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HAEMOLYMPH SUGAR LEVELS IN FORAGING HONEYBEES (*APIS MELLIFERA CARNICA*): DEPENDENCE ON METABOLIC RATE AND *IN VIVO* MEASUREMENT OF MAXIMAL RATES OF TREHALOSE SYNTHESIS

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Summary

Previous investigations of haemolymph sugar levels in honeybees have reported very different results, probably because different experimental conditions affected the activity levels of the animals. The present study investigated the dependence of haemolymph sugar levels in foraging honeybees on metabolic rate and whether the haemolymph sugar level is regulated. Free-flying foraging bees were trained to collect controlled amounts of sucrose solution of different concentrations (15 %, 30 % or 50 % sucrose w/w). Immediately after feeding, metabolic rate was measured over a given time depending on the sucrose concentration, then crop-emptying rate and haemolymph sugar levels were measured. Bees exhibiting a wide range of metabolic rates were compared to establish whether the observed differences in haemolymph sugar levels were due to limits in the supply of sugars from the crop or in the rate of trehalose synthesis in the fat bodies. Independent of the concentration of the sucrose solution supplied, haemolymph trehalose, glucose and fructose levels were constant for metabolic rates from 0 to 4.5 ml CO_2 h^{-1}. At higher metabolic rates, trehalose concentration decreased while that of glucose and fructose increased, with the exception of bees fed 15 % sucrose solution. As the supply of sugar from the crop *via* the proventriculus was sufficient to support even the highest metabolic rates, the observed pattern must result from an upper limit in the capacity of the fat body to synthesise trehalose. The maximal rate of conversion of glucose to trehalose in the fat body was therefore calculated to average 92.4 μg glucose min^{-1}. However, for bees fed 15 % sucrose solution both the rate of conversion of glucose to trehalose and the rate of sugar transport from the crop to the ventricle were limited, together resulting in a decrease in total haemolymph sugar levels for metabolic rates higher than 5 ml CO_2 h^{-1}.

Key words: haemolymph sugar, metabolism, honeybee, *Apis mellifera carnica*, trehalose, proventriculus.

Introduction

During foraging, honeybees are able to increase their rate of oxygen consumption by more than 70-fold between rest and flight (Kammer and Heinrich, 1978). In addition, the rapid processing of visual and other sensory information needs to be supported metabolically, so their metabolism must be extremely flexible to balance ATP synthesis and degradation to co-ordinate the catabolic pathways (Wegener, 1996; Candy et al., 1997).

Honeybees use almost exclusively sugars as substrates for flight (Sacktor, 1970; Rothe and Nachtigall, 1989) and their brain is also highly specialised for carbohydrate oxidation (Tsacopoulos, 1995). As there appears to be no active transport of substrates from the ventricle to the haemolymph (Crailsheim, 1988c), levels of substrates in the haemolymph must be kept sufficiently high to provide an adequate fuel supply. Indeed, total haemolymph sugar levels in the honeybee are among the highest recorded for any insect species (Fell, 1990).

Because honeybees store only limited amounts of glycogen in the flight muscle (Neukirch, 1982; Panzenböck and Crailsheim, 1997) and fat body (John, 1958; Panzenböck and Crailsheim, 1997), and use negligible quantities of amino acids as fuel (Micheu et al., 2000), they are almost exclusively dependent on intestinal and haemolymph energy supplies for most activities.

Previous investigations of haemolymph sugar levels in honeybees have been carried out under diverse and variable experimental conditions: assays were carried out with winter bees and summer bees, on bees ranging from previously starved to previously fed, from newly emerged to foragers and from active to immobilised. Consequently, haemolymph trehalose levels reported in the literature vary from 2 mg ml^{-1} (Bounias and Morgan, 1984) to 40 mg ml^{-1} (Bozic and Woodring, 1997), and glucose and fructose levels vary from 2 mg ml^{-1} (Abou-Seif et al., 1993) to approximately

15 mg ml^{-1} (Fell, 1990; Leta et al., 1996). Haemolymph sugar levels in bees have been reported to change in response to the concentration of sugar solution imbibed (Crailsheim, 1988b; Abou-Seif et al., 1993), season (Crailsheim, 1988b) and behavioural pattern (Bozic and Woodring, 1997). On the basis of this high recorded variability, it has been suggested that there is no haemolymph sugar homeostasis in insects (Candy et al., 1997), even though regulating hormones have been found in many insect species (Gäde, 1996).

For honeybees, a possible explanation for the recorded variability in haemolymph sugar levels is that the different experimental conditions cause different levels of activity, resulting in metabolic differences. Such metabolic differences could have an important impact on proventriculus activity and thus on crop-emptying rate (Roces and Blatt, 1999). Depending on both the sugar concentration of the fed solution and the maximal rate of solution flow through the proventriculus (Núñez, 1969; Roces and Blatt, 1999), there may be an insufficient sugar supply for bees displaying high metabolic rates. Thus, the sugar transport rate through the proventriculus (the energy input) must be compared with the bee's metabolic rate (the energy output) to obtain a comprehensive view of the factors determining haemolymph sugar levels.

