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The interplay among dietary fat, sugar, protein and açai (*Euterpe oleracea* Mart.) pulp in modulating lifespan and reproduction in a Tephritid fruit fly

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Abstract

Macronutrient balance is a critical contributor in modulating lifespan and health. Consumption of diets rich in fruits and vegetables provides numerous health benefits. The interactions among macronutrients and botanicals and how they influence aging and health remain elusive. Here we employed a nutritional geometry approach to investigate the interplay among dietary fat, sugar, protein and antioxidant- and polyphenolic-rich freeze-dried açai pulp in modulating lifespan and reproductive output in the Mexican fruit fly, *Anastrepha ludens* (Loew). Individual flies were cultured on one of the 24 diets made from a combination of 1) sugar and yeast extract (SY) at four ratios, 2) palmitic acid, a saturated fat, at two concentrations and 3) freeze-dried açai pulp at three concentrations. Fat addition decreased lifespan in females on the sugar only diet and the diet with a low SY ratio, while decreasing lifetime reproductive output in flies on the diet with the low SY ratio when compared to SY ratio-matched low fat controls. Açai supplementation promoted survival, while decreasing lifetime reproductive output, in flies on diets with high fat and high sugar but not other diets when compared to diet-matched non-supplemented controls. These findings reveal that the impact of fat and açai on lifespan and reproductive output depends on the dietary content of other macronutrients. Our results reveal the intricate interplay among macronutrients and nutraceuticals, and underscore the importance of taking macronutrient balance into consideration in designing dietary interventions for aging and health.

Keywords

Palmitic acid; Lifespan; Dietary intervention; Nutraceutical; Palm fruit; *Anastrepha ludens*

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1. Introduction

Dietary macronutrients are critical environmental factors that modulate lifespan and healthspan (Piper et al., 2011). Lifespan generally increases with decreasing intake of dietary protein or certain essential amino acids. The target-of-rapamycin (TOR) signaling pathway is one of the pathways mediating the effect of dietary protein in modulating lifespan (Zoncu et al., 2011). Increasing lines of evidence from nutritional geometry studies in invertebrate models have shown that lifespan and reproductive output are significantly influenced more by the ratio of carbohydrate to protein (C:P) in the diet than the concentration of protein or sugar alone (Piper et al., 2011). The C:P ratios for optimal lifespan and reproduction are often not the same, suggesting some trade-off between these two health parameters. Nutrient studies routinely use sugar as the carbohydrate source and yeast extract as the sole protein source for insect models. Yeast extract contains very low levels of fatty acids. In *Drosophila melanogaster*, two studies showed that the optimal C:P ratio for highest longevity is approximately 16:1, while for maximal lifetime egg production it is approximately 4:1 for females (Lee et al., 2008; Skorupa et al., 2008). In the Mexican fruitfly (mexfly), *Anastrepha ludens* (Loew), the sugar to yeast extract (SY) ratios for maximal longevity and lifetime reproductive output are 9:1 and 3:1 for females, respectively (Carey et al., 2008). Fat is another macronutrient in the diet. Consumption of high fat diets has been linked to decreased lifespan in mice and fly models (Birse et al., 2010; Boyd et al., 2011; Pearson et al., 2008) and to increased incidence of debilitating diseases, such as diabetes, cancer and cardiovascular diseases (Das 2010). However, not much is known about how fat interacts with dietary sugar and protein to modulate lifespan and health.

Fruits and vegetables, especially colorful ones, contain high amounts of bioactive phytochemicals. Fruits and vegetables provide many health benefits, such as reducing the incidence of cancer and cardiovascular diseases, delaying age-related decline of cognitive function, reducing inflammation and preventing microbial infection (Sabate and Ang 2009). Some botanicals, nutraceuticals and compounds identified from botanicals, such as nectarine and resveratrol, are effective in antagonizing the detrimental effects induced by high fat diets to lifespan and health (Boyd et al., 2011; Pearson et al., 2008). Açai (*Euterpe oleracea* Mart.), a fruit indigenous to the Amazon River floodplain, contains numerous phytochemicals that possess anti-oxidant, anti-inflammation, anti-cancer and anti-cardiovascular disease properties as demonstrated in cell and human disease animal models (Poulose et al., 2012; Schauss et al., 2006; Xie et al., 2011). We have previously shown that freeze-dried açai pulp extends lifespan in *D. melanogaster* maintained on a high fat diet (Sun et al., 2010). Considering the critical role of macronutrients in aging, it would be important to determine how the effect of açai pulp consumption on lifespan and health is affected by the macronutrient content in the diet. To this end, here we investigate the interplay among dietary fat, sugar, protein and açai on lifespan and lifetime reproductive output in mexflies using a nutritional geometry approach.

