Effect of diet quality and associated metabolic changes in adult stress response and life-history traits in *Drosophila ananassae*

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While investigating the role of larval nutrition in adult stress tolerance in *Drosophila ananassae* during the course of the previous study, we have assumed certain hypothesis behind the mechanisms underlying ecological adaptation of this species. Keeping this in view, the aim of the present study was to evaluate the mechanistic role of diets during stress response at cellular and metabolic level in *D. ananassae*. To gain insight into the molecular and physiological basis of variation in stress tolerance in flies developed on different nutritional regimes, we identified a novel factor that depositions of uric acid crystals in Malpighian tubules of flies has a regulatory role in tolerance to desiccation. To assess the role of diet in variation in the physiological process by immunostaining method, we checked (Na^+/K^+) ion-pump in Malpighian tubules. Results suggest that physiological activities of flies fed on carbohydrate food are higher as indicated by the elevated level of (Na^+/K^+)-ATPase ion transport. We also found significant dietary effects on egg production and egg-to-adult viability, suggesting a possible trade-off between the traits investigated. Expression of Hsp60 was also modulated by diet. Hsp60 was highly expressed in carbohydrate and protein-enriched medium compared to normal food.

Keywords: *Drosophila ananassae*, environmental stress, Malpighian tubules, nutritional regimes, physiology.

STARVATION, desiccation and exposure to extreme heat or cold are most stressful to insects among environmental challenges. There is a strong impact on the amount and quality of nutrients consumed by organisms on life-history traits and stress response[1–3]. The balance can be maintained by interplay between matter intake, digestion and the allocation of acquired energy to various functions such as maintenance, growth and reproduction[4]. For *Drosophila*, the ability to survive in dry habitats presents an especially formidable ecological and physiological challenge[5]. Desiccation resistance of an organism depends on both water content and rate of water loss. Mechanisms behind desiccation resistance are complex and species-specific.

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Uric acid has long been recognized as the major nitrogenous excretory product of insects. Two properties make it suitable for this purpose: it contains 33% nitrogen, and under the acid conditions which prevail in the terminal section of the excretory system[6], its solubility is quite low (11 mM at pH 5.5). Its elimination thus leads to the excretion of large amounts of nitrogen without incurring much loss of water. There are limits to the amount of uric acid or urate that can be accommodated within the body, and insects with a protein-rich diet have mechanisms for uric acid elimination[7]. Uricotelism is more energy-consuming than ammonotelism, but the advantage of uric acid is that, being highly insoluble in water, it can be precipitated in the excretory organ and then voided with minimal loss of water[8].

A population exposed to new and stressful environment, may acclimatize itself either by developing phenotypic compensation through increased competency in acclimation, or by evolving macromolecules that are either more tolerant to functional disturbance or better able to retain functional efficiency in the unfavourable environment[1,9–11]. In this respect, unsuitability or insufficient food resources are frequently encountered in nature and starvation is recognized as a major stress in natural populations of *Drosophila* species. Thermal and environmental stress induces the expression of *HSP* genes which assist in the maintenance, viability, prevention of protein unfolding and enhancement of cell survival. It has been reported that a variety of stresses, especially thermal stress, influence the process of spermatogenesis and oogenesis, and Hsp60C plays an essential role in spermatogenesis[12] as well as oogenesis[13] in *D. melanogaster*. The increase in the expression of Hsp60C in protein- and carbohydrate-rich diet would also be related to the change in egg production of females developed on protein and carbohydrate-rich medium.

D. ananassae, a cosmopolitan and domestic species belonging to the *ananassae* subgroup of the *melanogaster* species group is stenothermic and circumtropical in distribution[14–16]. India is a large tropical and subtropical subcontinent covering a large range of latitude and altitude. From south to north, the seasonal thermal amplitude shows a regular increase with progressively more

marked cold and warm seasons. Sisodia and Singh[17,18] found a high degree of variation in stress resistance at the population level in *D. ananassae*. In India, feeding habit and composition of food vary with latitude and the ratio of protein : carbohydrate also varies accordingly.

To understand the mechanisms behind diet-regulated stress tolerance in *D. ananassae*, we have selected Malpighian tubules (MTs) which are a good model to study the physiology of insects. We have selected another strain of *D. ananassae* and tested the effect of larval nutrition on resistance to desiccation, starvation, heat and cold tolerance, and life-history traits and then attempted to answer the following questions by selecting Malpighian tubules as a marker: (i) Is uric acid crystal deposition in Malpighian tubules an indicator of tolerance to desiccation resistance? (ii) Are the expressions of (Na^+/K^+)-ATPase ion transport altered in two different nutritional regimes? (iii) Is the expression of Hsp60 modulated by diet?

Keeping this in view, the aim of the present study was to evaluate the mechanistic role of diet during stress response at cellular and metabolic levels in *D. ananassae*.