The purpose of the present study was to investigate the dependence of haemolymph sugar levels on metabolic rate and whether haemolymph sugar levels are regulated. Foraging bees were trained to collect controlled amounts of sucrose solution of different concentrations. After feeding, metabolic rate, crop-emptying rate and haemolymph sugar levels were recorded. Bees exhibiting a wide range of metabolic rates were compared to investigate whether differences in haemolymph sugar levels are caused by limits in the supply of sugar from the crop or in the rate of trehalose synthesis in the fat body.

Materials and methods

Standardisation of honeybees

A queenright colony of honeybees *Apis mellifera carnica* (Pollm.) was kept in a two-frame observation hive in the vicinity of the bee station of the University of Würzburg, Germany. Experiments were carried out from the beginning of June to the end of August in the years 1996–1998.

It is well known that foragers react to increasing reward (sugar solution concentrations) with increasing flight speed (von Frisch and Lindauer, 1955; Gmeinbauer and Crailsheim, 1993), so that different feeding conditions have an impact on the foraging motivation of the bees. Therefore, an experimental design was used that guaranteed relatively undisturbed foraging behaviour of the bees. They were trained to fly 80 m from the hive to a feeding station located in the laboratory. The bees reached the feeding station through a 12 cm×15 cm hole in a window, which led into a wooden, L-shaped tunnel (110 cm long, 12 cm wide, 15 cm high) with a Plexiglas top. Green and yellow reference marks were drawn on the bottom and on both sides of the tunnel. Trained bees flew directly to the end of the L, where the feeder was located. The feeder consisted of a glass container filled with a polystyrene block in which a tiny glass cup was located.

During the training stage, all incoming foragers were allowed to feed *ad libitum*. During the experiments, only one bee was allowed to enter the tunnel at a time. It found exactly 30 µl of the same sucrose solution used in the training stage. As this quantity does not represent the maximal crop load (60–70 µl), the bee always collected all the solution provided. While the bee imbibed the sugar solution (approximately 20–40 s), the feeder with the bee was carefully placed into a respirometric chamber (height 44 mm, diameter 61 mm, volume 128 cm) in which it walked freely after feeding and did not try to fly. Only bees that did not interrupt feeding while being placed into the respirometric chamber were used in the experiments.

The concentrations of the fed solutions were reported as percentage sucrose equivalents (g solute per 100 g solution) (following Bolton et al., 1979). The concentrations used during the experiments, 15, 30 and 50 % sucrose (w/w), are in the range of nectar sugar concentrations that bees encounter naturally (Baker and Baker, 1978).

Gas exchange measurements

After food collection, the CO_2 production of each bee was measured over 15 min for bees fed 15 % sucrose, over 30 min for those fed 30 % sucrose, and over 60 min for bees fed 50 % sucrose. These times were chosen on the basis of preliminary experiments, in which half of the crop contents were found to have passed through the proventriculus after these intervals. Consequently, at the end of the CO_2 measurements the bees would still have sugar solution in their crop and therefore any changes in haemolymph sugar levels were not the result of exhaustion of the crop contents during the measurement period.

Open-flow respirometry was used to measure CO_2 production of bees after feeding. CO_2-free air was drawn through the respirometric chamber at a flow rate of 300 ml min^{-1}, which was controlled by a mass-flow controller. The high-resolution respirometry system used (Sable System TR-3, resolution 0.01 p.p.m. CO_2), including temperature control and correction to S.T.P.D. conditions, has been described elsewhere (Lighton, 1990). To obtain measurements over a wide range of metabolic rates, the respirometric chamber was placed in a water bath with temperatures ranging from 10 to 39 °C. Preliminary experiments showed that foraging bees tried to maintain high metabolic rates after feeding even at relatively low temperatures. Metabolic rates were expressed in ml CO_2 h^{-1} per bee, not as mass-specific rates, to allow comparisons, because the measurements of sugar transport rates through the proventriculus (see below) were calculated for the whole animal. The range of body masses of unfed bees varied between 71 and 85 mg (79.1±4.9 mg, mean ± S.E.M., N=22).

Measurement of crop emptying and sugar transport rates

As honeybee foragers flying to a familiar feeding place carry

only as much sugar solution in the crop as they need for their flight (Beutler, 1950; Sacktor, 1970; Brandstetter et al., 1988), training ensured that both the crop and rectum of workers arriving at the feeding station were empty. This was confirmed by dissections of control bees arriving at the feeder. Since bees were fed with a known quantity of sucrose solution, it was possible to determine the flow rate through the proventriculus by measuring the amount of solution in both the crop and rectum after specific time intervals. Since the concentration of the sucrose solution was known, the sugar transport rates through the proventriculus could then be calculated.

