Research Article

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In-house validation of high fat diet induced obesity models

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ABSTRACT

Background: Obesity results from a prolonged imbalance between energy intake and energy expenditure, as depending on basal metabolic rate, heat production, thermogenic effects of the diet and physical activity. Diet-induced obesity (DIO) in rodents can be achieved by different regimens and approaches. Diets providing a high fat intake have been established as a "gold standard" to generate obese rodent models and have proven to initiate pathologies similar to those encountered in humans. However, this dietary treatment is far from being standardized and its relevance has been criticized on the basis of findings in humans that total energy intake rather than fat *per se* determines body fat accumulation in humans. Hence, varieties of high fat diet regimens have been introduced by providing a choice of several palatable food items of variable composition, appearance and texture in addition to a non-purified diet. Further male rats have been considered in developing all types of obesity models and no rationale has been provided as of this discrimination and negligence of using female rats. Another pitfall in the obesity model development is that of the choice of the strain of the rats used for the study. The basis on which the specific strain is being chosen still remains controversial. This present study aimed at comparing and validating different diet induced obesity models utilizing different high fat diet regimens in different strains and gender. **Methods:** Sprague-Dawley and Wistar male and female rats were offered different high fat diet regimens for 7 weeks to induce obesity. Marketed high fat diet group served as a standard. Evaluation parameters like food intake, calorie intake, water intake, % increase in body weight, plasma total cholesterol, HDL-C, LDL-C, VLDL-C, triglyceride, oral glucose tolerance test and insulin tolerance test were performed to assess the efficacy. **Results:** Diet specific, strain specific and gender specific variation was observed in case of diet induced obesity models. Marketed diet produced better lipid profile and also produced signs of diabetes and thus can be used for obesity-diabetes studies. Vegetable ghee::coconut oil diet produced higher increase in lipid profile in shorter span of dietary manipulation of 4 week and also gave confirmation of equal amount of fat ingested by the entire group. In-house prepared HFD produced better results at the end of 7 week of dietary manipulation. Strain wise Sprague-Dawley rats produced better results and gender wise males showed greater increase in lipid profile than that of females. **Conclusions:** The results of the study provide clear evidence that vegetable ghee::coconut oil (3:2) diet serves as a better model of hyperlipidemia/obesity as it requires shorter time of dietary manipulation and economically also is more feasible. Marketed diet produced better results but takes longer time to develop and also causes glucose intolerance and insulin resistance thus can serve as a model of obesity and diabetes. Strain wise and gender wise Sprague-Dawley rats and males respectively served as a better model for obesity. The reason for this can be attributed to the metabolic activity as concluded from the calorie intake values that persists in Sprague-Dawley rats and that in males. Further study is required to derive proper conclusions.

KEYWORDS: High fat diet, calorie intake, validation of diet, type of fat, gender, strain

INTRODUCTION

Genetic and environmental factors play a role in the development of obesity, and diet is one of the main environmental factors that contribute to this disease. Human studies have shown that increased fat intake is associated with body weight gain which can lead to obesity and other related metabolic diseases. Contributing factors to recently prevalent health problems include hypercholesterolemia, hypertriglyceridemia and obesity, all physiological alterations that can be due to changes in diet.

To understand the genetic and environmental basis of obesity, animal models have proven useful by allowing manipulations technically or ethically not feasible in humans. [1]

These models of obesity have allowed insights into some critical pathways but their overall relevance is nonetheless questionable since common obesity cannot be attributed to a single gene or single pathway. Thus, the polygenic nature of obesity calls for a more realistic approach to generate animal models of obesity.

Animal rodent models are therefore useful tools for studying obesity as they will readily gain weight when fed high-fat diets. [2, 3]

Dietary manipulations in rodent species are effective models to explore and mimic the nutritional activities in humans as well as interventions for disease states.

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The percentage of components within each diet, duration of treatment, model species, gender and age are necessary to consider when performing an *in vivo* study.

Purified high fat diets used to induce obesity and obesity-related complications such as diabetes and metabolic syndrome typically have from 40-60% of energy derived from fat.

As a reference point, the normal pellet diets have in the range of 4-7% fat by weight, or about 10-18% of energy from fat. Adding fat (at the expense of carbohydrate) is done to increase the kcal density. Diets with higher fat levels will have a greater kcal density, since fat contains 9 kcal/g vs. 4 kcal/g for carbohydrate or protein. In the formulation of a high fat diet, it is common to adjust other nutrients (vitamins, minerals, protein) relative to energy content. A number of different fat sources can be used (solid fats, oils), and diets can be designed to target a specific fat level or fatty acid profile.

A growing body of literature reviews rodents as models of human obesity, though there are many confounding factors including species, strain, gender, age, type of diet, level of fat and duration of diet. In order to rationalize the effect of these variables it was necessary to carry out a validation study regarding the type of the high fat diet used, the metabolic energy supplied by the diet and the mode of diet administration. Further it was essential to consider the time factor and the economic factor while developing a suitable model of obesity as per the need.

In this study we have developed and compared in-house high fat diet induced obesity models and compared them in various strains and gender, using commercially available high fat diet as standard.

MATERIALS AND METHODS

1. ANIMALS AND DIETARY MANIPULATION.

All experiments were performed in compliance with and were approved by the Institutional Animal Ethics Committee and according to CPCSEA guidelines.

