Influence of oxygen partial pressures on protein synthesis in feeding crabs

ELENI MENTE,1 ALEXIA LEGEAY,2 DOMINIC F. HOULIHAN,1 AND JEAN-CHARLES MASSABUAU2

1Department of Zoology, University of Aberdeen, Aberdeen AB24 2TZ, United Kingdom; and 2Laboratoire d’Ecophysiologie et Ecotoxicologie des Systèmes Aquatiques, Unité Mixte de Recherche 5805-OASU, Université Bordeaux 1 and Centre National de la Recherche Scientifique, Arcachon 33120, France

Influence of oxygen partial pressures on protein synthesis in feeding crabs. Am J Physiol Regul Integr Comp Physiol 284: R500–R510, 2003; 10.1152/ajpregu.00193.2002.—Many water-breathing animals have a strategy that consists of maintaining low blood PO2 values in a large range of water oxygenation level (4–40 kPa). This study examines the postprandial changes in O2 consumption, arterial blood PO2, and tissue protein synthesis in the shore crab Carcinus maenas in normoxic, O2-depleted, and O2-enriched waters to study the effects of this strategy on the O2 consumption and peptide bond formation after feeding. In normoxic water (21 kPa), the arterial PO2 was 1.1 kPa before feeding and 1.2 kPa 24 h later. In water with a PO2 of 3 kPa (arterial PO2 0.6 kPa), postprandial stimulation of protein synthesis and O2 consumption were blocked. The blockade was partial at a water PO2 of 4 kPa (arterial PO2 0.8 kPa). An increase in environmental PO2 (60 kPa, arterial PO2 10 kPa) resulted in an increase in protein synthesis compared with normoxic rates. It is concluded that the arterial PO2 spontaneously set in normoxic Carcinus limits the rates of protein synthesis. The rationale for such a strategy is discussed.

Address for reprint requests and other correspondence: E. Mente, Dept. of Zoology, Univ. of Aberdeen, Tillydrone Ave., Aberdeen AB24 2TZ, United Kingdom (E-mail: e.mente@abdn.ac.uk and massabau @ecotox.u-bordeaux.fr).
account for up to 40% of the O2 consumption in a variety of aquatic species.

C. maenas is among those water breathers that live chronically with a near-hypoxic arterial PO2 (1–1.5 kPa). The postprandial doubling in O2 consumption 24 h after a meal is not generated through an increase in blood flow rate (25). In crustaceans, the microcirculation in the hepatopancreas (9), a major site of protein synthesis (18), is so well developed that these low arterial PO2s can be considered as quite close to the extracellular PO2. This tissue therefore represents an excellent opportunity to determine the O2 sensitivity of protein synthesis in vivo.

The aims of this study were to examine the postprandial changes in O2 consumption, arterial blood PO2, and tissue protein synthesis in the crab C. maenas in normoxia, hypoxia, and hyperoxia and to investigate how the low physiological oxygenation status in situ may influence in vivo protein synthesis.

**Materials and Methods**

**Animals and general conditions.** Common shore crabs, C. maenas, were collected locally from Arcachon Bay in France. They were transported and kept for at least 3 wk in the Marine Biological Station to allow acclimatization (30). The seawater had a constant salinity of 30–32‰, and pH and temperature were 8.0–8.3 and 15°C, respectively. All the crabs were in the size range 39–67 g and were in the intermoult stage. During the acclimatization period the crabs were fed on mussels, *Mytilus edulis* (41.0 ± 7.5% protein dry wt) twice a week.

**Experimenal conditions.** The experimental tanks were isolated from vibrations with antivibrating benches. Salinity was 32‰, and temperature was 15.0 ± 0.5°C. The PO2 was set at 0.10 ± 0.01 kPa and the pH at 7.80 ± 0.50 depending on the water titration alkalinity by using a pH-PCO2-stat (7). Shadow areas and a patch of dim light were provided to synchronize all individuals before feeding. Animals were fed with mussel flesh (2.8 ± 0.1% of the animal’s fresh weight) in normoxic water (water PO2 = 20–21 kPa) before any change in water PO2, O2 consumption, the rate of protein synthesis, arterial blood PO2, and lactate concentrations were determined before and after these changes.

**O2 consumption measurements.** Experiments were performed on 42 crabs weighing 53 ± 1 g. The O2 consumption of individual animals (μmol·min⁻¹·kg⁻¹) was measured in an open-flow respirometer (vol 160 ml) maintained at 15°C. The technique used was similar to that described in Ref. 29. Its main characteristics were 1) the respirometer was equipped with a pH-PCO2-stat, 2) a laboratory-made automatic device (continuously monitored PO2 in the exit water and adjusted the water flow through the respirometer to clamp PO2 inspired by the animals at either 21, 4, or 3 kPa), and 3) a rotor to ensure a homogeneous composition in the respirometer. It should be noted that the water PO2 was measured automatically with electrodes manipulated by remote control to ensure nonstressful conditions. Each crab was initially placed in an open tank (20 l, renewal rate 0.5 l/min) filled with water that had been equilibrated to a PO2 of 21 kPa and a PCO2 of 0.1 kPa. The crabs were allowed to settle for 24 h before recordings were made, and then they were transferred within 2–3 min to the respirometer (at time t₀) where the PO2 was 21 kPa. Reference preprandial O2 consumption measurements were made the next morning from 9:00 to 10:00 AM. Animals were allowed access to food at t₀ + 24 h for 30 min (from 10:00 until 10:30 AM). For feeding and to avoid contamination in the respirometer, the animals were temporarily transferred to a feeding chamber for 30 min (vol 400 ml, same water composition as that in the respirometer). They were then transferred back to the respirometer within approximately 2–3 min, and O2 consumption measurements were performed 2, 4, 6, 24, and 48 h later.

