ORIGINAL RESEARCH

Repurposing Disulfiram as an Anti-Obesity Drug: Treating and Preventing Obesity in High-Fat-Fed Rats

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Background and Objectives: A drug repurposing strategy is an approach for identifying new therapeutic uses for approved or investigational drugs. Thanks to the moderate cost of repurposing a drug compared to bringing new chemical entity to the market, drug repurposing is rapidly gaining ground. The aim of this work is to study the anti-obesity effect of disulfiram (DSF), an irreversible aldehyde dehydrogenase inhibitor approved by the Food and Drug Administration (FDA) to treat chronic alcoholism since 1951.
Methods: Thirty male Albino rats were randomly assigned to six groups. G1, the control group, was given a standard diet. G2, the positive control group, was given a high-fat diet (HFD). G3 was given an HFD, and DSF 50 mg/kg/day was administered orally from day one for six weeks. G4 was given an HFD, and DSF 200 mg/kg/day was administered orally from day one for six weeks. G5 was given an HFD for six weeks; then treatment started with 50 mg/kg/day DSF orally. G6 was given an HFD for six weeks; then treatment started with 200 mg/kg/day DSF orally for three weeks. The body weight, food consumption and blood glucose levels were monitored over the given time interval.
Results: Both doses of DSF significantly limited the body weight gain caused by an HFD for the treated animals. HF-fed rats received 50 and 200 mg/kg/day of DSF had their body weight increased by $51.93 \pm 7.89\%$ and $20.88 \pm 15.05\%$ respectively, whereas the body weight of control animals increased by $93.1 \pm 20.04\%$. DSF also significantly decreased the body weight of obese animals. At 50 and 200 mg/kg/day of DSF, HF-fed rats lost $16.74 \pm 8.61\%$ and $23.9 \pm 3.93\%$ respectively, as their untreated counterparts had their body weight increased by $11.85 \pm 3.79\%$ after three weeks of treatment, thus restoring a body weight matching those who received a standard diet.
Conclusion: FDA-approved disulfiram has a strong anti-obesity effect on HFD-fed rats.
Keywords: obesity, drug repurposing, Aldh1a1, disulfiram

Introduction

The pandemic of obesity is growing at a worrying pace, with an estimated direct and indirect cost of US $150 billion per year in the US alone.[1] Recent studies suggest that one out of five deaths is linked in one way or another to obesity.[1] Since 1975, the obesity prevalence has almost tripled.[2] Obesity is a main risk factor for many serious diseases, such as type II diabetes, fatty liver, cardiovascular diseases and cancer, among others.[3,4] Additionally, obesity has important psychological impacts on patients, resulting in a lower quality of life.[5] Although obesity is recognized by the World Health Organization as one of the major challenges in

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public health in the twenty-first century,[2] there is still no clear-cut solution.[6] To date, the Food and Drug Administration (FDA) has approved only five drugs or drug combinations for long-term obesity treatment: orlistat, lorcaserin, liraglutide, bupropion-naltrexone and phentermine-topiramate.[7] Four other drugs are FDA-approved for short-term use: benzphetamine, phentermine, phendimetrazine and diethylpropion.[7] Very recently, lorcaserin was withdrawn from the US market, as a safety clinical trial showed an increased occurrence of cancer.[8] Meanwhile, the European Medicines Agency has approved only three medicines, orlistat, bupropion-naltrexone and liraglutide, for obesity management in the European Union.[9] Thus, developing new pharmacotherapies to treat obesity is of imminent importance to fulfill a highly unmet medical need.

