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Comparisons of energy reserves among strains of *Tribolium castaneum* selected for resistance to hypoxia and hypercarbia, and the unselected strain

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Abstract

The quantities of energy reserves and their utilization were examined in adults of three strains of *Tribolium castaneum* (Herbst) before and during exposure to two modified atmospheres. It was shown that a strain selected for resistance to high carbon dioxide (CO_2) content (HCC) contained significantly greater triacylglycerol (TG) reserves than a strain selected for resistance to low oxygen (O_2) concentration (LOC) and an unselected strain. During exposure to HCC (65% CO_2, 20% O_2, balance nitrogen), the major energy sources were TGs, most of which were consumed during exposure; TG utilization by the unselected strain was more rapid than that by the HCC-selected strain. During exposure to LOC (0.5% O_2, 99.5% nitrogen), TGs were also utilized, but to a lesser extent, revealing an indication of more attenuated mobilization of energy reserves. Here, too, TG utilization by the unselected strain was more rapid than by the LOC-selected strain. The function of TGs in enabling the insects to maintain their water balance during exposure was considered.

Concentrations of polysaccharides and glucose were low in all strains and although they decreased during exposure to MAs, their contribution to metabolic energy supply during exposure was small. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: *Tribolium castaneum*; Resistance; Hypoxia; Hypercarbia; Energy reserves

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1. Introduction

A non-toxic and environmentally benign alternative to fumigation for the control of insects in stored products is the use of modified atmospheres (MAs). These are obtained by changing the proportions of the atmospheric gases within the storage container to atmospheres rich in carbon dioxide (CO_2), poor in oxygen (O_2) or a combination of both. Sensitivities of stored-product insects to MAs have been widely studied (Annis, 1987) and MA insect control procedures have been put into practice (Delmenico, 1993). Their potential for more widespread use has been reviewed by Bell and Armitage (1992), and Longstaff (1994), and further impetus is being provided by the forthcoming phase-out of methyl bromide (UNEP, 1995) and pressures to limit the use of phosphine, the remaining principal fumigant (Banks, 1994; EPA, 1998).

The development of insect resistance to fumigants has prompted laboratory studies on the acquisition of resistance to MAs (Bond and Buckland, 1979; Navarro et al., 1985). In a study using the flour beetle *Tribolium castaneum* (Herbst), (Donahaye, 1990a,b), a laboratory strain was selected for resistance to hypoxia and another for resistance to hypercarbia. Further studies showed that both selected strains were significantly heavier than the unselected strain (Donahaye, 1993). These findings together with those on rates of weight loss during exposure (Donahaye, 1992), led to this investigation into the nature of energy reserves, quantitative differences in these reserves between the strains, and rates at which these were utilized during exposure to MAs.

It is known that fat tissue represents a considerable proportion of the body weight of pupae and adults of the flour beetle. Villeneuve and Lemonde (1963) recorded that lipids form up to 20% of the wet body weight in *Tribolium confusum* du Val and that they are the major source of energy during metamorphosis. Gilbert (1967) noted that triacylglycerols (TGs) form an important energy source for insects. These undergo hydrolysis to produce fatty acids which release further energy by oxidation. Kennington and Cannell (1967) showed that during exposure of *Tribolium* pupae to anoxia, lipids did not provide an energy source; however, as soon as the insects were returned to air, they were utilized in quantity. The accepted belief is that carbohydrate reserves in insects are utilized by the glycolytic pathway during anaerobic metabolism, but little information is available in the literature on lipid and carbohydrate metabolism under stress from hypoxia or hypercarbia.

The function of TGs in the conservation of the water balance in insects has been discussed by Edney (1967) and by Wigglesworth (1972). Friedlander and Navarro (1979a) examined the influence of CO_2 on TG metabolism in *Ephestia cautella* (Walker) pupae, to determine whether water production from TG metabolism forms a regulatory mechanism to recompense for increased water loss due to the influence of CO_2 on spiracular openings. They found that TG metabolism was not sufficient to compensate for water losses under conditions of high CO_2 concentration and low ambient humidity.

Glycogen forms one of the major carbohydrate energy reserves in insects. Friedman (1970) noted that glycogen may form from 0.01% to more than 2% of the wet weight of insects. Other important carbohydrates stored by insects are chitin and trehalose. Villeneuve and Lemonde (1965) identified carbohydrate reserves in *T. confusum* pupae. They found trehalose in considerable quantities, while the most abundant aldohexose was 6-deoxyglucose. The polysaccharides they identified by electrophoresis were similar to amylose and amylopectin and different from glycogen.