**Blood analyses.** Experiments were carried out on 175 *Car- cinus* (weighing 58 ± 1 g). At least 3 days before the beginning of the experiments, animals kept in normoxic conditions were prepared for arterial blood sampling by drilling a hole in the carapace above the heart; a thin layer of cuticle was left in place and a piece of rubber was glued over it. All animals were fed with mussels, one mussel per crab (fresh weight 2 g) in normoxic water (salinity = 35‰; water PO2 = 20–21 kPa; water PCO2 = 0.1 kPa) and gently transferred 30 min later to test tanks with water at PO2 of 21, 4, or 3 kPa. Individuals (n = 6 per water oxygenation level) were sampled only once. Arterial blood samples (150 μl) were collected by gently removing crabs from the water and puncturing the heart through the rubber membrane with capillary glass tubes equipped with a needle. Samples were obtained within the first minute of emersion. This sampling technique was critically assessed in Ref. 30. After sampling, blood was stored on ice to prevent clotting and to slow down metabolic reactions. Arterial PO2 was determined within 2–3 min on 100-μl samples with an E5046 Radiometer polarographic electrode thermostated at 15°C. The electrode was calibrated with a zero PO2 solution (S4150 Radiometer) and seawater-equilibrated with a precision gas mixture (O2 fraction = 4%). As shown in Ref. 30, Fig. 2, this calibration procedure, which improves analysis quality in the low range, does not preclude the measurement of high blood PO2 values. l-Lactate concentra-

**Protein synthesis measurements.** Six starved crabs were transferred to experimental tanks (control group) and exposed in normoxia (at time t₀) where water PO2 was 21 kPa. The next morning (t₀ + 24 h), they were injected with radiolabel, returned to water, and killed 60 min later. Samples of hepatopancreas, heart, claw, and leg muscles were taken for analyses (see below). The same morning previously starved crabs kept in normoxia were given a single meal of *Mytilus edulis* (t₀ + 24 h) equivalent to 2.8 ± 0.1% of the animal’s fresh weight. The animals were transferred after 30 min to experimental tanks in normoxia (water PO2 = 21 kPa, n = 21), hypoxia (water PO2 = 4 kPa, n = 23; or PO2 = 3 kPa, n = 24), or hyperoxia (water PO2 = 60 kPa, n = 6). In each water oxygenation condition the animals were injected with a radiolabel at 2, 5, 24, and 48 h postfeeding except in hyperoxia where they were only injected at 24 h. As in reference condition, samples of hepatopancreas, heart, claw, and leg muscles were taken 60 min after injection.
Rates of protein synthesis in tissues were determined after the injection of a single "flooding dose" of [3H]phenylalanine (21). The injection solution consisted of 135 mM phenylalanine and 1-[2,6-3H]phenylalanine (Amersham) at 100 μCi/ml (3.7 MBq/ml) at a dose of 1.0 ml·100 g⁻¹·live wt⁻¹. The injection was made into the blood sinus at the base of the third walking leg. No O₂ consumption measurements were made on these animals because of the elevation of the O₂ consumption that occurs through handling (21).

After injection the animals were returned to the normoxic, hypoxic, or hyperoxic conditions. After a mean incubation time of 1 h (21), the animals were killed by destroying the brain. Two hundred-milligram samples of hepatopancreas, heart, claw, and leg muscles were taken, individually wrapped in plastic bags, immediately frozen in liquid nitrogen, and stored at −80°C for further analysis. Dissections took place in within 5 min (from killing the animal to the removal of the final tissue sample). The whole body was also frozen.

Tissue samples were homogenized while still frozen in 2% perchloric acid (PCA), thus separating the "free" intracellular (PCA soluble) and "protein-bound" (insoluble) phenylalanine fractions, and the precipitate was treated as described in Ref. 8. The frozen whole bodies were broken up and homogenized in 200 ml 0.2 M PCA. The resulting homogenate was thoroughly mixed and a subsample weighed into a tarred centrifuge tube for further analysis as described above. The phenylalanine-specific radioactivity of the free pool, the free-pool phenylalanine concentration, and protein-bound phenylalanine specific radioactivity were determined as described in Refs. 20 and 42.

