

Juvenile hormone regulation of longevity in the migratory monarch butterfly

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Monarch butterflies (*Danaus plexippus*) of eastern North America are well known for their long-range migration to overwintering roosts in south-central Mexico. An essential feature of this migration involves the exceptional longevity of the migrant adults; individuals persist from August/September to March while their summer counterparts are likely to live less than two months as adults. Migrant adults persist during a state of reproductive diapause in which both male and female reproductive development is arrested as a consequence of suppressed synthesis of juvenile hormone. Here, we describe survival in monarch butterflies as a function of the migrant syndrome. We show that migrant adults are longer lived than summer adults when each are maintained under standard laboratory conditions, that the longevity of migrant adults is curtailed by treatment with juvenile hormone and that the longevity of summer adults is increased by 100% when juvenile hormone synthesis is prevented by surgical removal of its source, the corpora allatum. Thus, monarch butterfly persistence through a long winter season is ensured in part by reduced ageing that is under endocrine regulation, as well as by the unique environmental properties of their winter roost sites. Phenotypic plasticity for ageing is an integral component of the monarch butterflies' migration–diapause syndrome.

Keywords: senescence; reproductive diapause; juvenile hormone; migration; phenotypic plasticity; *Danaus plexippus*

1. INTRODUCTION

The life cycle of the monarch butterfly *Danaus plexippus* is intimately coupled with its migratory behaviour. Adults of the eastern North America population overwinter in the transverse neovolcanic belt of south-central Mexico and return to the southern USA in early spring (Calvert & Brower 1986). Successive cohorts progressively recolonize more northern locations through spring and summer until individuals of the August/September cohort develop as migrant phenotypes and return to Mexico (Malcolm *et al.* 1993). The nature of this life cycle indicates that the adults exhibit phenotypic plasticity for their rate of ageing. Summer adults are likely to live a relatively short duration, perhaps two to five weeks, as the population generation time of summer cohorts is estimated to be in the order of only 40 days (Cockrell *et al.* 1993). In contrast, the migrant adult cohort must persist from early autumn through to spring, which is five- to sixfold longer than their summer counterparts. The migrant cohort travels south and overwinters in a state of reproductive diapause, which is characterized by the arrest of oogenesis, vitellogenesis and accessory gland development. Winter persistence may involve both the special environmental conditions of the roost and retarded ageing, which is conferred as part of the reproductive diapause syndrome. Here, we show that survival is extended in diapause monarch butterflies and that suppressed synthesis of juvenile hormone contributes to the extended longevity observed in the diapause–migrant syndrome.

The reproductive diapause of migrant adults in eastern North America is thought to be initiated by the short day lengths perceived in August and September (Barker &

Herman 1976). Relative to summer adults, migrants are characterized by a large wingspan and body mass, high lipid content, and arrested accessory gland (both sexes) and ovarian development (females) (Herman 1985). The migrant adults feed intensively for several weeks following eclosion before their southern flight to winter roosts (Urquhart 1960). Their roosts occur along a narrow altitudinal range of boreal *Abies religiosa* forests with specific environmental conditions that are likely to foster overwintering persistence: cool and moist air, substrate upon which to cluster and low levels of disturbance (Calvert & Brower 1986; Masters *et al.* 1988; Anderson & Brower 1993). Overwintering adults feed occasionally, but are physiologically characterized by a low body temperature (Brower & Calvert 1985; Masters 1993) and minimal reproductive tract mass (Herman *et al.* 1989). At these sites, adults enter a post-diapause phase in late December (males) and January (females) such that reproductive development can be initiated under long-day, warm conditions (Herman *et al.* 1989). The return migration begins in March accompanied by accelerating vitellogenesis, accessory gland synthesis and mating behaviour.

Monarch butterflies' reproductive diapause is centrally regulated by suppressed production of juvenile hormone. Both neck ligation and allatectomy (surgical removal of the corpora allatum, which synthesizes juvenile hormone) elicit traits of the diapause syndrome in adults raised under summer conditions, namely reduced accessory gland volume, arrested oogenesis and increased fat body lipid content (Herman 1975). These traits are rescued in part by therapy with juvenile hormone I or juvenile hormone II (Lessman *et al.* 1982). Conversely, incubation of migrant adults under summer-like conditions can initiate reproductive development, as does treatment of migrant adults with exogenous juvenile hormone

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(Herman 1975). The juvenile hormone titres of summer adults based on direct and indirect measures are 100 times greater than the measures from migrant adults that are wild caught in November through to January (Herman *et al.* 1981; Lessman & Herman 1983). Juvenile hormone is elevated in both sexes among the northward flying adults of March (Herman 1985).