Friedlander and Navarro (1979b) measured glycogen concentrations after exposure of *E. cautella* pupae to hypoxia and hypercarbia and found that hypercarbia increased glycogen utilization. This utilization is more accentuated under hypoxia which is associated with the production of lactate. Glucose levels were not influenced by hypercarbia but rose significantly during exposure to hypoxia. There is a particular interest in understanding the mechanism of utilization of energy reserves in insects under modified atmospheres. In this context, polysaccharides and triacylglycerols are generally considered as the principal energy reserves. Adler (1994) demonstrated that lactate was produced by *Sitophilus granarius* (L.) under anoxic conditions. However, after 24 h, lactate production decreased to almost zero, suggesting the inhibition of this metabolic pathway. It was suggested that the inhibition of glycolysis at low lactate levels is associated with a low energy yield and thereby in atmospheres that combine anoxia and hypercarbia the lethal action is enhanced under the influence of CO_2. Although without O_2, glycolysis is dominant, in a few cases, the breakdown of certain amino acids can be utilized for the production of the energy conserving adenosine triphosphate (ATP). Also, nutritive compounds such as lipids, proteins and carbohydrates can be metabolised with a maximum energy yield in the presence of O_2; but the energy yield of anoxic metabolism is comparatively low. This anaerobic metabolism gives rise to acidic compounds. Therefore, in the application of MAs, a particular interest exists in determining the levels of O_2 and CO_2 or their combination that can prevent oxidative metabolism and thereby cause insect death.

In the following experiments an attempt was made to elucidate the nature of the main energy reserves, namely polysaccharides and triacylglycerols, in adults of the three strains of *T. castaneum*, and their importance during exposure to the stresses of hypoxia and hypercarbia.

2. Materials and methods

2.1. Insects

Three strains of *T. castaneum* were used. One strain had been selected for resistance to a low O_2 content (LOC) atmosphere of 0.5% O_2 in nitrogen (N_2) at 95% r.h., and another to a high carbon dioxide content (HCC) atmosphere of 65% CO_2, 20% O_2 and 15% N_2 at 95% r.h.. A third strain, the original unselected laboratory strain sensitive to both MAs, was also employed. The insects were reared at 26°C on finely ground wheat and 2.5% brewer's yeast (Donahaye 1990a).

2.2. Triacylglycerol measurements

Concentrations before exposure to MAs: Adults, 15–20 days old, in four groups of 100, were taken from cultures of all three strains at the 23rd generation, weighed, and analyzed for TGs in the following manner:

Total lipid extraction was carried out by the method of Bligh and Dyer (1959), with slight modifications. TG concentration was measured by means of a "Sigma" kit according to the method of Bucolo and David (1973). The TG concentration was expressed in mg trioleoylglycerol per 100 insects on a dry weight basis (by extrapolation using results of water content analyses at the 21st generation), or mg/100 mg of insect material on a wet basis.

A set of 10 analyses of glycerol concentration in insects after exposure for 24 h to HCC and LOC using the method of Weiland (1975), resulted in an average of 0.3 μmol/g or 0.027% of insect wet weight. Therefore correction for glycerol in calculations of TG concentration was ignored.

Concentrations during exposure to MAs: The exposure experiments were carried out in a standard apparatus designed to mix the components of modified atmospheres in the required proportions, adjust the gas mixtures to 95% r.h. by passing them through wash-bottles containing sulphuric acid, and supply them to sets of 100 ml Erlenmeyer flasks at a uniform flow rate of 15 ml/min. per flask, in which the insects were exposed (Donahaye, 1990a). Adults 15–20 days old were taken from cultures of the 24th generation of all three strains. They were divided into groups of 100, weighed and placed in exposure flasks. Four groups of the HCC-selected and the unselected strains were exposed to HCC, and four groups of the LOC-selected strain and the unselected strain were exposed to LOC for each of the following exposure times: 0, 1, 2, 4, 6, 8, 10, 12, and 14 days. At the end of each exposure time, the insects were removed from the MA exposure apparatus and re-weighed to determine loss in weight. Then TG analysis was carried out as above.

By subtraction of recorded TG concentrations after exposure, from the TG concentrations recorded for insects before exposure, the percent losses in TG, in mg/100 insects, were calculated for each exposure period.