Materials and methods

Stock investigated

The Gwalior (GL) stock of *D. ananassae* used in the present experiment was established from flies collected from fruit and vegetable baits in Gwalior, Madhya Pradesh (lat. 26.22°, long. 78.18°), India in October 2010. Prior to the experiment, the flies were kept in simple culture medium. For maintaining the stock, simple culture medium containing agar-agar, dried yeast, maize powder, crude sugar, nipagin, propionic acid and plain water was used. In our laboratory, we maintain different species of *Drosophila* in simple culture medium. For making one-fourth unit of simple culture medium, 600 ml water and 71 g of the above-mentioned food ingredients in different proportions were used. The carbohydrate-enriched medium was prepared by mixing sucrose and culture medium in the ratio 1 : 4 (14.2 : 56.8 g) before adding water. The protein-enriched medium was prepared by mixing casein and simple culture medium in the ratio 3 : 2 (42.6 : 28.4 g) before adding water. Eggs were collected and transplanted to two types of food media: a carbohydrate-enriched medium and a protein-enriched medium. All vials contained approximately 7 ml of medium were pasted with dried yeast solution. Twenty eggs were kept in each vial. The eggs hatched and larvae developed at 25°C and 12 h L/D cycles. Virgin flies were collected and aged for 6–7 days before starting the experiments. To measure desiccation, starvation, chill-coma, heat shock, egg-to-adult viability, egg production and counting of ovariole number, we have followed the procedure of Sisodia and Singh[19]. Fifteen pairs of flies were transferred to fresh culture bottle in each generation to maintain stocks for each nutritional regime. All experiments were performed carefully. Flies used for experiments were of the same age and maintained under the same laboratory conditions with different nutritional regimes.

Body weight and lipid content

The method of Hoffmann and Parsons[20] was used to measure lipid content. Females and males were treated in groups of 20 separately, and two groups were tested per sex per nutritional group.

Immunostaining

Tissues were dissected out in 1× PBS and fixed in freshly prepared 4% paraformaldehyde for 20 min followed by washing thrice in 1× PBST (PBS, 0.1% Triton X-100) and processed for immunostaining as described earlier[21,22]. The primary antibodies used were: α-subunit of anti-mouse (Na^+/K^+)-ATPase and anti-rabbit Hsp60C (1 : 50, 1 : 100, a kind gift from Madhu G. Tapadia). Secondary antibodies used were anti-mouse and anti-rabbit Alexafluor 488). Chromatin was counterstained with DAPI (1 µg/ml in 1× PBS). All tissues were mounted in antifadent, DABCO (Sigma Aldrich) and analysed under Zeiss LSM 510 Meta Confocal microscope. The images were processed with Adobe Photoshop.

Determining uric acid deposition

For imaging of uric acid deposited in the MTs, larvae from control, protein and carbohydrate food were seen under polarized microscope. Images were taken with 10× objective and a digital zoom of 6× using a Nikon digital camera fitted on the microscope.

Statistical analysis

The Kaplan–Meir method was used to estimate this curve from the observed survival time without the assumption of an underlying probability distribution[23]. Comparison of two survival curves was done using a statistical hypothesis test called the log rank test. Two-way ANOVA was used to test the variation in stress response of flies developing on different nutritional regimes for different traits like desiccation, starvation, chill-coma recovery, heat shock survival, egg-to-adult viability and lipid content. Homogeneity of variance was also tested for different traits. Comparisons of egg production and difference in ovariole number in females developed from two different nutritional regimes were analysed using Student's *t*-test.

