4-hydroxy-2-nonenal (HNE), a toxic lipid peroxidation product, is associated with oxidative damage in cells and involved in various diseases including the initiation and progression of cancer. Cancer cells have a high, adaptable metabolism with a shift from oxidative phosphorylation to glycolysis and rely on high levels of glucose and glutamine as essential nutrients for cell growth. Here we investigated whether the toxic effects of HNE on the mitochondrial membrane potential (MMP) of cancer cells depends on their metabolic state by deprivation of glucose and/or glutamine. The addition of 16 μM HNE to N18TG2 neuroblastoma cells incubated in glucose medium led to a severe reduction of MMP, which was similar to the MMP of cells fed with both glucose and glutamine. In contrast, HNE addition to cells starved in glutamine medium increased their MMP slightly for a prolonged time period and this was accompanied by increased cellular survival. We found that β-oxidation of HNE did not cause the increased MMP, since the aldehyde dehydrogenase was distinctly more active in cells with glucose medium. However, after blocking fatty acid β-oxidation in cells starved in glutamine medium with etomoxir, which inhibits carnitine palmitoyltransferase 1, HNE addition induced a strong reduction of MMP similar to cells in glucose medium. Surprisingly, the effect of more toxic 4-oxo-2-nonenal was less pronounced. Our results suggest that in contrast to cells fed with glucose, glutamine-fed cancer cells are capable of β-oxidizing fatty acids to maintain their MMP to combat the toxic effects of HNE.