A drug repurposing strategy, also called repositioning, is an approach for identifying new therapeutic uses for approved or investigational drugs.[10] These new uses are usually outside the original medical indication.[11] Because of the high attrition rate, escalating costs and long time-frames for new drug discovery, drug repurposing is rapidly gaining ground.[10] The average cost of a successful drug repurposing is estimated to be US $300 million, about a tenth of that of bringing a new chemical entity to the market.[12] Since the repurposed drug successfully passed the safety assessment in preclinical studies and clinical trials, the risk of failure is significantly lower.[10] The redirection of sildenafil, which was originally developed as an antihypertensive agent to treat erectile dysfunction, is a great example of successful drug repurposing. The approval of thalidomide in 2006 to treat multiple myeloma is yet another example.[10] Drug repurposing could also be a useful approach during outbreaks of emerging diseases such as the recent attempts to use the FDA-approved human immunodeficiency virus (HIV) type 1 aspartate protease inhibitor, lopinavir,[13] or the combination of the anti-malaria agent hydroxychloroquine with the macrolide antibiotic azithromycin to overcome the current pandemic of coronavirus disease 2019 (COVID-19).[14]

Disulfiram (DSF), Figure 1, is an irreversible aldehyde dehydrogenase inhibitor. Disulfiram blocks the alcohol metabolism at the acetaldehyde stage, leading to the accumulation of the latter in the blood. This in turn produces a highly unpleasant reaction, which includes low blood pressure, tachycardia, facial flushing, vomiting and vertigo when the patient treated with disulfiram ingests even small amounts of alcohol.[15] In 1951, disulfiram was the first drug
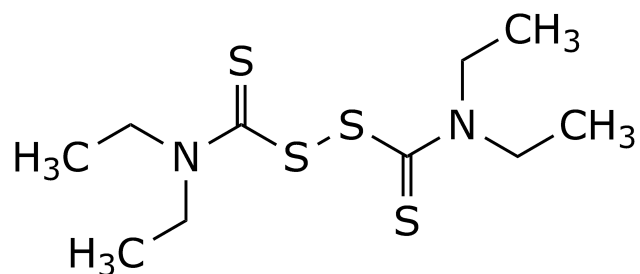
Figure 1 Chemical structure of disulfiram.

to be approved by the FDA to treat chronic alcoholism. Many studies since then have shown the potential of repurposing disulfiram as an anticancer agent.[16] Currently, disulfiram is progressing through different stages of clinical trials to treat diverse types of malignant tumors, such as metastatic breast cancer, glioblastoma and recurrent pancreatic carcinoma. Disulfiram has also shown therapeutic potential as a latency-reversing agent in treating AIDS patients.[17,18] More recently, disulfiram was shown to be beneficial in treating Lyme disease and babesiosis.[19]

In addition to inhibiting Aldh2, the isoform responsible for alcohol metabolism, disulfiram even more potently inhibits Aldh1a1,[20] the cytosolic isoform that irreversibly converts retinaldehyde (Rald) into retinoic acid.[21,22] The expression of Aldh1a1 is significantly higher in obese patients compared to lean subjects.[23] Furthermore, the disruption of expression or function of this enzyme in rats rendered them resistant to diet-induced obesity.[24] Moreover, Aldh1a1 deficient adipocytes were shown not only to effectively reduce body weight of mice fed a high-fat diet (HFD),[25,26] but also they markedly reduced waist circumference, body weight, and fat mass when implanted into visceral fat depots of obese large animal models such as dogs.[27] Additionally, Aldh1a1-deficient mice are viable and do not show any growth or survival defects.[28]

Thus, inhibiting the function of Aldh1a1 is clearly an attractive approach to treating obesity. In this study, we showed that disulfiram can not only effectively reduce the body weight of obese rats but that it also prevents weight gain induced by an HFD in rats.

Materials and Methods
Diet
Standard diet (SD) was purchased from Grains Silos & Flour Mills Organization, SA, Item No. F1005. SD is composed of 20% protein, 4% fat and 50% carbohydrates.

The high-fat diet was prepared weekly in the lab according to the following prescription: 40% cow fat, 10% eggs, 10% sugar and 40% standard diet.

Animal Experiments

Thirty male Albino rats (210 ± 20 g) were purchased from the King Fahad Medical Research Center, Jeddah, KSA. The rats were kept at 25 ± 2 °C in a 12 h light-and-dark cycle condition for five days' acclimation. Then the rats (8 weeks of age) were divided randomly into six groups (G1–G6) of five animals:

G1: Control group: was given a standard diet (SD).