The fractional rate of protein synthesis kₛ as a percentage of the total protein mass of each tissue or whole body per day was calculated as

$$k_s = \frac{(S/S_a) \times (1/t) \times 1440 \times 100}{1}$$

where Sₛ is the protein-bound phenylalanine specific activity at experimental time t (disintegrations·min⁻¹·nmol phenylalanine⁻¹), Sₐ is the free-pool phenylalanine specific activity at time t (disintegrations·min⁻¹·nmol phenylalanine⁻¹), t is the time between injection and killing of the animal (in min), and 1,440 is the number of minutes in a day. The kₛ was calculated for each whole body and for each tissue. Protein determinations were treated as described in Ref. 21. The RNA content and the RNA activity (kₛRNA, the amount of protein in g being synthesized per g of RNA per day) were calculated as described in Ref. 21.

Validation of the methodology. To check if hypoxia, through a putative change of blood flow rate, could alter the tissue distribution of radiolabel in the organism, another twelve crabs were randomly selected, transferred to the experimental tanks, and exposed to hypoxia (3 kPa). After an incorporation period of 30, 60, or 90 min, they were killed and samples were taken to measure the phenylalanine specific radioactivity of the tissue free pool and the incorporation of tritiated phenylalanine into tissue proteins.

Statistics. Data are expressed as individual values in histograms of frequency distribution and/or as means ± SE. Paired t-tests were used to test significant changes in O₂ consumption and blood O₂ status before and after feeding. Otherwise, differences between normoxic and hypoxic conditions were evaluated using the Mann-Whitney U test or Student’s t-test. ANOVA (followed where applicable by Tukey’s multiple comparison test) was used to compare tissue-specific stabilized free pool specific activities, protein synthesis rates, RNA to protein ratio, and translational efficiencies calculated after different times for each tissue. Linear regression analysis was used to establish protein labeling during the time course. P < 0.05 was considered to be statistically significant.

RESULTS

Postprandial O₂ consumption and supply in normoxia. After being starved for 5 days and after feeding, there was a peak in O₂ consumption (from 14.8 ± 0.7 up to 36.0 ± 3.3 μmol·min⁻¹·kg⁻¹) at 2 h after feeding (Fig. 1A). This was followed by a plateau from 6 to 24 h (O₂ consumption = 26.6 ± 1.9 μmol·min⁻¹·kg⁻¹ at 6 h; 26.9 ± 1.9 μmol·min⁻¹·kg⁻¹ at 24 h). The O₂ consumption returned to the reference value at 48 h after feeding. The reference arterial PO₂ in our resting and settled condition was only 1.1 ± 0.1 kPa despite an inspired PO₂ of 20–21 kPa (Fig. 1B). Four hours after feeding, the arterial PO₂ significantly increased to 1.6 ± 0.2 kPa (P < 0.05, Mann-Whitney test) from the reference value. Two hours later it decreased to 1.2 ± 0.1 kPa and remained at this value until 24 h after the meal. The low arterial PO₂s were not accompanied by any rise in blood lactate concentration, which demonstrated the absence of any noticeable anaerobic metabolism occurring during the specific dynamic action (SDA) (Fig. 1C).

Postprandial tissue protein synthesis rates in normoxia. The changes in fractional rates of protein synthesis after feeding are shown in Fig. 2. In all tissues the maximal rates were reached within 5 h after feed-
The fractional rates of protein synthesis were highest in the hepatopancreas where they doubled (ANOVA, \( P < 0.05 \)) from 5.4 \( \pm \) 0.3%/day before feeding to 10.9 \( \pm \) 0.3 and 11.6 \( \pm \) 2.2%/day at 5 and 24 h after feeding, respectively (not significantly different between 5 and 24 h, ANOVA, \( P > 0.05 \)). Rates of protein synthesis increased significantly (ANOVA, \( P < 0.05 \)) by threefold in the heart. Smaller increases occurred in the leg and claw muscle.

**Postprandial O\(_2\) consumption and supply in O\(_2\)-limited conditions.** The effect of exposing animals previously fed in normoxia to water with a PO\(_2\) of 4 kPa (Fig. 3A) was the abolition of the initial transient peak of O\(_2\) consumption. Instead the O\(_2\) consumption rose to a maximum value at 2 h after food intake and remained at around this value for 24 h (O\(_2\) consumption = 26.1 \( \pm \) 0.9 and 24.2 \( \pm \) 1.9 \( \mu \)mol\( \cdot \)min\(^{-1}\)\( \cdot \)kg\(^{-1}\) at 2 and 24 h after feeding, respectively; significantly different from the preprandial values, Fig. 3A; not different from the postprandial normoxic values at 4, 6, and 24 h). At a PO\(_2\) of 3 kPa (Fig. 3A), the situation was completely different. The postprandial increase was reduced to 18.8 \( \pm \) 1.2 \( \mu \)mol\( \cdot \)min\(^{-1}\)\( \cdot \)kg\(^{-1}\) 2 h after feeding (significantly different from the preprandial value). This value did not statistically change until 24 h after feeding when it increased to 19.3 \( \pm \) 1.2 \( \mu \)mol\( \cdot \)min\(^{-1}\)\( \cdot \)kg\(^{-1}\). Overall these results represent SDA responses at 6 h of 11.8 \( \mu \)mol\( \cdot \)min\(^{-1}\)\( \cdot \)kg\(^{-1}\) at 21 kPa, 13.0 \( \mu \)mol\( \cdot \)min\(^{-1}\)\( \cdot \)kg\(^{-1}\) at 4 kPa, and 3.3 \( \mu \)mol\( \cdot \)min\(^{-1}\)\( \cdot \)kg\(^{-1}\) at 3 kPa. In all situations the O\(_2\) consumption returned to the prefeeding level 48 h after feeding.