Reduced juvenile hormone during diapause may increase the butterflies' survival in several ways. When juvenile hormone is low, reproduction is curtailed and both the acute and future mortality costs of gonadal activity may be reduced. The acute mortality costs of reproduction are best described for *Drosophila melanogaster* in which courting males risk death proximal to their behavioural interactions and females suffer mortality upon receipt of seminal fluid (Partridge & Andrews 1985; Chapman *et al.* 1995). In addition, egg production appears to incur long-term mortality costs (Partridge *et al.* 1987; Sgro & Partridge 1999). Such a long-term mortality cost was shown to result in part from the allocation of resources towards egg production in the beetle *Callosobruchus maculatus* (Tatar & Carey 1995). Migrant adults of monarch butterflies may avoid these costs and thereby age at slower rates. A low juvenile hormone titre during reproductive diapause may further reduce senescence if this endocrine directly regulates the state of somatic maintenance, stress resistance or metabolic rate. High levels of somatic stress resistance are characteristic of dauer (Riddle *et al.* 1987) and of longevity extending mutants in the nematode *Caenorhabditis elegans* (Larsen 1993; Lithgow *et al.* 1994; Murakami & Johnson 1996), and of pupal and adult diapause in flies, including *Drosophila triauraria* and *D. melanogaster* (Adedokun & Denlinger 1984; Goto *et al.* 1998; Yocum *et al.* 1998). Mutation of the gene that encodes the insulin-like receptor in *D. melanogaster* leads to reduced synthesis of juvenile hormone and increases adult longevity (Tatar *et al.* 2001a). Here, we describe demographic studies of monarch butterflies in order to assess the effect of reproductive diapause upon longevity and the role juvenile hormone has in diapause-related ageing.

2. MATERIAL AND METHODS

(a) *Animals*

Adults from the field were collected near Minneapolis, MN, USA. Summer residents were captured between June and July 1987, and migrant adults were collected in late August and September 1988. Field-collected animals were returned to the laboratory, weighed, fed and treated. Reproductively active adults for manipulative studies of the corpora allatum and juvenile hormone treatments were reared outdoors on milkweed (*Asclepias syriaca* L.) from eggs laid by field-collected females in late May and June 1989.

In order to measure their lifespans, adult monarch butterflies were held in glassine envelopes and incubated at $25 \pm 1^\circ\text{C}$ under a 16 L:8 D photoperiod (summer photophase) at 65–95% relative humidity. In order to prevent abdominal tears, which are common in monarch butterflies held for long periods, the tarsal claws of the third leg were removed. Animals were fed every 2 days with 30% honey solution. Their lifespans were recorded from the day of capture or eclosion until death. Between 25 and 60 individuals per sex per cohort were aged under standard

conditions in order to assess the adult life expectancy of untreated, field-collected adults from summer and migrant cohorts.

(b) *Juvenile hormone treatments*

Juvenile hormone I (Sigma, St Louis, MO, USA), which has strong effects on monarch butterfly reproduction and juvenile hormone III (Sigma), which does not, were applied topically (Lessman *et al.* 1982). Both juvenile hormone solutions were bioassayed on neck-ligated monarch butterflies prior to use and were shown to have high and low activity, respectively. Juvenile hormone was dissolved in acetone at $50 \mu\text{g } 10 \mu\text{l}^{-1}$ and $10 \mu\text{l}$ of solution was applied to the abdomens of treated animals in each of the 6 weeks following eclosion. Control animals were treated weekly with $10 \mu\text{l}$ acetone only. Juvenile hormone treatments were used in two trials. First, field-caught migrant adults were treated with either juvenile hormone I solution or acetone control and subsequently scored for longevity (30–40 adults per sex per treatment). Second, in conjunction with the allatectomy trial, laboratory-reared summer adults were treated with juvenile hormone I, juvenile hormone III or acetone alone and subsequently scored for longevity (11–30 adults per sex per treatment).

