Undergraduate Students, Population, Health & Wellness

Hepatic Gene Expression Profile of Lipid Metabolism of Obese Mice After Treatment with Anti-Obesity Drugs

Maha Saeed Al-Qeraiwi[1], Manar Ahmed Al-Rashid[1]

Supervised by: Dr.Nasser Rizk, Miss. Amina Fadel, Dr. Abdelrahman Elgamal, and Miss. Dina Elsayegh

[1]Biomedical Science Department, College of Health Sciences, Qatar University, Doha, Qatar

[2]Biomedical Research center (BRC)

ABSTRACT

Obesity is a global disorder with multifactorial causes. The liver plays a vital role in fat metabolism. Disorder of hepatic fat metabolism is associated with obesity and causes fatty liver. High fat diet intake (HFD) to mice causes the development of diet-induced obesity (DIO). The study aimed to detect the effects of anti-obesity drugs (sulforaphane; SFN and leptin) on hepatic gene expression of fat metabolism in mice that were fed HFD during an early time of DIO.

Twenty wild types (WT) CD1 male mice aged ten weeks were fed a high fat diet. The mice were treated with vehicle; Veh (control group), and SFN, then each group is treated with leptin or saline. Four groups of treatment were: control group (vehicle + saline), Group 2 (vehicle + leptin), group 3 (SFN + saline), and group 4 (SFN + leptin). Body weight and food intake were monitored during the treatment period. Following the treatments of leptin 24 hour, fasting blood samples and liver tissue was collected, and Total RNA was extracted then used to assess the gene expression of 84 genes involved in hepatic fat metabolism using RT-PCR profiler array technique.

leptin treatment upregulated fatty acid beta-oxidation (Acsbg2, Acsm4) and fatty acyl-CoA biosynthesis (Acot6, Acsl6), and down-regulated is fatty acid transport (Slc27a2). SFN upregulated acyl-CoA hydrolase (Acot3) and long chain fatty acid activation for lipids synthesis and beta oxidation (Acsl1). leptin + SFN upregulated fatty acid beta oxidation (Acad11, Acam) and acyl-CoA hydrolase (Acot3, Acot7), and downregulated fatty acid elongation (Acot2). As a result, treatment of both SFN and leptin has more profound effects on ameliorating pathways involved in hepatic lipogenesis and TG accumulation and lipid profile of TG and TC than other types of intervention. We conclude that early intervention of obesity pa could ameliorate the metabolic changes of fat metabolism in liver as observed in WT mice on HFD in response to anti-obesity treatment.

INTRODUCTION

- Obesity is increasing worldwide.
- The prevalence of obesity in Qatar reached 41% in the last two decades.
- Obesity is a multifactorial disease, that affects many organs, primarily Liver.
- Obesity increases the risk of DM2, HTN, CVD, and fatty liver.
- Liver is the primary regulator of fat metabolism
- DIO mice models are used due to their high similarities to humans' obesity.

METHODOLOGY

The study protocol is shown in (**chart 1**) After two weeks on HFD, mice were acclimated for four days with daily ip saline injections. Following acclimation, mice were divided into two groups and received either ip vehicle (a cocktail of 30% Dimethyl sulfoxide (DMSO), 40% polyethylene glycol (PEG), and 30% phosphate buffered saline (PBS), n=10 mice) or SFN (5 mg/kg, n=10 mice) for six consecutive days. On the 7th day, 30 mins after vehicle/SFN injections, mice in either group received either saline or leptin (ip, 5 mg/kg BW).

At the termination of the experiment, fasting mice were decapitated post 24 hours of leptin treatment liver was collected and stored at a temperature of – 80 °C for further studies including gene expression for functional genomics.

RNA was extracted based on the procedure provided by the manufacturer with the kit (The RNeasy MinElute cleanup kit By QIAGEN. RT²-PCR is used for the gene expression profiling to analyze candidate gene panels and their metabolic pathways by using real time PCR. IPA software was used for pathway analysis.

