.1±2.1	20.0±5.3	63.5±4.8	72.1±11.2	70.5±5.7	73.4±4.2	82.7±4.4	Time effect P<0.0001
PoAA	16.8±3.1	20.7±3.8	88.2±18.4	74±8.1	69.8±7.3	83.7±6.3	78.2±9.2	Time×Group =NS
Hepatic sinusoidal glucagon (pg/ml)								
EuAA	53.2± 4.7	49.6± 9.0	144.6±16.0	138.7±16.5	134.0±11.3	142.7±13.7	124.0±16.2	Group effect = NS
PeAA	42.8±6.9	41.6±6.2	110.3±16.5	112.2±5.3	113.0±5.4	103.1±4.3	107.7±7.7	Time effect P<0.001
PoAA	30.7±4.2	32.4±3.8	122.3±15.7	90.6±7.8	96.5±10.5	98.2±7.8	106.6±13.6	Time×Group =NS

Data are expressed as means ± SE (n=9 in each group. The statistical evaluation of the data was performed by a 2-way repeated-measures analysis of variance to test the group and time effects and time × group interaction. When significant, the Bonferonni test was employed for post hoc analysis. Differences were considered significant when $P < 0.05$. NS= non significant

Table 3

Arterial and portal amino acid concentrations, hepatic aminoacid load and amino acid arterio-portal gradient in the three experimental groups EuAA, PeAA and PoAA

		Arterial concentration µM		Portal concentration µM		Hepatic load (µmol/kg/min)				ArterioPortal gradient µM	
		AA infusion		AA infusion		Basal		AA infusion		AA infusion	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
ALA	EuAA	459.2	42.0 ^{a}	542.8	44.7 ^{a}	7.0	0.8 ^{a}	9.1	1.0 ^{a}	−83.7	8.2 ^{a}
	PeAA	539.6	57.3 ^{b}	601.9	95.5 ^{b}	6.0	0.6 ^{a}	9.6	1.0 ^{a}	−62.3	13.8 ^{a}
	PoAA	470.2	40.4 ^{a}	777.4	101.6 ^{c}	7.8	1.0 ^{a}	11.0	1.1 ^{a}	−307.2	19.4 ^{b}
ARG	EuAA	157.8	13.5 ^{ab}	146.8	13.6 ^{a}	2.4	0.2 ^{a}	2.6	0.3 ^{a}	10.9	3.0 ^{a}
	PeAA	183.7	19.0 ^{a}	169.6	26.9 ^{a}	1.5	0.2 ^{b}	2.8	0.3 ^{a}	14.1	5.0 ^{a}
