Several insects are able to use a combination of carbohydrates and proline to power their flight, but the extent to which either substrate is oxidised varies widely. One end of the scale is exemplified by the tsetse fly *Glossina morsitans* (see review by Bursell, 1981) and different species of onitine and scarabaeine dung beetles (Gäde, 1997a, b), which have negligible stores of flight muscle glycogen and haemolymph carbohydrates and use proline almost exclusively as a substrate. At the other end of the scale, insects such as the blowfly *Phormia regina* use proline only at the beginning of flight to supply the Krebs cycle with intermediates (Sacktor and Wormser-Shavit, 1966; Sacktor and Childress, 1967). Proline oxidation plays a greater role during flight in the blister beetle *Decapotoma lunata*, although its importance is still minor compared with that of carbohydrates (Auerswald and Gäde, 1995). In the Colorado potato beetle *Leptinotarsa*...
decemlineata, proline is claimed to serve as the major energy source (Weeda et al. 1979).

The African fruit beetle Pachnodasinusuata was found to oxidise large amounts of proline as well as carbohydrates to power flight (Zebe and Gäde, 1993; Lopata and Gäde, 1994). This beetle occurs in large numbers in South Africa, where it feeds on a variety of flowers and fruits (Donaldson, 1979; Heinrich and McClain, 1986; Holm and Marais, 1992). The beetle’s flight apparatus consists of fibrillar muscles of which, amongst others, the dorso-longitudinal and basalar muscles perform the downstroke, during which most of the lift is generated (Schneider and Hoese, 1982). The dorso-ventral muscle is one of the upstroke muscles, which produce most of the drag.

P. sinuata is a large beetle that has already been the subject of flight experiments (Barnett et al. 1975; Zebe and Gäde, 1993; Lopata and Gäde, 1994). All previous experiments, however, were performed using a flight mill. This reduces the requirement for lift and wing-loading and, therefore, is different from free flight. In the present study, we used a different method of tethered flight which allowed the insect to generate lift (Schneider, 1989) to investigate a more natural metabolic situation in P. sinuata during flight. We investigated the role of proline and carbohydrates in supplying energy during flight and the contribution of the different compartments (flight muscles, haemolymph, fat body) of the insect to the substrate supply. Using the flight mill method, we investigated flights of longer duration, not examined in previous studies (see above). In addition, we used both methods of tethered flight to investigate whether the muscles involved in generating lift or drag show different metabolic patterns during flight, i.e. whether they are biochemically specialised to use either proline or carbohydrate oxidation for energy provision.

Materials and methods

Insects

Male fruit beetles Pachnodasinusuata flaviventris (Gory and Percheron) were caught in the vicinity of Cape Town and were kept as outlined previously (Zebe and Gäde, 1993). The body mass of the animals used averaged 1 g±23 %.

Flight experiments

The experimental conditions and the procedures for handling the animals were as outlined by Auerswald et al. (1998).

Lift-generating flight

In some beetles metabolite levels were measured after pre-flight warm-up. These experiments were performed as previously described in detail by Auerswald et al. (1998).

Flight without lift generation

Fruit beetles were fixed by the pronotum to a pipette tip using superglue; the pipette tip was then attached to the arm (length 29.5 cm) of a flight mill. Flight velocity was recorded by means of an infrared microprocessor tachometer (RS, UK) whose light beam was directed towards a reflector on top of the flight mill.

Samples for the determination of metabolite levels in the haemolymph and flight muscles were taken and stored as described by Auerswald et al. (1998). In addition, fat body samples were taken as follows: after removal of the gut, reproductive organs and Malphigian tubules from the abdomen, the tracheae and airsacs with the surrounding fat body tissue were dissected out and stored in the same way as the flight muscles. Dissection of the two tissues took approximately 2 min. To investigate the different flight muscle types (basalar, dorso-ventral and dorso-longitudinal; see Fig. 1), whole thoraces were frozen in liquid N2. Hitting the frozen sample with a pestle in a grinder cooled by liquid N2 caused the thorax to fall apart, and the different muscles (Fig. 1) were identified and then treated separately.