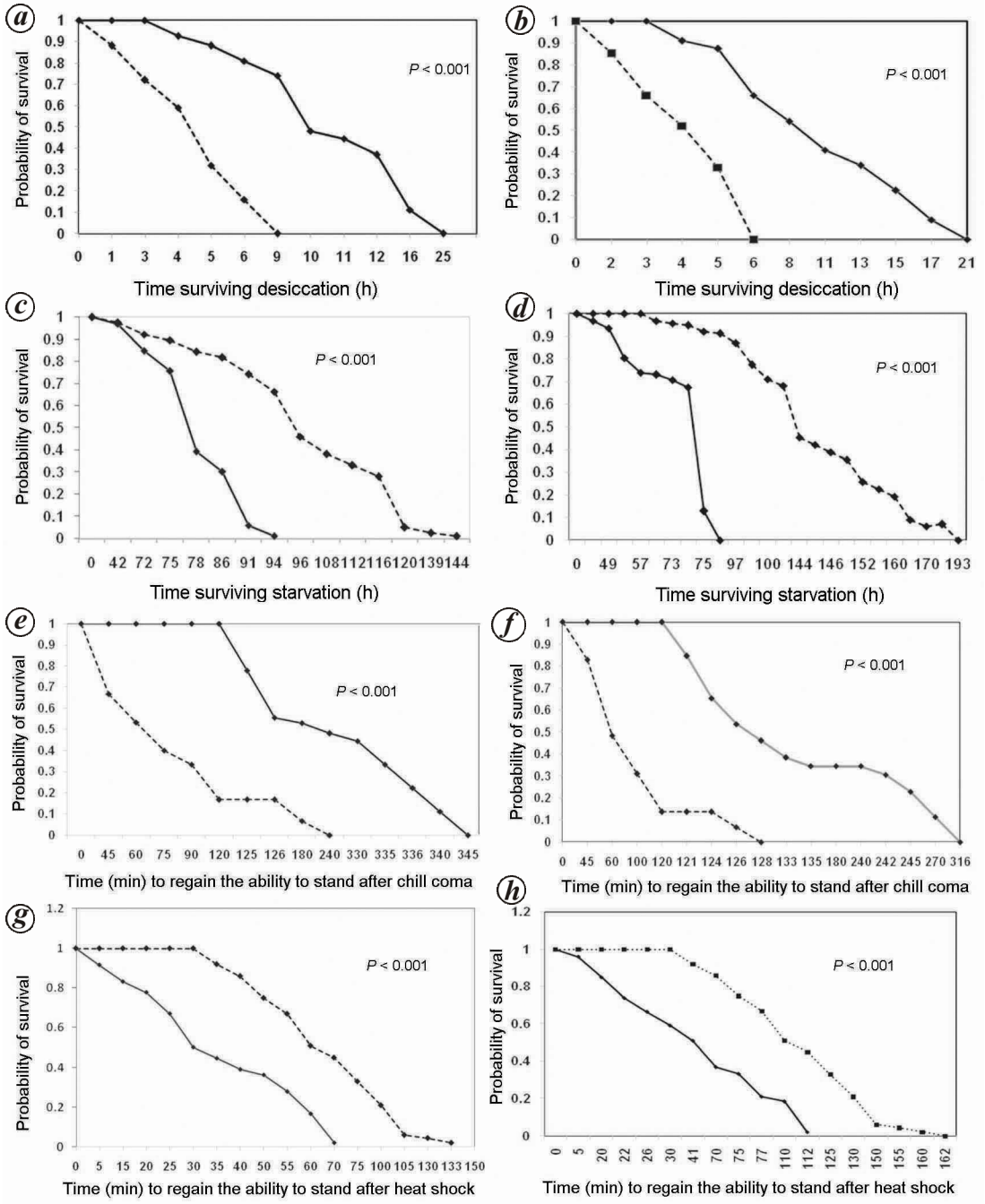

Figure 1. ***a***, ***b***, Survival curves for desiccation resistance in (***a***) males and (***b***) females derived from either protein (—) or carbohydrate (- - -)-enriched medium. ***c***, ***d***, Survival curves for starvation resistance in (***c***) males and (***d***) females derived from either protein (—) or carbohydrate (- - -)-enriched medium. ***e***, ***f***, Survival curves for chill-coma recovery in (***e***) males and (***f***) females derived from either protein (—) or carbohydrate (- - -)-enriched medium. ***g***, ***h***, Survival curves for heat-shock in (***g***) males and (***h***) females derived from either protein (—) or carbohydrate (- - -)-enriched medium.

Results

Stress tolerance

Desiccation resistance was affected by nutritional regimes. Male and female flies developing on the protein-enriched medium have higher desiccation resistance than flies developed on carbohydrate-enriched medium. There is highly significant variation in survival time of flies developed on two nutritional regimes in both the sexes (Figure 1 *a* and *b*). We observed higher starvation resistance in flies developed on carbohydrate-enriched medium than those developed on protein-enriched medium. There is highly significant variation in survival time of two types of flies in both the sexes (Figure 1 *c* and *d*). Chill-coma recovery time of the flies was significantly

Table 1. Two-way ANOVA for different traits in either protein or carbohydrate-enriched medium in *D. ananassae*

Trait	Source	d*f*	*F*	*P*
Desiccation	Fly nutrition	1	119.09	<0.001
	Fly sex	1	2.86	>0.05
	Fly nutrition × fly sex	1	1.57	>0.05
	Error	116		
Starvation	Fly nutrition	1	81.38	<0.001
	Fly sex	1	12.74	<0.001
	Fly nutrition × fly sex	1	13.63	<0.001
	Error	116		
Heat shock	Fly nutrition	1	42.32	<0.001
	Fly sex	1	0.170	>0.05
	Fly nutrition × fly sex	1	0.095	>0.05
	Error	116		
Chill-coma recovery	Fly nutrition	1	21.51	<0.001
	Fly sex	1	20.24	<0.001
	Fly nutrition × fly sex	1	19.92	<0.001
	Error	116		
Lipid content	Fly nutrition	1	19.28	<0.001
	Fly sex	1	13.57	<0.001
	Fly nutrition × fly sex	1	7.25	<0.01
	Error	126		
Egg-to-adult viability	Fly nutrition	1	12.47	<0.001
	Fly sex	1	10.12	<0.01
	Fly nutrition × fly sex	1	9.67	<0.01
	Error	126		

affected by nutritional regimes. Flies developed on protein-enriched medium recovered more slowly than those developed on carbohydrate-enriched medium (Figure 1 *e* and *f*). We found a significant influence of nutritional regimes on heat shock survival. Flies developed on protein-enriched medium have fast recovery from heat shock than those developed on carbohydrate-enriched medium (Figure 1 *g* and *h*). Table 1 shows highly significant effect of nutrition on stress resistance. Except desiccation and heat tolerance, there are significant variations between the two sexes and significant interaction is also observed between nutritional regimes and sexes.

Expression of heat shock protein

To know whether the thermal tolerance of flies is due to change in heat shock protein, Hsp60C expression was examined in Malpighian tubules of flies fed on normal, carbohydrate-rich and protein-rich food. Hsp60C was expressed only in the stellate cells of Malpighian tubules (Figure 2 *a*). The nuclei were counterstained with DAPI showing presence of Hsp60C only in the cytoplasm and not in nucleus of Malpighian tubules (Figure 2 *a*′–*c*′) and clearly distinguishing the smaller nuclei of stellate cells and larger nuclei of principal cells. After feeding on protein-rich medium (Figure 2 *b*) and carbohydrate-rich medium (Figure 2 *c*), the level of Hsp60C increased significantly in comparison to flies fed on normal standard diet (Figure 2 *a*). The increase in fluorescence intensity was measured by line profile display of LSM 510 Meta software of confocal microscope (see graph in Figure 2). The level of Hsp60C is greater in carbohydrate-rich medium compared to protein-rich medium. This result shows that Hsp expression increases in protein- and carbohydrate-rich diet to cope with stress.