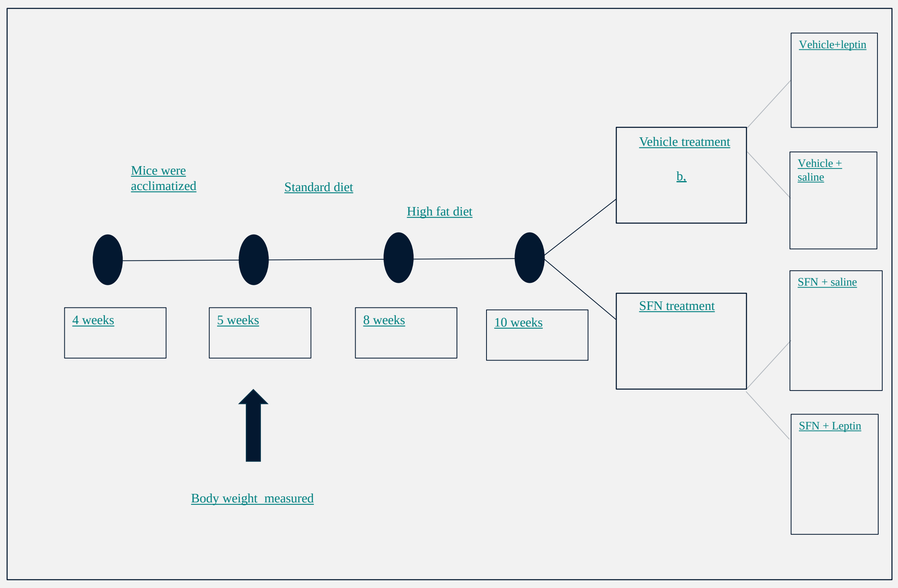

Chart 1: a timeline representation of the preparation and treatments that were given to CD1mice

RESULTS

Table 1: Body weight, and biochemical tests results of the four groups of mice (Vehicle, Leptin, SFN, Leptin + SFN)

	Vehicle (group A)		SFN (group B)		P-value (Anova)
Leptin	Vehicle (n = 5) (Control) (group A1)	Leptin (n = 5) (group A2)	SFN (n = 5) (group B1)	Leptin + SFN (n = 5) (group B2)	
Age (weeks):	10	10	10	10	
Body Weight (mg):	33.43 ± 1.21	34.11 ± 2.22	32.88 ± 3.17	34.27 ± 4.18	0.785
Leptin (ng/µL):	1.316 ± 0.2433	20.28 ± 4.72*	1.21 ± 0.13¥ ∞	13 ± 3.66 **	<0.0001
Insulin (ng/ml):	3.16 ± 2.6	6.83 ± 8.40	2.04 ± 0.71	1.55 ± 0.68	0.329
Glucose (mg/dl):	157.44 ± 6.06	154.17 ± 7.42	164.33 ± 10.50	158.50 ± 12.87	0.548
Triglycerides (mg/dl):	194.50 ± 16.61	182.90 ± 12.26	159.70 ± 11.96	133.50 ±51.83	0.0366
Total Cholesterol (mg/dl):	192.90 ± 41.02	154.60 ± 7.776	158.50 ± 13.9	136.50 ± 14.71*	0.0145
ALT (U/L):	24.23 ± 8.614	21.42 ± 6.199	24.14 ± 7.203	16.44 ± 3.167	0.2447

The data are presented as the mean and standard deviation for all results. * p and ** P values are compared to control, ¥ p value compared to group A2, and ∞ P value compared to B2. Two tailed P-value <0.05 is considered significant.

FIGURES:

