B.2 Specific Aims. The term 'epigenetics', literally means 'above the genome', and describes the study of stable modifications of gene expression potential [1]. DNA methylation is one molecular mechanism mediating epigenetic phenomena, and indicates the covalent transfer of a methyl group to the carbon at position 5 of cytosine residues [2], usually within regions of DNA in which cytosine occurs next to a guanine nucleotide (CpG sites). DNA methylation is linked to the regulation of gene expression and transcriptional silencing [3] and unusual patterns of DNA methylation in humans are related to disease, including many types of cancer [4]. Environmental exposures such as alcohol [5], stress [6], diet [6], and aerobic exercise [7] also influence DNA methylation. In particular, alcohol use has been associated with changes in methylation of several candidate genes, including MAOA [8], SNCA [9], DAT [10], ANP [11], NR2B [12], NGC [13], and POMC [14]. Thus, it has been proposed that alcohol use may lead to deleterious changes in methylation and gene expression that could in turn have downstream effects on the development and maintenance of substance use disorders [15]. In fact, the effects of alcohol abuse on DNA methylation may mediate the association between alcohol and cancer, as well as other biological and behavioral sequelae of alcohol abuse such as changes in brain structure and craving. For example, recent work in our lab suggests that methylation in the DRD2 gene is related to loss of control over alcohol consumption and reactivity to alcohol cues (see preliminary studies described below).

Conversely, aerobic exercise may exert protective effects against aberrant DNA methylation. In older adults, exercise attenuated age-dependent methylation changes in the ASC gene [16]. In breast cancer patients, aerobic exercise was associated with decreased methylation of the tumor suppressor gene L3MBTL [17]. Our group demonstrated that healthy adults who increased physical activity over a 12-month exercise program had decreased DNA methylation across a panel of markers associated with breast cancer [17]. More importantly for the present application, our pilot data also demonstrated an association between self-reported exercise during a 12-month exercise intervention and methylation change in the DRD2 CpG site over the 12-months.

Although the potential for aerobic exercise to produce positive outcomes for individuals with alcohol use disorders across a variety of domains has been suggested [18], little is known about the possible protective or reparative epigenetic effects of aerobic exercise in individuals at risk for aberrant DNA methylation changes resulting from long term alcohol abuse. Given that preliminary studies suggest that alcohol is associated with an increase in methylation of the DRD2 site and that exercise is associated with a decrease in methylation of this site, a logical yet untested extension is that exercise may mitigate the effect of alcohol on DRD2 methylation. Despite the advances in epigenetic research discussed above, previous studies of methylation in relation to environmental exposures have been primarily cross-sectional, so long-term effects of alcohol and exercise on methylation are not well understood. The proposed study compares methylation at the DRD2 site among heavy drinking regular exercisers, sedentary heavy drinkers, light drinking regular exercisers and sedentary light drinkers at a baseline and 6-month follow-up session. Baseline fitness will be estimated by conducting a test of maximal oxygen capacity (VO2 max) on all participants. Drinking and aerobic exercise behaviors will be assessed on a monthly basis during the 6-month period. Thus, the proposed study will examine main effects of alcohol consumption and aerobic exercise on DRD2 methylation, and the interaction between exercise and alcohol predicting DRD2 methylation, both cross-sectionally and longitudinally.

Specific aims:

1. Determine whether DNA methylation differences at the DRD2 site exist between heavy and light drinkers both cross-sectionally and longitudinally

   Hypothesis 1: Heavy drinkers will show higher methylation at baseline compared to light drinkers

   Hypothesis 2: Heavy drinkers will show greater increases in methylation from baseline to 6-month follow-up compared to light drinkers

2. Determine whether DNA methylation differences at the DRD2 site exist between exercisers and non-exercisers both cross-sectionally and longitudinally

   Hypothesis 3: Regular exercisers will show lower DRD2 methylation at baseline compared to non-exercisers

   Hypothesis 4: Regular exercisers will show greater decreases in DRD2 methylation from baseline to 6 month follow-up compared to non-exercisers

3. Determine the nature of the interaction between exercise participation and alcohol consumption in predicting DRD2 methylation both cross-sectionally and longitudinally

   Hypothesis 5: At baseline, DRD2 methylation differences in heavy drinking exercisers compared to heavy drinking non-exercisers will be greater than the DRD2 methylation differences in light drinking exercisers compared to light drinking non-exercisers.

   Hypothesis 6: The difference in change in DRD2 methylation from baseline to 6-months in heavy drinking exercisers compared to heavy drinking non-exercisers will be greater than the change in DRD2 methylation from baseline to 6-months in light drinking exercisers compared to light drinking non-exercisers.