When the water PO\(_2\) was 4 kPa, the arterial PO\(_2\) (Fig. 3B) decreased to 0.6 \( \pm \) 0.1 kPa 4 h after feeding compared with the normoxic reference value of 1.6 \( \pm \) 0.2 kPa (4 h postfeeding). The arterial PO\(_2\) did not
change statistically until 24 h after feeding (0.8 ± 0.1 kPa) compared with 1.2 ± 0.1 kPa in normoxic animals (24–48 h). When fed animals were transferred from water at a Po2 of 21 kPa to hypoxic water at 3 kPa (Fig. 3B), the resulting arterial Po2 was slightly lower than in water at 4 kPa. Two hours after feeding it was 0.5 ± 0.1 and 0.6 ± 0.1 kPa from 6 to 48 h (significantly lower values than at water of a Po2 of 4 kPa, P < 0.05).

The consequences of these hypoxic exposures is illustrated in Fig. 3C where blood lactate is used as a marker of the initiation of anaerobic metabolism. Remarkably, when the arterial Po2 decreased to 0.7 ± 0.1 kPa at a water Po2 of 4 kPa 2 h after feeding, there was only a minor, although significant, transient lactate rise up to 3.9 mmol/l compared with 0.2 ± 0.1 mmol/l in normoxic reference conditions (P < 0.05). When the arterial blood was at a Po2 of 0.5 ± 0.1 kPa in water with a Po2 of 3 kPa, there was a much larger rise in blood lactate that peaked at 4 h after feeding (blood lactate = 11.9 ± 3.7 mmol/l). This revealed that the arterial Po2 at the anaerobic threshold is in the range 0.5–0.7 kPa in fed C. maenas. Thus the minimum arterial Po2 required for an aerobic SDA response is in this range.

Postandpial tissue protein synthesis rates in O2-limited conditions: validation of the methodology. It was possible that hypoxia could have induced changes in blood flow rate that could have interfered with the distribution of the injected phenylalanine throughout the animal, resulting in poor flooding of the tissues and problems with the interpretation of the protein synthesis values. To eliminate this possibility, the time course of flooding of the free pools and rate of incorporation of radiolabel into proteins were determined at a water Po2 of 3 kPa in starved animals.

The mean free-pool phenylalanine radioactivities in the heart, claw, leg, and the hepatopancreas (Table 1) were elevated within 30 min of injection and did not change significantly at each time interval (ANOVA, P > 0.05). The phenylalanine-specific radioactivity of the solution injected was 1,856.3 ± 53.03 dpm/nmol phenylalanine (n = 4). In all tissues the values were significantly lower than the specific radioactivity of the injection solution (ANOVA, P < 0.05). Hypoxia (3 kPa) at 60 min after the injection had no significant effect on free pool specific activity, irrespective of tissue (Table 1). One hour after the flooding dose injection, in 3 kPa the free phenylalanine levels in the claw tissue were ninefold above the normal values of 200–700 nmol phenylalanine/g fresh wt (8), demonstrating a large increase in the phenylalanine free pool due to the flooding dose of the amino acid. There were significant linear correlations between protein-bound phenylalanine radioactivity (Sa) and time under hypoxia at 3 kPa in starved animals (Table 1). From the intercept of the regression line, it is possible to estimate how soon after injection the radiolabel began to be incorporated into body protein. The intercepts did not differ significantly from zero, confirming that protein labeling had begun immediately after the injection. The commonly accepted criteria for the successful measurement of protein synthesis by the flooding dose method have therefore been met in crabs living in water with a Po2 of 3 kPa. This also implies that the circulation and diffusional processes were not impaired even in the extremely hypoxic conditions we studied.

Tissue protein synthesis rates. The influence of exposing the crabs to 4 and 3 kPa, forcing the arterial Po2 down to 0.7 or 0.5 kPa, respectively, compared with the normoxic control is presented in Fig. 4. Protein synthesis rates were tissue specific. At a water Po2 of 4 kPa and arterial blood Po2 of 0.7–0.8 kPa (Fig. 3B), the time course of changes in the rates of protein synthesis in claw and leg muscle appeared simply delayed as they did not differ from the normoxic values from 10 to 48 h in fed animals (Fig. 4). In the hepatopancreas the situation was completely different. There was 1) a transient and significant decrease occurring from 2 to 5 h and 2) no increase in protein synthesis from 24 to 48 h compared with the unfed value. In the heart there was an intermediate status as the rates of protein synthesis reached the normoxic reference value for fed animals at 10 h and then was limited at 24 and 48 h (no difference with the reference unfed value).