Water content of insects, rates of water loss during exposure, and total dry weight losses were previously examined under HCC and LOC atmospheres for different exposure periods (Donahaye, 1992). In the present work these calculated TG concentrations were integrated with the dry weight losses recorded in the previous study.

2.3. Measurement of polysaccharides and glucose

Exposure of the three strains of insects to the MAs was similar to that for measurement of TGs, and exposure times were the same. Measurements of polysaccharides, without distinction between glycogen, amylose and amylopectin, and of concentrations of glucose, were carried out by the method of Keppler and Decker (1974). This method employs amyloglucosidase which specifically hydrolyses oligo- and polyglucosides of the amylose, and glycogen-type, whereas chitin as a source of polysaccharide was not included in the test.

Concentrations of polysaccharides are expressed in mg/100 mg initial wet-weight (based on the weight of insects before exposure). Concentrations of glucose are given in nmol of glucose/g initial wet-weight.

3. Results

3.1. Triacylglycerol measurements

Before exposure to MAs: *t*-tests on the results given in Table 1 show that on the basis of weight of TG/100 insects, the differences in TG between the HCC-selected and the LOC-selected or the unselected strains were significant, but the LOC-selected strain did not differ significantly from the unselected strain. However, when the TG of the insects was compared on the basis of 100 mg dry weight, the HCC-selected and the LOC-selected strains both differed significantly from the unselected strain.

During exposure to MAs: Calculated progressive percentage reduction in TG levels during exposure to the HCC and LOC atmospheres, together with mortality results are presented in Figs. 1 and 2 respectively. In order to ensure that the insects only utilized their initial energy reserves, they were exposed to the MAs without food. In both experiments selected and unselected strains were tested.

For the HCC atmosphere, significant differences were found in TG utilization between selected and unselected strains during exposure. For the unselected strain, the exposure time after which no individuals were able to recover was 4 days, whereas only 85% mortality of the HCC-selected strain was recorded after 14 days exposure to the HCC atmosphere.

For the LOC atmosphere, significant differences in TG utilization over the first 12 days of exposure were revealed, with a rapid decrease in TG of the unselected strain as mortality increased. At 14 days, mortality of the LOC-selected strain had risen to 70% and a similar TG level was recorded for both strains.

3.2. Concentrations of polysaccharides and glucose

Before exposure to MAs: One-way analysis of variance of recorded polysaccharide levels of the three strains failed to reveal a significant difference between them (ranging between 2.54 mg/g for the unselected, 2.99 mg/g for the LOC-selected, and 2.84 mg/g for the HCC selected strains). However, there was a significant difference (Student's multiple range test) between the initial glucose concentrations of the HCC-selected strain, which averaged 130.5 nmol/g, and the

Table 1
Average triacylglycerol concentrations in adults of three strains of *Tribolium castaneum* at the 23rd generation of selection

Strain	Adult wt (mg/100 insects)[a]	mg TG/100 insects	mg TG/100 mg dry wt
HCC-selected	202.2 a[b]	20.69 a	10.50 a
LOC-selected	208.5 b	13.83 b	6.76 b
Unselected	184.8 c	14.36 b	8.70 c

[a] Donahaye (1993).
[b] In each column, different letters indicate significant differences at $p < 0.01$.

LOC-selected strain, which averaged 91.1 nmol/g, while the unselected strain averaged 117.3 nmol/g.

During exposure to MAs: Figs. 3 and 4 show that the initial quantities of polysaccharide were very low, representing from 0.25% to 0.3% of the wet weight of the insect, and formed a minor energy source in comparison with TGs. During exposure to HCC or to LOC, polysaccharide concentrations of both selected and unselected strains dropped to up to half their initial levels after 14 days exposure. This slow decrease indicates that the glycolytic pathway is not of major importance during exposure of *T. castaneum* to either of the MAs tested.

Simultaneous with the drop in polysaccharide levels, the glucose concentrations shown in Figs. 5 and 6 rose considerably during the first days of exposure, both to HCC and to LOC. In both cases the rise in glucose concentration was greater for the unselected than for the selected strains. After 14 days exposure, glucose levels had dropped to levels similar to those recorded initially.