Body weight and lipid content

We found a significant variation in lipid content in flies developed on two different nutritional regimes. Lipid content was higher in flies developed on carbohydrate-enriched medium than those developed on protein-enriched medium (Table 1). Body weight was higher in flies developed on carbohydrate-enriched medium than those developed on protein-enriched medium. Mean body weight for females and males developed on protein-rich diet was 0.029 ± 0.0008 and 0.019 ± 0.0005 mg respectively, whereas body weight for females and males developed on carbohydrate-rich diet was 0.035 ± 0.0021 mg and 0.026 ± 0.0024 mg respectively. Mean lipid content

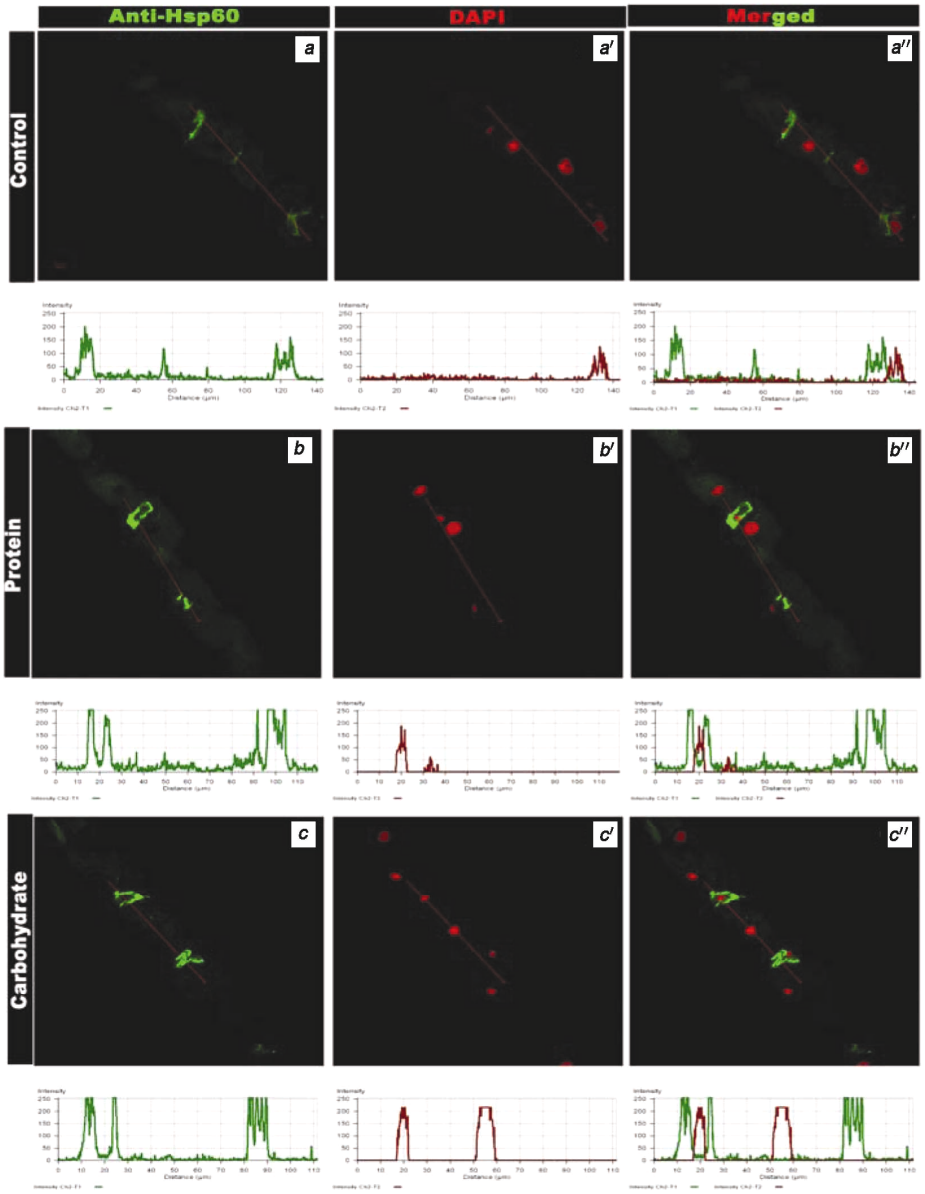

Figure 2. Enhanced level of Hsp60C in Malpighian tubules (MTs) of flies fed on protein and carbohydrate. Flies feeding on high protein and carbohydrate food cause upregulation of Hsp60C in MTs (***b***, ***c***) in comparison to control (***a***). The level of Hsp60C protein is more in MTs of flies fed on carbohydrate food (***c***) compared to those fed on protein-rich food (***b***). The nuclei were counterstained with DAPI (pseudocolour red – *a*′–*c*′) and merged images are an overlay of Hsp60C and DAPI (*a*″–*c*″). The graph below shows the difference in the mean intensities of fluorescence quantified by line profile display of confocal microscope (scale bar 20 µm).

for females and males developed on protein-rich diet was 0.004 ± 0.0008 and 0.002 ± 0.0005 mg respectively, whereas lipid content for females and males developed on carbohydrate-rich diet was 0.008 ± 0.0021 and 0.005 ± 0.0024 mg respectively.