At a water Po2 of 3 kPa and arterial blood Po2 of 0.5–0.6 kPa, and when the animals clearly relied on anaerobic metabolism, there was no increased synthesis of protein except the transient significant rise in the

Table 1. Time course of free pool and protein-bound phenylalanine-specific radioactivity of tissues of starved individual crabs exposed to hypoxia (3 kPa) at various times after injection of labeled phenylalanine

<table>
<thead>
<tr>
<th>Incubation Time, min</th>
<th>30</th>
<th>60</th>
<th>90</th>
</tr>
</thead>
<tbody>
<tr>
<td>S_a, dpm/nmol Phe</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatopancreas</td>
<td>1,237.9 ± 129</td>
<td>1,656.7 ± 91.7</td>
<td>1,377.5 ± 203</td>
</tr>
<tr>
<td>Heart</td>
<td>1,413.5 ± 28.8</td>
<td>1,525.2 ± 57.0</td>
<td>1,425.6 ± 46.0</td>
</tr>
<tr>
<td>Claw</td>
<td>1,234.7 ± 23.6</td>
<td>1,276 ± 35.5</td>
<td>1,334.5 ± 58.9</td>
</tr>
<tr>
<td>Leg</td>
<td>1,390.8 ± 20.7</td>
<td>1,509 ± 152.4</td>
<td>1,467.2 ± 39.2</td>
</tr>
<tr>
<td>S_b, dpm/nmol Phe</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatopancreas</td>
<td>y = 0.446 ± 0.022t (r^2 = 0.53, P &lt; 0.006, n = 12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>y = 0.543 ± 0.013t (r^2 = 0.45, P &lt; 0.05, n = 9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Claw</td>
<td>y = -0.347 ± 0.015t (r^2 = 0.49, P &lt; 0.01, n = 12)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; n is no. of samples. Regression equations given as y = a + bx, where y is protein-bound Phe-specific activity (S_a), b is slope, a is intercept on y-axis, and x is labeling time (min). S_a, free pool Phe-specific radioactivity; dpm, degradations/min.
claw at 5 h. In the hepatopancreas the rate of protein synthesis was reduced to very low values (48 h = 0.8 ± 0.2%/day).

Tissue RNA to protein concentrations and RNA translational efficiencies. The ranking of the tissues in terms of RNA to protein ratios was as previously described (21). There was no significant change in the concentration of RNA with hypoxia in the tissues either expressed in relation to wet weight or protein. Therefore, the amount of protein synthesized per unit RNA (kRNA) showed the same response as protein synthesis rates in hepatopancreas, heart, leg, and claw in normoxia and hypoxia (3 and 4 kPa) (Table 2).

Table 2. RNA translational efficiency of the tissues of C. maenas at 5 and 24 h after a single meal

<table>
<thead>
<tr>
<th>kRNA</th>
<th>Prefeeding Value</th>
<th>5 h After Feeding</th>
<th>24 h After Feeding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg protein·μg RNA⁻¹·day⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normoxia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatopancreas</td>
<td>2.631 ± 0.60</td>
<td>5.190 ± 0.50*</td>
<td>3.179 ± 0.77*</td>
</tr>
<tr>
<td>Heart</td>
<td>0.525 ± 0.05</td>
<td>1.787 ± 0.35*</td>
<td>1.548 ± 0.47</td>
</tr>
<tr>
<td>Claw</td>
<td>0.188 ± 0.02</td>
<td>0.472 ± 0.10</td>
<td>0.617 ± 0.09*</td>
</tr>
<tr>
<td>Leg</td>
<td>0.833 ± 0.17</td>
<td>1.315 ± 0.22*</td>
<td>1.189 ± 0.08</td>
</tr>
<tr>
<td>Hypoxia (4 kPa)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatopancreas</td>
<td>0.903 ± 0.33*</td>
<td>2.674 ± 0.45</td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>1.360 ± 0.12*</td>
<td>0.821 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>Claw</td>
<td>0.306 ± 0.02</td>
<td>0.423 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>Leg</td>
<td>0.927 ± 0.10</td>
<td>1.056 ± 0.13*</td>
<td></td>
</tr>
<tr>
<td>Hypoxia (3 kPa)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatopancreas</td>
<td>0.992 ± 0.32</td>
<td>0.456 ± 0.03*</td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>0.692 ± 0.09</td>
<td>0.888 ± 0.13</td>
<td></td>
</tr>
<tr>
<td>Claw</td>
<td>0.615 ± 0.10*</td>
<td>0.418 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>Leg</td>
<td>1.013 ± 0.09</td>
<td>0.857 ± 0.18</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 animals in each time point except for the 48-h group, which were n = 3 (21 kPa), n = 5 (4 kPa), and n = 6 (3 kPa). kRNA, RNA translational efficiency. *Significant difference (ANOVA followed by Tukey’s multiple comparison test; P < 0.05) from the prefeeding reference value.
protein synthesis at 24 h after feeding. At that time there should be no more food in the foregut (32), and Figs. 2–4 suggest a plateau in the parameters studied. There were no statistically significant relationships between arterial blood PO2 and fractional rates of protein synthesis in the claw, leg, and heart muscles, but there was a clear relationship between blood PO2 and fractional rates of protein synthesis in the hepatopancreas (Fig. 5). Therefore we performed an experiment during which we transferred fed Carcinus into hyperoxic water where PO2 was so high (60 kPa) that it exceeded the animals’ regulation capacity (28). The result of this experiment where the mean arterial PO2 increased to 10.9 ± 1.8 kPa (measured 24 h after the transfer, n = 20) is presented in Fig. 6. For clarity, data were combined with previous results from Fig. 5. In all tissues the distribution of the experimental points followed a single exponential equation of the type $y = y_0 + a(1 - b^x)$ with a good resolution power ($0.906 < R^2 < 0.999$), which shows that the arterial PO2 explained 90–99% of the total variability. Three different situations were observed. First, in the claw muscle and the heart, the improved O2 conditions in hyperoxia allowed a large increase in protein synthesis (189 and 280%, respectively, $P < 0.05$). Second, in the hepatopancreas the change was much smaller as the $k_s$ was 13.4 ± 1.1%/day at an arterial PO2 of 1.2 ± 0.1 kPa and 16.7 ± 1.3%/day at arterial PO2 of 10.9 ± 1.8 kPa, which represents a 20% increase. Third, in the leg muscle, the arterial PO2 change did not lead to any $k_s$.