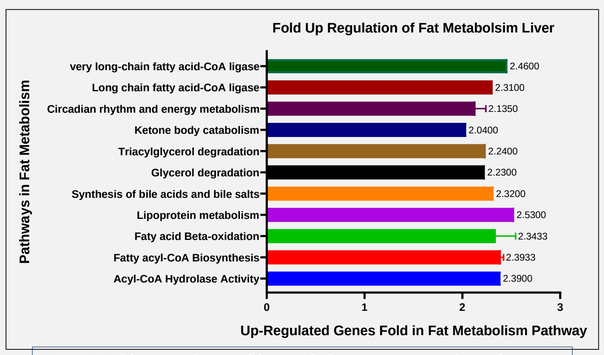

Figure1: Fold up-regulation of fat metabolism in Leptin vs Control group

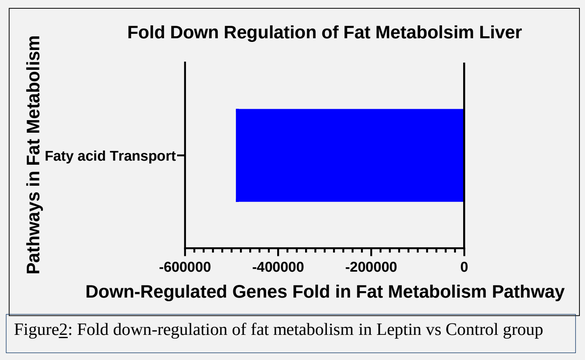

Figure2: Fold down-regulation of fat metabolism in Leptin vs Control group

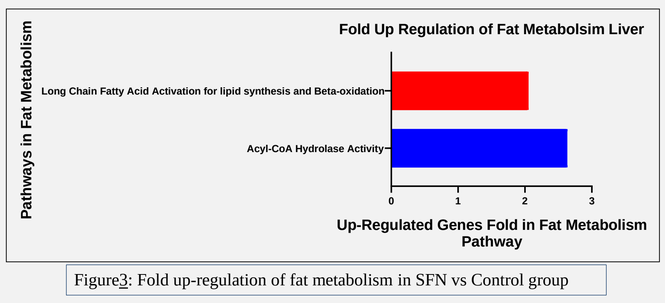

Figure3: Fold up-regulation of fat metabolism in SFN vs Control group

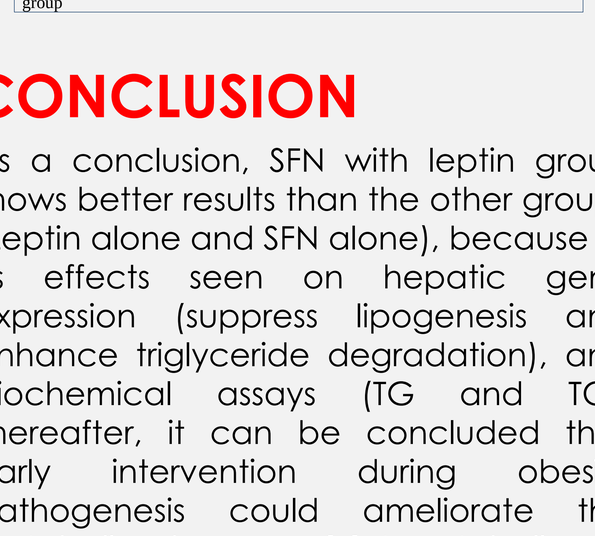

Figure4: Fold up-regulation of fat metabolism in Leptin + SFN vs Control group

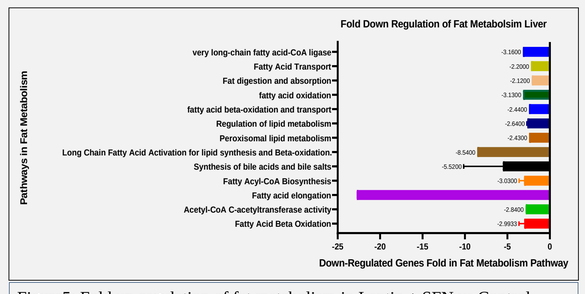

Figure5: Fold up-regulation of fat metabolism in Leptin + SFN vs Control group

CONCLUSION

As a conclusion, SFN with leptin group shows better results than the other groups (Leptin alone and SFN alone), because of its effects seen on hepatic gene expression (suppress lipogenesis and enhance triglyceride degradation), and biochemical assays (TG and TC). Thereafter, it can be concluded that early intervention during obesity pathogenesis could ameliorate the metabolic changes of fat metabolism in liver as observed in WT mice on HFD in response to anti-obesity treatment.

REFERENCES

- Trends in adult body-mass index in 200 countries from 1975 to 2014: a pooled analysis of 1698 population-based measurement studies with 19.2 million participants. (2016). *Lancet, 387*(10026), 1377-1396. doi:10.1016/s0140-6736(16)30054-x
- WHO. (2017). Qatar Stepmise report 2012: chronic disease risk factor surveillance. Doha: Supreme Council of Health; 2015 (http://www.sch.gov.qa/publications/publications, accessed 24 December 2015)
- Gao, M., Ma, Y., & Liu, D. (2015). High-fat diet-induced adiposity, adipose inflammation, hepatic steatosis and hyperinsulinemia in outbred CD-1 mice. *PLoS One, 10*(3), e0119784-e0119784. doi:10.1371/journal.pone.0119784
- Guo, Y., Darshi, M., Ma, Y., Perkins, G. A., Shen, Z., Haushalter, K. J., . . . Taylor, S. S. (2013). Quantitative proteomic and functional analysis of liver mitochondria from high fat diet (HFD) diabetic mice. *Mol Cell Proteomics, 12*(12), 3744-3758. doi:10.1074/mcp.M113.027441

ACKNOWLEGEMENTS

Special thanks goes to Qatar foundation (Research grant: NPRP 9-03-075), and College of Health Sciences-Qatar University (Research grant: QUST-1-CHS-2019-3) for their funds, thanks to the institutional Animal Care & Use Committee (QU-IACUC) for their ethical approval, and thanks to the laboratory animal research center (LARC) for providing the mice.

Thanks to Dr. Nasser Rizk for his supervision, help, and endless support. Thanks to those who are behind the success of this research: Dr. abdelrahman elgamal, Miss. Dina, Miss. Amina Saleh, Miss. Amina Aldous, Miss. Ovelia, and Miss. Aisha.

Lastly we would like to thank the biomedical research center (BRC), and the biomedical department labs in the science building for allowing us to access their labs to work on our research.