1.1 Materials:

Standard marketed high fat diet (24F) was purchased from porvimi nutrilab diets®, banglore. Regular acting insulin was from Wosulin-R®, Wockhardt ltd. Aurangabad. The feed ingredients such as casein, dl-methionine, lard, vanaspati ghee, coconut oil, corn starch, soyabean oil, vitamin and mineral mix were obtained from the commercial sources and were of analytical grade. Normal pellet diet was obtained from amrut pranav agro ltd. Baroda, Gujarat.

1.2 Animals:

Male Sprague-Drawley (SD) and Wistar male rats of body weights ranging from 100-250 g were procured from Torrent Research Centre. The experiment protocol was approved by the Institutional Animal Ethics Committee (Registration number: KBIPER/2012/302) and had the prior approval of the project through the same committee. The animals were housed in standard polypropylene cages (three rats/cage) and maintained under controlled room temperature (22±2 ?C) and humidity (55±5%) with 12:12 h light and dark cycle. All the rats were fed with commercially available balanced rat normal pellet diet (NPD) (Amrut feeds, baroda, gujarat) and water ad libitum, prior to the dietary manipulation. Experimental design and animal handling were according to the guidelines of Institutional Animal Ethics Committee.

Table 1 Study groups and treatment

Groups	Dietary manipulation	No. of Animals
Group-1	In house prepared high fat diet (0 – 7 week) (SD rats)	6
Group-2	In house prepared high fat diet (0 – 7 week) (Wistar rats)	6
Group-3	Vanaspati ghee + coconut oil (3:2) (10 ml/kg) (0 – 7 week) (Wistar rats)	6
Group-4	Marketed standard high fat diet (24F) (0 – 7 week) (Wistar rats)	6
Group-5	Marketed standard high fat diet (24F) (0 – 7 week) (Female rats)	6
Group-6	Vanaspati ghee + coconut oil (3:2) (10 ml/kg) (0 – 7 week) (Female rats)	6

Sprague Dawley and Wistar rats were housed.

Group 1 and 2: - were fed with in-house prepared high fat diet. (Composition of in-house prepared high fat diet given in table no.2). It contains 26.39% of fat supplying 52.56 %kcal from fat and having a metabolic energy of 5261 kcal/kg.

Table 2. Nutritional information of In-house prepared HFD

	gm %	kcal %
Protein	13.745	12.16
Carbohydrate	39.34	34.82
Fat	26.39	52.56
	kcal/gm	5.24
Ingredients	**Weight (gm)**	**kcal**
Normal pellet diet (NPD)	200	724
Casein	135	540
Lard	260	2340
Sucrose	245	980
DL-methionine	3	12
Corn starch	150	600
Vitamin and mineral mix	5	20
Salt	1	0
Soyabean oil	5	45
Total	**1004**	**5261**

Groups 3 and 4: - Edible coconut oil and vanaspati ghee were procured from the market and a mixture of the two was prepared in a ratio of 2: 3 respectively v/v. [4,5,6,7] This high fat diet, at a dose of 10 ml/kg body weight (8.13 kcal/ml), was fed to the animals, per orally, daily in addition to normal pellet diet (NPD) (commercial rat pellets from Pranav Agro Industries Ltd., India).

The standard pellet diet consisted of crude protein (22.06%), crude oil (4.04%), crude fiber (4.0%), ash (10.0%) and sand silica (0.15%) and supplies energy of 3620 kcal/kg. [8]

Group 5 and 6: - Animals were fed with commercially available market preparation of high fat diet containing 24 % fat supplying energy 48%kcal from fat and having a metabolic energy of 4880 kcal/kg. [9]

2. EVALUATION PARAMETERS:

1.Physical parameters

i. Daily food intake
ii. Daily calorie intake
iii. Daily water intake
iv. Weekly body weight

2.Biochemical parameters

i. Oral Glucose Tolerance Test [10]
ii. Insulin Tolerance Test [11]
iii. Plasma Total Cholesterol
iv. Plasma Triglyceride
v. HDL Cholesterol
vi. LDL Cholesterol {Fiedman's formula: TC-(TG/5-HDL)}
vii. VLDL Cholesterol{Fiedman's formula: TG/5}

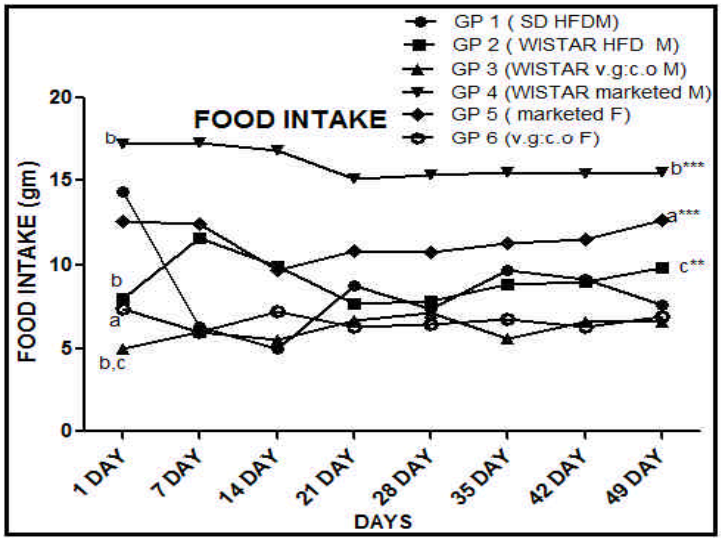
Statistical analysis:

All the data were analyzed using Graphpad version 5.0 and evaluated using one way and two way ANOVA applying tukey's test and bonferroni test respectively.