Fig. 5. Relationships between mean arterial blood PO2 values and protein synthesis rates in various tissues 24 h after a single meal in crabs exposed to water PO2 ranging from 3 to 21 kPa. *Significantly different from the prefeeding value.

Fig. 6. Influence of superoxygenated blood (water PO2 = 60 kPa) on the efficiency of protein synthesis rate in various tissues 24 h after a single meal. Same data as in Fig. 5 for water PO2 ranging from 3 to 21 kPa. PO2 and PO2 at which 50 and 99% of the reaction were occurring in vivo. *Significantly different from the prefeeding value.
improvement. The calculated arterial \( P_{O_2} \)s at which 50 and 99% of the reaction were occurring in vivo (\( P_{50} \) and \( P_{99} \), respectively) were 1.1 and 5.9 kPa in the claw muscle, 1.3 and 5.9 kPa in the heart, 0.8 and 2.2 kPa in the hepatopancreas, and 0.6 and 0.9 kPa in the leg muscle.

**DISCUSSION**

In this study we present evidence that in normoxic water, i.e., in unlimited \( O_2 \) supply conditions, a crustacean exhibiting the low blood oxygenation strategy can adjust its arterial \( P_{O_2} \) to a very low level despite the increased \( O_2 \) demand associated with protein synthesis and the possibility to maximize protein synthesis efficiency in hepatopancreas, heart, and claw muscles with slightly higher \( P_{O_2} \). A comparison of various rates of protein synthesis determined in steady state 24 h after feeding at various experimentally manipulated arterial \( P_{O_2} \) (including the use of superoxygenated blood) suggested that in reference normoxic conditions the spontaneously set arterial \( P_{O_2} \) is limiting the rate of protein synthesis in the claw and heart muscles as well as in the hepatopancreas but not in the leg muscle. In the hepatopancreas, which is the major site of protein synthesis, the rate of synthesis was only 80% of its maximum value although a \( P_{O_2} \) change from 1.2 to 2.2 kPa would be enough to achieve 100% of the maximum rate. Consequently, the present findings suggest that the low blood oxygenation strategy reported in water breathers can contribute very significantly to the energy budget, which raises the question why these animals maintain such low oxygenation levels in their internal milieu.

To recall the origin of this strategy, we already suggested that these low \( P_{O_2} \)s might reflect the level of \( O_2 \) in the environment when animals first evolved, about 2 billion years ago, in the Proterozoic age (27). An analysis of the literature also clearly shows that in reference normoxic conditions the spontaneously set arterial \( P_{O_2} \) is limiting the rate of protein synthesis in the claw and heart muscles as well as in the hepatopancreas but not in the leg muscle. In the hepatopancreas, which is the major site of protein synthesis, the rate of synthesis was only 80% of its maximum value although a \( P_{O_2} \) change from 1.2 to 2.2 kPa would be enough to achieve 100% of the maximum rate. Consequently, the present findings suggest that the low blood oxygenation strategy reported in water breathers can contribute very significantly to the energy budget, which raises the question why these animals maintain such low oxygenation levels in their internal milieu.

To recall the origin of this strategy, we already suggested that these low \( P_{O_2} \)s might reflect the level of \( O_2 \) in the environment when animals first evolved, about 2 billion years ago, in the Proterozoic age (27). An analysis of the literature also clearly shows that in reference normoxic conditions the spontaneously set arterial \( P_{O_2} \) is limiting the rate of protein synthesis in the claw and heart muscles as well as in the hepatopancreas but not in the leg muscle. In the hepatopancreas, which is the major site of protein synthesis, the rate of synthesis was only 80% of its maximum value although a \( P_{O_2} \) change from 1.2 to 2.2 kPa would be enough to achieve 100% of the maximum rate. Consequently, the present findings suggest that the low blood oxygenation strategy reported in water breathers can contribute very significantly to the energy budget, which raises the question why these animals maintain such low oxygenation levels in their internal milieu.

