Understanding cellular cholesterol homeostasis is crucial in fully understanding cholesterol as an established risk factor for heart disease. How cholesterol feeds back to achieve its own homeostasis has been explained by two alternate models. The first and prevailing model is that cholesterol is sensed directly by binding to a specific protein, Scap (SREBP-cleavage activating protein). The alternative view is that sterols induce a change in the physical properties of the membrane bilayer in the environs of the sensing proteins. Since the enantiomer of cholesterol (ent-Chol) is a mirror image of natural or native cholesterol (nat-Chol), with both molecules sharing major physicochemical properties, ent-Chol is a valuable tool in distinguishing between these two models. We compared the effects of ent-Chol with nat-Chol on several measures of cholesterol homeostasis, including activation of the master transcriptional controller of cholesterol metabolism (Sterol Regulatory Element Binding Protein, SREBP-2) by Western blotting of precursor and mature forms of SREBP-2, as well as by measuring SREBP-2 target gene expression. Importantly, we found that at equivalent concentrations within the cell, ent-Chol suppressed activation of SREBP-2 almost as effectively as nat-Chol. These findings indicate that cholesterol exerts at least some of its effects on cholesterol homeostasis via altering membrane properties.