RESULT AND DISCUSSION

5.1 Food intake

Food intake (gm) was measured on weekly basis for each group and averaged for per animal. GP 5 (marketed female) showed significantly higher food intake than with GP 6 (v.g::c.o female) ($p<0.05$). GP 4 (marketed Wistar male) showed significantly higher food intake than GP 2 (HFD Wistar male), GP 3 (v.g::c.o Wistar male) and GP 5 (marketed female) ($p<0.05$). GP 2 (HFD Wistar male) showed significantly higher food intake than with GP 3 (v.g::c.o Wistar male) ($p<0.05$).

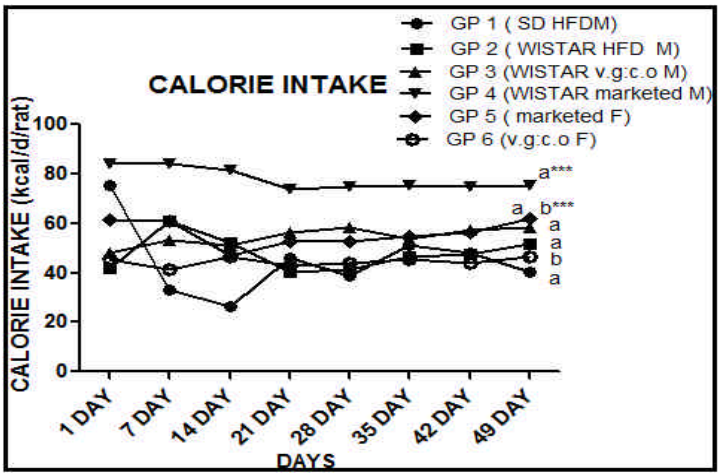


All the values are expressed as Mean±SEM
*a*** = GP 5 showed significant difference with GP 6 ($p<0.05$)*
*b*** = GP 4 showed significant difference with GP 2, GP 3 and GP 5 ($p<0.05$)*
*c** = GP 2 showed significant difference with GP 3 ($p<0.05$)*

Fig 5.1 Effect of various High fat diets on weekly food intake (gm/ d/rat) vs. time (days)

5.2. Calorie intake

Calorie intake (kcal/kg) was measured on weekly basis for each group and averaged per animal. GP 4 (marketed Wistar male) showed significantly higher calorie intake than GP 1 (HFD SD male) 2 (HFD Wistar male), 3 (v.g::c.o Wistar male), 5 (marketed female) ($p<0.05$). GP 5 (marketed female) showed significantly higher calorie intake than GP 6 (v.g::c.o female) ($p<0.05$).

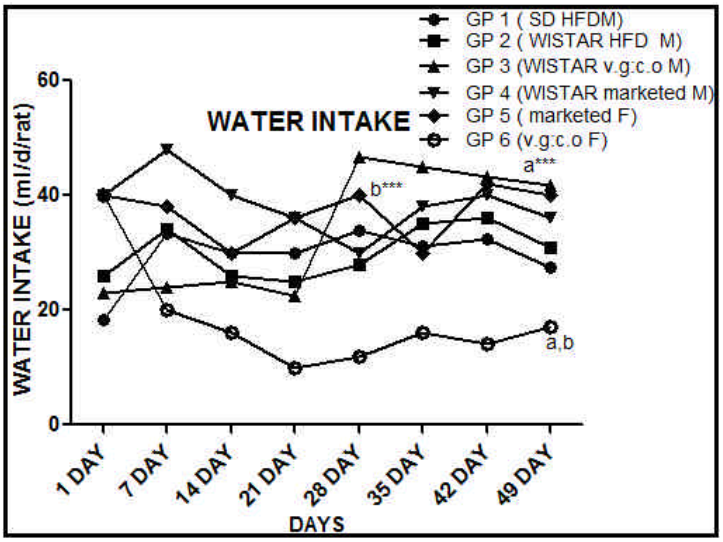


All the values are expressed as Mean±SEM
*a*** = GP 4 showed significant difference with GP 1, 2, 3, 5 ($p<0.05$)*
*b***= GP 5 showed significant difference with GP 6 ($p<0.05$)*

Fig. 5.2 Effect of various HFD on Calorie intake (kcal/d/rat) vs. time (days)

5.3 Water intake

Water intake (ml) was measured on weekly basis for each group and averaged per animal. GP 3(v.g::c.o Wistar male) showed significant difference with GP 6(v.g::c.o female) ($p<0.05$). GP 2(HFD Wistar male) showed significant difference with GP 6(v.g::c.o female) ($p<0.05$).