To recall the origin of this strategy, we already suggested that these low \( P_{O_2} \)s might reflect the level of \( O_2 \) in the environment when animals first evolved, about 2 billion years ago, in the Proterozoic age (27). An analysis of the literature also clearly shows that in reference normoxic conditions the spontaneously set arterial \( P_{O_2} \) is limiting the rate of protein synthesis in the claw and heart muscles as well as in the hepatopancreas but not in the leg muscle. In the hepatopancreas, which is the major site of protein synthesis, the rate of synthesis was only 80% of its maximum value although a \( P_{O_2} \) change from 1.2 to 2.2 kPa would be enough to achieve 100% of the maximum rate. Consequently, the present findings suggest that the low blood oxygenation strategy reported in water breathers can contribute very significantly to the energy budget, which raises the question why these animals maintain such low oxygenation levels in their internal milieu.
5 h after a single meal with respect to the prefeeding values. The current work agrees with that of Ref. 21 in *C. maenas*, which found that the hepatopancreas, gill, heart, proventriculus, leg muscle, and claw muscle protein synthesis rates increased 3 h after feeding in normoxic conditions; this increase occurred between 2 and 5 h after the meal in the present study. The ranking of the tissues in terms of their protein synthesis rates was similar to that previously reported from crustacean studies (21) and is in agreement, so far as the tissues are comparable, with the results from fish (11, 37). Tissues such as hepatopancreas and gills have higher rates of synthesis than muscle and heart, which in turn have higher rates than skeletal muscle. The liver is an important organ in the metabolism and utilization of nutrients from the diet and makes a significant contribution to protein metabolism in the whole body due to its high rate of protein turnover (11). The reduction in fractional protein synthesis rate during hypoxia was most marked in the hepatopancreas. The hepatopancreas responds to hypoxia by a downregulation of protein synthesis when the arterial *PO*₂ is 0.5 kPa. This downregulation can be assumed to be an energy-saving process in a tissue, which is a major contributor to meal-stimulated protein synthesis (at least after a period of starvation) (34). Survival of vertebrates under anoxic or hypoxic conditions is achieved by a drastic reduction in ATP-consuming process, coupled with an increased ATP production via anaerobic pathways, allowing maintenance of cellular ATP concentrations and cell function (17). In contrast, in anoxia- and hypoxia-intolerant animals, cellular ATP concentrations rapidly decrease during anoxia, leading to loss of ion gradients, a rise in intracellular Ca²⁺ concentrations, and multiple cellular damaging processes, leading to death (17). Therefore as part of the general downregulation of metabolism, there is a decrease in protein synthesis in anoxia/hypoxia-tolerant animals on exposure to these conditions, since protein synthesis is an energetically expensive process accounting for a large proportion of cellular energy consumption (3). Mammalian tissues have been found also to reduce rates of protein synthesis in conditions of hypoxia (reviewed in Ref. 20). In isolated hepatocytes of rainbow trout, protein synthesis started to fall below an extracellular *PO*₂ of 2 kPa (36). During anoxia, protein synthesis rates of isolated liver cells of the turtle *Chrysemys picta bellii* were reduced by 92% (reviewed in Ref. 20). Crucian carp living in complete anoxia reduce liver protein synthesis rates by 90% or more compared with normoxic values; however, rates of protein synthesis in the brain are maintained in anoxia at normoxic rates (42). The decrease in protein synthesis rates during hypoxia (compared with the normoxic values) in this study suggested that invertebrates as well as fish downregulate protein synthesis in a tissue-specific manner when exposed to hypoxic conditions.

It seems unlikely that food processing in the gut makes a major contribution to the SDA because work in place showed no detectable increase in *O₂* consumption after an indigestible meal of kaolin (4). In contrast, the synthesis of macromolecules, especially proteins, can be very costly in energetic terms and so could be a substantial contributor to postprandial metabolism. A significant proportion of the SDA should be accounted for by an elevation of protein synthesis (23). Protein synthesis is an important determinant of *O₂* consumption after feeding in cod (26). On the basis of protein synthesis costs derived from the literature (2), it may contribute as much as 44% of the observed postprandial rise in *O₂* consumption.

An interesting observation in the present study was that at arterial *PO*₂ of 0.7 kPa (inspired *PO*₂ of 4 kPa), *O₂* consumption had doubled from 6 to 24 h postfeeding while the rate of protein synthesis was limited to the reference prefeeding level in normoxia. This shows that at this arterial *PO*₂, the major *O₂*-consuming process (which is the mitochondria pool) was still consuming *O₂* at its normal rate. The ability to supply ATP at the rate required by the energy-demanding process as well as the *O₂*-sensitive key step that impaired the protein synthesis remains to be studied.