Egg production, ovariole number and egg-to-adult viability

We found significant difference in egg production of females developed on different nutritional regimes when tested on simple culture medium (*t*-test: 7.42, $P < 0.001$). Average female egg production/day was 54 in case of females developed on carbohydrate-enriched medium, while for protein-fed females the average egg production was 82. There was significant difference in ovariole number between females developed on two types of food (*t*-test: 6.51, $P < 0.001$). Females developed on protein-rich food had higher ovariole number than those developed on carbohydrate-rich food. So there is positive correlation between number of eggs laid and ovariole number. We also found trade-offs between egg production, ovariole number and egg-to-adult viability (Figure 3 *a* and *b*).

Assuming that the sex ratio of the eggs collected is 50 : 50, Table 1 shows that there is a significant effect of nutritional regimes and sex on egg-to-adult viability. We also found significant interaction between sex and nutritional regime on egg-to-adult viability. On an average 35% more females developed on protein-enriched food, while on average 40% more males developed on carbohydrate-enriched medium. Viability is greater in carbohydrate-rich diet (Figure 3 *c*).

Expression of ion channel protein (Na^+/K^+)-ATPase

(Na^+/K^+)-ATPase is critical to a variety of physiological processes like osmoregulation, cell-volume regulation, transport of certain amino acids and sugar, and maintenance of membrane excitability. (Na^+/K^+)-ATPase is a membrane-bound protein which translocates the ions using energy released by ATP hydrolysis. When the animals undergo different stress conditions, their Malpighian tubules are exposed to different osmolalities depending on the feeding state[24,25]. To assess the physiological effect, we checked (Na^+/K^+)-ATPase pump in Malpighian tubules of flies fed on different foods. Malpighian tubules are the excretory organ of *Drosophila* enriched in ion channels for osmoregulation and detoxification and maintain homeostasis of the body. (Na^+/K^+)-ATPase is expressed on the basolateral surface of the Malpighian tubules[26]. We observed that expression of (Na^+/K^+)-ATPase was strongly increased when flies were fed on carbohydrate food (Figure 4 *c*) in comparison to control fed on normal food (Figure 4 *a*). The (Na^+/K^+)-ATPase expression was also modestly increased in flies fed on protein medium (Figure 4 *b*) in comparison to control (Figure 4 *a*). Chromatin was counterstained with DAPI (pseudocolour red – *a′–c′*) and merged images were an

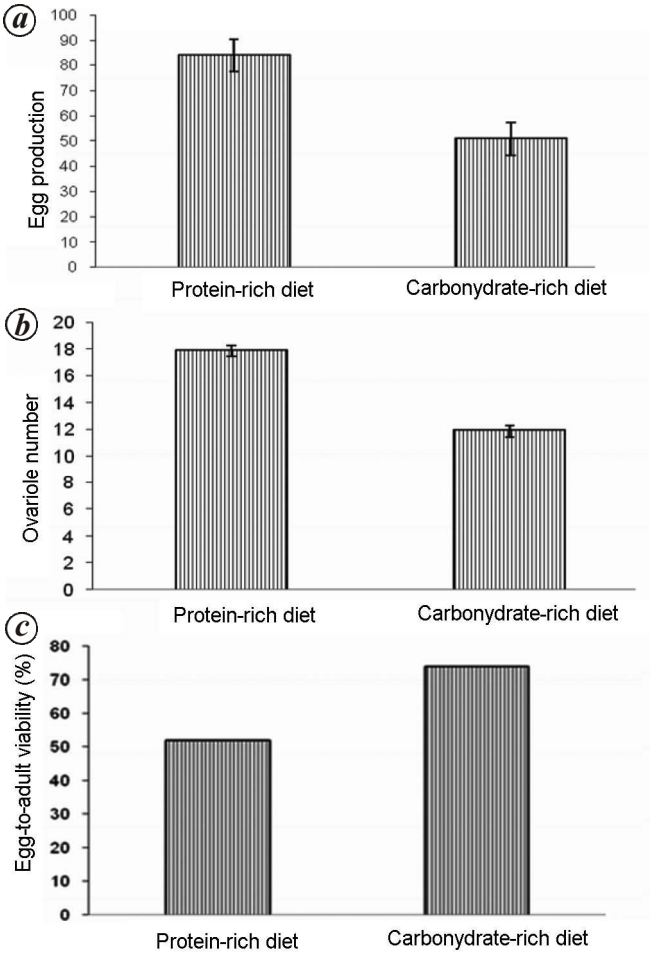

Figure 3. Ovariole number, egg production and egg-to-adult viability of flies developing on protein-rich and carbohydrate-rich diet. Bar represents mean ± SE.

