1 **Does early-life diet affect longevity? A meta-analysis across experimental**

2 **studies**

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13   **Abstract**

14   Life-history theory predicts that nutrition influences lifespan due to trade-offs between

15   allocating resources to reproduction, growth and repair. In spite of occasional reports that

16   early diet has strong effects on lifespan, it is unclear whether this prediction is generally

17   supported by empirical studies. We conducted a meta-analysis across experimental studies

18   manipulating pre- or post-natal diet and measuring longevity. We found no overall effect of

19   early diet on lifespan. We used meta-regression, considering moderator variables based on

20   experimental and life-history traits, to test predictions regarding the strength and direction of

21   effects that could lead to positive or negative effects. Prenatal dietary manipulations reduced

22   lifespan, but there were no effects of later diet, manipulation type, development mode or sex.

23   The results are consistent with the prediction that early dietary restriction disrupts growth and

24   results in increased somatic damage, which incurs lifespan costs. Our findings raise a

25   cautionary note, however, for placing too strong an emphasis on early dietary effects on

26   lifespan, and highlight limitations of measuring these effects under laboratory conditions.

27

Introduction

Conditions in early development can influence a suite of life history traits later in life, including the pace of ageing and total lifespan [1–3]. King penguin chicks which experience rapid catch-up growth have shorter telomeres [4], for example, and red deer born under harsh environmental conditions show faster senescence [5]. An important feature of early development is the amount and type of food received, which has immediate effects on growth and can influence later traits. Several studies have manipulated nutrition in early life – providing diets to pregnant mothers or to young before maturity – and measured offspring survival. These studies have traditionally been conducted on laboratory rodents [6], although there are an increasing number of manipulations on a range of species [7,8]. Despite occasional reports of strong effects [9,10], which have raised concerns in the health sciences [11], it is not yet known how general these effects are across biological systems.

Life-history theory provides a framework for understanding how and when early-life diet should influence lifespan. Individuals face trade-offs when allocating resources among traits that enhance growth and reproduction, versus those, such as somatic repair, that increase longevity [12]. Individuals who experience resource limitation in early life may invest in earlier reproduction, incur higher levels of damage, and pay a cost of reduced lifespan [13]. Alternatively, those individuals with low resources during development may experience slower growth, delayed reproduction and live a longer life [14].

Whether restricted diet in early life *per se*, rather than nutritional limitation across development, extends or reduces lifespan depends on several factors. Reducing total energy content might extend lifespan through increasing allocation towards somatic repair [15]; whereas limiting key nutrients for healthy development, such as protein, might impose damage during development and reduce lifespan [16]. The diet experienced beyond early development is likely important. A switch from low to high nutrition can result in catch-up

55 growth, which accrues costs later in life [17]. In contrast, being maintained on a low-nutrition

56 diet could enhance lifespan-extending effects if individuals allocate more to repair [9]. There

57 may also be sex differences in how individuals respond to dietary challenges [7,10]. Increased

58 allocation to growth and reproduction may reduce lifespan to a greater extent for the sex

59 experiencing stronger selection for condition-dependent traits or incurring higher energetic

60 costs to reproduction. Continuous developers might have higher plasticity when conditions

61 improve, compared to organisms with metamorphosis, where adult size is established by

62 larval diet.

63   Here, we conduct a meta-analysis, selecting studies in which diet was manipulated in

64 early development – at any period from early embryonic stages until age of first reproduction

65 – and later longevity was recorded. We used meta-regression [18] to test hypotheses regarding

66 the causes of heterogeneity across studies (Table 1).