### RNA concentrations and translational efficiency

Overall, in this study there was no reduction in tissue *RNA*-to-protein ratios in hypoxia. Studies on mammals have also shown no reduction in tissue *RNA*-to-protein ratios in hypoxia-exposed rats (38). It would be expected, perhaps, that there would be a downregulation of RNA synthesis under hypoxia concurrent with the reduction in protein synthesis as an energy-saving strategy. However, Smith et al. (42) showed that there was little agreement between *RNA* and protein synthesis rates within individual tissues in anoxia, suggesting that RNA synthesis may comprise a fixed cost of protein synthesis that probably cannot be reduced under *O₂*-limited conditions (36). A number of studies in endo- and ectotherms have shown a linear relationship between *RNA* concentration, expressed as *RNA* to protein ratio, and fractional rates of protein synthesis (21, 33). In the present study there was a linear correlation between protein synthesis rates and *RNA* to protein ratio between tissues. The rates of protein synthesis relative to the *RNA* concentration were radically elevated after a meal in normoxia. Changes in *RNA* activity (*k*ₐ) after a meal have also been found in fish (33) and rat muscle (15). In the rat, hypoxia has been shown to reduce *k*ₐ by 20%-35% in several tissues but not in skeletal muscle (38).

### Energetic cost of protein synthesis

It has been suggested that the postprandial increase in *O₂* consumption after feeding is a reflection of the energy cost of a surge in protein synthesis, with the total animal’s *O₂* consumption representing the sum of the increases in *O₂* demand of the individual tissues (18). The contribution that protein synthesis makes to *O₂* consumption can be calculated from the energy cost of protein synthesis. Increasing rates of protein synthesis resulted in reduced cost (36). When the minimal and experimentally determined costs of protein synthesis were used, this process can account for from 24 to 52% of the total *O₂* consumption in normoxic conditions in this study.
O₂ consumption devoted to protein synthesis falls in hypoxia 4 kPa from 19 to 31% and in hypoxia 3 kPa from 15 to 22%. Overall, the percentage of the energy consumption used for protein synthesis in *C. maenas* is comparable with some of the values obtained for some fish and fish cells (reviewed in Ref. 41). Except for the results from fish cells, calculation of the contribution that protein synthesis makes to whole animal O₂ consumption produces values ranging from 20 to 40%: *C. maenas*, 19–37%; octopus, 35–51%; mytilus, 20%; cod, 20–40%; salmonids, 20–40%; and tilapia, 37% (reviewed in Ref. 41).

Why is the blood oxygenation status limiting protein synthesis after feeding? In fed and normoxic *Carcinus*, the arterial P<sub>o2</sub> was only 1.2 kPa despite the tissue apparent P<sub>90</sub> of 5.9 kPa in claw muscle and heart, 2.2 kPa in the hepatopancreas, and 0.9 kPa in the leg muscles (Fig. 6). It is only in the latter tissue that the rate of protein synthesis was not O₂ limited in normoxic animals, possibly due to a good match between a relatively low rate of synthesis and a well-developed microcirculation for a proportionally small amount of tissue (16). It remains then to discuss why the blood oxygenation was set at such a low value at the expense of higher rates of protein synthesis. We suggest an analysis based on a strategy in terms of costs and benefits at the whole body level. We list here three hypotheses, which are not mutually exclusive. First, we know that O₂ is not only used in mitochondria for ATP production and that changes of local P<sub>o2</sub> in crustaceans can play a role for example as a neuromodulator-like substance (31) or in modulating fast muscle activity (12). Moreover, in mammalian tissue where local P<sub>o2</sub> is also in the 1- to 3-kPa range, it has been proposed that the production of reactive oxygen species (ROS) can act as signaling molecules enabling cells to regulate electrical activity (1) and O₂-dependent gene expression (10). In addition, most of the metabolic reactions in which O₂ participates directly could be expressed (10). In addition, most of the metabolic reactions in which O₂ participates directly could be expressed (10). In addition, most of the metabolic reactions in which O₂ participates directly could be expressed (10).

In conclusion, postprandial stimulation of protein synthesis and O₂ consumption (the SDA response) under hypoxia and hyperoxia were used as a test bed to investigate the effects of the low blood O₂ strategy in the green crab *C. maenas*. P<sub>o2</sub> in the arterial blood, O₂ consumption, and in vivo protein synthesis rates were measured to define the in vivo P<sub>50</sub>, that is the P<sub>o2</sub> value permitting 50% of the maximum reaction rate. We found that in vivo P<sub>50</sub> ranged from 0.6 to 1.3 kPa, which is in the same order of magnitude as the actual arterial P<sub>o2</sub> in normoxic *Carcinus*. The results suggest that even in air-equilibrated water, i.e., in conditions where O₂ supply is not limited by any environmental constraint, the blood oxygenation status in resting green crabs can be set at such low values that it limits the rate of protein synthesis in most tissues. This is the first time that such a bioenergetic strategy has been described.

We gratefully acknowledge Dr. R. Smith. This research was carried out while E. Mente had a grant from the Commission of the European Communities under Contract No. FAIR GT96/1292 and A. Legeay had a grant from the French Ministry of Research and Education.

REFERENCES

34. McMillan DN and Houlihan DF. Protein synthesis in trout liver is stimulated by both feeding and fasting. Fish Physiol Biochem 10: 23–34, 1992.