Vitamin D is a fat soluble vitamin, essential in multiple physiological functions. Studies suggest that vitamin D deficiency is associated with obesity and vice versa, low vitamin D levels might be an independent predictor of obesity. A randomized, double-blind, placebo-controlled trial was conducted among 125 overweight and obese Caucasian volunteers with vitamin D deficiency or insufficiency. Volunteers were randomly assigned to either oral vitamin D (Dlux 3000) supplement (intervention, n=76) or placebo (control, n=49), on a daily basis for 3 months following a weight loss diet. Fat mass, weight, BMI, RMR and serum 25(OH)D were monitored on baseline and each month. DNA samples were extracted from buccal swabs and genotyped for the rs2228570 (VDR), rs1544410 (VDR), rs731236 (VDR), rs1800544 (ADRA2A), rs1801252 (ADRB1), rs1042713 (ADRB2) and rs4994 (ADRB3) polymorphisms. Significant improvement in vitamin D status and reduction in weight, BMI and fat percentage were observed in the intervention group (p<0.05). In the intervention group, carriers of the rs2228570 T allele showed greater vitamin D level improvement compared with the homozygous C allele (p=0.067). Furthermore, heterozygous (CT) for the rs731236 showed lesser weight loss (p=0.068) and for the rs1042713, a significant lower decline in fat percentage was observed for homozygous AA carriers compared with the heterozygous (p=0.051). In the control group, differences in weight loss (p=0.055) and BMI (p=0.045) were observed between rs1544410 AA and GG homozygous. We finally concluded that Vitamin D oral spray supplementation improved 25(OH)D levels and obesity markers in overweight and obese volunteers with vitamin D deficiency or insufficiency. Genetic polymorphisms seem to influence vitamin D supplementation response and obesity markers.