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Oxidative costs of reproduction: Oxidative stress in mice fed standard and low antioxidant diets

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HIGHLIGHTS

- Does high intake of antioxidants explain lack of oxidative damage in reproduction?
- Breeding mice were fed low antioxidant or standard laboratory diet.
- Breeding mice increased food intake 4-fold.
- Oxidative damage was decreased in breeders independent of dietary antioxidant intake.
- These results lend support to the oxidative shielding hypothesis.

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ABSTRACT

Lactation is one of the most energetically expensive behaviours, and trade-offs may exist between the energy devoted to it and somatic maintenance, including protection against oxidative damage. However, conflicting data exist for the effects of reproduction on oxidative stress. In the wild, a positive relationship is often observed, but in laboratory studies oxidative damage is often lower in lactating than in non-breeding animals. We hypothesised that this discrepancy may exist because during lactation food intake increases many-fold resulting in a large increase in the intake of dietary antioxidants which are typically high in laboratory rodent chow where they are added as a preservative.

We supplied lactating and non-breeding control mice with either a standard or low antioxidant diet and studied how this affected the activity of endogenous antioxidants (catalase, superoxide dismutase; SOD, and glutathione peroxidise; GPx) and oxidative damage to proteins (protein carbonyls, PC) in liver and brain tissue. The low antioxidant diet did not significantly affect activities of antioxidant enzymes in brain or liver, and generally did not result in increased protein damage, except in livers of control mice on low antioxidant diet. Catalase activity, but not GPx or SOD, was decreased in both control and lactating mice on the low antioxidant diet. Lactating mice had significantly reduced oxidative damage to both liver and brain compared to control mice, independent of the diet they were given. In conclusion, antioxidant content of the diet did not affect oxidative stress in control or reproductive mice, and cannot explain the previously observed reduction in oxidative stress in lactating mammals studied in the laboratory. The reduced oxidative stress in the livers of lactating mice even under low antioxidant diet treatment was consistent with the 'shielding' hypothesis.

1. Introduction

The evolution of life histories has been explained by the presence of limited resources, which result in a trade-off between survival (somatic maintenance) and reproduction [1,2]. The existence of a trade-off implies that resources are limited, or the capacity to utilise resources is physiologically constrained (e.g. [3–6]). This may be particularly important during periods when energy demands are high, such as during lactation, during which mammals appear constrained by their capacity to dissipate heat [7–10]. It has been suggested that reproduction may lead to an increase in oxidative stress thus affecting ageing (Oxidative stress theory of ageing, [11], but see [12,13]). Oxidative stress occurs when there is an imbalance between the production

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of reactive oxygen species (ROS) and the capacity of the antioxidant protection and repair systems. ROS are primarily produced in mitochondria during the process of oxidative phosphorylation when oxygen can react prematurely with an electron to form the superoxide anion. ROS is deleterious to an organism as it can cause damage to macromolecules, i.e., DNA, proteins and lipids. ROS can be removed by exogenous antioxidants obtained via the diet (e.g., carotenoids, Vitamin C and E), and by endogenously produced antioxidants, i.e., superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx). Some ROS escape detoxification and cause damage, which may be repaired (DNA) or removed by turnover (proteins), but some damage accumulates leading to an increasing risk of malfunction.

It has been hypothesised that during reproduction selective resource allocation reduces investment in protection and repair, resulting in an increase in oxidative damage, reducing survival and fecundity. Many studies attempting to determine if oxidative stress is the proximal cost of reproduction produced ambiguous results [14–25]. Supportive evidence has been found in birds and mammals, e.g., a study investigating changes in antioxidant activity in response to brood size manipulations in zebra finches showed reduced antioxidant protection in serum with increased reproductive effort [14]. Recently, a study in the North American red squirrel found increased oxidative damage to serum proteins during lactation [21], which was reduced when populations were given supplementary feeding. Contradictory results have also been found, showing no or negative relationships between oxidative stress and reproductive effort, e.g. oxidative damage was found to be reduced in the livers of reproductive compared to non-reproductive female house mice [22,26] and bank voles [24] and breeding and non-breeding zebra finches did not differ in their resistance to oxidative stress (include tissue) [17], although a negative relationship between the number of eggs laid and oxidative resistance was found in that same study.

