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The effects of folate intake on DNA and single-carbon pathway metabolism in the fruit fly *Drosophila melanogaster* compared to mammals

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ABSTRACT

Mechanisms of vitamin function in non-mammals are poorly understood, despite being essential for development. Folate and cobalamin are B-vitamin cofactors with overlapping roles in transferring various single-carbon units. In mammals, one or both is needed for nucleotide synthesis, DNA methylation, amino acid conversions and other reactions. However, there has been little investigation of the response to folate or cobalamin in insects. Here, we manipulated folate intake and potentially cobalamin levels in the fruit fly *Drosophila melanogaster* with chemically-defined diets, an antibiotic to reduce bacterially-derived vitamins, and the folate-interfering pharmaceutical methotrexate, to see if single-carbon metabolites and DNA synthesis rates would be affected. We found that similar to mammals with low folate intake, fruit fly larvae had significantly slower growth and DNA synthesis rates. But changes to single carbon-metabolites did not mirror that of mammals with abnormal folate or given MTX. Five of the nine metabolites measured were not significantly affected (methionine, serine, glycine, methylglycine, and dimethylglycine) and three (cystathionine, methylgycine, and methylmalonic acid) were only decreased in larvae consuming methotrexate. Metabolites expected to be elevated if flies used cobalamin from microbial symbionts were not affected by dietary sulfaquinoxaline. Our data support the role of folate in nucleotide synthesis in *D. melanogaster* and that microbial symbionts provide functioning folates. We could not confirm how folate intake affects single carbon pathway metabolites, nor whether *Drososphila* use microbially-derived cobalamin. Further work should explore which cofactors are used in fruit flies in these important and potentially novel pathways.

1. Introduction

Vitamin requirements in insects are similar to those in mammals, so it is generally assumed that the consequences of vitamin deficiencies will also be similar (Dadd, 1985). However, folic acid (B-9) and cobalamin (B-12) are important vitamins whose specific roles are not well known in insects. Here we examine the effect of folate (the active forms of folic acid) manipulation, possible cobalamin levels and metabolism on growth, development, DNA synthesis rates, and folate and cobalamin pathway intermediates in the fruit fly *Drosophila melanogaster*.

Both folate and cobalamin are cofactors that transfer single-carbon units (e.g. CH_3, CHO) in numerous reactions, thus they are often considered together (Fig. 1). Single-carbon metabolism includes nucleotide synthesis (the purine ring and thymidine from uridine, see Fig. 1), so without sufficient folate, enough thymidine cannot be made, and DNA is more likely to have uracil misincorporations (Blount et al., 1997). These roles of folate in nucleotide synthesis are likely why antifolates, frequently used in cancer and anti-inflammatory treatments, slow cell growth (Wagner, 2001). Insects also appear to require folates for nucleotide synthesis, but the evidence is scarce, indirect, and only available for two species (Grzelakowska and Zielinska, 1965; Sang, 1959; Zielinska and Grzelakowska, 1965).

Other folate or cobalamin dependent reactions convert or catabolize amino acids (Fig. 1). In mammals, this includes formation of the universal methyl donor, S-adenosyl-methionine (SAM). Thus, folate and/or cobalamin deficiencies decrease SAM levels, and thus DNA methylation levels which disrupt gene regulation in mice (Friso and Choi, 2002). The roles of these vitamins in DNA metabolism may explain the correlation between abnormal folate intake and cancer, neural tube defects and other disorders (Bender, 2003; Reynolds, 2006). Many insects, including *D. melanogaster,* have methylated DNA, although mechanisms of epigenetic regulation in some insects, including *D. melanogaster*, appear quite different than mammals (Lyko et al., 1999, 2000). Overall, there is little evidence that folates are cofactors for any single-carbon reaction in

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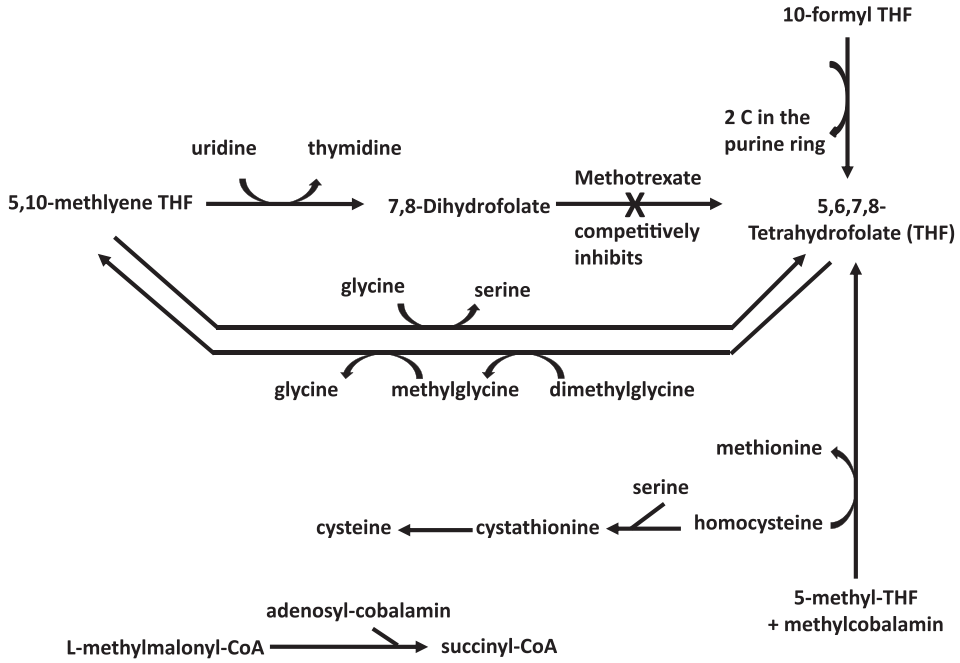