(c) *Allatectomy*

The allatectomy surgery followed standard procedures for allatectomy on the day of eclosion (Barker & Herman 1973). Adults were anaesthetized with CO_2 and secured to a surface where the head could be bent forward and downward. The dorsal neck membranes were slit longitudinally and pulled open with hooked pins. The exposed corpora allatum was removed with forceps and, owing to its close association, the corpora cardiacum was sometimes ablated as well. The wound was treated with 1:1:1 penicillin, phenylthiourea and streptomycin and closed with a piece of Gelfoam absorbable gelatin. Approximately 50% of subjects that had been operated on survived with vigorous feeding to 1 week and were subsequently included in the longevity assays. Sham-operated individuals were handled identically, but without the removal of tissue. At death, allatectomized animals were dissected in order to confirm the arrest of ovary and male accessory gland development.

3. RESULTS

(a) *Survival of field-caught summer and migrant adults*

Migrant adults sampled from field populations in Minnesota were significantly longer lived upon transfer to laboratory conditions than were samples of field-caught summer adults (figure 1). However, the sample of individuals collected in the summer may have initially contained older individuals than the sample collected in the autumn. Body mass may be a crude proxy for age if it can be shown that mass and life expectancy are associated within a cohort. Therefore, an accelerated failure time model (Weibull distribution) was used for comparing longevity among cohorts while controlling for body mass as a continuous covariate. Mass was significantly associated with survival among males but not among females such that an increase of 0.01 mg predicts a 0.2% increase in longevity ($\exp(\beta_{\text{body weight}}) = 1.002$, $\chi^2 = 12.18$ and $p = 0.005$). However, the survival times among individuals of the migrant cohort remained 41–52% greater than

Table 1. Reproductive status as evaluated at death for adults reared under summer-like conditions and treated as controls (intact or sham operated) or with removal of the corpora allatum (allatectomy).

(Significant differences among sample means (\pm s.e.) are indicated by different letter superscripts for each trait measure.)

	male ejaculatory duct (mg)	female mature oocytes (n)	ovary mass (mg)
intact control	31.9 \pm 3.4 ^a	204.3 \pm 15.8 ^a	64.90 \pm 4.71 ^a
sham-operated control	27.8 \pm 1.4 ^a	153.3 \pm 22.2 ^b	62.60 \pm 6.4 ^a
allatectomy	19.2 \pm 1.6 ^b	0.0 \pm 0.0 ^c	2.24 \pm 0.2 ^b

that of the summer cohorts after accounting for mass in both males and females (males, $\exp(\beta_{\text{cohort}}) = 1.412$, $\chi^2 = 11.70$ and $p = 0.006$, and females, $\exp(\beta_{\text{cohort}}) = 1.524$, $\chi^2 = 10.77$ and $p = 0.001$). These data indicate that migrant monarchs might have an intrinsic capacity for outliving summer adults. The treatment of non-reproductive migrant adults with juvenile hormone supports this finding. When treated with juvenile hormone I and transferred to summer laboratory conditions, field-caught migrant adults terminate reproduction and have a reduced life expectancy relative to acetone-treated controls (figure 2).

(b) Survival of laboratory-reared adults that were allatectomized or treated with juvenile hormone

The survival of the butterflies among the control cohorts did not vary significantly (figure 3*a,c*): treatment with acetone, juvenile hormone III or sham operation did not affect their longevity relative to untreated, intact adults. The life expectancy of controls with the cohorts combined was 78.6 ± 1.9 days for females and 67.6 ± 3.7 days for males. Allatectomy nearly doubled the butterflies' life expectancy in both males and females, to 120.3 ± 1.2 and 131.2 ± 1.0 days, respectively. In contrast, topical application of juvenile hormone I reduced the butterflies' life expectancy by one-half, to 39.0 ± 1.7 days for females and 32.5 ± 1.8 days for males. The impact of treatment upon mortality was primarily caused by variation in the intercepts of the mortality trajectory among females (figure 3*b*) and through variation in both the intercept and slope among males (figure 3*d*). Data on the reproductive effects of allatectomy but not those of juvenile hormone are available (table 1). Allatectomized adults had reduced reproductive development at death relative to sham and intact controls, particularly among females who had ovaries with levels that resembled those of newly eclosed adults.