	PoAA	121.9	8.1 ^{b}	233.7	35.4 ^{b}	1.8	0.1 ^{ab}	2.9	0.3 ^{a}	−111.9	17.5 ^{b}
ASN	EuAA	78.2	6.5 ^{a}	70.1	6.7 ^{a}	1.3	0.1 ^{a}	1.3	0.2 ^{a}	8.1	3.4 ^{a}
	PeAA	107.7	10.2 ^{b}	101.8	15.3 ^{b}	1.2	0.1 ^{a}	1.7	0.2 ^{b}	5.9	2.4 ^{a}
	PoAA	60.4	4.7 ^{a}	128.3	18.7 ^{b}	1.3	0.1 ^{a}	1.7	0.2 ^{b}	−67.9	7.7 ^{b}
ASP	EuAA	7.3	1.8 ^{a}	8.7	1.9 ^{a}	0.2	0.0 ^{a}	0.1	0.0 ^{a}	−1.3	0.8 ^{a}
	PeAA	17.7	2.3 ^{b}	17.9	2.3 ^{b}	0.2	0.0 ^{a}	0.3	0.0 ^{b}	−0.2	1.2 ^{a}
	PoAA	12.2	1.5 ^{ab}	21.2	2.9 ^{b}	0.2	0.0 ^{a}	0.3	0.0 ^{b}	−9.0	1.6 ^{b}
CYS	EuAA	36.6	4.1 ^{a}	37.2	3.2 ^{a}	0.5	0.0 ^{a}	0.6	0.1 ^{a}	−0.7	1.7 ^{a}
	PeAA	55.7	6.2 ^{b}	53.1	7.7 ^{b}	0.6	0.1 ^{a}	0.9	0.1 ^{a}	2.5	1.6 ^{a}
	PoAA	24.1	5.1 ^{a}	54.3	9.4 ^{b}	0.4	0.1 ^{a}	0.7	0.1 ^{a}	−30.2	5.7 ^{b}
GLN	EuAA	754.1	32.1 ^{a}	648.3	28.7 ^{a}	13.4	1.2 ^{a}	12.7	1.0 ^{a}	105.7	8.3 ^{a}
	PeAA	1005.6	67.6 ^{b}	934.4	111.6 ^{b}	15.8	1.3 ^{a}	16.4	0.6 ^{ab}	62.4	19.3 ^{a}
	PoAA	781.3	25.5 ^{a}	989.7	125.2 ^{b}	17.5	1.7 ^{a}	19.1	2.2 ^{b}	−208.4	32.6 ^{b}
GLU	EuAA	74.7	4.2 ^{a}	80.8	4.5 ^{a}	0.7	0.1 ^{a}	0.9	0.1 ^{a}	−6.0	1.6 ^{a}
	PeAA	131.4	13.8 ^{b}	133.4	16.4 ^{b}	0.7	0.1 ^{a}	1.4	0.2 ^{b}	−2.1	3.5 ^{a}
	PoAA	79.4	5.8 ^{a}	132.5	17.5 ^{b}	0.8	0.1 ^{a}	1.5	0.1 ^{b}	−53.1	8.6 ^{b}
GLY	EuAA	319.3	34.9 ^{a}	266.6	32.0 ^{a}	4.6	0.4 ^{a}	4.7	0.5 ^{a}	52.7	13.8 ^{a}
	PeAA	313.6	21.6 ^{a}	301.5	41.5 ^{b}	4.1	0.4 ^{a}	5.0	0.5 ^{a}	12.2	11.4 ^{b}
	PoAA	206.2	16.8 ^{b}	317.4	47.2 ^{b}	4.6	0.4 ^{a}	4.6	0.5 ^{a}	−111.2	22.3 ^{c}
HIS	EuAA	57.3	3.2 ^{a}	56.2	3.4 ^{a}	1.0	0.1 ^{a}	1.0	0.1 ^{a}	1.1	1.6 ^{a}
	PeAA	87.9	13.2 ^{b}	86.2	11.0 ^{b}	1.0	0.1 ^{a}	1.4	0.2 ^{b}	1.7	1.8 ^{a}
	PoAA	59.4	6.1 ^{a}	87.8	12.9 ^{b}	0.9	0.1 ^{a}	1.3	0.1 ^{b}	−28.4	4.1 ^{b}