2. Material and Methods

2.1. Fly strain and culture

Mexican fruitflies, *Anastrepha ludens* (Loew), were obtained as pupae from the MOSCAFRUT mass rearing facility at Metapa, Chiapas, Mexico. Pupae were placed inside population cages until emergence. Adult flies were exposed to one of the 24 diets sorted at random. These diets were made from a combination of sugar and yeast extract (SY) (MP Biomedicals, Solon, OH, USA) at four ratios (3:1, 9:1, 24:1 and sugar only) and freeze-dried açai pulp at three concentrations of (0, 2 and 4%) with or without fat (2% palmitic acid, Sigma-Aldrich, St. Louis, MO, USA). Açai pulp was described previously (Sun et al., 2010) and was kindly provided by Dr. Alexander G. Schauss who obtained it from EcoFruits

International (South Jordan, UT, USA). Specifically, diets were prepared by dry-mixing sugar and yeast extract at a specified ratio that were then dissolved in autoclaved water in a 1:2 (weight/volume) ratio. The final concentration of total sugar and yeast extract is approximately 40% (w/v). For high fat diets, 2 g of palmitic acid plus 1 ml of Tween-80, as an emulsifier, were added to each 100 ml of the SY solution. The proper amount of açai pulp was added to the SY or SY with fat diets, which were briefly pre-heated in a 60°C water bath for one minute, and then the solution was filtered to sort out açai solid particles.

2.2. Lifespan and reproductive output assays

Lifespan and reproduction assays were performed as described previously with minor modifications (Zou et al., 2009). Briefly, adults were sorted by sex and placed in clear Plexiglass® condominium cages (size 4×4×10 cm per cage). During the first 10 days of adult life, a pair (one female and one male) was placed in each condominium cage for mating, and then one fly was removed from the cage. Each cage contained a black silicon membrane for females to lay eggs. Individual females and males were placed in alternate units to avoid mixing eggs from any two females. Individual flies were exposed to one of the above 24 diets sorted at random. A 6-μl droplet of one of the 24 diets and another 6-μl droplet of water were provided on a glass slide daily to each fly. The number of dead flies and eggs laid by each female were recorded daily. The percentage of fertile flies in each diet condition was calculated by the number of flies that laid at least one egg by the total number of females. The lifespan and reproduction experiments were run with 100 individuals per sex per treatment, making a total of 4,800 flies.

2.3. Statistical analysis

Data were analyzed with StatView version 5.0 software (SAS, Cary, NC, USA) or SPSS Statistics version 19 (IBM, Armonk, NY, USA). Mantel-Cox logrank tests were performed for lifespan data and Student's *t*-test analyses were run for lifetime reproductive output data. Lifespan and reproductive output values are expressed as means ± standard error. For any comparison, $p < 0.05$ was considered as statistical significance.

3. Results

3.1. Fat interacts with sugar and protein to affect lifespan differentially in males and females

To investigate the interplay among dietary fat, sugar and protein on lifespan, we measured lifespan of *mex*flies on eight diets containing sugar and yeast extract at four ratios, 3:1, 9:1, 24:1 and sugar only, with or without 2% palmitic acid, a saturated fat. Consistent with previously published results (Carey et al., 2008), without addition of palmitic acid, mean lifespan was the highest in both males and females on the SY9:1 and 3:1 diets, and the lowest in both males and females on sugar only diet (Table 1 and Fig. 1A and B). Addition of palmitic acid decreased lifespan in females on the SY3:1 and sugar only diets ($p < 0.01$) and marginally on the SY24:1 diet ($p = 0.054$) relative to the SY ratio-matched low fat controls. Fat addition did not statistically significantly affect lifespan in females on SY 9:1 diet nor males under any of the four SY dietary conditions, although there is a trend for fat to reduce lifespan in males on SY24:1 and sugar only diets ($p = 0.077$ and 0.071 , respectively), when compared to the SY ratio-matched low fat controls (Table 1 and Fig. 1A and B). These findings suggest that the impact of fat on lifespan depends on gender and dietary sugar and protein content. Further, fat is detrimental to lifespan in females on a sugar only diet and a high protein diet.