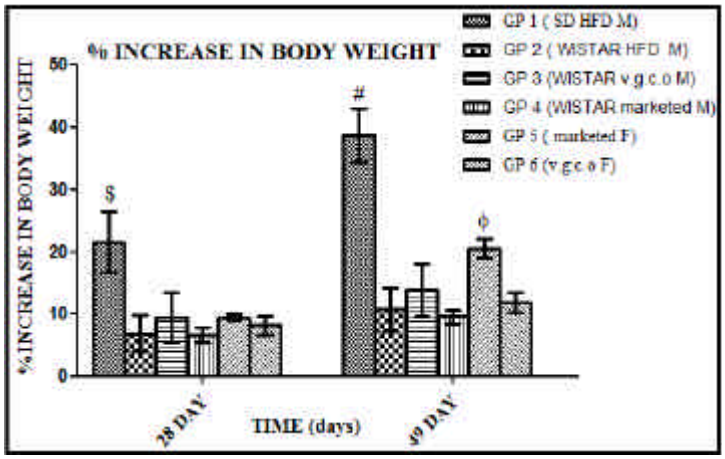


All the values are expressed as Mean±SEM.
*a*** = GP 3 showed significant difference with GP 6 ($p<0.05$)*
*b*** = GP 2 showed significant difference with GP 6 ($p<0.05$)*

Fig. 5.3 Effect of various HFD on water intake (ml/d/rat) vs. time (days)

5.4 Body weight

Body weight of the rats was measured weekly for 7 weeks. % increase in body weight was calculated for each group and represented in terms of mean±SEM. GP 1 (SD HFD M) showed significantly higher weight gain than with GP 2(Wistar HFD M) at the end of 4 ($p<0.01$) and 7 week ($p<0.001$). GP 5(Marketed Female) showed significantly higher weight gain than with GP 4(Wistar marketed M) ($p<0.01$) and GP 6 (v:g::c:o Female) ($p<0.05$)

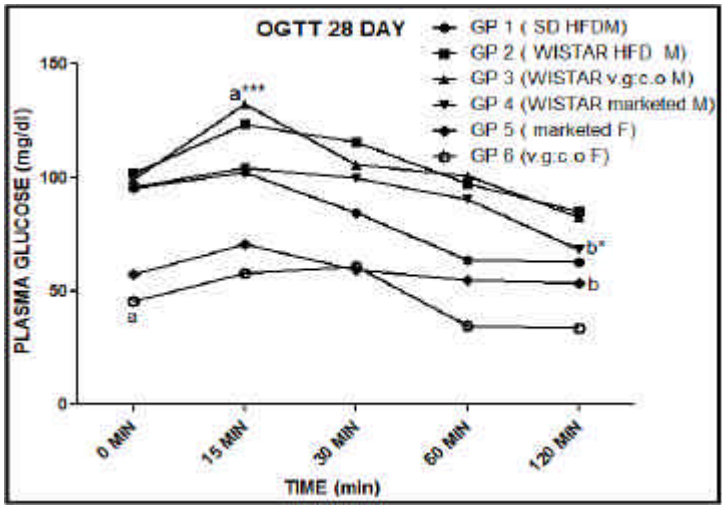


All the values are expressed as Mean±SEM.
$ = GP 1 showed significant difference with GP 2 ($p<0.01$)
 # = GP 1 showed significant difference with GP 2 ($p<0.001$)
? = GP 5 showed significant difference with GP 4 ($p<0.01$) and GP 6 ($p<0.05$)

Fig. 5.4 Effect of various HFD on % increase in body weight vs. time (days)

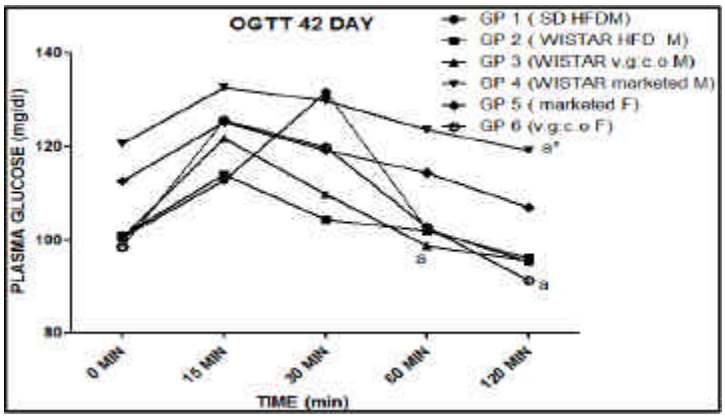
5.5 Oral Glucose Tolerance Test

OGTT was performed at 0 week, 4th week and 6th week. GP 3(v.g::c.o Wistar M) showed significantly higher tolerance than GP 6(v.g::c.o F) ($P<0.05$) at 28 day. GP 4(Wistar marketed M) showed significantly higher tolerance than difference with GP 2(Wistar HFD M) and GP 3(v.g::c.o Wistar M) at 42 day.



a*** = GP 3 showed significant difference with GP 6 ($P<0.05$)
b* = GP 4 showed significant difference with GP 5 ($P<0.05$)

Fig.5.5.1 Effect of various HFD on Oral Glucose Tolerance Test (28 day)



a = GP 4 showed significant difference with GP 2 and GP 3*

Fig.5.5.2 Effect of various HFD on Oral Glucose Tolerance Test (42 day)

5.6 Insulin Tolerance Test

No significant difference ($p>0.05$) was observed between the groups in response to insulin at 15 days of diet modification whereas significant difference ($p<0.05$) was observed between GP 4 (marketed Wistar M) and GP 3(v.g::c.o Wistar M) at 49 days of diet modification.