Preparation of samples and measurement of metabolite levels

Haemolymph

Samples (1 μl) of haemolymph were either blown immediately into 100 μl of concentrated H2SO4 for the measurement of levels of total lipids (Zöllner and Kirsch, 1962) or carbohydrates (Spik and Montreuil, 1964) or pipetted into 60 μl of 80% acetonitrile for amino acid analysis (see below).

Tissue samples

Perchloric acid extracts from frozen tissues were made according to the method of Zebe and Gäde (1993).

Measurement of glycogen

Glycogen was extracted as previously described (Zebe and Gäde, 1993) and analysed by the modified anthrone method (Spik and Montreuil, 1964) using glucose as a standard.

![Fig. 1. Flight muscles of Pachnodasinusuata seen from the front of the metathorax: BM, basalar muscle; DVM, dorso-ventral muscle; DLM, dorso-longitudinal muscle; SM, subalar muscle; ODM, oblique dorsal muscle; StPM, sternopleural muscle; TCxM1, tergocoxal muscle 1.](image-url)
The majority of the beetles flew for between 1 and 5 min. Occasionally, a beetle flew for up to 20 min, but this occurred in less than 5 % of the flights (N=42).

Concentrations of metabolites in the haemolymph

Mean resting levels of proline and alanine in the haemolymph were 98 and 8 μmol ml\(^{-1}\), respectively. The proline concentration dropped during the first 30 s of flight and levelled off at approximately 50 μmol ml\(^{-1}\) (Fig. 2A). Mean alanine concentration increased to 42 μmol ml\(^{-1}\) during the first 30 s of flight and to 62 μmol ml\(^{-1}\) after 5 min of flight (Fig. 2A).

Proline and alanine concentrations were inversely related during rest after a flight of 5 min (Fig. 2A). Pre-flight levels had not been reached after 60 min of rest. The sum of the concentrations of the two amino acids was more or less stable during flight and subsequent rest at approximately 110 μmol ml\(^{-1}\), with the exception of the value at 30 s of flight, which was 90 μmol ml\(^{-1}\).

The mean concentration of haemolymph carbohydrates at rest was approximately 7 mg ml\(^{-1}\). The concentration increased during the first 30 s of flight to approximately 9 mg ml\(^{-1}\) and then dropped significantly (Fig. 2B). After 5 min of flight, a value of less than 6 mg ml\(^{-1}\) was reached. During 60 min of subsequent rest after 5 min of flight, the concentration rose to 7.5 mg ml\(^{-1}\), a return to pre-flight levels.

Mean haemolymph lipid levels showed a small but significant (P<0.001) increase of 1.5±1.1 mg ml\(^{-1}\) from an initial value of 10.0±3.1 to 11.5 mg ml\(^{-1}\) in 13 beetles which stopped flying after 11.0±3.8 min. After 60 min of subsequent rest, the concentration of lipids in the haemolymph was 14.0±6.0 mg ml\(^{-1}\) (data not shown).
Metabolite concentrations in flight muscles

The most dramatic changes in flight muscle metabolite concentrations occurred during the first 30 s of flight (Fig. 3A,B). The mean resting proline concentration of 55 μmol g⁻¹ fresh muscle mass declined to 31 μmol g⁻¹ during the first 30 s of flight. In contrast to levels in the haemolymph, proline levels dropped further to reach 13 μmol g⁻¹ after 5 min of flight. The resting alanine concentration of 11 μmol g⁻¹ increased to 36 μmol g⁻¹ during the first 30 s of flight (Fig. 3A). Again in contrast to the situation in the haemolymph, the level continued to increase, reaching 55 μmol g⁻¹ after 5 min of flight. Pre-flight levels of proline and alanine were re-established after 1 h of rest following a 5 min flight. The sum of the concentrations of proline and alanine was within the range 64–70 μmol g⁻¹ throughout the experiment.