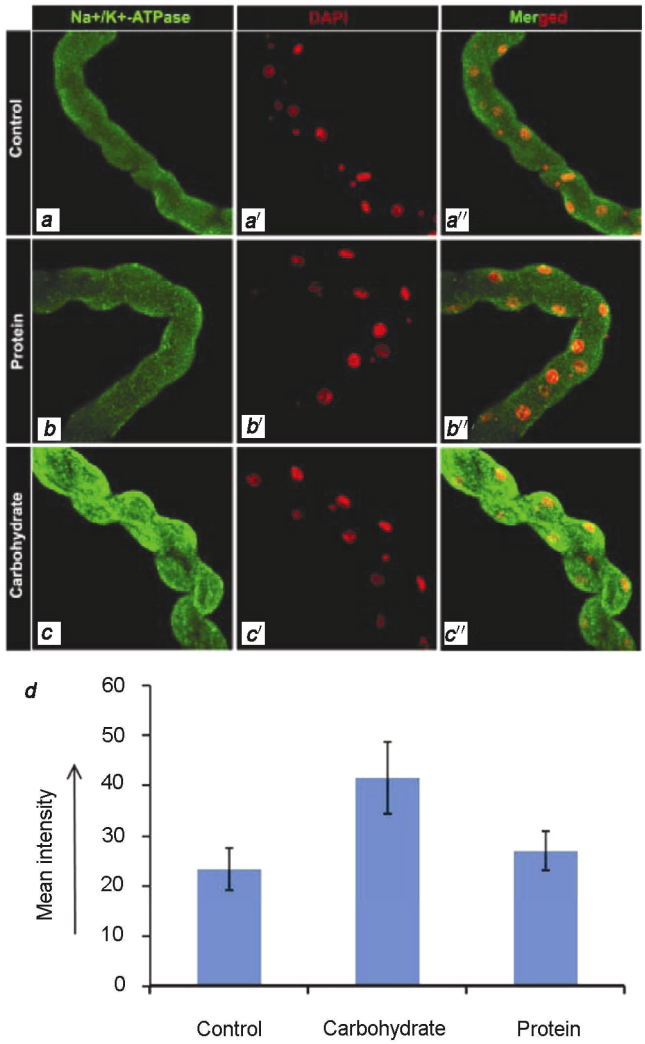

Figure 4. Enhanced level of (Na^+/K^+)-ATPase in MTs of flies fed on protein and carbohydrate food. Flies feeding on high protein and carbohydrate food causes upregulation of (Na^+/K^+)-ATPase in MTs (***b***, ***c***) compared to control (***a***). The level of (Na^+/K^+)-ATPase increases more in MTs of flies fed on carbohydrate food (***c***) compared to those fed on protein-rich food (***b***). The MTs were counterstained with DAPI (pseudocolour red – ***a′–c′***) and merged images are an overlay of (Na^+/K^+)-ATPase and DAPI (***a″–c″***) (scale bar 20 µm). Difference in the mean intensities of fluorescence quantified and represented as a bar diagram.

overlay of DAPI and (Na^+/K^+)-ATPase (*a″–c″*). These results suggest that flies fed on carbohydrate food becomes more active in their physiological activity of ion transport as indicated by the elevated level of (Na^+/K^+)-ATPase.

Deposition of uric acid

In the light of elevated level of (Na^+/K^+)-ATPase pump in carbohydrate- and protein-enriched medium, we wanted to study the physiology of the Malpighian tubules. Fluid secretion is the main physiological action of the Malpighian tubules, which can be directly judged by the deposition of uric acid crystals[27,28]. These are the major nitrogenous excretory product of insects, clearly visible in birefringence light. We observed more deposition of uric acid crystals in protein-fed larvae (Figure 5 *c*) in comparison to control (Figure 5 *a*) and carbohydrate-fed larvae (Figure 5 *b*). This result suggests that metabolism of flies is affected when fed on protein-rich medium.

Discussion

Although in this study we have selected another strain of *D. ananassae*, which was collected from a different geographic locality, we found similar results to our previous findings[19]. On the basis of our results for stress response as well as life-history traits in two types of medium, we cannot rule out that not only plastic response but also selection may be involved in explaining our results.

Crystals of uric acid deposition and (Na^+/K^+)-ATPase activity

Flies developed on protein-rich diet show higher desiccation resistance compared to those developed on carbohydrate-rich diet. Our findings are similar to those of Andersen *et al.*[29], where *D. melanogaster* flies developed on protein diet showed higher survival in desiccation stress. Our results strongly support our previous study[17] in which North Indian populations where protein-rich diets is available for the flies have higher desiccation tolerance compared to South Indian populations where carbohydrate-rich diet is available for the flies. In the Indian subcontinent, it is an interesting fact that the composition of food varies with latitude and altitude for not only *Drosophila*, but human as well.

In this study we have examined the mechanisms behind the higher desiccation resistance of flies developed on protein-enriched medium. We observed that deposition of crystals of uric acid in the Malpighian tubules was higher in flies developed on protein-enriched medium than those developed on carbohydrate and standard food medium. In our study, expression of ion channel protein (Na^+/K^+)-ATPase has been modulated in flies fed on carbohydrate- and protein-rich medium. Flies developed on carbohydrate-rich medium have higher expression of (Na^+/K^+)-ATPase activity and due to higher ion channel proteins water loss is also higher during desiccation in these flies. However, the condition is just the reverse in case of flies developed on protein-enriched medium, where expression of ion channel proteins is less than carbohydrate-enriched medium and due to uric acid deposition water loss is minimum, which increases the tolerance of desiccation resistance. The possible explanation behind this is that uric acid minimizes water loss during desiccation and increases the tolerance of desiccation stress. As a result, survival is higher in flies developed on protein-enriched medium. Previous reports on desiccation resistance have shown the possibility that the end-product of protein metabolism is uric acid, which had a protecting effect on the increasing osmotic pressure during desiccation by reducing water loss from the cells[29].

