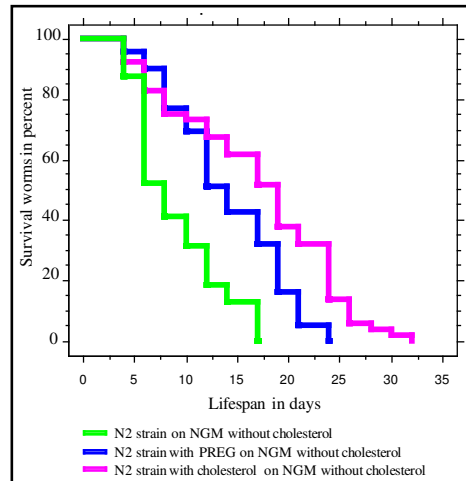


Supplemental material

Figure S1

The effect of PREG on animals grown in a cholesterol-deprived medium



To investigate the possibility that the shortened lifespan of animals cultured in the absence of cholesterol was caused by lack of a steroidal derivative of cholesterol, we cultured the animals in the presence of several steroids on a deprived-cholesterol medium.

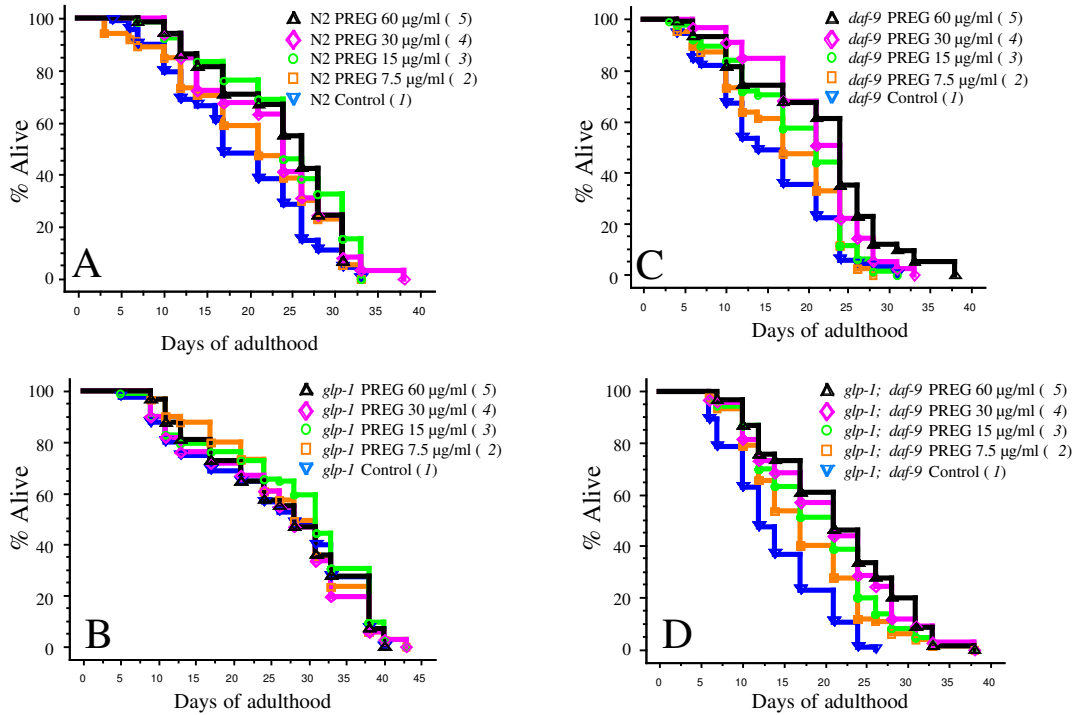
Lifespan studies were at 20°C. Animals hatched from gravid hermaphrodites (grown on NGM medium) shifted to a cholesterol-deprived medium. In this last medium, we have replaced agar (pail agar granulated, Biovalley) by agarose (Medium EEO, Sigma). No bactopectone was added and this medium contains the regular salts MgSO₄ and NaCl. In these conditions, PREG was not detected (corresponding to the GC/MS threshold detection that is <0.1 fg/worm).

Worms from first generation have a short lifespan on a cholesterol-deprived medium: mean lifespan 9 ± 0.5 days (n=64). PREG supplementation at final concentration of 7.5 µg/ml, allowed the worms to live longer than our control: mean lifespan 14 ± 0.7 days (n=63). However, this increase was lower than after cholesterol supplementation at 7.5 µg/ml: 16.9 ± 0.8 days (n=55). (P<0.0001).

This suggests that pregnenolone can provide or compensate for at least some of the longevity benefits conferred by cholesterol, but that either cholesterol itself, or another derivative of cholesterol, produces a longer lifespan independently of pregnenolone.