3.2. Açai supplementation promotes survival of flies under high sugar and high fat diets

We have previously shown that a freeze-dried açai pulp promotes survival in *D. melanogaster* on a high fat diet but not a standard SY diet (Sun et al., 2010). To determine the interplay among açai and macronutrients on lifespan, we measured lifespan of mexflies on the SY or SY plus 2% palmitic acid diets supplemented with 0%, 2% or 4% açai. Under the dietary conditions without addition of palmitic acid, supplementation of either 2% or 4% açai increased survival of males on sugar only diet ($p < 0.05$) but not the other three SY diets when compared to the diet- and gender-matched non-supplemented controls (left parts in Fig. 1A and B, Table 1 and supplemental Table S1). Under the dietary conditions with addition of 2% palmitic acid, supplementation of either 2% or 4% açai promoted survival in both males and females on sugar only diet, and supplementation of 4% but not 2% açai increased survival of males on the SY24:1 diet and decreased survival of females on the SY3:1 diet, when compared to gender- and diet-matched non-supplemented controls (right parts in Fig. 1A and B, Table 1 and supplemental Table S2). Açai supplementation at either 2% or 4% did not alter lifespan in males on the SY3:1 and SY9:1 diets with 2% palmitic acid, and females on the SY3:1, 9:1 and 24:1 diets with 2% palmitic acid. These findings suggest that the prolongevity effect of açai depends on the macronutrient content in the diet, and açai supplementation alleviates the detrimental effect of fat on lifespan under dietary conditions with a high sugar to protein ratio or sugar only.

3.3. The effects of fat and açai on lifetime reproductive output

To determine the effect of fat and açai on health fitness, we measured lifetime reproductive output for female mexflies. Consistent with previously published results, flies on the SY3:1 diet had the highest lifetime egg production relative to the SY9:1, 24:1 and sugar diets (Fig. 2, Table 1 and supplemental Table S3). Addition of 2% palmitic acid reduced lifetime egg production in flies fed the SY3:1 diet but not the other three diets, when compared to the diet-matched low fat controls (Fig. 2). Supplementation of 4% açai reduced the lifetime egg production in flies on the sugar only diet with 2% palmitic acid but not under any other dietary conditions, when compared to diet-matched non-supplemented controls (Fig. 2). Supplementation of 2% açai had little or no effect on the lifetime egg production in flies under any dietary condition relative to diet-matched non-supplemented controls (Fig. 2 and supplemental Table S3). Our findings suggest that fat reduces lifetime reproductive output, one parameter of health fitness, but does not necessarily affect longevity in flies on the diet with a low ratio of sugar-to-protein. On the other hand, açai supplementation may promote survival, while reducing lifetime reproductive output, in flies on sugar only diet.

4. Discussion

In this study, we investigated the interplay among sugar, yeast extract (representing dietary protein), palmitic acid (representing dietary fat) and açai on lifespan and lifetime reproductive output in mexflies. Our findings from this nutritional geometry study reveal the interactive nature of macronutrients and botanicals in modulating lifespan and health fitness. Macronutrient imbalance is a major risk factor for health and many diseases, such as metabolic syndrome, cancer and heart disease (Das 2010). Consistent with this notion, we have found that palmitic acid, a saturated fat, is detrimental to lifespan in female mexflies, and to a lesser extent, in males, on the sugar only diet. Interestingly, addition of dietary protein generally alleviates the damaging effects of palmitic acid on lifespan. However, addition of too much protein augments the detrimental effect of fat on both lifespan and lifetime reproductive output. Diets high in fruits and vegetable are generally considered to be good for health and can antagonize the detrimental effects of fat (Sabate and Ang 2009). Our data indicate that the prolonged survival induced by açai supplementation is associated with reduced lifetime reproductive output in flies on the high sugar and high fat diet. Açai

supplementation is not effective in promoting survival or altering reproductive output in most other diets. Considering that reproduction generally increases with protein and decreases with fat, it is unlikely that the extra protein and fat provided by açai play any significant role in modulating lifespan and reproduction. Açai supplementation provides negligible amount of extra carbohydrate. Our study supports the importance of considering macronutrient balance and evaluating interactions among macronutrients and botanicals as they impact on animal health. Our findings also support the notion that lifespan and reproductive output is uncoupled and conditioned on diet. We tested only one concentration of palmitic acid and reproductive output as the only health fitness variable of flies besides lifespan in this study. Future study is warranted to include additional concentrations and different types of fat and additional health parameters, such as locomotor activity, in order to disentangle the intricate relationship among macronutrients and dietary interventions on aging and health.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Effects of fat and açai on lifespan and health depend on macronutrient content.
- Fat decreases lifespan in flies on the sugar only diet and on a high protein diet.
- Fat decreases lifetime reproductive output in females on a high protein diet.
- Açai promotes survival in flies on high fat and high sugar diets.
- Açai decreases reproductive output in flies on a high fat and high sugar diet.

Fig. 1.

The effect of palmitic acid addition and açai supplementation on lifespan. (A) Mean lifespan of males on diets each with a combination of one of four sugar-yeast extract (SY) diets, 0% or 2% palmitic acid, and 0%, 2% or 4% açai. (B) Mean lifespan of females on the same diet as in (A). The SY ratios refer to the ratios of sugar to yeast extract in the diet. Error bars represent standard errors. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ for comparison between 2% palmitic acid-fed flies and SY diet-matched controls or between açai supplemented flies and diet-matched non-supplemented controls by the logrank test.