4. DISCUSSION

Migrant adults in reproductive diapause are longer lived than summer adults when life tables are constructed under controlled environmental conditions in order to exclude extrinsic mortality risks. These demographic assays are based on field-caught individuals and could be biased by their age at collection if older individuals were collected in the summer sample. Body mass among summer females correlates with life expectancy as measured from the time of collection, but statistical control of body mass as a proxy for age at collection did

not eliminate the mortality differences between the summer and migrant cohorts. This analysis indicates that migrant adults might have an increased capacity for survival relative to summer adults, although we recognize that in these data differences in their age at collection were not experimentally controlled. In addition, the survival of summer versus migrant adults was determined using cohorts that developed under different field conditions and different years. Larval diet or other environmental experiences unrelated to diapause may produce the observed differences in adult longevity.

The experimental manipulation of juvenile hormone indicated that retarded ageing during reproductive diapause might be an intrinsic aspect of the diapause syndrome. Topical application of juvenile hormone I to migrant and summer adults reduced their longevity. It is conceivable that topical application of juvenile hormone shortens the butterflies' life span as a pharmacological side-effect rather than as a physiological consequence of normal hormone action. The results of the allatectomy trials are therefore particularly important because they demonstrate a large increase in longevity upon removal of the juvenile hormone source in both males and females. The differences in survival between treatments for both males and females are primarily caused by a reduction in the intercept of log mortality, although the rate of change in demographic ageing (log mortality slope) is accelerated in treated males. Variance in the log mortality intercept is a common feature of both environmental and genetic manipulations of ageing that is observed in invertebrate model systems as well as in human demographic samples (Finch 1990; Promislow *et al.* 1999).

Reproductive diapause is regulated by juvenile hormone in part or as a whole in many insects other than monarch butterflies, for example in potato beetles, acridid grasshoppers, blowflies and *Drosophila* (Denlinger 1985; Pener 1997; Tatar & Yin 2001). Extended longevity associated with reproductive diapause is observed in both grasshoppers and *D. melanogaster*. Acridid grasshoppers of arid climates reproductively diapause through the dry season. Allatectomy applied to non-diapause acridid males elicits several diapause traits including accessory gland arrest, behavioural changes and altered integument colour (Pener & Greenfield 1992). Allatectomized males of *Anacridium aegyptium*, *Schistocerca gregaria* and *Locusta migratoria* each increase their adult life expectancy by 100–200% relative to sham or non-operated controls (Pener 1972). *D. melanogaster* may overwinter in adult reproductive diapause in temperate climates, as occurs in the endemic *Drosophila littoralis* (Lumme *et al.* 1974) of

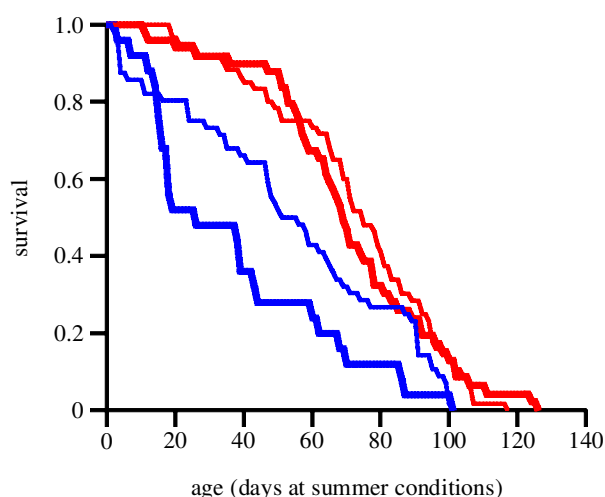


Figure 1. The survival (l_x) of field-caught adults after they were introduced to summer-like laboratory conditions. The median residual life expectancy (\pm s.e.) of migrant adults in 1988 (red lines) was 74.5 ± 1.8 days for males (thin line) ($n_0 = 60$) and 68.8 ± 1.8 days for females (thick line) ($n_0 = 49$). The median residual life expectancy (\pm s.e.) upon transfer to the laboratory of summer adults in 1987 (blue lines) was 55.0 ± 3.7 days for males (thin line) ($n_0 = 56$) and 25.5 ± 2.5 days for females (thick line) ($n_0 = 25$). Mortality differs significantly (log-rank test) among the female cohorts ($\chi^2 = 17.2$ and $p < 0.0001$) and among the male cohorts ($\chi^2 = 31.38$ and $p < 0.0001$).