67

68 **Methods**

69 We conducted a comprehensive literature search on Google Scholar and SCOPUS for studies

70 linking early-life diet with longevity, based on keywords (ageing, "compensatory growth",

71 "catch-up growth", damage, development, "developmental programming", "early life",

72 growth, lifespan, longevity, maternal, "maternal diet", oxidative, senescence, stress, survival,

73 telomere), and surveying papers cited by or in several key reviews. We only included studies

74 that conducted a dietary manipulation on pregnant females or offspring before the age of

75 sexual maturity. For studies which provided survival curves, we extracted the log hazards

76 ratio, ln(HR), based on differences in percentage of experimental and control individuals alive

77 at 75%, 50% and 25% of control group survival. However, not all studies report survival

78 curves and we therefore repeated our analysis using mean longevity. Where data were

79 provided separately for groups of individuals, for example by sex, we calculated multiple

80   effect sizes. In total, our search yielded 50 effect sizes of ln(HR) from 18 studies, and 77

81   effect sizes of mean longevity from 21 studies across 14 species (Table S1).

82   We used meta-regression to investigate whether the effect of early diet on longevity

83   was mediated by manipulation type, post-treatment diet, sex, stage of manipulation, vertebrate

84   versus invertebrate and whether catch-up growth was observed (Table 1). We conducted

85   Bayesian mixed-effects meta-analysis (BMM) using the library MCMCglmm [19] in the

86   statistical environment R (version 2.15 [20]). We first fit an intercept-only model to examine

87   an overall effect of early diet on longevity. As ln(HR) provides a measure of risk of death, a

88   negative effect indicates that diet manipulation extends lifespan. We then fit a model

89   including all moderators and examined their 95% higher posterior densities (HPD, or credible

90   interval). Any moderators whose HPD did not overlap zero were considered statistically

91   significant. We tested for publication bias by inspecting funnel plots and conducting Egger's

92   regression [21]. We calculated marginal and conditional R^2 to establish the total variance

93   explained by fixed effects or both fixed and random terms in each model, respectively [22].

94   We included study as a random term. Further details are provided in the electronic

95   supplementary material (ESM).

96

97   **Results**

98   We found no overall effect of early diet on subsequent risk of death (HPD: -0.150, 0.125, Fig.

99   1a). The lack of effect may be due to heterogeneity among studies, for example by combining

100   studies where reducing calories increased lifespan and others where reducing specific

101   nutrients reduced lifespan. Heterogeneity in the data was moderate (I^2 = 57.7%, Table S2 in

102   ESM). In our meta-regression to examine differences between studies, we found no

103   significant effects of moderators on risk of death (Fig. 1b). The marginal R^2 was only 0.04

104   (Table S2 in ESM).

105   We did not find an overall effect of early diet on mean longevity (HPD: -0.200, 0.101,

106   Fig. 1c), and these data had low heterogeneity ($I^2 = 32.5\%$, Table S2 in ESM). Several

107   moderators had significant effects (Fig. 1d), although the marginal R^2 was only 0.08 (Table

108   S2 in ESM). Early dietary restriction extended longevity to a greater extent in vertebrates than

109   in invertebrates (HPD: 0.219, 0.944), and when there was no catch-up growth (HPD: 0.039,

110   0.624). Longevity was reduced when dietary restriction occurred before birth (HPD: -1.343, -

111   0.471), and in studies combining both sexes (HPD: -1.086, -0.152).

112   To understand contrasting results in models analysing ln(HR) and longevity, we

113   repeated our analysis on those studies measuring both. Only the effect of pre- versus post-

114   natal stage on longevity remained significant (HPD: -1.069, -0.024). Publication bias was

115   weak or absent.

116

117   **Discussion**

118   The impact of early-life nutrition has recently come to the forefront of concerns regarding

119   healthy ageing [23]. Life history theory provides explanations for why early diet restriction

120   should influence lifespan [13,15]. However, we find that experimental studies generally fail to

121   demonstrate these effects. A plausible explanation for the lack of an overall effect is that

122   positive and negative effects cancel out. Indeed, there are evolutionary rationales for

123   expecting opposite patterns across studies. We found little evidence, however, that these

124   factors explain the overall lack of an effect of early diet on mortality risk and longevity. The

125   general conclusion of narrative reviews, that early nutrition affects later-life mortality [6],

126   thus appears to be driven by a small number of key studies (e.g. [8,9], Fig S2).