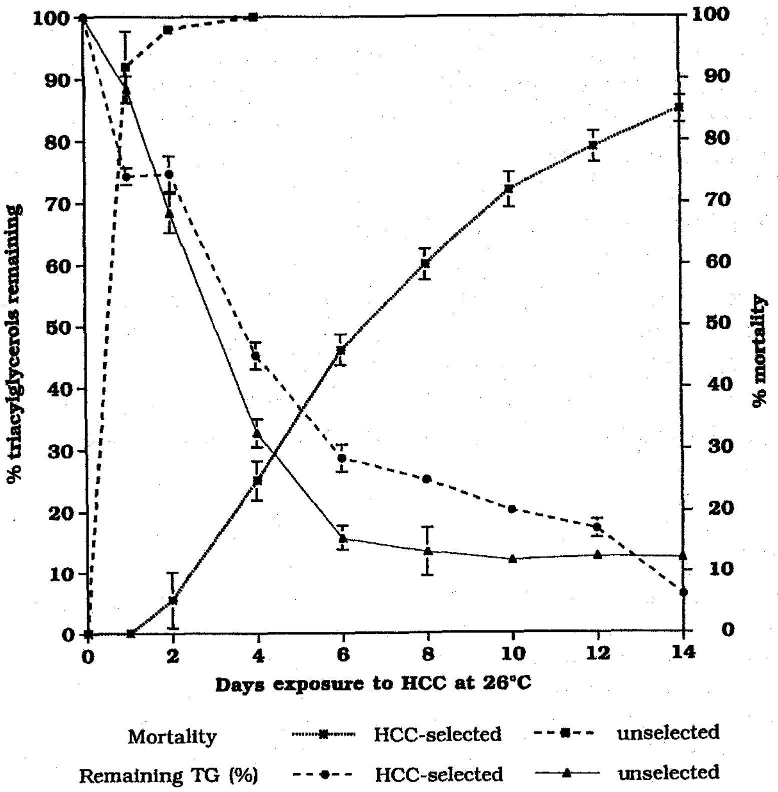

Fig. 1. Rates of utilisation of triacylglycerols and mortality of *Tribolium castaneum* during exposure of the high (65%) carbon dioxide concentration-selected, and unselected strains to the high carbon dioxide concentration atmosphere.

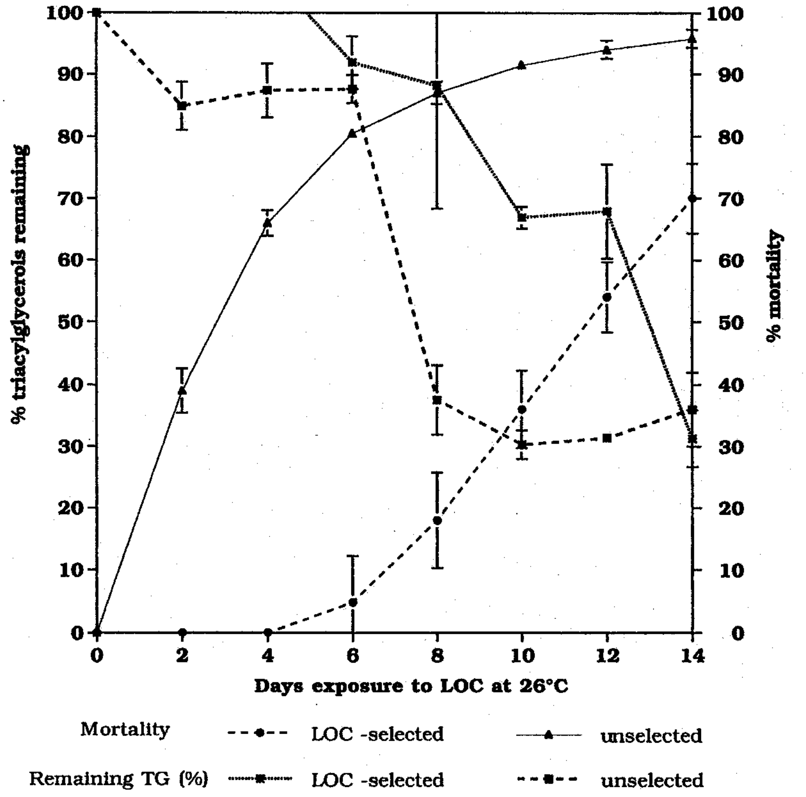

Fig. 2. Rates of utilisation of triacylglycerols and mortality of *Tribolium castaneum* during exposure of the low (0.5%) oxygen concentration-selected, and unselected strains to the low oxygen concentration atmosphere.