Finland and *D. triauraria* of Japan (Kimura 1988; Kimura *et al.* 1992). Overwintering adult *Drosophila* must somatically persist much longer than their summer counterparts. *D. melanogaster* age at negligible levels during induced diapause in laboratory conditions (Tatar *et al.* 2001b). The reproductive diapause of *D. melanogaster* can be terminated by exogenous application of the juvenile hormone analogue methoprene: single doses are sufficient for initiating vitellogenesis even while adults are maintained under diapause-inducing conditions (Saunders *et al.* 1990). In addition, juvenile hormone analogue treatment reverses the somatic traits that are characteristic of *D. melanogaster* diapause: it reduces the level of resistance to a free radical generator (methyl viologen) and to heat shock and it promotes accelerated demographic ageing (Tatar *et al.* 2001b).

An extended life span during diapause may result indirectly through the reduced costs of gonadal activity and directly through the induction of specific physiology that is adapted for somatic maintenance. A reduction in reproductive activity increases longevity in many insects, as demonstrated through both environmental and genetic manipulations (Bell & Koufopanou 1986; Partridge & Barton 1993; Tatar 2001). Reproduction indirectly accelerates ageing when nutrients are competitively allocated to egg production at the expense of somatic maintenance (Tatar & Carey 1995). Conversely, reproduction may directly promote ageing if the metabolism of reproductive activity generates free radicals (Sohal & Weindruch 1996) or if somatic stress response systems are suppressed during reproduction because they antagonize reproductive function (Silbermann & Tatar 2000). Thus, stress resistance and a reduced metabolic rate in monarch

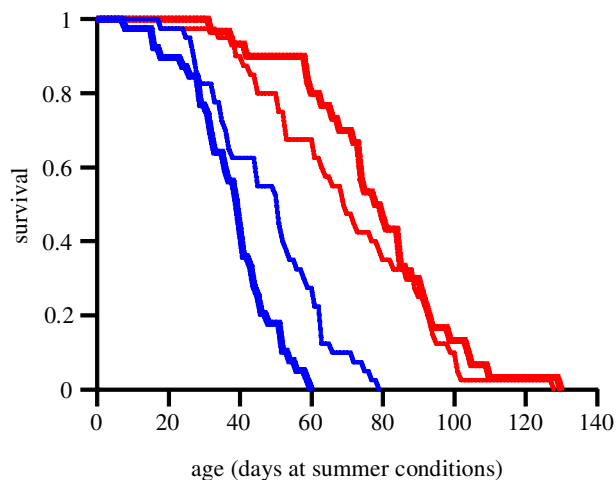


Figure 2. The survival (l_x) of field-caught migrant adults with juvenile hormone I treatment and without treatment (acetone control) after they were introduced to summer-like laboratory conditions. The median residual life expectancy (\pm s.e.) of the juvenile hormone I-treated cohort (blue lines) was 50.3 ± 1.1 days for males (thin line) ($n_0 = 40$) and 39.1 ± 1.0 days for females (thick line) ($n_0 = 39$). The median residual life expectancy (\pm s.e.) in the acetone control cohorts (red lines) was 69.0 ± 1.6 days for males (thin line) ($n_0 = 40$) and 79.0 ± 2.7 days for females (thick line) ($n_0 = 30$). Mortality differs significantly (log-rank test stratified by sex) between the juvenile hormone-treated and control cohorts ($\chi^2 = 75.01$ and $p < 0.0001$).

butterflies may be a mechanism for preserving the soma during adult diapause, in addition to the sparing of resources from reproduction. The balance of physiological activity devoted towards reproduction versus somatic maintenance for each of these mechanistic constraints is likely to involve endocrine regulation; multiple tissues and cell types must be integrated so that the adult life history can be appropriately tuned to environmental conditions. In this way, juvenile hormone, as suggested by Dingle & Winchell (1997), may provide a key regulatory endocrine for moderating the plasticity of life history. Slow senescence in monarch butterflies may be coupled with traits of non-reproduction through the downregulation of juvenile hormone. The ability of migrant adults to survive during the winter period will be due in part to reduced senescence, as well as to the special climatic conditions of their roost sites.