G2 and G5: Positive control group: was given a high-fat diet (HFD).

G3: was given high a fat-diet, and DSF 50 mg/kg/day was administered orally from day one for six weeks.

G4: was given an HFD, and DSF 200 mg/kg/day was administered orally from day one for six weeks.

G6: was given an HFD for six weeks; then treatment started with 50 mg/kg/day DSF orally.

G7: was given a high-fat diet for six weeks; then treatment started with 200 mg/kg/day DSF orally.

All the groups had free access to water and the designated diet.

At the end of week six, HFD-fed rats were redistributed into three new groups, G5, G6 and G7 of five animals each in order to ensure a close body weight average (415 ± 10 g).

All protocols were approved by the Biomedical Ethics Committee of the Faculty of Medicine, King Abdulaziz University. The experiments were carried out according the regulations of Saudi National Committee for Bioethics.

Drug Administration

Disulfiram USP was purchased from Xenex Laborotories Inc, Coquitlam, CA. Catalogue No. DI175.

The DSF dose was calculated according to the rat body weight. DSF was dispersed in vegetable oil (1 mL) and was given daily to the rats by gavage.

Oral Glucose Tolerance Test (OGTT)

OGTT was performed at the end of the study. The rats were made to fast for over 5 h, and a baseline blood draw from the lateral tail vein was collected for plasma fasting glucose. The animals were gavaged with a glucose solution of 2 g/kg body weight, and blood droplets from the tail vein were thereafter collected to measure glycemia at 30, 60, 90 and 120 min.[29,30] Glucose levels were measured by Bayer Contour® TS Blood Glucose Test Strips and Bayer Contour® TS Blood Glucose Monitoring System.

Statistical Analysis

Descriptive statistics were performed for all variables. The data are expressed in mean ± SD. Normality tests were done with a Shapiro–Wilk test for all data to check for normality.

For the primary outcome analysis, ANOVA or the Kruskal Wallis test (depending on the normality) was done to compare between all groups. Post hoc analysis was performed using either a Tukey HSD test if equal variance was assumed or a Games-Howell test if equal variances were not assumed. Paired t-test or Mann Whitney test (depending on the normality) was used to check for the statistical significance difference within every group. A significance level of $P < 0.05$ was used to detect any statistical differences. No sample was excluded from the analysis. Secondary analysis for OGTT was done using both area under the curve (AUC) and ANOVA to test and visualize the difference between different group in response to the oral glucose.

Statistical analysis was carried out using SPSS 24 (IBM SPSS Inc. Chicago, IL, USA). Graphs were created using GraphPad Prism 7 (GraphPad Software Inc., San Diego, CA, USA).

Results
Body Weight

Over the first six weeks of the study, using paired t test we can notice that the body weight of the rats receiving a standard diet (G1) increased by 59.8 ± 7.94% (from 200.0 ± 31.93 g to 315.6 ± 27.15 g) ($P < 0.001$). But the rats on HFD (G2) gained 93.1 ± 20.04% (from 232.6 g ± 22.45 to 448.0 ± 52.03 g) ($P < 0.001$). Meanwhile, the body weight gain of HFD-fed rats receiving 50 mg/kg/day of DSF (G3) was only 51.93 ± 7.89% (from 231.6 ± 19.23 g to 358.8 ± 33.26 g) ($P < 0.001$). On the other hand, a high dose of DSF, 200 mg/kg/day (G4), limited the body weight gain caused by HFD to only 20.88 ± 15.05% (from 230.4 ± 12.14 g to 279.0 ± 41.22 g) ($P = 0.036$), Figure 2.

