Undergraduate students- Population, Health and wellness

The expression of bile acid Receptor TGR5 in Adipose Tissue in Diet-induced obesity mice

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ABSTRACT

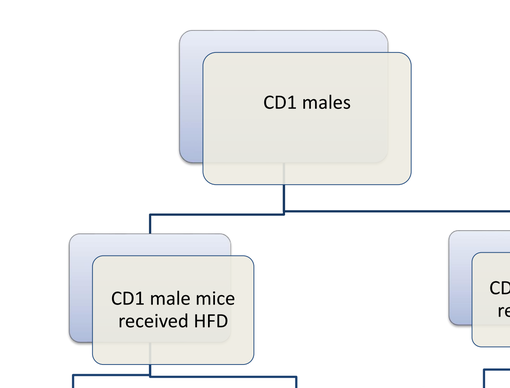
Bile acids are significant physiological factors for digestion, solubilization, absorption, toxic metabolites and xenobiotics. In addition, bile acids are responsible of signal transduction as well as metabolic regulation that activate several receptors such as farnesoid X receptor (FXR) and the membrane G-protein receptor 5 (TGR5). Activation of TGR5 by bile acids is associated with prevention of obesity as well as ameliorating the resistance to insulin via increasing energy expenditure. The objective of this research is to investigate TGR5 gene expression level in different fat depots including visceral or epididymal adipose tissue (eWAT), brown adipose tissue and inguinal adipose tissue (iWAT) and to study the response of TGR5 gene expression to the anti-obesity treatment (SFN). Three groups of male CD1 mice were used in this study; lean group fed with SCD, DIO mice on HFD and DIO obese mice treated with anti-obesity treatment. Body weight (BW) and phenotype data were evaluated by weekly including blood samples for analysis of glucose, insulin, leptin, triglycerides (TG). Total RNA was extracted from different fat depots and RT-PCR profiler array technology was used to in order to assess the mRNA expression of TGR5 and leptin. There was significant downregulation of TGR5 gene expression level in obese (DIO) mice and remarkable upregulation of TGR5 gene expression after successful weight loss in DIO mice treated with SFN in time dependent manner at 1 weeks and 4 weeks of ip applications. In conclusion, obesity is associated with decrease in expression of TGR5 in different fat depots and treatment with anti-obesity drug (Sulforaphane) causes stepwise upregulation of TGR5 gene expression in epididymal white adipose tissue parallel stepwise decrease in body weight. Increase of expression of TGR5 in DIO mice in eWAT is accompanied by improvement in glucose homeostasis and insulin action.

INTRODUCTION

Obesity arise when energy intake exceeds energy output causing an imbalance of energy metabolism. It is now known that three types of adipose tissue are known; white adipose tissue, brown adipose tissue and beige adipose tissue. The functional roles of these types are; WAT stores energy in the form of lipid , and predispose to fat accumulation and consequently obesity, and the BAT increase energy expenditure as heat by thermogenesis via the action of uncoupling protein one (UCP1) as this protein functions to generate heat instead of ATP in mitochondria. The role of browning of white adipose tissue (beige fat) is illustrated by many studies that demonstrated a role in energy metabolism close to the BAT function. Several factors are affecting the BAT and browning of WAT such as aging, sex, genetic, cold exposure, and adrenergic stimulation. Recently some studies have shown that bile acids also activate BAT. Obesity is a chronic disorder that increasing globally, and recent data indicated that 20% of the populations would be obese. The state of Qatar is top nation in Gulf countries with a high prevalence of obesity (39.5% males, 43.2% females) (16). Bile acids (BA) formed by the liver are new class of signaling molecules that could play a significant role in energy metabolism. Maruyama et al. identified a cell membrane G-protein coupled receptor (GPCR) that was acted upon by bile acids. This receptor is commonly termed the membrane-bile acid receptor (M-BAR), or TGR5, rather than the G-protein bile acid- activated receptor (GP-BAR1). It is imperious that GP-BAR1 is the official gene name symbol for Homo sapiens the NIH gene database and by the HUGO Gene Nomenclature Committee. A single exon on chromosome 1C3 in moue and 2q34 in humans encodes TGR5. TGR5 is universally expressed in humans and animals including a comprehensive distribution profile in all tissue with various degree of variations, with high expression in liver, intestine, endocrine glands, brown adipose tissue, brain, spleen.