After gas exchange measurements the bee was gently caged and anaesthetised with CO_2 until the proboscis was extended, i.e. the individual was completely anaesthetised but did not regurgitate its crop contents. This procedure took less than 10 s. To measure the amounts of fluid contained in both the crop and rectum, the bee was fixed ventrally onto a wax plate after it had been anaesthetised, its abdomen was dissected and the haemolymph was absorbed with a piece of filter paper. The crop was carefully pulled out of the intestine with tweezers. The crop contents were squeezed out by pressing the crop against pre-weighed filter paper, so that the crop tissue remained between the tweezers. The moistened paper was put into a small pre-weighed vial and weighed on a microbalance (Mettler UMT5) to the nearest 0.001 mg. Crop content mass (in mg) was converted into a volume by dividing by the density of the fed sugar solution. To measure the rectum fluid content, an incision was made in the rectum wall, and the fluid was absorbed with pre-weighed filter paper. Again, the moistened paper was put into a small pre-weighed vial and weighed. Since the rectal fluid contained no sugars, its density was assumed to be 1 g ml^{-1}. Measurements of rectal fluid production were observed to correspond well with crop-emptying rates, thus providing a control for the fluid volume measurements and confirming that no changes in haemolymph volume that would affect measurements of haemolymph sugar levels occurred during the experiments (see also Roces and Blatt, 1999). The whole dissection procedure from the beginning of anaesthesia lasted less than 3 min.

Haemolymph sampling and determination of sugar levels

To obtain a sample of haemolymph, the protruding head of the anaesthetised bee was deflected ventrally, the exposed dorsal neck membrane was punctured with a pin and 0.5 µl of haemolymph was collected with a microcapillary. Immediately after collection, each single haemolymph sample was placed in 400 µl of distilled water and kept at −20 °C until analysis.

High-performance liquid chromatography (HPLC) was used to measure trehalose, glucose and fructose concentrations. Sugars were separated on a carbopac PA1 column at a flow rate of 0.9 ml min^{-1}. The HPLC running buffer consisted of 80 mmol l^{-1} NaOH. Sugar concentrations were determined by isocratic ion chromatography with pulsed amperometric detection (4500i, Dionex, Idstein, Germany). The system was calibrated after every fourth sample with a standard solution containing 50 µmol l^{-1} each of trehalose, glucose, fructose and sucrose. Measurements were performed with a sensitivity of 3 µCoulomb. The detection limit for measurements was 5 µmol l^{-1}. Chromatograms were processed with Winpeak software (Chromatography Data System, Biotronik, Maintal, Germany). Results were converted into mg ml^{-1}.

Results

Metabolic rate showed a negative linear relationship with ambient temperature for bees walking inside the respirometric chamber after feeding (Fig. 1). At low temperatures, metabolic rates were nearly as high as those recorded from flying honeybees (from 7 to 13 ml CO_2 h^{-1}; Nachtigall et al., 1995; Balderrama et al., 1992). Metabolic rates ranging from 0.5 to 9.5 ml CO_2 h^{-1} were recorded in the present study.

In order to compare the crop-emptying rate with the metabolic expenditure, the amount of sugar passing through the proventriculus in a given period was compared with that required to support the bee's metabolic expenditure over the same period. Metabolic expenditure was calculated directly from CO_2 production, since both flight and walking in bees is fuelled by carbohydrate catabolism (respiratory quotient, RQ=1; Rothe and Nachtigall, 1989), and given that 1 l of CO_2 is produced by the catabolism of 1.23 g of carbohydrate (Eckert, 1993).

Fig. 2 shows the sugar transport rate of bees fed 30 µl of 15 %, 30 % or 50 % sucrose solution. The 'normlines' plotted indicate the expected relationship if the bees meet all their metabolic demands using sugar passed through the proventriculus. For bees fed 15 % sucrose solution (Fig. 2A) sugar transport rates mostly lay above the normline, i.e. the foragers passed slightly more sucrose through the proventriculus than was needed to meet metabolic demands (comparison of linear regressions for measurements and normline: slopes were significantly different, ANCOVA,

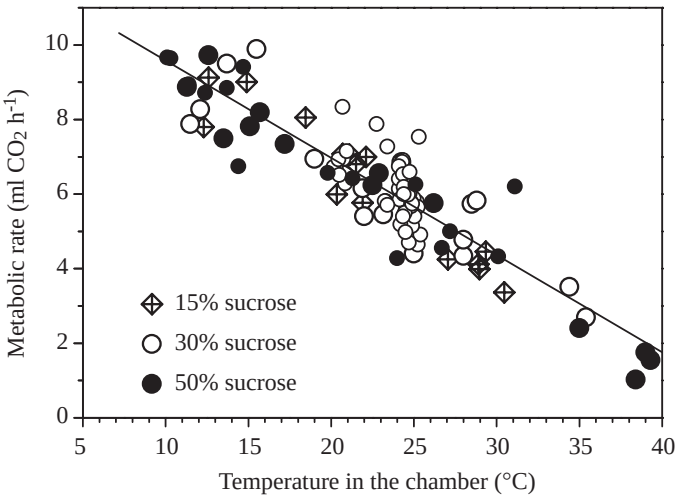

Fig. 1. Metabolic rate $\dot{V}_{CO_2}$ of honeybees *versus* temperature *T* of the respirometric chamber, $\dot{V}_{CO_2}$=12.19–0.261*T*; *N*=110, *r*=–0.91, *P*<0.0001). Each point represents a single bee fed either 15 %, 30 % or 50 % sucrose solution.