At week six, rats fed with HFD were redistributed into three groups, G5, G6 and G7, to ensure a close body weight average. By using paired t test we can notice that G5, which continued receiving HFD, had their body weight increased 11.85 ± 3.79% (from 422.5 ± 58.38 g to 473.3 ± 78.25 g) ($P = 0.021$) over a period of three weeks, whereas the body
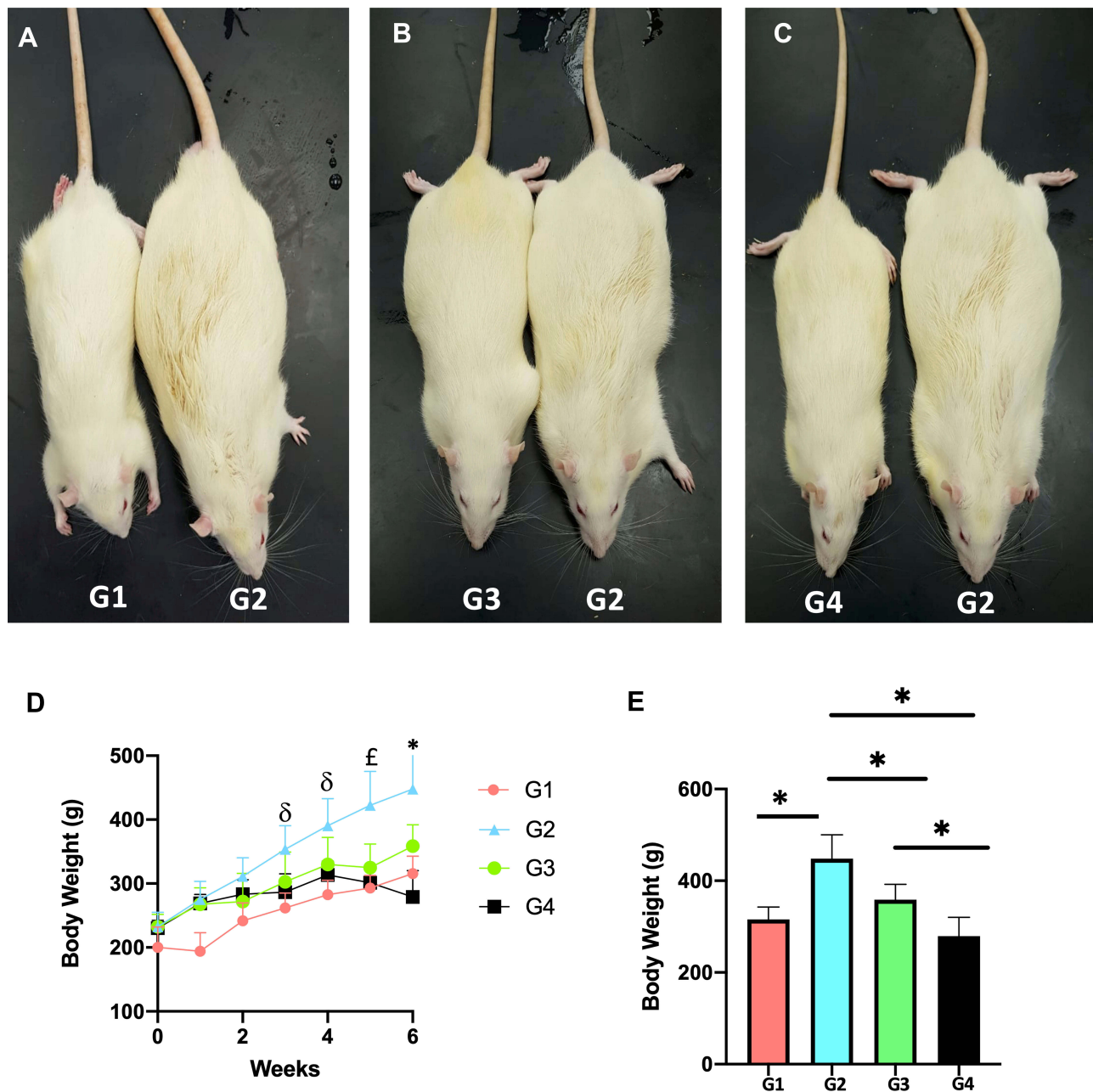
Figure 2 Effect of high-fat diet/disulfiram on rats at week 6. Dorsal view of: (**A**) G1 (SD) vs G2 (HFD), (**B**) G3 (HFD + 50mg DSF started at W0) vs G2 (HFD), (**C**) G4 (HFD + 200mg DSF started at W0) vs G2 (HFD). (**D**) Body weight of G1-G4 over the 6 weeks of the study. (**E**) Body weight of G1-G4 at week 6. Data are presented as mean ± SEM (n = 5 rats per group). ANOVA F=11.62 $P < 0.001$. $^{\delta}P < 0.05$ between (G1 and G2) and (G2 and G4), $^{£}P < 0.05$ between (G1 and G2), (G2 and G3) and (G2 and G4), $^{*}P < 0.05$ between (G1 and G2), (G2 and G3), (G2 and G4) and (G3 and G4).