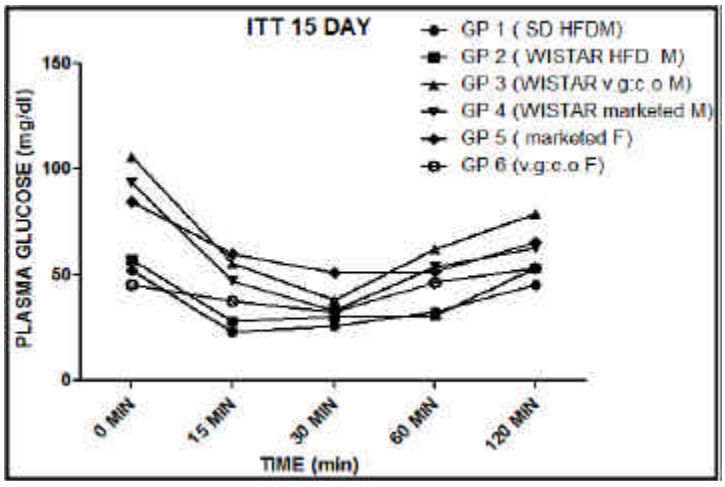
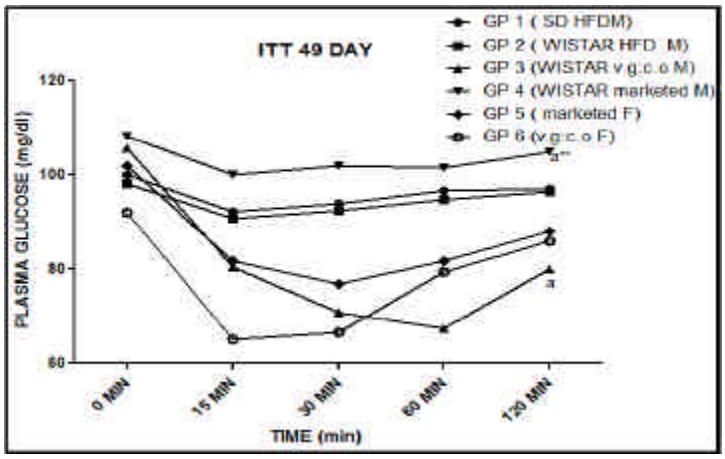


Fig 5.6.1 Effect of various HFD on insulin tolerance test (15 day)

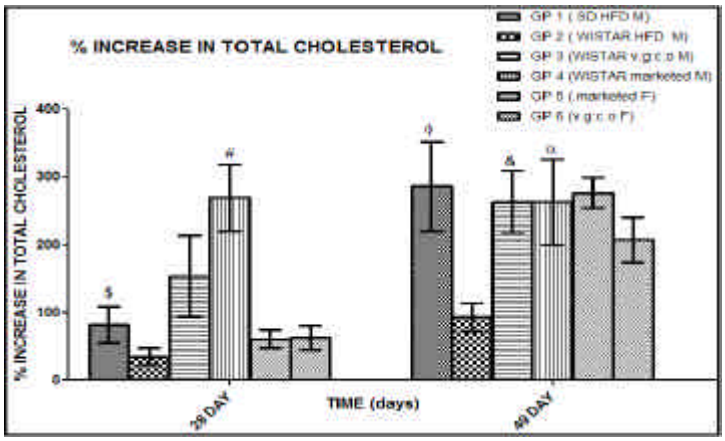


*a** = GP 4 showed significant difference with GP 3 ($P<0.05$)*

Fig 5.6.2 Effect of various HFD on insulin tolerance test (49 day)

5.7 Plasma Total Cholesterol

Plasma TC (mg/dl) was measured at day 0, week 4 and week 7. GP 1 showed significantly higher TC than GP 2 ($p<0.01$). GP 4 showed significant difference with GP 2 ($p<0.01$) and GP 5 ($p<0.001$). GP 3 showed significant difference with GP 2 ($p<0.05$). GP 1 showed significant difference with GP 2 ($p<0.05$). GP 4 showed significant diffference with GP 2 ($p<0.05$).

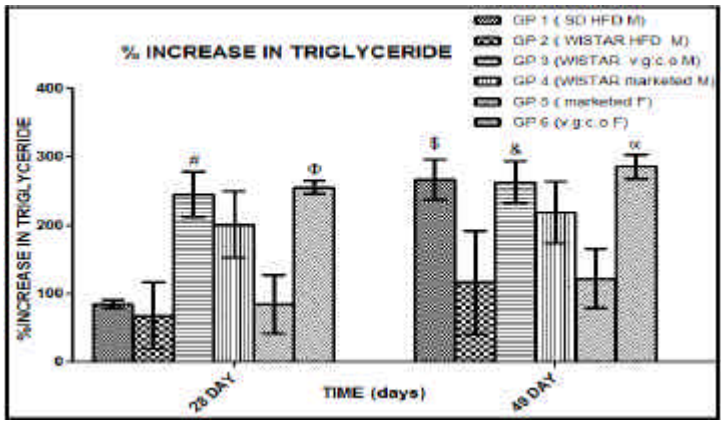


All the values are expressed as Mean±SEM.
$ = GP 1 showed significant difference with GP 2 ($p<0.01$)
 # = GP 4 showed significant difference with GP 2 ($p<0.01$) and GP 5 ($p<0.001$)
&= GP 3 showed significant difference with GP 2 ($p<0.05$)
? = GP 1 showed significant difference with GP 2 ($p<0.05$)
a = GP 4 showed significant diffference with GP 2 ($p<0.05$)

Fig 5.7.1 Effect of various HFD on % increase in total cholesterol vs. time (days)

5.8 Plasma Triglyceride

Plasma triglyceride (mg/dl) was measured at week 0, week 4 and week 7. GP 1 (SD HFD M) showed significantly higher increase in TG compared to GP 2(Wistar HFD M) ($p<0.05$). GP 3(Wistar v.g::c.o M) showed significantly higher increase in TG compared to GP 2 (Wistar HFD M) ($p<0.05$). GP 6(v.g::c.o F) showed significant difference with GP 5(marketed F) ($p<0.01$).