Fig. 1. Aspects of single carbon metabolism as generally understood from mammals. Methotrexate competitively inhibits dihydrofolate reductase, which converts 7,8-dihydrofolate to 5,6,7,8-tetrahydrofolate.

insects, whereas in mammals, it is known that many single-carbon metabolites are affected by folate and/or cobalamin intake (Table 1).

Studies on folate in insects have provided conflicting results: in various species it has been reported to stimulate growth, have no effect, or be toxic (Noland et al., 1949; Sang, 1956; Saxena and Kaul, 1974). Newer information suggests that some of these inconsistencies may occur because insects can receive folates from microbial symbionts. In *D. melanogaster*, when antibiotics are consumed, mono-, di-, and triglutamated folate levels are reduced, larval growth rate slows, and larval development time increases without dietary folic acid. But without antibiotics or dietary folic acid, flies can use bacterially-derived folates and interestingly grow more quickly than flies fed folic acid, even though they have the same body folate levels (Blatch et al., 2010). Here, we investigate possible explanations for this pattern.

Cobalamin has not been considered a vitamin for insects, since they survive on diets made without it (Dadd, 1985; Sang, 1956) and an analysis of 23 different insect species spanning eight orders of the Class found that most had very low to non-detectable levels of cobalamin (Wakayama et al., 1984). It is possible, however, that cobalamin requirements are lower than most other vitamins and insects use minute amounts of it obtained from microbial symbionts.

This study had two primary aims. The first was to examine potential effects of folate intake on DNA synthesis and single-carbon metabolites in *D. melanogaster*. The second was to see if there is any indirect evidence that fruit flies use bacterially-derived cobalamin, by comparing previously observed metabolite changes in cobalamin-deficient humans to flies with altered diets. To investigate these two aims, we exposed flies to chemically-defined diets, and/or antibiotics to decrease vitamins obtained from microbial symbionts, and by using the antifolate methotrexate (a dihydrofolate reductase inhibitor that strongly decreases folate availability, see Fig. 1). We measured levels of growth, DNA synthesis, and nine single-carbon cycle metabolites.

Table 1
Single carbon metabolite level and DNA synthesis rate changes in humans or mammals due to low folate or cobalamin intake or MTX.

Metabolite	Change in level observed in mammals with			Change in Drosophila[j]
	Low folate	Low cobalamin	MTX	
Cystathionine	↑[a]	↑[a]	Unknown	↓ w/MTX
Dimethylgycine	↑[b]	No change[b]	Unknown	No change
Glycine	↑[d]	↑[e]	Unknown	No change
Homocysteine	↑[b]	↑[b]	↑[c,f]	Undetectable
Methionine	No change[b]	No change[b]	↓[c]	No change
Methylcitrate	No change[b]	↑[b]	Unknown	No change
Methylglycine	↑[b]	No change[b]	Unknown	↓ w/MTX
Methylmalonic acid	No change[b]	↑[b]	Unknown	↓ w/MTX
Serine	↑[g]	↑[e]	Unknown	No change
DNA synthesis rate	↓[h]	↓[h]	↓[i]	↓ w/MTX or low folate

[a] (Stabler et al., 1993).
[b] (Allen et al., 1993a) and (Allen et al., 1993b).
[c] (Broxson et al., 1989).
[d] (Dickson et al., 2005).
[e] (Ebara et al., 2001).
[f] (Svardal et al., 1988).
[g] (Cuskelly et al., 2001).
[h] (Bender, 2003).
[i] (Rhee et al., 1992).
[j] This study; changes observed on 0FSM diet (no folic acid, 5 ng/mL sulfaquinoxaline, and 5 μg/mL methotrexate) compared to all other diets used.

2. Methods

2.1. Fly rearing and handling

Fruit fly colonies on a standard yeast-based media were maintained as previously described (Blatch et al., 2010). Larvae were generated for the DNA content analysis by allowing fifteen adult females and ten adult males, per vial, to lay eggs for one day, in twenty vials per dietary treatment group (described below). Larvae in these vials were collected at the wandering stage, and five larvae were grouped together as one sample, and weighed. Adult males were collected from the same vials within one day of emergence, grouped as five males per sample, and weighed. Growth rates were calculated as larval mass divided by age in days since oviposition. Fifteen larval and adult male samples were collected per treatment group. Samples were stored at −70 °C until DNA extraction.