weight their counterparts receiving standard diet nonsignificantly increased by $2.47 \pm 6.84\%$ (from 315.6 ± 27.15 to 323.4 ± 35.14g) ($P = 0.317$) over the same period of time. Meanwhile, rats fed with HFD and received 50 mg/kg/day of DSF, G6, lost $16.74 \pm 8.61\%$ (from 414.2 ± 68.54 g to 351.3 ± 92.60 g) ($P = 0.009$) from their weight during the same time interval. Moreover, 200 mg/kg/day of DSF caused a body weight reduction of $23.9 \pm 3.93\%$ (from

417.4 ± 21.65 g to 321.8 ± 8.18 g) ($P = 0.003$) in G7 over the same period, Figure 3.

Comparison Between Groups at Week Six and Week 9

There was a statistically significant difference between the four groups after six weeks of intervention using the one-way ANOVA (F (3,18) = 11.620, P < 0.001). Levene's test was
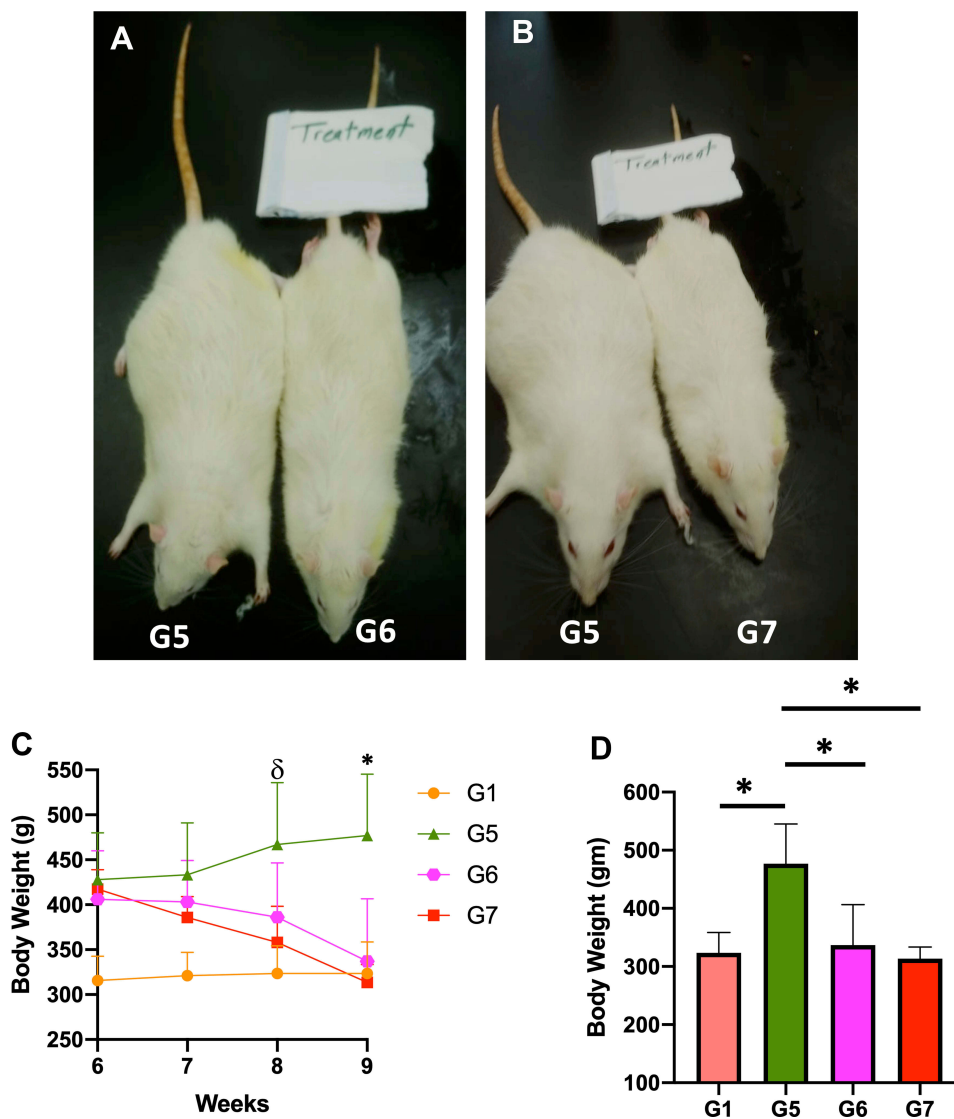
Figure 3 Effect of disulfiram on rats at week 9. Dorsal view of: (**A**) G5 (HFD) vs G6 (HFD + 50mg DSF started at W6), (**B**) G5 (HFD) vs G7 (HFD + 200mg DSF started at W6). (**C**) Body weight of G1, G5-G7 over the 3 weeks of the study. (**D**) Body weight of G1, G5-G at week 9. Data are presented as mean ± SEM (n = 5 rats per group). ANOVA F=7.779, P= 0.02. $^{\delta}P < 0.05$ between (G1 and G5) and (G5 and G7), *P < 0.05 between (G1 and G5), (G5 and G6), and (G5 and G7).