The cause of the variation in responses has been debated [12,13,27] and various suggestions explaining the discrepancies have been offered, including differences in experimental design, biological markers, tissues, species, laboratory vs. field conditions. Indeed, depending on the marker used or the tissue measured contradicting results can be found within the same individuals [28,29]. One possibility for the lowered damage during reproduction observed noticeably in lactating rodents' livers in captivity ([22,24]) is that this does not reflect a change in protection strategy but may rather be an artefact of elevated food intake in lactation [30] combined with the very high levels of antioxidants that are added to rodent chow as a preservative (Speakman et al. submitted). During lactation in mice at room temperature, food intake increases by about a factor of 4 to 5 reaching a plateau around day 10–18 of lactation (Johnston et al., 2001). In laboratory conditions, where animals are fed on standardised diets, the intake of exogenous antioxidants thus increases proportionately to food intake. This increased intake of exogenous antioxidants may mitigate detrimental effects of increased ROS production during peak lactation, and may explain why negative effects of lactation on oxidative stress in the liver have been observed [22,24]. Since the liver is the first organ to experience such absorbed antioxidants it would be particularly prone to such impacts, possibly explaining why damage to the liver often goes down in such studies [22,24,28,29] when damage to other tissues goes up [24,28,29]. To test this hypothesis we formulated a low antioxidant diet that had 5-times less antioxidants compared to the standard diet. At peak lactation therefore intake of antioxidants of lactating mice on the low antioxidant diet would thus be similar to the intake of antioxidants in non-reproductive control animals on the standard diet. Hence, if endogenous antioxidant overload was an issue, these animals would be expected to have increased oxidative stress noticeably in their livers, compared to both lactating and control mice fed the standard diet.

2. Materials and methods

2.1. Animals & housing

Sixty-five MF1 mice, 15 male and 50 female, were obtained from Harlan laboratories (Bicester, UK) at 10 weeks of age, and housed in shoebox cages (NKP Cages, M3 Mouse Cage, 48" × 15" × 13") with sawdust shavings and paper wool for bedding. After 2 weeks of acclimation, mice had ad libitum access to food (CRM(P) standard laboratory chow) and water and were housed under a 12:12 LD cycle and 21 °C (±1 °C). We used MF1 mice because of our extensive prior knowledge of its reproductive energetics [31].

2.2. Experimental protocol

Female mice were randomly assigned to one of 4 groups ensuring an even body weight distribution: 1. control on standard diet (n = 11), 2. control on low antioxidant diet (n = 10), 3. reproductive mice on standard diet (n = 15) or 4. reproductive mice on low antioxidant diet (n = 15). The low antioxidant diet (D12072502) used was specifically formulated by Research Diets (New Brunswick, USA) for this study based on the D12450B diet (10%, 70% and 20% of kJ from fat, carbohydrates and protein respectively) with a reduced antioxidant content by removing Vitamin E from the vitamin mix and using a different type of fat source that did not contain antioxidants, i.e., tocopherol-stripped corn oil was used in the low antioxidant diet as the main fat source instead of soybean oil and lard that may contain some antioxidants. See Table 1 for detailed dietary content and Supplementary Table 1 for a detailed description of the vitamin mixes used.

Reproductive mice were housed in pairs (one each from the standard and low antioxidant diet groups) with a single male for 7 days to induce pregnancy. When the male was removed both females were housed individually for the remainder of the study. Control mice were housed individually from the start of the experiment and not exposed to a male mouse. Reproductive mice were fed standard lab chow (CRM(P)) until day 1 of lactation (day 0 = date of birth), when the new diet, with standard or low antioxidants was introduced. The new diet was introduced initially as a 50–50% mix of CRM(P) and the assigned diet, and from day 2 till the end of lactation only the assigned diet was provided. Control mice were introduced to the new diets in the same way on the first day of the first reported lactation in reproductive mice. Food intake and body mass were measured daily throughout the experimental period, except on the day of parturition (day 0: [8]). In addition, litter mass and litter size was recorded from day 1 of lactation onwards in reproductive mice. On day 18 of lactation all mice were

Table 1
Nutritional information for standard and low antioxidant diet.

Product #	Standard D12450B		Low antioxidant D12072502	
	g	kJ	g	kJ
Protein	19%	10%	19%	10%
Carbohydrate	67%	70%	67%	70%
Fat	4%	20%	4%	20%
Total (kJ/g)	**15.9**	**100%**	**15.9**	**100%**
Soybean oil	25	941	0	0
Corn oil, tocopherol stripped	0	0	45	1694
Lard	20	753	0	0
Mineral mix S10026	10	0	10	0
Dicalcium phosphate	13	0	13	0
Calcium carbonate	5.5	0	5.5	0
Potassium citrate, 1 H_2O	16.5	0	16.5	0
Vitamin mix V10001	10	167	0	0
Vitamin mix V13401 (−VitE)	0	0	2	33
Choline bitartrate	2	0	2	0

Diets were formulated by Research diets, New Brunswick, USA. g = weight in grams and kJ shows energy content in kJ.