METHODOLOGY

CD1 male mice (n=20) were fed with a high fat diet (60%) for 12 weeks to generate diet induced obese (DIO) mice with body weights between 45-50 gm. Another group used as lean and fed with standard chow diet for 12 weeks. Thereafter, lean mice received vehicle and DIO mice received either SFN (5mg/kg BW) (n=10) or vehicle (n=10) daily by intraperitoneal injections for four weeks. Three groups of male CD1 mice were employed in this study; lean group , DIO group, and DIO treated the group with anti-obesity treatment (SFN). Body weight (BW) was evaluated daily during the study. Glucose tolerance test (1g/kg BW, IP) was evaluated in the third week of treatment. At the end of 4 weeks of the injection, samples of blood and adipose tissues of different depots were collected. The expression levels of TGR5 and leptin genes were analyzed by qRT-PCR. Blood was also used for glucose, triglycerides, leptin, and insulin measurements.



RESULTS

Body weight changes and biochemical parameters:

Table1: Body weight, and biochemical data of the three groups of studied of CD1 male mice (Lean-Vehicle, Obese-Vehicle, and Obese- treated SFN,)

	Lean (group A) (n=8)	Obese (group B) (n=8)	Obese- treated SFN (group C) (n=8)	P-value (Anova)
Age (weeks):	19	19	19	
Body Weight (mg):				
Before treatment	29.05 ± 1.47		51.45 ± 3.27	<0.0001
After treatment	33.91 ± 2.52	50.77 ± 3.01[&] 52.73± 2.63[&*]	43.52±4.09	<0.0001
Leptin (ng/µL):	1.32 ± 0.24	8.11 ± 2.95*[&]	4.23 ± 2.08	<0.0001
Insulin (ng/ml):	3.16 ± 2.14	5.93 ± 1.71*[&]	4.66 ± 0.28	0.029
Glucose (mg/dl):	127.35 ± 6.12	194.17 ±7.42*&	147.44 ± 6.06	<0.0001
Triglycerides (mg/dl):	164.54 ± 18.72	182.90 ± 12.26	175.47 ± 15.12	0.066

The data are presented as the mean and SD for all results. [&] p value is compared to group A (lean), and the * P value is compared to group C (obese-SFN treated group). Two tailed P-value <0.05 is significant.

FIGURES:

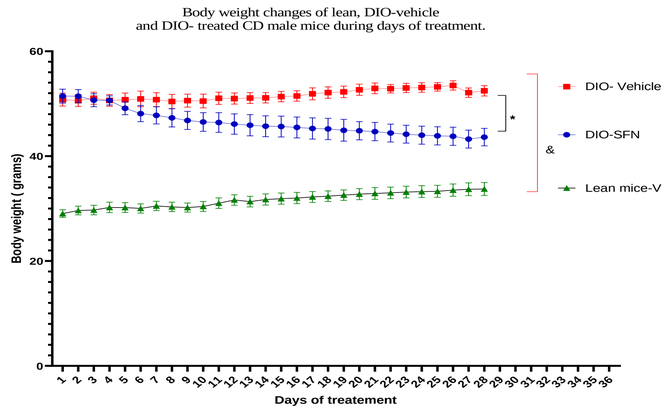


Figure1: Body weight changes of lean, DIO-vehicle, and DIO- treated SFN of CD1 male mice during days of treatment.