127   While it is tempting to draw conclusions about the evolutionary basis for early diet

128   effects on lifespan, studies testing these effects are almost always conducted under laboratory

129   conditions. In the laboratory, causes of mortality typical of natural conditions are absent, and

130   individuals experience predictable food, no predation and a different reproductive regime to

131   that in the wild. Thus, evidence for weak or absent effects in laboratory studies may simply be

132   due to the fact that intrinsic damage may not be sufficiently strong to cause increased

133   mortality risk [24].

134   Nevertheless, our analysis identified predictors of the effect of early-life diet on mean

135   longevity. We found prenatal diet manipulations had stronger negative effects compared to

136   postnatal manipulations. Thus, at least in live-bearing species, mothers do not fully buffer

137   their offspring from nutritional stress. This is also consistent with observations that variation

138   in biomarkers of ageing, such as telomere length, primarily accrue early in life [4]. Early diet

139   extends lifespan in vertebrate but not invertebrate species, potentially because juvenile and

140   adult function are decoupled through metamorphosis. Our results may thus be more easily

141   interpreted in light of mechanistic theory concerning the link between diet, damage reduction

142   and lifespan [25] than broader life history explanations.

143   Our analyses suggest weak general evidence that reduced nutrients early in life

144   influences lifespan. Whatever effects exist, and we have theoretical reasons to believe that

145   they should, may be specific to the study system. This conclusion is similar to a recent

146   extensive meta-analysis on lifespan-enhancing effects of diet restriction [26]. This study

147   found that protein restriction had stronger life-extending effects than caloric restriction, yet

148   replication in our study was not sufficient to make this comparison. Indeed, animals show

149   plasticity in growth and development across their life, such that single effects of diet

150   restriction may be weak and context-dependent. Insofar as laboratory conditions are

151   informative, the overall evidence as it stands does not provide strong support that food

152   restriction during development causes major effects on adult intrinsic mortality or lifespan.

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237   **Table and figure legends**

238

239   **Table 1.** Rationale for predictor variables in meta-regression.

240

241   **Figure 1.** Funnel plot (a,c) of effect sizes against power, with counter-shaded confidence

242   intervals (90%, 95% and 99% CI); and forest plot (b,d) of HPD intervals (posterior mean and

243   95% CI) in the meta-analysis on ln(HR) and mean longevity.

244

245   **Table 1**

Predictor	Rationale
Manipulation type (diet quality or quantity)	If dietary restriction extends lifespan, expect positive effect for reduced quantity but not quality of food. In contrast, if certain nutrients have carry-over effects for individual quality, expect quality effect to be stronger.
Post-treatment diet (control or restricted)	If dietary restriction extends lifespan, expect stronger positive effect if adults are on restricted diet too. If there is a cost of dietary mismatch, expect stronger negative effects when juveniles are on a restricted diet then adults are on a high-food diet.
Sex	If restriction reduces lifespan due to allocation trade-offs between growth and reproduction, predict stronger effect in males due to condition-dependent sexual selection; or in females if they experience high costs of reproduction.
Manipulation stage (pre- or post-natal)	Predict stronger effect of pre-natal diet due to disruption of sensitive stages in development; alternatively predict weaker effect if mothers buffer offspring from nutritional stress.
Vertebrate vs Invertebrate	Expect positive or negative effect sizes to be stronger in invertebrates because of indeterminate growth, hence less plasticity in response to early diet.
Evidence for catch-up growth (yes or no)	Expect weaker effect if individuals compensate for effect of manipulation through catch-up growth. Alternatively, if catch-up growth incurs costs, expect stronger effect under catch-up growth.

246

247

Figure 1

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