not significant (P=0.146). For post hoc analysis, the results of the Tukey HSD test for multiple comparison showed a statistically significance difference between G1 and G2 (P = 0.001). Additionally, there was no statistically significant difference neither between G1 and G3 (P = 0.402), nor between G1 and G4 (P = 0.490). However, there was a statistically significant difference between G3 and G4 (P = 0.042), Figure 2E.

After redistribution of the HFD groups at week six, there was a statistically significant difference between the four groups after three weeks of intervention using the one-way ANOVA (F (3,18) = 7.779, P = 0.02). Levene's test was not significant (P = 0.095). For post hoc analysis, the results of the Tukey HSD test for multiple

comparison showed a statistically significance difference between G1 and G5 (P = 0.005) but neither between G1 and G6 (P = 0.814), nor between G1 with G7 (P = 0.987). On the other hand, there was a statistically significant difference between G5 and G6 (P = 0.025), and between G5 and G7 (P = 0.003). Furthermore, there was no statistically significant difference between the 50 mg G5 and G7 (P = 0.627), Figure 3D.

Food and Water Intake

Food and water intakes were calculated for three separate days in the middle of the study. DSF administration had no statistically significant effect on the food intake of the treated animals at either dose. However, DSF significantly

Table 1 Food and Water Consumption

	Groups Mean Values						ANOVA
	G1	G2	G3	G4	F value	P value	Multiple Comparison
Food g/100g BW/Day	8.49 ± 0.373	5.75 ± 0.522	6.08 ± 0.836	4.11± 0.389	F (3, 8) = 10.31	0.004	G1, G3; P= 0.04 G1, G4; P= 0.003
Water mL/100g BW/Day	12.58	5.95	8.20	7.31	F (3, 8) = 41.55	<0.001	G1, G2; P <0.001 G1, G3; P <0.001 G1, G4; P <0.001 G2, G3; P= 0.03 G2, G4; P= 0.021