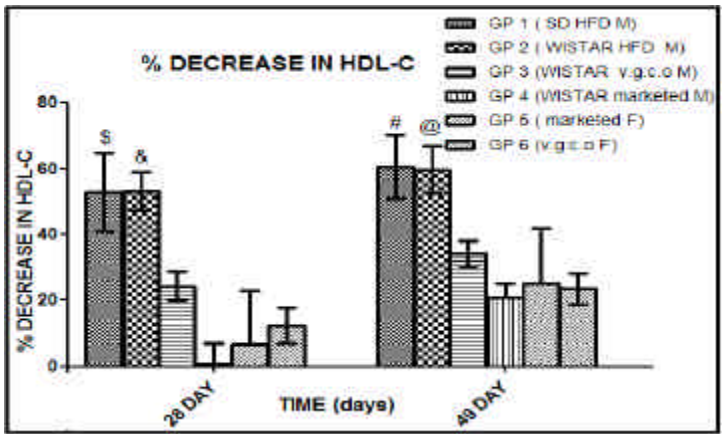


All the values are expressed as Mean±SEM
$ = GP 1 showed significant difference with GP 2 ($p<0.05$)
 # = GP 3 showed significant difference with GP 2 ($p<0.05$)
& = GP 3 showed significant difference with GP 2 ($p<0.05$)
F = GP 6 showed significant difference with GP 5 ($p<0.01$)
a = GP 6 showed significant difference with GP 5 ($p<0.01$)

Fig. 5.8.1 Effect of various HFD on % increase in plasma triglyceride vs. time (days)

5.9 Plasma HDL cholesterol

Plasma HDL-C was measured at week 0, week 4 and week 7. GP 1(SD HFD M) showed significantly higher decrease in plasma HDL-C compared to GP 4 (Wistar marketed M).

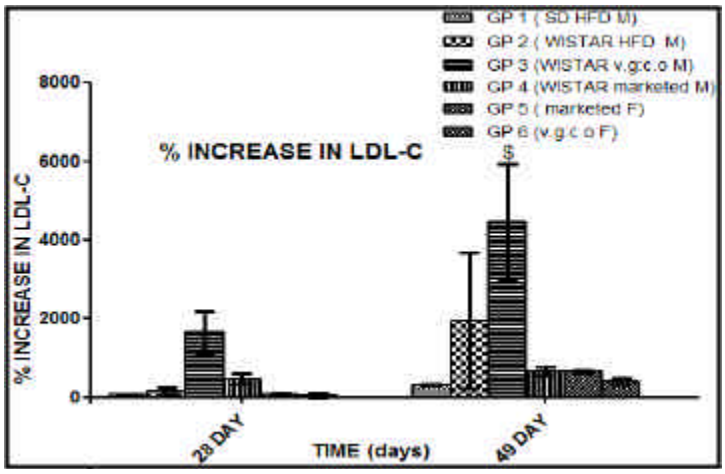


All the values are expressed as Mean±SEM
$ = GP 1 showed significant difference with GP 4 ($p< 0.01$)
 # = GP 1 showed significant difference with GP 4 ($p<0.05$)
& = GP 2 showed significant difference with GP 4($p<0.01$)
@= GP 2 showed significant difference with GP 4 ($p<0.05$)

Fig. 5.9.1 Effect of various HFD on % Decrease in plasma HDL-C vs. time (days)

5.10 Plasma LDL cholesterol

Plasma LDL-C was calculated using Fredman's formula. GP 3(Wistar v.g::c.o M) showed significantly higher increase in LDL-C compared to GP 2(Wistar HFD M) ($p<0.01$), GP 4(Wistar marketed M) ($p<0.001$) and GP 6(v.g::c.o F) ($p<0.001$).



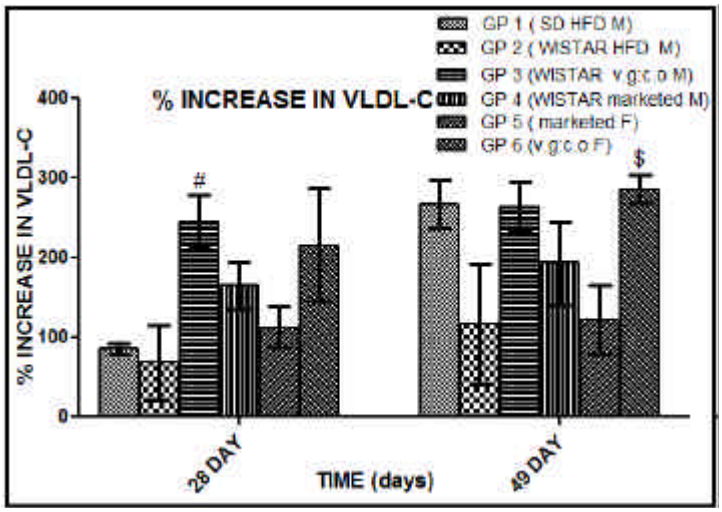
values are expressed as Mean±SEM
$ = GP 3 showed significant difference with GP 2 ($p<0.01$), GP 4 ($p<0.001$) and GP 6 ($p<0.001$)

Fig. 5.10.1 Effect of various HFD on % increase in LDL-C vs. time (days)

5.11 Plasma VLDL cholesterol

Plasma VLDL cholesterol was calculated using Fredman's formula. GP 3 showed significantly higher increase in VLDL-C compared GP

1(SD HFD M) ($p<0.05$) and GP 2 (Wistar HFD M) ($p<0.01$). GP 6(v.g::c.o F) showed significantly higher increase in VLDL-C compared to GP 5 (marketed F) ($p<0.05$.)