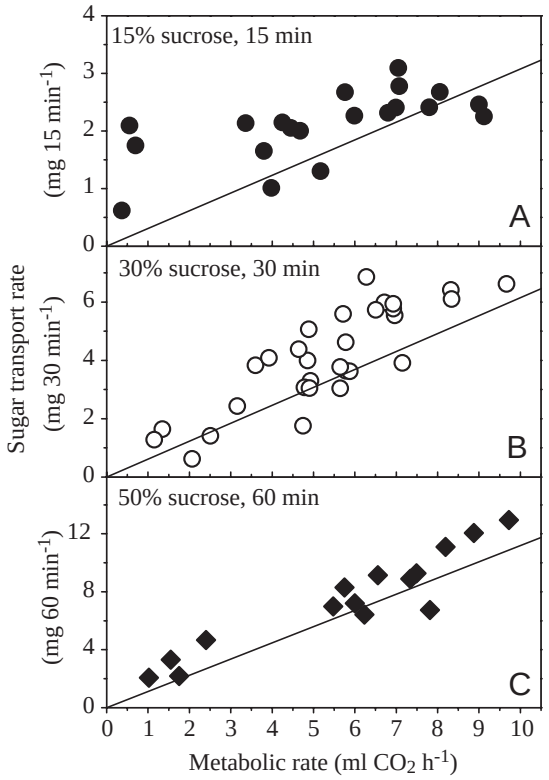

Fig. 2. Relationship between the sugar transport rate through the proventriculus and the CO_2 production of bees fed 30 µl of one of three different sucrose solutions. Each point represents a single bee. The lines in the graphs are not regression lines but are normlines, representing the expected sugar transport rate, calculated from the measured CO_2 production, assuming all the metabolic expenditure to be met from the imbibed sucrose solution. (A) Bees fed 15 % sucrose solution that remained in the gas exchange chamber for 15 min. (B) Bees fed 30 % sucrose solution that remained in the gas exchange chamber for 30 min. (C) Bees fed 50 % sucrose solution, measured over 60 min.

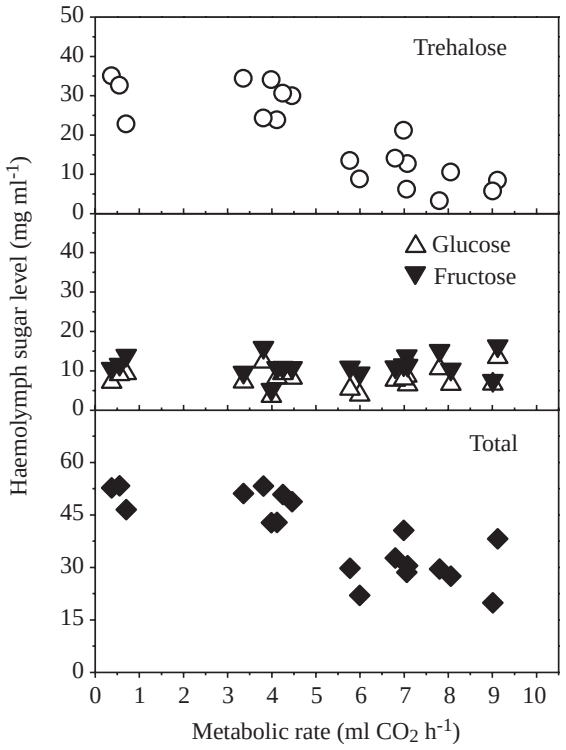

Fig. 3. Haemolymph levels of various sugars *versus* metabolic rate for bees fed 15 % sucrose solution (same individuals as in Fig. 2A). Haemolymph samples were taken 15 min after feeding ended. Each symbol represents one bee. Note the different scales on the ordinates. Total haemolymph sugar levels (C_{Tot}) decrease significantly with increasing metabolic rate $\dot{V}_{CO_2}$ above 4.5 ml CO_2 h^{-1} (see text).

$F_{(1,36)}$=16.95, P<0.001, so that significance of intercept differences cannot be tested). Those bees with the highest metabolic rates (8 ml CO_2 h^{-1} and above), however, showed sugar transport rates similar or even lower than predicted. However, note that the maximal fluid transport rate of the proventriculus in honeybees is 48 µl h^{-1} (Roces and Blatt, 1999), therefore the maximal amount of sugar that can pass through the proventriculus in bees fed 15 % sucrose solution is 7.63 mg h^{-1}, which could not support metabolic rates higher than 6.2 ml CO_2 h^{-1}.