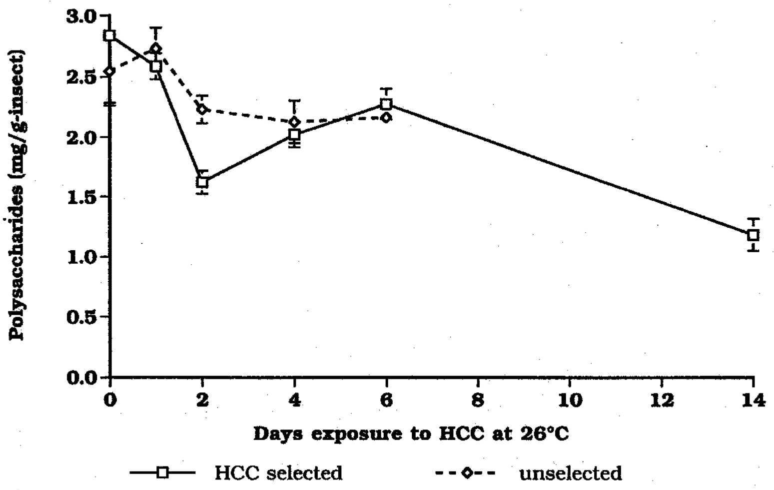

Fig. 3. Changes in polysaccharide concentration of *Tribolium castaneum* during exposure of the high (65%) carbon dioxide-selected, and unselected strains to the high carbon dioxide concentration atmosphere.

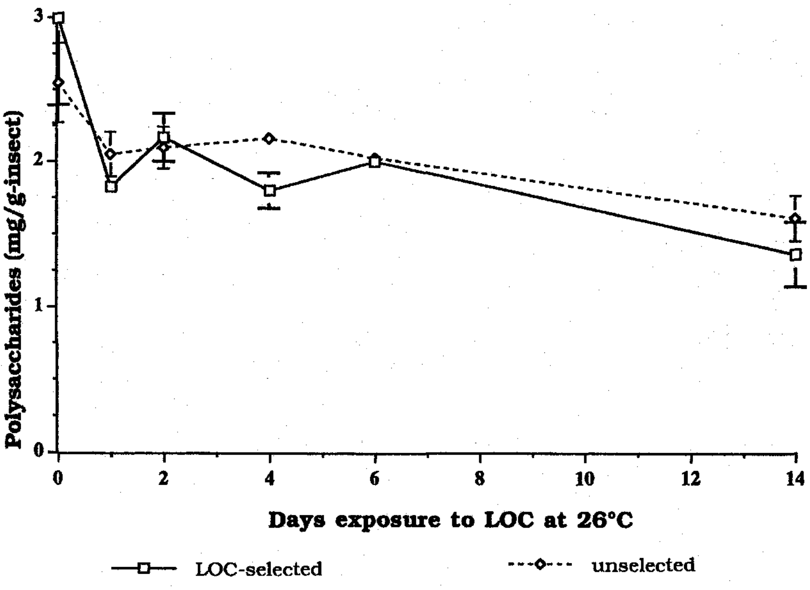

Fig. 4. Changes in polysaccharide concentration of *Tribolium castaneum* during exposure of low (0.5%) oxygen-selected, and unselected strains to the low oxygen concentration atmosphere.