All the values are expressed as Mean±SEM
 # = GP 3 showed significant difference with GP 1 ($p<0.05$) and GP 2 ($p<0.01$)
$ = GP 6 showed significant difference with GP 5 ($p<0.05$)

Fig. 5.11.1 Effect of various HFD on % increase in VLDL-C vs. time (days)

Table 3 Result summary table

Parameter	wk	GP 1	GP 2	GP 3	GP 4	GP 5	GP 6
% Increase in body weight	4	21.60± 4.968 $	6.910±2.929	9.552± 4.022	6.616± 1.172	9.55±0.560	8.186± 1.468
	7	38.705±4.336 #	10.863± 3.502	13.970± 4.218 @	9.612± 1.160	20.606± 1.525 F	11.876± 1.599
% Increase in Total cholesterol	4	82.215± 26.478$	35.086± 12.225	153.595± 59.77	269.41± 48.767#	60.682± 13.622	63.115± 17.893
	7	285.97± 65.98?	93.073± 20.99	263.91± 45.486&	263.12± 63.064a	276.88± 21.911	207.61± 33.486
% Increase in Triglyceride	4	84.95±6.216	67.97±48.11	245.15± 32.976#	201.46± 48.332	84.624± 42.95	255.640± 9.677F
	7	266.77± 29.990$	116.29± 75.607	263.58± 30.784&	218.50± 45.367	122.22± 43.380	286.09± 17.413a
% Decrease in HDL -C	4	52.91± 11.83$	53.24± 5.791&	24.41±4.320	0.698± 6.25	6.672±16.44	12.411± 5.46
	7	60.515± 9.57#	59.56± 7.203@	34.16± 3.750	20.79±4.53	25.11± 16.91	23.53± 4.548
% Increase in LDL -C	4	73.722±13.96	162.356± 91.54	1652.7± 531.02	471.55± 131.020	80.62± 37.469	46.98± 48.589
	7	318.01± 35.88	1960.4± 1721.34	4465.8± 1471.9$	669.35± 122.148	660.92± 39.545	395.14± 82.472
% Increase in VLDL -C	4	84.957±6.217	67.96±48.11	244.82± 33.45#	164.47± 28.620	112.29± 25.62	216.11± 70.93
	7	266.77± 29.99	116.29± 75.607	264.03± 30.75	192.97± 52.01	122.57± 43.29	285.93± 17.38$

DISCUSSION

There has been a growing body of literature using rodents as models of human obesity, even though there are many confounding factors including species, strain, gender, age of the animal, type of diet, level of fat, and type of control diet. In the current investigation, we have tried to rationalize these issues, thus validating the high fat diet induced obesity models, which further help to design studies with tightly controlled conditions and therefore improve our understanding of obesity and related diseases.

Different type of dietary manipulation was carried out in two strains i.e., Sprague Dawley and Wistar rats and in both the gender. Then the groups were analyzed by comparing parameters such as food intake, calorie intake, water intake, body weight gain, lipid profile, glucose tolerance and insulin resistance.

High fat diet acts as a source of saturated fat resulting in increase in body weight and increase in blood lipid concentration. Food intake and body weight are direct measures of obesity. [12] It was found that GP 5 (marketed female) showed significantly higher food intake than with GP 6 (v.g::c.o female) ; GP 4 (marketed Wistar male) shows significantly higher food intake than GP 2 (HFD Wistar male), GP 3 (v.g::c.o Wistar male); GP 2 (HFD Wistar male) showed significantly higher food intake than with GP 3 (v.g::c.o Wistar male) indicating that food intake varies with diet composition. Furthermore GP 4 (marketed Wistar male) showed higher food intake compared to GP 5 (marketed female) indicating gender wise differences in response to same diet.

Thus food intake of marketed group was the highest and that of the in-house prepared HFD and vegetable ghee:coconut oil group ate NPD the lowest.

However food intake varies depending upon the composition and mode of administration of the diet. Hence it is important to consider calorie intake to obtain a better comparison. The calorie intake of marketed diet was significantly higher than in-house prepared diets evident from its higher food intake as the kilocalories of all diets were almost equal. Male rats showed higher calorie intake than female rats. Second in calorie intake ranks the vegetable ghee::coconut oil (3:2) diet. Moreover one can obtain an equal amount of calorie intake in all the animals with this diet as it is given in a fixed dose of (10 ml/kg) and also gives an assurance of animals being consuming the high fat diet. While considering the body weight gain, SD strain fed with in-house HFD were observed to have a higher weight gain than Wistar strain during 4 week and 7 week supporting the strain preference for SD. Surprisingly, female group fed with marketed diet showed higher weight gain than male fed the same diet indicating female rats can be employed for obesity studies particularly for screening of anti-obesity drugs concerning body weight. The vegetable ghee:coconut oil diet showed slightly difference in weight gain with that of in-house HFD indicating both produce almost similar result.