Glycogen stores in the flight muscles amounting to 77 μmol glucose equivalents g⁻¹ fresh muscle mass declined most rapidly within the first 10 s of flight to a value of 48 μmol g⁻¹ (Fig. 3B). Thereafter, glycogen levels were depleted at a less dramatic rate, reaching 12 μmol g⁻¹ after 5 min of flight. After 60 min of rest, the glycogen concentration had almost returned to its pre-flight level.

Endothermic warm-up

Endothermic warm-up of *P. sinuata* is energy-expensive and metabolic changes occur during this process (Auerswald et al. 1998). Therefore, metabolite concentrations were determined just after the beetles had warmed up. These data were later taken into account for the calculation of rates of substrate consumption (see Discussion) to separate the energetic costs of flight from those of heat generation (see Table 1). The proline concentration after warm-up was 77.6±13.3 μmol ml⁻¹ (N=5) in the haemolymph and 46.8±4.8 μmol g⁻¹ muscle mass (N=5) in the flight muscles, while the respective alanine concentrations were 29.5±16.2 μmol ml⁻¹ (N=5) and 21.5±4.0 μmol g⁻¹ (N=5). The glycogen concentration in the flight muscles after warm-up was 71.9±6.1 μmol glucose equivalents g⁻¹ fresh muscle mass (N=5), and no changes in the haemolymph carbohydrate and lipid levels occurred during warm-up. These data are not depicted in the respective figures.

Rates of oxygen consumption and respiratory quotient

Because flight velocity was not directly measurable, we used the rate of oxygen consumption as an indicator of flight performance. Mean $V_O_2$ was highest shortly after the start of flight at 104 ml g⁻¹ body mass⁻¹ h⁻¹ after 2 min, but fell steadily with flight duration (Fig. 4). After 12 min of flight, $V_O_2$ was 92 ml g⁻¹ body mass⁻¹ h⁻¹. However, the respiratory quotient remained at approximately 0.9 throughout flight (Fig. 4).

Flight without lift generation

Flight duration

All the beetles (N=48) flew for at least 45 min, and more than 10 % flew for approximately 2 h; the longest flight recorded lasted 165 min.

![Fig. 3. Metabolite concentrations in the flight muscle tissue of the complete flight muscles of *Pachnoda sinuata* during different durations of lift-generating flight and subsequent rest after 5 min of flight. (A) Proline (broken line) and alanine (solid line); (B) glycogen. Values are given as means ± s.d. (N=5–8). For an explanation of the symbols, see Fig. 2.](image)

Concentrations of metabolites in the haemolymph

In resting beetles, the following mean concentrations of metabolites were found: proline 129 μmol ml⁻¹, alanine 14 μmol ml⁻¹, total carbohydrates 9 mg ml⁻¹ and lipids 9 mg ml⁻¹.

The proline concentration in the haemolymph decreased during the first 15 min of flight to 40 μmol ml⁻¹ and reached a plateau of 30–40 μmol ml⁻¹ after approximately 30 min of flight (Fig. 5A). The alanine concentration rose to 74 μmol ml⁻¹ after 15 min of flight and then to 90–100 μmol ml⁻¹ in beetles that flew for 30 min or longer (Fig. 5A). Pre-flight levels of proline were reached after 60 min of rest following a flight of 30 min, while pre-flight alanine values were reached after 15 min of rest. The sum of the concentrations of proline and alanine was more or less stable throughout the experiment (between 120 and 145 μmol ml⁻¹), with the exception of the value at 15 min of flight, which was 114 μmol ml⁻¹. During the subsequent resting period, the sum of the concentrations was lower, ranging from
Flight substrates of fruit beetle 2337