A similar pattern was observed for bees fed 30 % and 50 % sucrose solution (Fig. 2B,C). However, while bees fed 30 % passed significantly more sucrose through the proventriculus than needed to meet metabolic demands [comparison of linear regressions for measurements and normline: slopes did not differ statistically (ANCOVA, $F_{(1,58)}$=2.23, P>0.1), but the intercepts were statistically different (ANCOVA, $F_{(1,59)}$=23.19, P<0.0001)], those fed 50 % passed only as much as needed (slopes and intercepts were statistically similar; ANCOVA, $F_{(1,38)}$=0.023, P>0.4 and ANCOVA, $F_{(1,39)}$=1.22, P>0.2, respectively). Compared with bees fed 15 % sucrose solution, the sugar transport rate through the proventriculus of bees fed 30 % or 50 % sucrose solution is sufficient to meet the highest metabolic rates. To support a metabolic rate of 10 ml CO_2 h^{-1}, the proventriculus must transport 12.3 mg h^{-1} sugar or 36.38 µl h^{-1} of 30 % sucrose solution, which is below the maximal recorded transport rate of 48 µl h^{-1} (Roces and Blatt, 1999).

Haemolymph sugar levels of the individuals plotted in Fig. 2A are presented in Fig. 3. Bees fed 15 % sucrose solution showed a constant trehalose concentration of approximately 30 mg ml^{-1} for metabolic rates ranging from 1 to 4.5 ml CO_2 h^{-1} (regression analysis, where C is the haemolymph sugar concentration and $\dot{V}_{CO_2}$ the metabolic rate, C_{Tre}=30.75–0.36$\dot{V}_{CO_2}$, N=9, r=–0.129, P>0.5). At higher metabolic rates, the trehalose concentration was observed to significantly decrease, reaching 5 mg ml^{-1} for bees with metabolic rates of 9 ml CO_2 h^{-1} (regression analysis, $C_{Tre,tot}$=37.02–3.34$\dot{V}_{CO_2}$, N=19, r=–0.822, P<0.001). In contrast there was no relationship observed between metabolic rate and haemolymph sugar levels for glucose (7.9±2.5 mg ml^{-1}, mean ± S.D.; regression analysis, $C_{Glu,tot}$=7.77+0.03$\dot{V}_{CO_2}$, N=19, r=0.033, P>0.5) or fructose (mean ± S.D. = 11.4±2.7 mg ml^{-1}; regression analysis, $C_{Fru,tot}$=10.98+0.08$\dot{V}_{CO_2}$, N=19, r=0.082, P>0.5). These

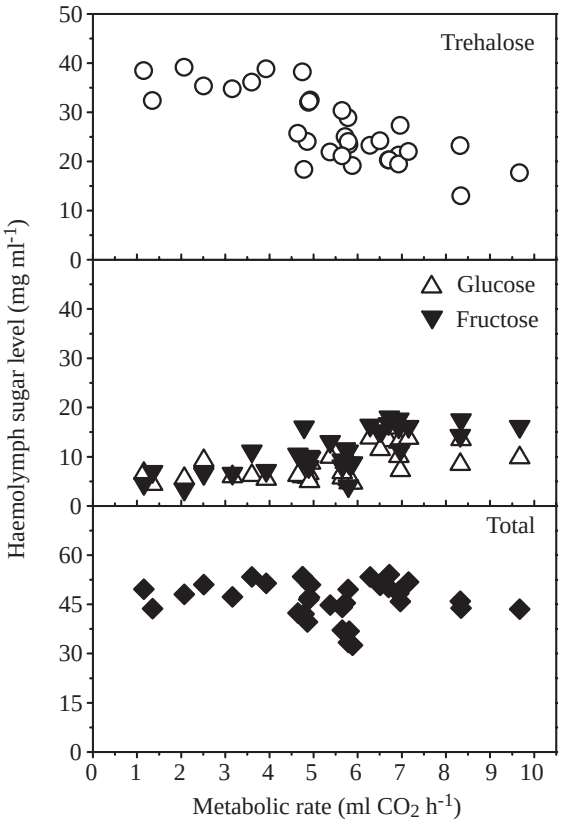

Fig. 4. Haemolymph sugar levels *versus* metabolic rate for bees fed 30 % sucrose solution (same individuals as in Fig. 2B). Haemolymph samples were taken 30 min after feeding ended. Each symbol represents one bee. Note the different scales on the ordinates.