Figure S2

PREG effects on the lifespans of wild type (N2), *glp-1(e2141ts)*, *daf-9(rh50)*, and *glp-1(e2141ts); daf-9(rh50)* mutants



This experiment is a repetition of that described in Fig. 2 of the text. Supplementations with PREG were performed on standard NGM medium. 100 µl ethanol was added to the medium of control experiments (1). Increasing concentrations of PREG in 100 µl ethanol were added to the medium in experiments 2 to 5. The final concentrations on plates were: 7.5 µg/ml (2), 15 µg/ml (3), 30 µg/ml (4) and 60 µg/ml (5). The four strains were grown under identical conditions. Eggs were hatched at 20°C, shifted to 25°C from L1 stage to L3 stage (25°C is the non-permissive temperature of the *glp-1(e2141ts)* mutation), then animals were switched back at 20°C. The lifespan study was initiated at the young adult stage at 20°C. Every two days the animals were transferred to new plates to keep the amount of PREG ~ constant.

A) N2 strain: lifespan extension was the same (15%-20%) at all concentrations of PREG. Mean lifespans were 18.9 ± 0.9 days for N2 control (1) (n=80); 20.6 ± 1.1 days for N2 with PREG 7.5 μ g/ml (2) (n=68) (p = 0.1151); 24 ± 1 days for N2 with PREG 15 μ g/ml (3) (n=54) (p = 0,0002); 22.8 ± 0.9 days for N2 with PREG 30 μ g/ml (4) (n=63) (p = 0.0058) and 23.5 ± 0.9 days for N2 with PREG 60 μ g/ml (5) (n=70) (p = 0.0004). **B)** *glp-1(e2141ts)* strain: lifespan were the same at all concentrations of PREG. Mean lifespans were 25.9 ± 1.2 days for *glp-1* control (1) (n=79); 27.6 ± 1 days for *glp-1* with PREG 7.5 μ g/ml (2) (n=86) (p = 0.7529); 27.9 ± 1.2 days for *glp-1* with PREG 15 μ g/ml (3) (n=78) (p = 0.2342); 25.9 ± 1.2 days for *glp-1* with PREG 30 μ g/ml (4) (n=79) (p = 0.7813) and 26.6 ± 1 days for *glp-1* with PREG 60 μ g/ml (5) (n=85) (p = 0.6333). **C)** *daf-9(rh50)* strain: lifespan extensions increased with increasing concentration of PREG. Mean lifespans were 15.4 ± 0.8 days for *daf-9* control (1) (n=88); 17.2 ± 0.8 days for *daf-9* with PREG 7.5 μ g/ml (2) (n=81) (p = 0.2031); 18.8 ± 0.8 days for *daf-9* with PREG 15 μ g/ml (3) (n=82) (p = 0.0071); 21.1 ± 0.7 days for *daf-9* with PREG 30 μ g/ml (4) (n=82) (p < 0.0001) and 21.5 ± 0.9 days for *daf-9* with PREG 60 μ g/ml (5) (n=76) (p < 0.0001). **D)** *glp-1(e2141ts); daf-9(rh50)* strain: lifespan extensions increased with increasing concentration of PREG. Mean lifespans were 13.8 ± 0.6 days for *glp-1; daf-9* control (1) (n=87); 17.5 ± 0.8 days for *glp-1; daf-9* with PREG 7.5 μ g/ml (2) (n=85) (p = 0.0002); 19.2 ± 0.8 days for *glp-1; daf-9* with PREG 15 μ g/ml (3) (n=87) (p < 0.0001); 20.4 ± 0.9 days for *glp-1; daf-9* with PREG 30 μ g/ml (4) (n=77) (p < 0.0001) and 21.3 ± 0.9 days for *glp-1; daf-9* with PREG 60 μ g/ml (5) (n=77) (p < 0.0001).

Table S1**Animals lacking the DAF-12 nuclear hormone receptor cannot respond to PREG**

	Strains	Temperature (in celsius)	Mean lifespan (in days)	n	p-value (lifespans compared with N2)
Experiment 1	N2	25	13.2 ± 0.4	70	
	<i>glp-1</i>	25	21 ± 0.9	68	< 0.0001
	<i>glp-1; daf-12</i>	25	12.7 ± 0.4	72	0.026
	<i>glp-1; daf-12</i> + PREG (15µg/ml)	25	12.7 ± 0.5	69	0.013
Experiment 2	N2	25	13.9 ± 0.6	60	
	<i>glp-1</i>	25	17.5 ± 0.7	69	< 0.0001
	<i>glp-1; daf-12</i>	25	14.3 ± 0.7	68	0.061
	<i>glp-1; daf-12</i> + PREG (15µg/ml)	25	15.1 ± 0.8	67	0.018
Experiment 3	N2	20	18.2 ± 0.9	77	
	<i>glp-1</i>	20	26.7 ± 1.1	55	< 0.0001
	<i>glp-1; daf-12</i>	20	15.3 ± 0.7	75	0.001
	<i>glp-1; daf-12</i> + PREG (15µg/ml)	20	15.6 ± 0.7	72	0.002