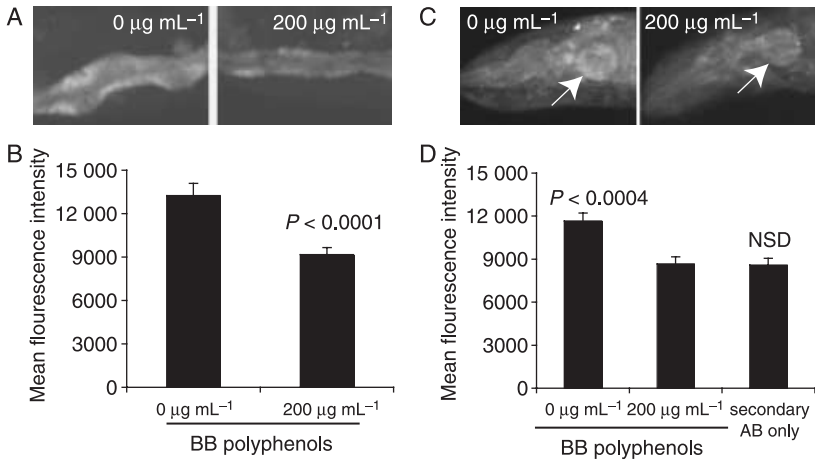

Fig. 2 Markers of aging and oxidative damage are reduced in animals treated with BB polyphenols. (A) Intestinal autofluorescence from lipofuscin in representative day 16 animals with 0 μg mL^{-1} (left) or 200 μg mL^{-1} blueberry polyphenols (right). (B) Mean fluorescence intensity from intestinal lipofuscin in day 16 adults treated with indicated amounts of blueberry polyphenols; 0 μg mL^{-1}, n = 24 animals; 200 μg mL^{-1}, n = 26 animals. (C) Representative images of pharynxes from BB-treated or control animals on adult day 14 immunostained with antisera specific for 4-HNE. Arrows designate terminal bulb. Similar results were obtained for 4-HNE immunofluorescence in the somatic gonad. (D) Mean fluorescence intensity of 4-HNE immunofluorescence in pharynx terminal bulbs from day 14 adults. Third bar shows background fluorescence measured in animals stained with secondary antibody only. *P*-values are *t*-test vs. 200 μg mL^{-1}; NSD, no significant difference; n = 18 animals (0 μg mL^{-1}); 16 animals (200 μg mL^{-1}); n = 4 animals (secondary antibody control).

in BB-treated animals, compared with controls (Fig. 2A,B). 4-Hydroxynonenol (4-HNE) is a lipid peroxidation product that accumulates with aging in many animals (Chiarpotto *et al.*, 1995). In *C. elegans*, a correlation between 4-HNE levels and aging has not yet been demonstrated, although levels of 4-HNE-modified protein have been correlated with lifespan (Ayyadevara *et al.*, 2005). BB treatment also reduced levels of 4-HNE in 14-day adults (Fig. 2C,D).

BB treatment delayed aging-related increase in heat-shock protein mRNA levels

We next examined the effects of BB treatment on a transcriptional marker of aging. Several independent analyses of gene expression during aging in *C. elegans* have revealed that heat-shock protein mRNA levels increase with age (Lund *et al.*, 2002; Golden & Melov, 2004). Using real-time PCR, we confirmed that expression of *hsp-12.6, -16.1, -16.49* and *-70* increased markedly in control animals between days 0 and 4 of adulthood under our laboratory conditions (Fig. 3). BB treatment blocked this increase and mRNA levels for these *hsp*s remained at basal levels through to adult day 4. From this experiment, we conclude that BB polyphenols delayed changes in *hsp* expression that are normally associated with aging in *C. elegans*, consistent with the observation that BB treatment delayed morphological features of aging.

Proanthocyanidin components of blueberries enhance longevity

Blueberries contain a mixture of different polyphenol compounds that can be separated into three primary fractions enriched in either anthocyanins (ATC), proanthocyanidins (PAC)

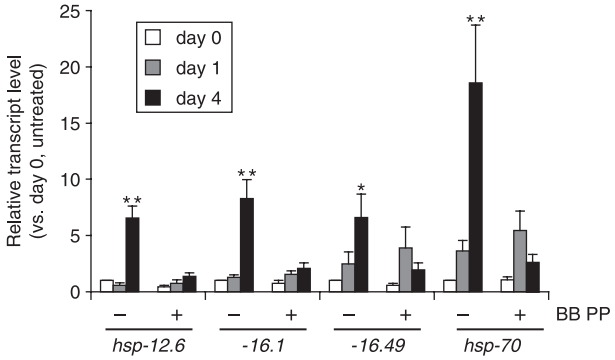

Fig. 3 BB polyphenols reduced aging-related increase of inducible *hsp* transcripts. Expression levels of small heat-shock proteins, relative to actin, were determined by RT-PCR in populations treated with 200 μg/ml of blueberry polyphenol (BB PP) at 25 °C. Graph shows mean of two independent experiments with SEM; $^{**}P < 0.01$ vs. day 0 within treatment; $^{*}P < 0.05$ vs. day 0 within treatment.

or hydroxycinnamic esters, mainly chlorogenic acid (CA). Major components of each of these fractions have been shown to confer significant antioxidant activity and ATC can protect cells against oxidative stress *in vitro* (Youdim *et al.*, 2000; Zheng & Wang, 2003). To determine which fraction(s) delayed aging, we assayed their effects on *C. elegans* lifespan. Neither the ATC-enriched fraction nor purified CA had any significant effect on longevity (Fig. 4A) (control, 12.0 ± 0.34 days; ATC, 11.7 ± 0.36 days, P = 0.96 vs. control; CA, 11.7 ± 0.39 days, P = 0.99). However, treatment with the PAC-enriched fraction increased lifespan to a similar extent as the starting BB polyphenol mixture or the remixed fractions (Fig. 4A,B) (PAC, 14.4 ± 0.36 days, $P < 0.0001$ vs. control; start, 14.8 ± 0.36 days, $P < 0.0001$; remix, 14.0 ± 0.48 days, $P < 0.0001$; complete statistics for lifespan trials with PAC-enriched fraction are presented in Table 2). Thus, components