Table 1. Rates of oxygen consumption calculated from data on rates of substrate utilisation

<table>
<thead>
<tr>
<th>Rate of consumption during the first 30 s of flight*</th>
<th>Rate of O2 consumption</th>
<th>% of total VO2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proline</td>
<td>31.6 µmol g⁻¹ tissue mass min⁻¹</td>
<td>79 (µmol O2 g⁻¹ tissue h⁻¹)</td>
</tr>
<tr>
<td>Glycogen</td>
<td>64.3 µmol g⁻¹ tissue mass min⁻¹</td>
<td>385.8 (µmol O2 g⁻¹ tissue h⁻¹)</td>
</tr>
<tr>
<td>Proline+glycogen</td>
<td>95.2</td>
<td>51.2</td>
</tr>
</tbody>
</table>

Flight muscle

The mean level of total carbohydrates in the haemolymph dropped from the initial value of 9.4 mg ml⁻¹ to 4.8 mg ml⁻¹ during the first 30 min of flight (Fig. 5B). Subsequently, the concentration of carbohydrates increased to reach pre-flight levels after 90 min of flight. When beetles that had flown for 30 min rested, pre-flight values were reached after 120 min.

The mean haemolymph lipid concentration rose almost linearly during the first 60 min of flight from 9.4 to 16.3 mg ml⁻¹ and remained at this level (Fig. 5C). In beetles that rested after 30 min of flight, no significant return towards pre-flight levels occurred.

Metabolite concentrations in flight muscles

Flight muscles contained approximately 59 µmol g⁻¹ fresh muscle mass proline and 5 µmol g⁻¹ alanine at rest. During the first 15 min of flight, proline concentration dropped to 22 µmol g⁻¹ and the level remained at approximately 20 µmol g⁻¹ after longer flight times (Fig. 6A). Concomitantly, alanine concentration increased to 38 µmol g⁻¹ during a flight of 15 min and reached a plateau of more than 40 µmol g⁻¹ after flights of 30 min or longer. Pre-flight levels of the two amino acids were regained after 60 min of rest following a 30 min flight. The sum of proline and alanine concentrations is very stable, at 60–65 µmol g⁻¹, throughout flight and subsequent rest.

A mean glycogen concentration of approximately 65 µmol g⁻¹ fresh muscle mass was measured in flight muscles of resting beetles. These stores were largely depleted during the first 15 min of flight (to 16 µmol g⁻¹; Fig. 6B). There was a negligible further decrease to approximately 10 µmol g⁻¹ during 45 min of flight. Recovery of stores after 30 min of flight took approximately 120 min.

Metabolite concentrations in the fat body

The changes in the amino acid levels in the fat body during flight were less pronounced than those in the flight muscles, and a significant change was measured only for alanine (Fig. 7A). However, during subsequent rest after 30 min of flight, the concentrations of the two amino acids increased (alanine) and decreased (proline) significantly compared with the value at the end of flight (Fig. 7A). The total concentration of proline and alanine ranged from 44 to 50 µmol g⁻¹ fresh tissue mass during flight and subsequent rest.

The mean level of glycogen in the fat body (155 µmol g⁻¹) was much higher than in the flight muscles. Depletion of the stores took longer than in the flight muscles. After 30 min of flight, a concentration of approximately 60 µmol g⁻¹ was reached, a value that did not change with longer flight durations (Fig. 7B). Pre-flight levels were restored after 120 min of rest following 30 min of flight.

Flight velocity

As a measure of performance during flight mill flight, we recorded flight velocity (results not depicted). The highest flight velocity (approximately 6 km h⁻¹) was observed immediately after the initiation of flight. Velocity decreased consistently until it reached a stable level of approximately 3 km h⁻¹ after approximately 30 min of flight.