		Arterial concentration μM		Portal concentration μM		Hepatic load (μmol/kg/min)				ArterioPortal gradient μM	
		AA infusion		AA infusion		Basal		AA infusion		AA infusion	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
ISO	EuAA	63.3	6.6 [a]	56.1	5.7 [a]	1.1	0.1 [a]	1.0	0.1 [a]	7.2	2.4 [a]
	PeAA	169.5	24.2 [b]	152.8	28.1 [b]	1.3	0.2 [a]	2.6	0.4 [b]	16.7	5.5 [b]
	PoAA	89.4	6.3 [a]	177.1	24.2 [b]	1.3	0.1 [a]	2.3	0.2 [b]	−87.8	8.0 [c]
LEU	EuAA	232.6	14.7 [a]	219.0	16.3 [a]	3.4	0.3 [a]	3.8	0.3 [a]	13.5	6.7 [a]
	PeAA	412.3	45.8 [b]	392.1	65.2 [b]	3.0	0.3 [a]	6.5	0.8 [b]	20.2	8.0 [a]
	PoAA	211.5	19.1 [a]	382.2	55.8 [b]	2.7	0.3 [a]	5.2	0.6 [b]	−170.6	17.1 [b]
LYS	EuAA	214.2	14.7 [a]	199.5	20.0 [a]	2.7	0.3 [a]	2.4	0.3 [a]	9.8	4.9 [a]
	PeAA	364.9	26.4 [b]	361.5	46.0 [b]	2.8	0.2 [a]	5.2	0.3 [b]	3.0	11.3 [a]
	PoAA	231.0	19.2 [a]	390.6	50.3 [b]	2.6	0.6 [a]	5.4	0.5 [b]	−159.6	20.6 [b]
MET	EuAA	55.6	2.9 [a]	50.5	2.8 [a]	0.8	0.1 [a]	0.9	0.1 [a]	5.1	2.0 [a]
	PeAA	99.9	14.5 [b]	93.9	18.0 [b]	0.9	0.1 [a]	1.5	0.2 [b]	5.9	3.2 [a]
	PoAA	44.4	4.5 [a]	87.8	13.2 [b]	0.9	0.1 [a]	1.5	0.2 [b]	−43.5	4.7 [b]
PHE	EuAA	50.0	2.8 [a]	45.1	2.7 [a]	0.9	0.1 [a]	0.8	0.1 [a]	4.9	2.1 [a]
	PeAA	109.4	6.9 [b]	107.5	14.6 [b]	0.9	0.1 [a]	1.8	0.1 [b]	1.9	2.4 [a]
	PoAA	49.2	5.3 [a]	112.6	15.8 [b]	0.9	0.1 [a]	1.5	0.2 [b]	−63.3	7.5 [b]
PRO	EuAA	120.5	9.0 [a]	126.6	7.8 [a]	2.0	0.3 [a]	2.2	0.4 [a]	−3.1	2.6 [a]
	PeAA	357.3	32.6 [b]	362.5	57.6 [b]	2.5	0.2 [a]	5.9	0.6 [b]	−5.2	8.9 [a]
	PoAA	183.0	12.0 [c]	350.5	49.6 [b]	2.8	0.3 [a]	4.7	0.5 [b]	−167.5	15.8 [b]
SER	EuAA	126.5	7.2 [a]	113.8	6.1 [a]	2.0	0.2 [a]	2.0	0.2 [a]	12.7	2.8 [a]
	PeAA	233.8	33.7 [b]	210.6	36.0 [b]	2.0	0.3 [a]	3.6	0.5 [b]	23.2	5.2 [b]
	PoAA	114.2	7.4 [a]	258.1	39.0 [b]	2.1	0.2 [a]	3.3	0.3 [b]	−143.9	17.9 [c]
THR	EuAA	199.0	13.7 [a]	233.3	15.6 [a]	3.9	0.4 [a]	3.9	0.4 [a]	−20.1	4.4 [a]
	PeAA	346.3	47.0 [b]	355.8	57.2 [b]	2.9	0.3 [a]	5.9	0.9 [b]	−9.5	9.0 [b]
	PoAA	258.2	22.3 [a]	380.8	50.1 [b]	3.6	0.6 [a]	5.4	0.7 [b]	−122.6	15.4 [c]
TRP	EuAA	51.5	5.8 [a]	46.2	5.0 [a]	0.6	0.1 [a]	0.5	0.1 [a]	3.2	2.0 [a]
	PeAA	120.0	17.1 [b]	118.2	20.0 [b]	1.0	0.1 [a]	2.0	0.3 [b]	1.8	2.3 [a]
	PoAA	146.5	14.7 [b]	185.3	30.3 [c]	1.2	0.1 [a]	2.7	0.3 [c]	−38.8	8.5 [b]
TYR	EuAA	53.8	4.6 [a]	48.7	4.4 [a]	0.9	0.1 [a]	0.9	0.1 [ab]	5.1	2.7 [a]
	PeAA	42.1	4.6 [a]	35.3	4.9 [a]	0.7	0.1 [a]	0.6	0.1 [a]	6.8	1.1 [a]

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		Arterial concentration µM		Portal concentration µM		Hepatic load (µmol/kg/min)				ArterioPortal gradient µM	
		AA infusion		AA infusion		Basal		AA infusion		AA infusion	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
VAL	PoAA	30.8	2.9 [b]	66.9	10.0 [b]	0.8	0.1 [a]	0.9	0.1 [b]	−36.0	4.0 [b]
	EuAA	217.0	10.8 [a]	202.5	9.2 [a]	4.1	0.2 [a]	3.6	0.3 [a]	14.5	4.0 [a]
	PeAA	465.9	24.2 [b]	442.8	54.7 [b]	3.7	0.3 [a]	7.3	0.3 [b]	23.0	6.9 [a]
	PoAA	309.4	23.1 [c]	462.4	65.2 [b]	3.5	0.4 [a]	6.6	0.6 [b]	−153.0	14.7 [b]

Data are expressed as means ± SE (n=9) in each group. The basal data are the mean of the values at 90 and 120 min. The AA infusion data are the mean of the determinations between 150 and 300 min. The statistical evaluation of the data was performed by a one way measures analysis of variance. Bonferroni adjusted data are shown. Differences were considered significant when $P < 0.05$. Values with different letters are significantly different within the same column, $P < 0.05$.