Fig. 2.

The effects of palmitic acid addition and açaí supplementation on lifetime reproductive output in females. The SY ratios refer to the ratios of sugar to yeast extract in the diet. * $p < 0.05$ for comparison between 2% palmitic acid-fed flies and SY diet-matched controls by the Student's t -test. ** $p < 0.01$ for comparison between açaí supplemented flies and diet-matched non-supplemented controls by the Student's t -test. Error bars represent standard errors.

Table 1

The interplay among sugar, yeast extract, palmitic acid and açai pulp on lifespan and reproduction.

Sex	SY diet ^a (ratio)	Palmitic acid (w/v)	Açai (v/w)	Lifespan (d) (Mean±SE)	p value for lifespan ^b	% of fertile females	Lifetime egg production/fly	p value for egg production ^b
Male	SY3:1	0%	0%	56.5±2.7				
			2%	55.5±2.5	0.793			
			4%	52.6±2.5	0.248			
	SY9:1	0%	0%	57.8±2.7				
			2%	53.5±2.7	0.311			
			4%	55.5±2.6	0.536			
	SY24:1	0%	0%	45.7±2.5				
			2%	47.7±2.4	0.607			
			4%	47.9±2.5	0.667			
	Sugar only	0%	0%	32.4±1.3				
			2%	37.3±1.6	0.035			
			4%	37.4±1.5	0.011			
Female	SY3:1	2%	0%	56.0±2.7	0.886 ^c			
			2%	54.6±2.9	0.87			
			4%	53.6±2.6	0.608			
	SY9:1	2%	0%	56.6±2.5	0.443 ^c			
			2%	51.3±2.6	0.256			
			4%	53.6±2.4	0.433			
	SY24:1	2%	0%	40.5±2.4	0.077 ^c			
			2%	42.3±2.4	0.516			
			4%	48.1±2.7	0.014			
	Sugar only	2%	0%	29.5±1.1	0.071 ^c			
			2%	33.0±1.4	0.019			
			4%	35.3±1.4	0.001			
Female	SY3:1	0%	0%	55.5±2.3		89%	686.3±63.1	
		2%	2%	58.0±2.5	0.358	92%	652.7±66.6	0.715

Sex	SY diet ^a (ratio)	Palmitic acid (w/v)	Açai (v/w)	Lifespan (d) (Mean±SE)	p value for lifespan ^b	% of fertile females	Lifetime egg production/fly	p value for egg production ^b
			4%	47.1±2.3	0.032	84%	650.7±69.0	0.704
	SY9:1	0%	0%	58.5±2.9		68%	266.3±30.4	
			2%	56.0±2.6	0.441	72%	266.8±33.8	0.99
			4%	59.5±3.0	0.968	70%	326.5±38.3	0.222
	SY24:1	0%	0%	50.0±2.9		24%	70.6±11.8	
			2%	54.2±3.0	0.287	36%	67.4±10.1	0.84
			4%	51.0±2.8	0.962	30%	64.2±11.2	0.697
	Sugar only	0%	0%	32.9±1.4		13%	39.1±7.9	
			2%	35.6±1.5	0.233	9%	34.3±6.7	0.673
			4%	36.6±1.7	0.099	12%	20.5±5.0	0.064
	SY3:1	2%	0%	43.8±1.9	0.001^c	94%	506.5±47.1	0.0227^c
			2%	40.3±2.3	0.354	86%	490.0±53.2	0.816
			4%	40.8±2.0	0.43	89%	486.9±48.3	0.772
	SY9:1	2%	0%	55.9±2.3	0.276 ^c	68%	293.3±33.1	0.5475 ^c
			2%	50.9±2.6	0.419	68%	300.8±32.9	0.874
			4%	54.9±2.6	0.86	67%	278.1±31.5	0.74
	SY24:1	2%	0%	43.7±2.5	0.054 ^c	36%	91.4±19.7	0.4262 ^c
			2%	43.6±2.5	0.975	37%	77.5±13.7	0.562
			4%	44.9±2.8	0.451	33%	66.6±10.9	0.286
	Sugar only	2%	0%	28.7±1.1	0.01^c	13%	32.5±5.2	0.4974 ^c
			2%	35.1±1.4	<0.0001	16%	33.9±11.7	0.92
			4%	37.1±1.5	<0.0001	16%	16.8±2.2	0.006

Note:

^aThe ratio of the SY diet refers to the ratio of sugar to yeast extract.

^bp values were calculated by comparing açai-fed flies to gender- and diet-matched non-supplemented controls using logrank analysis for lifespan and Student's *t*-test for egg production.

^cp values were from comparing fat-fed flies to gender- and diet-matched low-fat controls using logrank analysis for lifespan and Student's *t*-test for egg production.