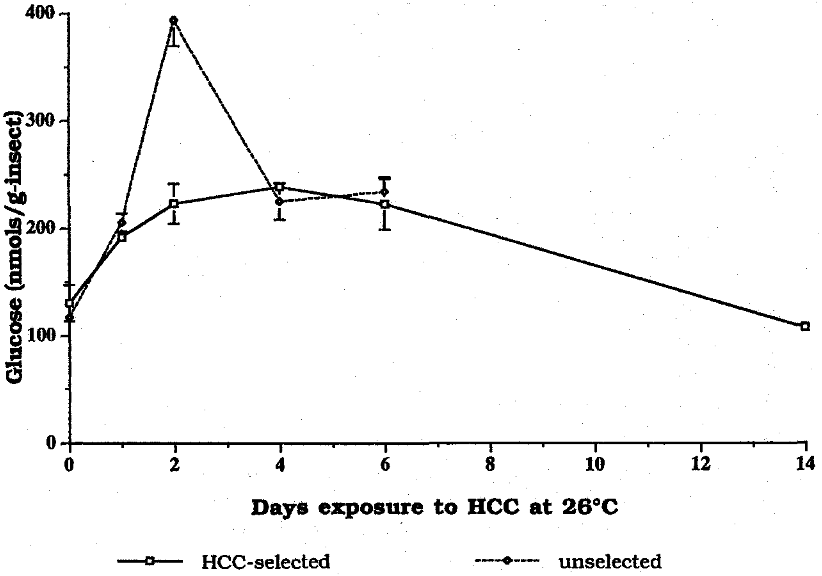

Fig. 5. Changes in glucose concentration of *Tribolium castaneum* during exposure of the high (65%) carbon dioxide-selected, and unselected strains to the high carbon dioxide concentration atmosphere.

4. Discussion

The results of TG concentrations in *T. castaneum* before exposure to MAs may be compared to those of Villeneuve and Lemonde (1963), who measured free lipids (petroleum ether extract) and recorded 25.2 mg/100 adult insects of *T. confusum* (4–5 days old), or 12.1 mg/100 mg insect dry wt. The finding that the heavier LOC-selected strain did not show a proportional increase in TG content, (in contrast to the HCC-selected strain), was not explained by tests to show the presence of other energy reserves. However a possible conjecture is that an increased resistance to desiccation may be imparted by enhanced cuticular characteristics or by increased body size which reduces the ratio of surface area to volume.

From Fig. 1 it can be seen that exposure to HCC was accompanied by rapid utilization of TGs. For the unselected strain about 85% of the TGs had disappeared within 6 days. More prolonged exposure did not decrease TGs further, probably because total mortality occurred after about 6 days (taking into account the fact that mortality analysis does not determine the times at which death occurs, but rather the times at which the insects reach a point of no-recovery). For the HCC-selected strain the decrease in TG concentration was slower. After 14 days of exposure, nearly 5% of the TGs remained and mortality had reached 85%.

To summarize, Table 2 was prepared to enable comparison between the average initial TG concentrations and dry weights (DW) in adults of the HCC-selected and unselected, and the LOC-selected and unselected strains, with those after exposure to the HCC and LOC atmospheres respectively, at the 10th day. The Table shows that for the 10-day exposure period, the total dry weight loss of the HCC-selected strain was just over 30% with the major part being caused by reduction in TGs. Also shown is an additional dry weight loss of 13%

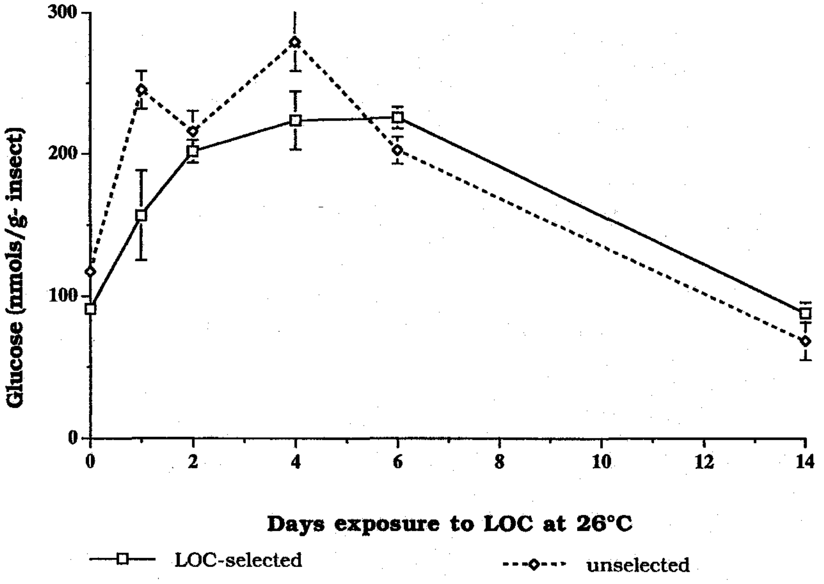

Fig. 6. Changes in glucose concentration of *Tribolium castaneum* during exposure of the low (0.5%) oxygen-selected, and unselected strains to the low oxygen concentration atmosphere.

indicating that energy from another unidentified source was being utilized by the HCC-selected strain, whereas in the unselected strain all dry weight loss was accountable by utilization of TGs.