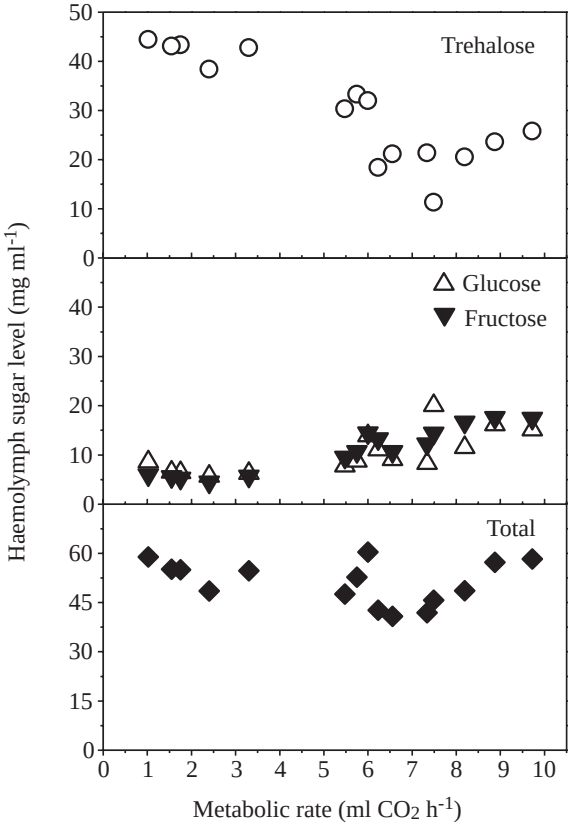

Fig. 5. Haemolymph sugar levels *versus* metabolic rate for bees fed 50 % sucrose solution (same individuals as in Fig. 2C). Haemolymph samples were taken 60 min after feeding ended. Each symbol represents one bee. Note the different scales on the ordinates.

patterns led to a significant decrease in total haemolymph sugar levels for metabolic rates higher than 4.5 ml CO_2 h^{-1} (C_{Tot}=55.95–3.24$\dot{V}_{CO_2}$; N=19, r=–0.79, P<0.0001).

Bees fed 30 % sucrose solution (Fig. 4) also showed a constant trehalose concentration for metabolic rates up to 4.5 ml CO_2 h^{-1} (regression analysis, C_{Tre}=35.43+0.39$\dot{V}_{CO_2}$, N=7, r=0.168, P>0.5). Beyond this point trehalose concentration decreased with increasing metabolic rate (regression analysis, $C_{Tre,tot}$=42.42–2.91$\dot{V}_{CO_2}$, N=33, r=–0.78, P<0.001; Fig. 4). Glucose and fructose levels were constant at approximately 6 mg ml^{-1} for metabolic rates up to 4.5 ml CO_2 h^{-1} (regression analysis, C_{Glu}=6.01+0.07$\dot{V}_{CO_2}$, N=7, r=0.048, P>0.5; C_{Fru}=3.14+1.37$\dot{V}_{CO_2}$, N=7, r=0.606, P>0.1), above which levels of both monosaccharides increased, reaching 15 mg ml^{-1} at the highest metabolic rates (regression analysis, $C_{Glu,tot}$=3.35+0.92$\dot{V}_{CO_2}$, N=33, r=0.553, P<0.001; $C_{Fru,tot}$=2.25+1.66$\dot{V}_{CO_2}$, N=33, r=0.748, P<0.001). The opposite patterns exhibited by levels of trehalose and the monosaccharides gave a constant total haemolymph sugar concentration, independent of metabolic rate (C_{Tot}= 48.01–0.32$\dot{V}_{CO_2}$, N=33, r=–0.110, P>0.5).

For bees fed 50 % sucrose solution (Fig. 5), the trehalose levels remained stable for the lowest metabolic rates (regression analysis, C_{Tre}=44.77–1.18$\dot{V}_{CO_2}$, N=5, r=–0.445, P>0.4). As for bees fed the lower concentrations of sucrose, trehalose levels then decreased with metabolic rate, from the mean value of 43.5 mg ml^{-1} to approximately 15 mg ml^{-1} (regression analysis, $C_{Tre,tot}$=47.55–3.23$\dot{V}_{CO_2}$, N=15, r=–0.848, P<0.001). Levels of glucose (6.6±1.1 mg ml^{-1}) and fructose (5.4±0.6 mg ml^{-1}) were initially relatively constant for metabolic rates up to 3.5 ml CO_2 h^{-1}, increasing up to 17 mg ml^{-1} at the highest metabolic rates ($C_{Glu,tot}$=4.38+ 1.09$\dot{V}_{CO_2}$, N=15, r=0.721, P<0.005; $C_{Fru,tot}$=2.29+1.58$\dot{V}_{CO_2}$, N=15, r=0.946, P<0.001). The opposite patterns shown by levels of trehalose and the monosaccharides gave a constant total haemolymph sugar concentration independent of metabolic rate (C_{Tot}=57.63–0.86$\dot{V}_{CO_2}$, N=15, r=–0.291, P>0.2). Unfortunately, no measurements were performed between 3.5 and 5 ml CO_2 h^{-1} for the 50 % sucrose group.

Figs 3–5 show, that independent of the concentration of the sucrose solution, the trehalose levels were stable at low metabolic rates (1–4.5 ml CO_2 h^{-1}). At these metabolic rates mean trehalose levels were 29.7±4.9 mg ml^{-1} for bees fed 15 %, 36.4±2.5 mg ml^{-1} for bees fed 30 %, and 43.4±2.3 mg ml^{-1} for bees fed 50 % sucrose solution. The differences between the groups are statistically significant. (ANOVA, $F_{(2,18)}$=19.42; after Newman–Keuls comparisons, P<0.01).