Starvation resistance and lipid content

The present study provides evidence for higher starvation resistance for flies developed on carbohydrate-rich diet compared to those developed on protein-rich diet. Greater starvation resistance requires physiological changes which are likely to trade-off with other fitness-related traits. Starvation resistance is governed by several factors, but their general importance is uncertain. There is also evidence for an association between starvation resistance and carbohydrate metabolic reserves, particularly as the association between starvation and energy reserves is strongest when both carbohydrate and lipid components of these reserves are considered[30]. Our previous results suggest that flies collected from different geographic localities differ in their resistance to starvation because of differences in their tendency to store body lipid[17]. In the present study, we have quantified the lipid content of flies developed on two types of food medium and we found that flies developed on carbohydrate-rich medium have higher lipid reserves compared to those developed on protein-enriched medium. So there is positive correlation between lipid content and starvation tolerance in *D. ananassae*[17]. In *D. melanogaster*, flies feed on a diet supplemented with coconut oil showed significantly increased triglyceride and glucose levels and shorter lifespan. Under starvation condition the flies on the high fat diet showed increased resistance, due to their increased triglyceride and energy stores[31]. Shreve *et al.*[32] reported that in *D. melanogaster* raising larvae on a cholesterol-augmented diet significantly increased the amount of cholesterol in the cell membrane of the adult flies and resulted in increased cold resistance. Ballard *et al.*[33] determined the relationship between starvation resistance, body lipid content and lifespan in five recently collected

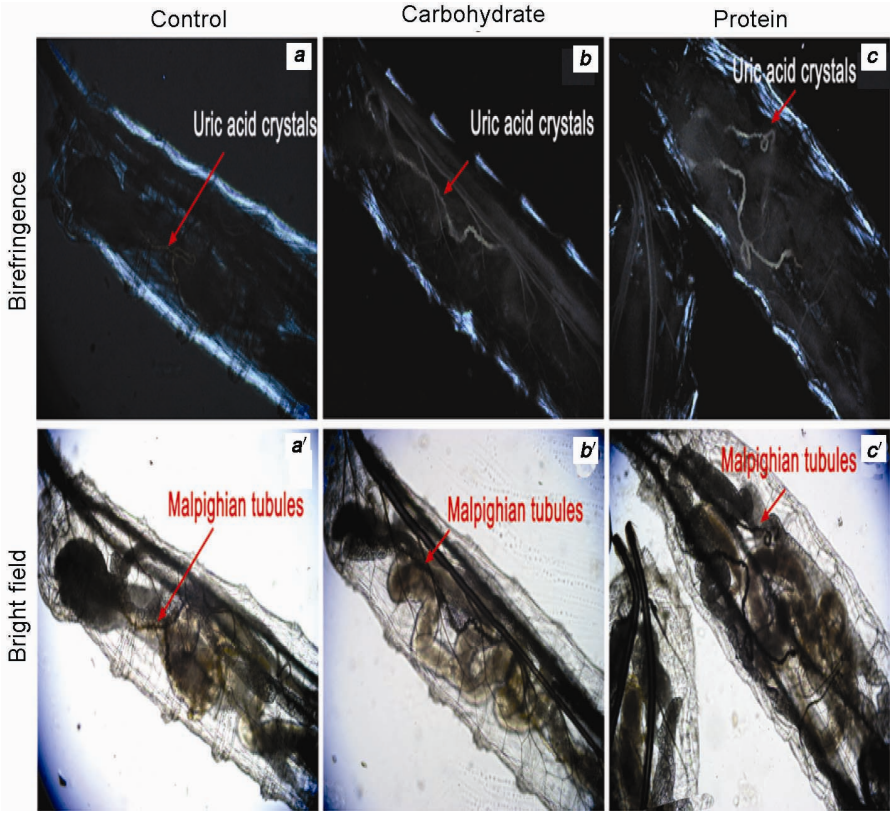

Figure 5. Protein-rich food increases uric acid deposition in MTs. Flies fed on protein-rich food show highly increased deposition of uric acid crystals in the MTs (***c***) in comparison to control (***a***) and carbohydrate-fed flies (***b***). Arrow denotes the uric acid crystals seen in Birefringence (***a***–***c***) light. Bright-field microscopy images corresponding to control (***a′***), carbohydrate (***b′***) and protein (***c′***) show the MTs.