A further aspect of the difference in TG utilization between the two strains lies in their difference in ability to maintain water balance during exposure (Donahaye, 1992, 1993). Clearly, the ability to control the loss of metabolic water through steady and moderate utilization of TGs in the HCC-selected strain, prolongs its survival during exposure. In comparison, the unselected strain consumed its TG reserves under HCC within the first 6 days, and underwent rapid and continuous desiccation in spite of the high ambient humidity of 95%. The comparison in DW loss at the 10th day exposure (Table 2) also shows the effective use of TG reserves and the ability to control water loss in the HCC-selected but not in the unselected strain.

Losses in TG of both the LOC selected and unselected strains during exposure to LOC were much more moderate and although they were clearly a source of energy reserve during exposure, with utilization by the unselected strain being more rapid, there was no clear indication that mortality ensued when TG reserves were fully utilized. Calculations indicate

Table 2
Comparison of average triacylglycerol concentrations and dry weights (DW) in adults of the HCC-selected and unselected, and the LOC-selected and unselected strains, exposed to the HCC atmosphere and LOC atmosphere respectively, at the 10th day of exposure (all data based on 100 insects)

	Initial weight (mg) ± SD	Final weight (mg) ± SD	Loss in weight (mg)	% loss in weight
	(a) HCC atmosphere (65 vol% CO_2, 20 vol% O_2, 15 vol% N_2 at 26°C)			
HCC-selected strain:				
DW without TG	77.18 ± 1.323	65.18 ± 1.365	13.36	13.00
TGs	20.69 ± 3.211	2.90 ± 0.413	16.44	79.46
Total DW	97.87 ± 1.323	68.01 ± 1.721	29.80	30.45
Water	98.7 ± 1.334	65.5 ± 1.679	33.2	33.6
Unselected strain:				
DW without TG	65.88 ± 0.454	67.48 ± 4.886	−0.83	−3.75
TGs	14.34 ± 1.085	1.72 ± 0.138	11.92	82.72
Total DW	80.29 ± 0.454	69.20 ± 4.989	11.09	13.84
Water	99.73 ± 0.564	47.62 ± 2.138	52.12	52.26
	(b) LOC atmosphere (99.5 vol%N_2, 0.5% vol O_2 at 26°C)			
LOC-selected strain:				
DW without TG	79.49 ± 1.200	74.61 ± 1.516	3.22	2.73
TGs	13.83 ± 2.184	8.62 ± 0.349	6.86	44.33
Total DW	93.32 ± 1.200	83.23 ± 1.213	10.09	10.8
Water	117.46 ± 1.511	109.36 ± 1.359	8.10	6.89
Unselected strain:				
DW without TG	65.22 ± 1.176	58.81 ± 2.588	8.00	5.43
TGs	14.36 ± 1.003	2.73 ± 0.639	9.98	69.22
Total DW	79.52 ± 1.176	61.55 ± 2.684	17.98	22.62
Water	98.78 ± 1.460	77.74 ± 2.782	21.04	21.32

that both strains also used other sources of energy reserve during exposure (Table 2). Weight loss experiments (Donahaye, 1992) showed that water loss during exposure to LOC was not critical. It may be assumed therefore that the TGs were a source of energy during aerobic metabolism under exposure to LOC, and that the more rapid utilization of TG in the unselected strain was due to its higher metabolic rate during exposure.

In conclusion, it was shown previously (Donahaye, 1992) that for exposure to hypercarbia, and even to hypoxia of 0.5% O_2, metabolism of the three strains was mainly aerobic and that duration of survival was correlated with their rates of metabolism during exposure and ability to conserve water. The present findings show that TGs form the principal energy source for metabolism during exposure. The greater quantities of TG in the HCC resistant strain and its lower rate of metabolism, enable it to survive longer under hypercarbia, and this appears to be an important mechanism of resistance. Under hypoxia, as mortality progressed, energy reserves of TG still remained relatively high and their depletion does not appear to limit survival of either strain. Possibly mortality ensues when individuals are no longer able to maintain aerobic metabolism.

An immediate drop in polysaccharide level during the first 48 h is apparent for all strains exposed to HCC and LOC. For the HCC-selected strain there is a temporary recovery after 48 h (Fig. 3) which indicates glycogen production from other polysaccharide sources, possibly chitin. However, this point needs further attention. The levels of glucose only provide information on the relation between its rate of production and its rate of consumption. This can explain why the level of glucose rose when polysaccharide levels fell, and then later declined.

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