As indicated above, trehalose levels decreased for metabolic rates greater than 4.5 ml CO_2 h^{-1}, and, with the exception of bees fed 15 % sucrose, glucose and fructose concentrations increased. As the supply of sugar from the crop was not limited (see Fig. 2), it indicates that the rate of conversion of glucose to trehalose in the fat body was not high enough to maintain constant haemolymph trehalose levels at metabolic rates higher than 4.5 ml CO_2 h^{-1}. The upper limit to the rate of trehalose synthesis therefore occurs at 5.54 mg glucose h^{-1}, i.e. 92.4 μg glucose min^{-1}.

Discussion

Dependence of sugar transport rates on metabolic rates

A linear dependence between sugar transport rate and metabolic rate of the foragers was detected in all investigated groups. The amount of sugar leaving the crop was similar or slightly higher than that required to meet the bees' energy demands. Sugars leaving the crop reach the haemolymph *via* the ventricle; the transport rate from ventricle to haemolymph is very fast (Crailsheim, 1988a). Increasing consumption of haemolymph sugars with increasing metabolic rates was met by increasing sugar transport rates through the proventriculus, leaving the total haemolymph sugar levels unchanged. Bees fed 15 % sucrose could not sustain metabolic rates higher than 6.2 ml CO_2 h^{-1} in this way, because the maximal transport rate through the proventriculus is 48 μl h^{-1} (Roces and Blatt, 1999), which would not allow the supply of enough sugar at higher metabolic rates. Therefore, these bees must use haemolymph trehalose to meet their energy demands, thus leading to the observed decrease in trehalose and therefore in total haemolymph sugar levels. The highest flow of sugars through the proventriculus is in addition expected to prevent an increase in the monosaccharide levels at higher metabolic rates, as observed in our experiments, because not enough sugars can be passed to support the bee's metabolic expenditure.

Dependence of haemolymph sugar levels on metabolic rates

For bees fed 30 and 50 % sucrose solution, the crop can deliver sufficient sugars to support the highest metabolic demands of the bees. Both glucose and fructose levels increased with increasing metabolic rates (see Results), indicating that there was no delay in the movement of sugars from the ventricle to the haemolymph. The most probable explanation for the decrease in trehalose levels at the highest metabolic rates (above 4.5 ml CO_2 h^{-1}) therefore is a limitation of the rate of trehalose synthesis in the fat body: a maximum value of 5.54 mg glucose h^{-1} was calculated. Núñez et al. (Núñez et al., 1974) showed that bees fed 50 % sucrose solution had lower haemolymph trehalose levels and higher glucose and fructose concentrations than bees fed 7.5 % sucrose. Although they did not measure metabolic rates, they suggested a limit to the rate of trehalose synthesis in the fat bodies of bees fed 50 % sucrose solution. More recently, Woodring et al. (Woodring et al., 1994) suggested that haemolymph trehalose levels decreased in maximally active bees, because the fat body was unable to synthesise new trehalose as quickly as it was consumed, but no data were presented to allow comparison with the present results.

Decreasing trehalose levels and a concomitant increase in concentration of glucose and fructose was also found by Abou-Seif et al. (Abou-Seif et al., 1993), working with caged bees assayed after 2 h starvation periods. Unfortunately, detailed descriptions of the experimental treatment of the bees were lacking. However, it is noteworthy that haemolymph sugar levels at the beginning of their experiment were in the same range as those of our bees exhibiting low metabolic rates.

Fell (Fell, 1990) noted that mean haemolymph sugar levels in the literature were generally similar, but that they showed high individual variability. Given the present results, it is likely that these are due to metabolic differences. In experiments on summer bees, Fell (Fell, 1990) reported haemolymph concentrations of 20 mg ml^{-1} for trehalose, 16 mg ml^{-1} for glucose and 11 mg ml^{-1} for fructose, values which suggest that the samples were taken from bees with rather high metabolic rates.

Bozic and Woodring (Bozic and Woodring, 1997) reported lower trehalose levels, but higher glucose and fructose concentrations in dancing than in resting bees. As it is very likely that dancing bees have higher metabolic rates than resting bees, these results correspond well with the present findings; their haemolymph sugar concentrations were in the same range as in the present study.

The present results show clearly that, when investigating haemolymph sugar levels of honeybees, and presumably of all other insects, measurement of the metabolic rate is important. If a comparison between two groups is made, one should take into account the effects of different handling protocols on the metabolism of the animals. For honeybees it is known that metabolic rate is dependent on temperature (Crailsheim et al., 1999), and that it increases with increasing crop load (Wolf et al., 1989). In addition, it has been suggested that a motivational drive controls the metabolic expenditure of foraging bees and modulates the effects of the carried load, leading to changes in metabolic expenditure as a function of the reward flow rate, independent of the crop load (Núñez and Giurfa, 1996; Moffatt, 2000; Balderrama et al., 1992).

