Bumblebees provide critical commercial and wild pollination services throughout the world. However, populations of many wild bumblebee species are declining, while commercial mass rearing operations are also reporting reduced survival, fertility and health. In wild populations, the majority of the losses occur during the winter diapause, a sensitive period during life cycle associated with physiological changes in hormones, fats, metabolism and immunity. Similarly, treatment of managed bumblebees with CO2 to bypass diapause results in variable losses and there is evidence for both short- and long-term negative effects on queen performance and survival. The underlying molecular mechanisms associated with diapause or the impacts of CO2 on queens have not been characterized. Here we studied the behavior, physiology and genome-wide gene expression patterns of *Bombus terrestris* and *Bombus impatiens* queens as they transition through emergence, mating, diapause and colony founding, either under artificial diapause or CO2 treatment. Genes involved in core nutrient storage and metabolic pathways, including insulin signaling, seem to play a major role in the process, similar to other non-social insects, despite differences in social organization and patterns of diapause (complete vs. reproductive diapause). However, pathways that have been modified during the evolution of eusociality, such as vitellogenin and juvenile hormone, do not show a typical pattern of expression. We further explore the critical role of the insulin pathway in regulating diapause by RNA interference of FoxO, a downstream molecule in the insulin signaling pathway. CO2 treatment affected the expression levels of several genes and was associated with lower lipid mass in fat body, higher flying activity and mortality. Understanding the physiological and molecular processes underlying diapause and CO2 treatment can lead to recommendation to improve commercial and wild bumblebee management.