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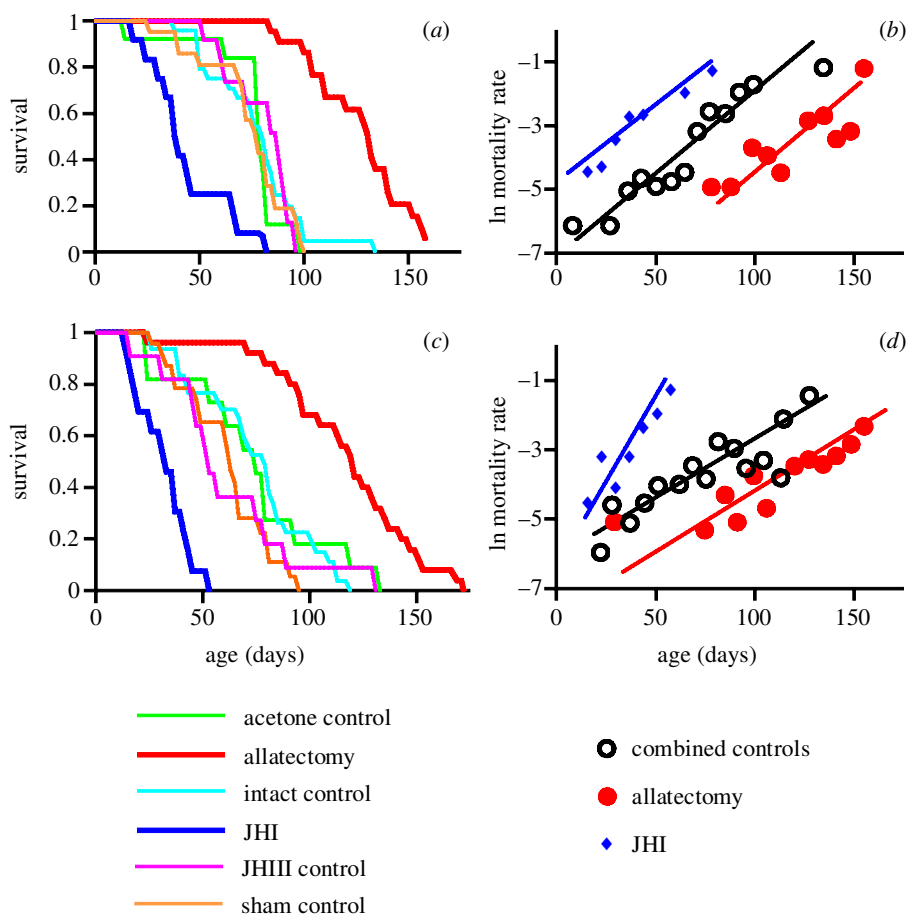


Figure 3. The survival (l_x) and the log mortality rate ($\ln\mu_x$) in the cohorts of laboratory-reared ageing monarch butterflies. (a,b) Females and (c,d) males. Control cohorts are shown individually for survival and with all observations combined for mortality (open circles): intact, 24 females and 30 males; acetone only, 13 females and 11 males; juvenile hormone III, 12 females and 11 males; sham operated, 21 females and 23 males, juvenile hormone I treated (blue line and filled diamonds), 12 females and 13 males and allatectomized (red line and filled circles), 22 females and 25 males. (b,d) The parameters for the plot of the Gompertz mortality model $\mu_x = ae^{bx}$ were estimated by maximum likelihood from the distribution of deaths with the WINMODEST program (Pletcher 1999). WINMODEST was used for evaluating differences in the Gompertz parameter estimates a and b of the treatment cohorts relative to the combined control cohort (likelihood ratio test). The intercepts among females (a) vary among all comparisons (smallest $\chi^2 = 4.58$ and $p < 0.05$). Among males, the slope b of juvenile hormone I is significantly greater than the control ($\chi^2 = 7.04$ and $p < 0.01$), but the intercepts do not differ ($\chi^2 = 0.0075$); the allatectomies and controls differ only in intercept a ($\chi^2 = 6.32$ and $p < 0.01$).

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