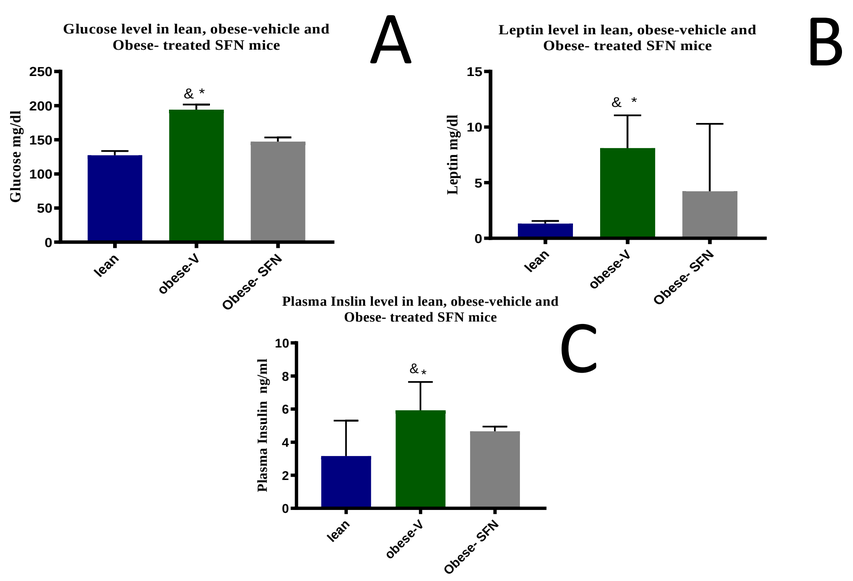


Figure2: A: Bars shows the mean and SD of blood glucose level in lean, obese-vehicle, and obese-treated SFN mice groups. **B: :** Bars shows the mean and SD of leptin level in lean, obese-vehicle, and obese-treated SFN mice groups. **C:** Bars shows the mean and SD of blood insulin level in lean, obese-vehicle, and obese-treated SFN mice groups.

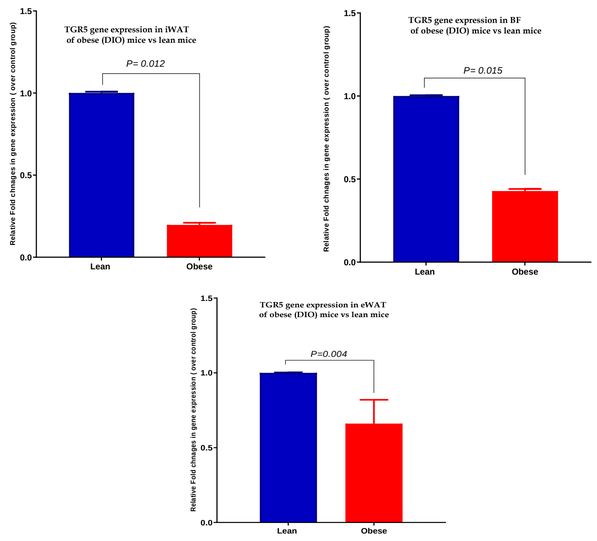


Figure3: Expression of TGR5 in depots of adipose tissues in CD1 male mice.

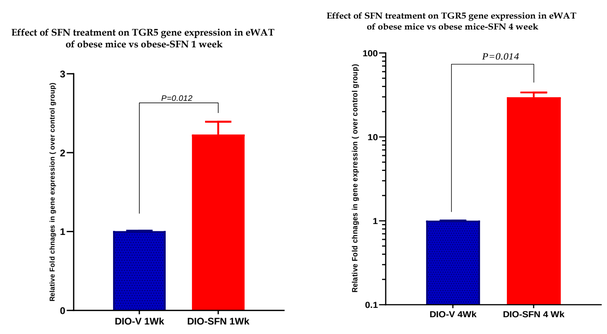


Figure4: Expression of TGR5 in eWAT after SFN treatment for 1 week and 4 weeks in CD1 male mice

CONCLUSION

This study has demonstrated that obesity-induced by HFD in DIO mice is associated with a decrease in expression of TGR5 in epididymal white adipose tissue (visceral), brown fat and beige fat (iWAT). Treatment with anti-obesity drug using sulforaphane causes stepwise upregulation of TGR5 gene expression in epididymal white adipose tissue with parallel stepwise decrease in body weight of DIO mice with marked decrease in leptin expression in adipose tissue. Moreover, the increase of expression of TGR5 in DIO mice in eWAT is accompanied by improvement in glucose homeostasis and insulin action.

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