Muscle types

To determine whether the functional specialisation of the different muscles is accompanied by a biochemical/physiological specialisation, we measured metabolic changes separately in the dorso-longitudinal (DLM), dorso-ventral (DVM) and basalar (BM) muscles (see Fig. 1) after 30 s of lift-producing flight and after different durations of flight without lift production (Fig. 8). Resting levels of the substrates did not differ between the various muscles. No significant differences in levels between muscles were measured after 30 s of lift-generating flight (Fig. 8A). When lift was minimised, however, significant differences in levels occurred between the muscle types (Fig. 8B). These differences...
were largest in animals that flew for only 5 min. Proline levels were highest in the DVM in these animals and, in addition, the alanine level was significantly lower than in the DLM. Minor differences in levels occurred after both 10 and 20 min of flight without lift, but these differences were less pronounced than after a 5 min flight.

**Haemolymph volume and tissue mass**

To estimate the participation of proline and carbohydrates and the different parts of the body to the supply of energy during flight, respectively, we calculated the respective rates of oxygen consumption. It was necessary to know the haemolymph volume. For 12 beetles (ranging from 610–1400 mg body mass), a significant linear relationship between body mass (m, mg) and haemolymph volume (V, µl) was measured: \( V = 0.113m + 50.69 \), \( P<0.001 \). The mean haemolymph volume for the beetles with a mean body mass of 1020±237 mg was 165.8±29.5 µl. Flight muscles were found to represent 8.2±1.9 % (\( N=5 \)) of total body mass and the fat body tissue 4.1±3.8 % (\( N=6 \)). Thus, a beetle with a mean fresh mass of 1020 mg contained 83.6 mg of flight muscles, 41.8 mg of fat body tissue and 166 µl of haemolymph.

**Discussion**

In the present study, we quantify the pattern of metabolic changes in the African fruit beetle *P. sinuata* using a method that simulates natural flight (Schneider, 1989); our previous studies had analysed the situation only during flight without lift generation (Zebe and Gäde, 1993; Lopata and Gäde, 1994).

During lift-generating flight, we could distinguish two metabolic phases. The first phase lasted from take-off until approximately 30 s of flight. It is characterised by a steep decrease in the haemolymph and flight muscle proline levels and a mirror-image increase in the concentration of alanine concentration, the end product of partial proline degradation (Bursell, 1981; Weeda *et al.* 1979). The concentration of glycogen in the flight muscles is rapidly reduced, while carbohydrate levels in the haemolymph increase during this
These carbohydrates are probably released from the fat body by the action of a neuropeptide from the corpus cardiacum (Lopata and Gäde, 1994) which was identified as an octapeptide of the large AKH/RPCH family, termed Mem-CC (Gäde et al. 1992; Gäde, 1996). Flight performance in this early flight period is high, as indicated by the high rates of oxygen consumption. It is noteworthy that, at the end of this phase, minimum values of the total concentration of proline and alanine occur in the haemolymph (but not in the flight muscles). This indicates a deficit of Krebs cycle intermediates early in flight (since proline is utilised but no comparable amount of alanine arrives in the haemolymph) and that proline resynthesis in the fat body is not yet generating proline at the maximum rate. Thus, a steady state between rates of proline oxidation and resynthesis is not reached.

In the second phase, metabolite levels appear to stabilise. Proline and alanine concentrations in the haemolymph remain constant, while the concentrations in the flight muscles reach a plateau and then increase again at a slower rate. Resynthesis of proline from alanine in the fat body probably takes place at a maximum rate, which should be approximately 1.5 μmol ml⁻¹ haemolymph min⁻¹ (calculated for the first 15 min of rest after flight; Fig. 2A). Glycogen stores in the flight muscle decrease at a much lower rate, while haemolymph carbohydrate concentration is reduced during this phase. The carbohydrates released from the fat body in the first phase now become available and is oxidised. Flight performance is also markedly lower than during the first phase. The second phase is therefore characterised by the fact that metabolism is in an equilibrium state: lower flight performance results in a lower demand for fuels and these are provided by using the carbohydrate reserves previously mobilised from the fat body. The stable respiratory quotient (RQ) during this phase suggests that proline is also contributing to the energy provision. Owing to the resynthesis of proline from alanine in the fat body (see Bursell, 1977; L. Auerswald and G. Gäde, unpublished data for P. sinuata fat body), rates of oxidation and synthesis are balanced and are responsible for the steady state observed during this phase.