Dependence of haemolymph sugar levels on the sucrose solution concentration

Trehalose levels remained constant over low metabolic rates within each feeding group, however, this mean level increased significantly with the sucrose solution concentration between groups. As the bees appear to adjust sugar transport rates through the proventriculus to equal metabolic rate, this phenomenon can not be explained by assuming that more sugars per unit solution enter the crop at higher sucrose solution concentrations. One possible explanation would be that the concentration of the sucrose solution affects the motivation of the foragers, and that this in turn influences the set point of the controlling system regulating trehalose levels.

These results correspond well with those of Crailsheim (Crailsheim, 1988c) and Abou-Seif et al. (Abou-Seif et al., 1993) who found for caged honeybees that individuals fed higher concentration sucrose solutions showed higher haemolymph sugar levels. Leta et al. (Leta et al., 1996) stressed that there was no correlation between the amount of sugar solution carried in the crop and haemolymph sugar concentration in individual bees, as they found constant levels of trehalose, glucose and fructose in colonies preparing to swarm and in those that were not.

Regulation of haemolymph sugar levels

Owing to their high haemolymph sugar levels in comparison to vertebrates, it is still widely believed that there is no need for haemolymph sugar homeostasis in insects (Candy et al., 1997). However, regulatory hormones similar to those of vertebrates have been found in almost all insects investigated to date (Gäde et al., 1997; Van der Horst et al., 1999). In particular, honeybees with their large amount of food readily available in the hive or carried in the crop and their almost total lack of body reserves would not seem to need sugar-mobilising hormones. However, Woodring et al. (Woodring et al., 1993) found a factor in the corpora cardiaca (CC) of honeybees *Apis mellifera* that temporarily elevated total haemolymph sugars levels in *Periplaneta americana* and lipid concentrations in *Acheta domesticus*; but which had no effect on total haemolymph sugars when injected in either fed or unfed honeybees. However, a significant increase in trehalose levels 30 and 60 min after injection of CC extract, both in active bees moving about in glass vials and in immobilised bees, was found in a later study (Woodring et al., 1994). No change in glucose or fructose levels were observed. Both Maier et al. (Maier et al., 1990) and Woodring et al. (Woodring et al., 1994) detected an increase in trehalose levels 30 min after glucagon injection; again no change in concentration of glucose and fructose was detected. Lorenz et al. (Lorenz et al., 1999) showed that *Manduca sexta* adipokinetic hormone (Mas-AK) is present in the CC of the honeybee strain *Apis mellifera ligustica* but missing in *A. m. carnica*. Winter bees of both strains showed a weak trehalosemic response to injections of synthetic Mas-AKH after 60 min, whereas summer bees of both races did not. Note, however, that experimental periods of 30 or 60 min might be too long to observe a response, owing to the high metabolic rates of honeybees.

O'Connor and Baxter (O'Connor and Baxter, 1985) also detected an insulin-like material in the head of *A. mellifera*. However, Maier et al. (Maier et al., 1988) noted that although the protein structure of this material is well explored, its biological role needs elucidation.

In the present study, for bees fed 30 or 50 % sucrose solution, total haemolymph sugar levels remained constant independent of metabolic rate, suggesting regulation of haemolymph sugar levels. Only in bees fed 15 % sucrose solution did the total haemolymph sugar concentration decrease significantly (for metabolic rates higher than 4.5 ml CO_2 h^{-1}). We also showed that trehalose concentration was stable for low metabolic rates (1–4.5 ml CO_2 h^{-1}) in all three groups, but that the mean value depended on the concentration of the fed solution, suggesting that trehalose levels can be adjusted to the feeding conditions. To what extent hormones are involved, and whether these observations are evidence of haemolymph sugar homeostasis or rheostasis, a state in which homeostatic regulation is present but there is a change in the regulated level (Mrosovsky, 1990), cannot be answered yet.

Regulation of haemolymph sugar levels could function as follows: with increasing metabolic rates the consumption of trehalose increases. This leads to a feedback signal to the proventriculus, which then releases more sucrose solution into the ventricle. The cleavage of sucrose in the ventricle allows both glucose and fructose to enter the haemolymph. Glucose is transformed to trehalose in the fat body, while the fructose is transformed into glucose in the haemolymph by a hexokinase and phosphoglucoisomerase (Candy et al., 1997). At low metabolic rates, bees are able to maintain constant trehalose levels, but at higher metabolic rates trehalose synthesis is not fast enough to balance the trehalose consumption. However, as trehalose levels decrease and levels of glucose and fructose increase at high metabolic rates, the total haemolymph sugar concentration remains constant, providing the transport rate through the proventriculus is not limiting.

Further work needs to be carried out to elucidate the feedback loops involved in the regulation of haemolymph sugar levels in honeybees, but in such work careful standardisation of procedures for handling the bees is needed, and their metabolism should be monitored.

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