D. simulans populations from four distinct geographic localities. They observed a negative relationship between lifespan and starvation resistance in both males and females, suggesting a fitness cost to increasing lipid reserves. However, a reduced rate of respiration could increase starvation resistance. Harshman and Schimd[34] found no correlated change in respiration rate in lines selected for female starvation resistance in *D. melanogaster*. Aggrawal[35] studied physiological basis of sex-specific as well as between-population divergence for starvation resistance in *D. leontia*. Females stored higher levels of body lipids and glycogen contents, and utilized both of these energy resources under starvation stress, whereas the starved males metabolized only body lipids as a source of energy. In *Drosophila*, energy storage patterns are species-specific. Marron *et al*.[36] measured lipid, carbohydrate, and protein content in 16 *Drosophila* species from arid and mesic habitats. In five species, rates of lipid and protein metabolism were similar during starvation and desiccation, but carbohydrate metabolism was several-fold higher during desiccation. Thus, total energy consumption was lower in starved flies than desiccated ones. Cactophilic *Drosophila* did not have greater initial amounts of reserves than mesic species, but may have lower metabolic rates that contribute to stress resistance.

Cold tolerance and lipid content

The faster recovery from chill-coma when flies are fed a carbohydrate-enriched diet has been explained by suggesting different physiological interpretations. Carbohydrate is well known to increase the fat content of the flies[37]. In *Drosophila* species, there is positive correlation between body lipid content and resistance to cold temperature[38], and starvation[18,34] and desiccation stress[39]. Andersen *et al*.[29] found that *D. melanogaster* flies grown on carbohydrate-enriched medium have faster recovery from chill-coma than those grown on protein-enriched medium. Thus it is likely that the faster recovery from chill-coma of flies raised on carbohydrate-enriched medium is due to higher lipid deposits. However, the physiological basis for how fat deposits improve chill-coma recovery is not fully understood. It has been suggested that higher lipid deposits may affect the quality and quantity of the cuticular hydrocarbons influencing water loss and update of the cell[40]. Aggrawal *et al*.[41] found evidence that drought acclimation facilitates increased cold-tolerance in a series of low-temperature treatments (0°C, –2°C, and –4°C) in *D. melanogaster*. They observed a dramatic increase in desiccation resistance associated with low-humidity acclimation, consistent

with changes in bulk water, dehydration tolerance and levels of energy metabolites.

Heat tolerance

We found that flies developed on protein-enriched medium have higher heat resistance than those grown on carbohydrate-enriched medium. Very few flies developed on carbohydrate-rich diet have revived after heat shock. Hsp70 is upregulated in flies developed on protein-enriched medium compared to those developed on protein deficient medium[29].

The expression of HSP60C was significantly enhanced in carbohydrate- and protein-rich diet compared to control, but the enhanced expression is more in carbohydrate-rich medium (Figure 2 *c*). The possible explanations for this are that flies fed on carbohydrate are sensitive to heat as very few flies have revived after heat shock and after thermal treatment they required overexpression of HSP to cope with thermal stress, whereas flies fed on protein-enriched medium have the capacity to tolerate thermal stress much better than those fed on carbohydrate medium. Kanazawa *et al.*[42] studied the effects of feeding on a high sucrose diet on body weight gain, plasma triglyceride and stress tolerance in rats. There was enhanced gene expression of heat shock proteins (HSP70 and 27) and suppression of NO_x production in the brain, which was induced by a high sucrose diet given for one week resulting from response to stress, although the standard diet did not have a similar effect. It has been reported that a variety of stresses, especially thermal stress, influence the process of spermatogenesis and oogenesis, and Hsp60C plays an essential role in spermatogenesis[12] as well as oogenesis[13] in *D. melanogaster*. The increase in the expression of Hsp60C in protein- and carbohydrate-rich diet would also be related to the change in egg production of females developed on protein- and carbohydrate-rich medium.

Life-history traits

Two sexes have different requirements during development and growth. We found a higher developmental success for females on protein-rich medium, while developmental success for males was higher in carbohydrate-rich medium. Our results are consistent with the findings of Andersen *et al.*[29], which proves that *Drosophila* species have similar type of sex-specific requirements. Egg production in females developed on protein-enriched medium is higher than those developed on carbohydrate-enriched medium. Egg-to-adult viability in *D. ananassae* shows interesting results because flies developing under protein-rich condition have reduced egg-to-adult viability, suggesting a trade-off between egg-to-adult survival and egg production. The trade-off was found for both diet types and was caused by antagonistic pleiotropy. In *D. melanogaster*, trade-off was found between egg-to-adult survival and body mass in protein-rich diet[43]. It has also been explained that this event is caused by antagonistic pleiotropy, whereby alleles coding for larger body size which is advantageous under protein-enriched conditions, also have a negative effect on physiological processes that affect survival.

Conclusion

As the physiology of *D. ananassae* is maintained by the excretory organ, the function of the Malpighian tubules was studied. There is a relation between nutritional diet and physiology of the Malpighian tubules. The physiology was observed by determining the upregulation of Na^+/K^+ pump in carbohydrate- and protein-fed flies. The final product of physiological action of the Malpighian tubules is urine formation, which was observed by visualizing deposition of uric acid crystals. It was found to be highly increased in protein-rich diet and modestly increased in carbohydrate-rich diet. These results clearly indicate that the flies fed on different diets show a difference in their physiological adaptation to recover from stress conditions. Our results bring out the concern about the role of diet and specifically the dietary protein : carbohydrate ratio in maintaining variation for these traits within and among populations. If organisms are faced with natural variation in macro nutrients (proteins, carbohydrates and lipids) availability, some selection pressure is operating behind the ability to use different food resources. Differences in metabolic rates of flies developed on different nutritional regimes give rise to specific physiological pathways, which lead to adaptation to environmental stress.

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