These two phases observed during flight seem to contrast with the three phases described by Zebe and Gäde (1993). However, these authors studied flight on a flight mill (without lift production). They found that during the first phase, which comprised the first 2 min of flight, proline was the exclusive fuel in the flight muscles. In the light of our new results, discussed in Auerswald et al. (1998), there is a very plausible explanation: previous studies (Zebe and Gäde, 1993) did not
Fig. 8. Metabolite concentrations in different flight muscles of *Pachnoda sinuata*. (A) At rest and after lift-generating flight (N=5; each extract contained pooled material from two individuals); (B) after flight without lift generation lasting for 5, 10 or 20 min (N=7). Values are given as means ± S.D. Significance was calculated using Student’s *t*-test. ‡*P*<0.05, ‡‡*P*<0.01 compared with the value for the dorso-ventral muscle (DVM). BM, basalar muscle; DLM, dorso-longitudinal muscle. Pro, proline; Ala, alanine; Glyc, glycogen.

take the pre-flight warm-up phase into account. It is now clear from metabolic and respiratory studies that proline is the exclusive fuel during warm-up (Auerswald *et al.* 1998); this warm-up phase was, however, included in the metabolic changes observed during 2 min of flight in our previous study (Zebe and Gäde, 1993).

All previous flight data for *P. sinuata* were obtained during flight without lift, but such studies investigated only relatively short periods of flight (15–20 min; Barnett *et al.* 1975; Zebe and Gäde, 1993; Lopata and Gäde, 1994). Our flight mill experiments in the present study lasted up to 2 h. Using our data and those of the studies cited above, flight on a flight mill can be characterised as follows: the two phases we found during lift-generating flight are also apparent, but the metabolic changes occur much more slowly. The rapid changes discussed for the first phase of lift-generating flight (30 s) take place over 4–8 min in the flight muscles and over 8–30 min in the haemolymph during flight on the flight mill. The increase in haemolymph carbohydrate levels, which occurred during the first phase of lift-generating flight, occurs only after more than 30 min of flight on the flight mill; at this time, fat body glycogen level reaches a low and stable value. The carbohydrates in the haemolymph are probably derived from mobilised fat body reserves. As indicated by measurements of flight velocity, flight performance reaches a low level at this stage.

Beetles flew for longer when lift was minimised. While the maximum flight duration of lift-generating flight was 20 min, some individuals flew for almost 3 h on the flight mill. This indicates that the energy expenditure of flight mill flight is much lower than that of lift-generating flight. This is in accordance with the observations of Heinrich (1971), who measured *V*O$_2$ values of 45–50 ml O$_2$ g body mass$^{-1}$ h$^{-1}$ during true free flight, but only 21 ml g body mass$^{-1}$ h$^{-1}$ during tethered flight without lift generation in the sphinx moth *Manduca sexta* (see also review by Casey, 1989).

The qualitative changes seen during both types of flight experiments are comparable. This means that, although flight mill experiments do not resemble natural conditions, investigations using this method may have merits. However, the quantitative differences caused by minimising lift production must be taken into account.