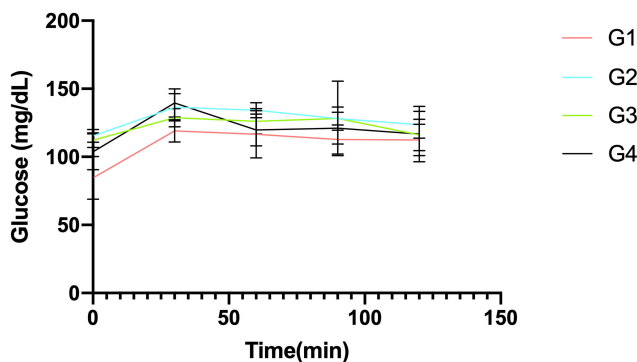**Note**: Data are presented as mean ± SEM (n = 5 rats per group).

increased the water consumption of treated rats compared to their counterparts receiving HFD only, Table 1.

Glycemic Response

OGTT was performed at week six to evaluate the effect of DSF on the glycemic response in HFD-fed rats, Figure 4. The total AUC of the glycemic response was calculated for groups G1–G4. The DSF decreased the AUC at both doses. However, this reduction was not statistically significant, Table 2.

Discussion

Retinoids and the enzymes controlling their metabolism form a complex system that controls important biological processes, such as fuel metabolism and adipogenesis.[23,24,31] Aldh1a1$^{-/-}$ mice are known to be resistant to diet-induced obesity, and they show better insulin sensitivity than their wildtype counterparts.[23,31] Disulfiram is a known inhibitor of aldehyde dehydrogenases.[15] It is an FDA-approved drug for treating chronic alcoholism. Disulfiram is considered a safe, well tolerated drug.[32] A single oral dose of 6 g or daily doses of up to 0.75 g administered for months are generally

Table 2 Glycemic Response. AUC for Oral Glucose Tolerance Test

Groups	G1	G2	G3	G4
Total Area	13,407	15,543	14,913	14,721
Std. Error	549.2	344.9	658.0	552.2
95% Confidence Interval	12,331 to 14,483	14,867 to 16,219	13,623 to 16,203	13,639 to 15,803

tolerated by human adults without symptoms.[33,34] Disulfiram is also characterized by a high oral LD$_{50}$ dose of up to 8.6 g/kg in rats.[35] Indeed, disulfiram is a potent inhibitor of both isoforms of aldehyde dehydrogenase Ald1a1 and Aldh2. But since Aldh1a1 has bigger substrate entrance tunnel than Aldh2, disulfiram, a rather large hydrophobic compound, has a higher affinity to Aldh1a1.[20] In this work, we investigated the potential use of disulfiram as a treatment for obesity.

Our results showed that despite the fact that DSF had no significant effect on food intake, it considerably limited the body weight gain of treated animals caused by an HFD. When administered at a moderate dose of 50 mg/kg/day to rats along with an HFD, DSF completely cancelled the obesogenic effect of the diet after six weeks of treatment. HFD-fed rats treated at higher doses of DSF, 200 mg/kg/day, showed a strong resistance to body weight increase. They gained less weight compared not only to their counterparts receiving HFD but also to those on a standard diet.

DSF also considerably decreased the body weight of obese animals at both doses of 50 and 200 mg/kg/day. After three weeks of DSF treatment, obese rats lost up to 24% of their weight, thus restoring a body weight matching that of those who received a standard diet.

Figure 4 Glycaemic response during oral glucose tolerance test. Data are presented as mean ± SEM (n = 5 rats per group).

All in all, disulfiram, an FDA-approved drug, showed promising anti-obesity activity on high-fat diet-fed rats at both tried doses. These findings suggest disulfiram as a potential treatment for obesity. Future studies should focus on the molecular mechanisms of disulfiram. Clinical trials on healthy obese adults could also be envisaged.

Acknowledgment

Disclosure

The authors report no conflicts of interest in this work.

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