High fat diet is not only related to obesity but also causes changes in glucose metabolism leading to glucose intolerance and ultimately diabetes. However a longer duration dietary manipulation is required

to obtain significant level of serum glucose to be served as diabetes model. Oral glucose tolerance shows a significantly higher tolerance observed in group served with marketed high fat diet. Thus if one wants to screen a particular compound for both obesity and diabetes then marketed diet serves a good source though significant level of plasma glucose corresponding to diabetes was not reached during 6 week study, this parameter needs to be further evaluated. In-house prepared high fat diets also showed minor glucose intolerance at the end of 6 week study. Whilst, vegetable ghee:coconut oil model did not produce any sign of glucose intolerance indicating that the model is effective for studies concentrating on hyperlipidemia only.

Insulin tolerance test was performed in order to get information whether the high fat diet produced resistance towards insulin or not further confirming diabetes development. It was reported that at the end of 7 week the marketed diet group developed significant resistance towards insulin in comparision to other groups while vegetable ghee:coconut oil diet group did not show any sign of resistance supporting the results of OGTT.

Dieatary high fat causes increase in serum cholesterol, VLDL cholesterol and decrease in HDL cholesterol.[13] Normal range of total cholesterol level in rats is between 80-100 mg/dl. Here SD strain fed with inhouse high fat diet showed significantly more increase in TC than that of Wistar strain. Marketed high fat diet showed the maximum rise in TC compared to other groups while female fed with marketed diet showed less increase in TC compared to male rats indicating gender difference in response to same diet. Vegetable ghee:coconut oil diet showed higher raise in TC compared to inhouse high fat diet at 4 weeks only which than remained stable at the end of 8 week indicating a 4 week manipulation of this diet is sufficient to produce hypercholestremia.

Obesity results in increased level of oxidized LDL-C which is found to cause endothelial damage, oxidative stress and inflammation, which further aggrevates obesity. Normal range of LDL-C level in rats is between 30-50 mg/dl. Oxidized LDL cholesterol is also risk factr for the development of atherosclerosis.[14] Wistar male rats given v.g::c.o diet showed significantly higher increase in LDL-C compared to Wistar male rats fed with in-house HFD, Wistar male rats given marketed diet and female rats v.g::c.o diet and the increase was significant in just 4 week dietary manipulation.

The hepatic overproduction of VLDL appears to be the primary and crucial defect of obesity. Synthesis of VLDL is promoted by an increase in the flux of free fatty acids in liver and ultimately the particles are converted to LDL. Increased NEFA and glucose flux in the liver are important regulators of hepatic VLDL production. VLDL particles are mainly cleared from circulation by the LDL receptor, also referred to as apo B/E receptor. The transcrioption of the LDLR gene is regulated by intracellular cholesterol concentration, hormones and gorwth factors. Any abnormality in these factors results in decrease clearance of VLDL. Thus, VLDL increases in obesity.[15] Normal range of VLDL-C level is between 10-15 mg/dl.

Wistar male rats given v.g::c.o diet showed significantly higher increase in VLDL-C compared to Wistar and SD male rats given in-house HFD M. Female rats given v.g::c.o diet showed significantly higher increase in VLDL-C compared to females given marketed diet.

Hypertriglyceridemia in combination with low concentrations of HDL-C is one of the most common atherogenic profile of lipid metabolism. Release of free fatty acis by lipoprotein lipase from increaed serum triglycerides causes lipotoxicity resulting in insulin-receptor dysfunction. Free fatty acids also produce oxidative stress. The release of excessive free fatty acids provokes lipotoxicity, as lipids and their metabolites create oxidative stress. This affects adipose as well as nonadipose tissue, accounting for its pathophysiology in many organs, such as the liver and pancreas resulting in the metabolic syndrome.[16] Normal range of triglycerides level in rats is between 70-80 mg/dl. SD strain showed significantly higher increase in TG compared to Wistar strain given in-house HFD. Vegetable ghee::coconut oil diet showed significantly higher increase in TG compared in-house HFD. In case of female rats, vegetable ghee::coconut oil diet showed higher increase in TG than marketed diet in just 4 weeks.

Obesity frequently associated with a low concentration, adverse distribution pattern and abnormal metabolism of HDL particles. HDL particles are the smallest lipoprotein particles, containing approximately 20% cholesterol ester and very little triglyceride. Obesity has been shown to adversely affect the levels of HDL subfractions principally associated with cardioprotection: HDL_2, apolipoprotein A-I and pre-$ß_1$.[17] Normal range of HDL-C level in rats is between 40-50 mg/dl. In-house HFD produced significant decrease in plasma HDL-C even as early as at the end of 4 week diet manipulation in both the strains while comparatively marketed diet showed only marginal decrease in HDL-C justifying its usefulness in screening of anti-obesity drugs targeting HDL-C as therapeutic target. Vegetable ghee:coconut oil HFD produced decrease in HDL-C which decreased more at the end of 7 week, but was much less compared to in-house HFD suggesting its usefulness for studies utilizing HDL-C as therapeutic target.

A study conducted at our institute utilized and obesity model of SD female rats fed with in-house HFD. The data were shared and compared with that of SD male rats given in-house HFD. It was observed that % increase in body weight and lipid profile were better achieved in case of males than that of female indicating gender specific variation in response to diet induced obesity model.

Another study conducted at our institute utilized SD male rats and were given vegetable ghee::coconut oil (1:1) diet showed a better achieved lipid profile and % increase in body weight in comparison to Wistar male rats used in the present investigation and given vegetable ghee:: coconut oil (3:2) diet, indicating strain specific variation. However due to different ratios used it requires further investigation.

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