Interestingly, haemolymph lipid concentration increases significantly during both types of flight, but no restoration of the pre-flight level occurs during subsequent rest. Similar data are reported for the Colorado potato beetle *Leptinotarsa decemlineata* during tethered flight (Mordue and de Kort, 1978; Weeda *et al.* 1979). However, the maximum increases of 1.5 mg ml$^{-1}$ after 11 min of lift-generating flight and 9.1 mg ml$^{-1}$ after 2 h of flight on the flight mill were small in *P. sinuata* compared with that of approximately 20 mg ml$^{-1}$ after 20 min of tethered flight in *L. migratoria* (Goldsworthy *et al.* 1972), which uses fat as the predominant fuel for flight. These results suggest that lipids are not used as a flight substrate in *P. sinuata*. This idea is supported by the fact that haemolymph lipid levels do not increase during starvation in *P. sinuata* (L. Auerswald and G. Gäde, unpublished results) as they do in *L. migratoria* (Jutsum *et al.* 1975). In addition, in contrast to the locust, where AKH peptides initiate the release of lipids for flight (see Goldsworthy and Mordue, 1989; Gäde, 1992), haemolymph lipid levels in *P. sinuata* were not influenced by the fruit beetle’s own AKH peptide, Mem-CC (Lopata and Gäde, 1994). During flight, the RQ did not fall, as would be expected if fatty acids were oxidised during prolonged flight. Thus, taken together, these data suggest that lipids are not oxidised by the flight muscles, but that they might be used for other purposes.

We assumed that, during flight where lift is minimised, the muscles involved in generating lift should show a slower depletion of substrates compared with lift-producing flight. We therefore measured metabolic changes separately in three different muscles of the flight apparatus. As expected, metabolic changes during lift-generating flight in the lift-generating downstroke muscles (BM and DLM) and in the DVM, one of the upstroke muscles responsible for producing drag, did not differ, because all the muscles are used. However, during flight where lift is minimised, we again found no large
differences. Unexpectedly, the DVM was found to have used less proline than the DLM and BM and to have generated less alanine than the DLM. This was an unexpected result, suggesting a major participation of the downstroke muscles (BM and DLM) in generating both lift and drag. This pattern of substrate use, in addition to the similar resting values of metabolites in the various muscles, excludes a biochemical specialisation of certain muscles to oxidise one or other fuel.

Our results allow us to calculate the contributions of the different fuels (carbohydrates and proline) as well as the contribution of the major compartments (flight muscles, haemolymph) to the overall energy consumption for a ‘model beetle’ (as outlined in the Results) during the first 30 s of lift-generating flight by using the rate of substrate utilisation and the equation for the partial oxidation of proline (see Auerswald and Gäde, 1995). The data shown in Table 1 demonstrate clearly that proline and carbohydrates contribute to a similar extent (44.4 versus 55.6 %, respectively) to the energy required for flight. A major part of flight substrates in P. sinuata is supplied by the flight muscle tissue itself (54 %; Table 1). The haemolymph also supplies a large amount of energy (46 %), but this is a relatively smaller amount than we found in the blister beetle Decapotoma lunata (75 %; Auerswald and Gäde, 1995). This difference is mainly caused by the fact that P. sinuata has a much lower relative haemolymph volume (163.7 μl g⁻¹ body mass) than D. lunata (347.2 μl g⁻¹ body mass). The sum of the two compartments amounts to a body-mass-specific rate of oxygen consumption of 95.2 ml g⁻¹ h⁻¹. Because we did not measure metabolic changes in the fat body during lift-generating flight, we assumed from the data obtained for flight mill flight that the rate of substrate depletion in the fat body is similar to that in flight muscle tissue. Despite the low percentage of body mass due to the fat body, $V_O_2$ is then well above 100 ml g⁻¹ body mass h⁻¹, taking the rate of resynthesis of proline into account (see Figs 2A, 3A for resting values after flight). This value is in good agreement with our results obtained from measuring $V_O_2$ in vivo (approximately 104 ml O₂ g⁻¹ body mass h⁻¹, Fig. 4).

In conclusion, we have shown that proline and carbohydrates are both major substrates for energy supply during flight in P. sinuata. In addition, we have demonstrated differences in substrate use between the two flight methods as well as the high energy demand required of lift-generating flight.

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References


