

## ORIGINAL RESEARCH

**Effect of feeding saturated and unsaturated fats on lipid peroxidation and weight gain in rabbits****<sup>1</sup>Dr. Sangeeta Gupta, <sup>2</sup>Dr. Garima Sehgal, <sup>3</sup>Dr. Seema, <sup>4</sup>Dr. Tejinder Singh, <sup>5</sup>Dr. Vidushi Gupta**<sup>1</sup>Assistant Professor, Department of Physiology, Govt. Medical College, Amritsar, Punjab, India<sup>2</sup>Assistant Professor, Department of Biochemistry, Oxford Medical College Hospital & Research Centre, Bangalore, India<sup>3</sup>Professor & HOD, Department of Physiology, S.S Tania Medical College, Hospital & Research Centre, Sriganaganagar, India<sup>4</sup>Assistant Professor, Department of Biochemistry, Govt. Medical College, Amritsar, Punjab, India<sup>5</sup>Professor & HOD, Department of Physiology, Dayanand Medical College, Ludhiana, Punjab, India**Corresponding Author:**Dr. Tejinder Singh**Email:** [v\\_tajinder79@yahoo.com](mailto:v_tajinder79@yahoo.com)

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**Abstract****Background:** Fat is an essential nutrient in our balanced diet. The present study was designed to study the effect of saturated and unsaturated fats on lipid peroxidation and weight gain in rabbits.**Material and methods:** The present study was conducted in Dayanand Medical College & Hospital, Ludhiana. Study was conducted on 30 albino rabbits of either sex and weighing between 1-1.5 kg. They were divided into control group and study group. The rabbits in study group were further divided into two groups who were fed on coconut oil and soyabean oil. Blood sample will be collected from 10 rabbits and estimation of serum MDA levels and measurement of weight will be done at the start of study and then at the end of three months without feeding fat diet. They will be fed on standard pelleted (Gold Mohur) diet.**Results:** The finding of our study revealed decrease in serum MDA levels after feeding coconut oil for 12 weeks as compared to control group but this decrease is not significant ( $P=0.2499$ ). Whereas, serum MDA levels were significantly increased in rabbits fed on soyabean oil as compared to control group ( $P=0.0318$ ). Rabbits in soyabean oil group when compared with coconut oil group showed significantly higher levels of MDA i.e., more lipid per oxidation. ( $P=0.001203$ ). There was less gain in weight in coconut oil group as compared to those in control group though this is not significant ( $P=0.7641$ ). Rabbits in soyabean oil group showed more gain in weight when compared to those in control group and this gain was significant ( $P=0.0021$ ). Rabbits in soyabean oil group showed more gain in weight when compared to those in coconut oil ( $P=0.0021$ ).**Conclusion:** The results of our study showed that weight gain was maximum in rabbits who have consumed soyabean oil & least in coconut oil group. Hence, the results of our study showed that unsaturated fats cause more lipid peroxidation and more gain in weight as compared to saturated fats.**Keywords:** Coconut oil, Soyabean oil, MDA level

## Introduction

Fat is an essential nutrient in our balanced diet. Firstly, fat is most concentrated source of energy and one gram of fat gives 9 Kcal of energy. Essential fatty acids are those fatty acids which are not synthesized by body and need to be supplied through diet.<sup>1</sup> Dietary fatty acids largely vary in carbon chain length, degree of saturation and isomeric configuration of double bonds.<sup>2</sup>

Fatty acids are unsaturated and saturated depending upon whether they contain double bond or not respectively. Saturated fatty acids are further classified according to number of carbon atoms they have. These are formic, acetic, propionic, capric (decanoic), lauric and myristic acid if they have 1,2,3,10,12 and 14 carbon atoms respectively. Whereas unsaturated fats are further subdivided according to the number of double bonds they have i.e., monounsaturated and polyunsaturated containing two or more double bonds.<sup>3</sup>

There are basically three parameters to adjudge any oil as the healthiest cooking oil. i.e ratio of saturated/monounsaturated /polyunsaturated fatty acid, ratio of essential fatty acids (omega 6/omega3) and presence of natural antioxidants.

The world health organization (WHO) recommends polyunsaturated fatty acid (PUFA)/Saturated fatty acids ratio of 0.8 to 1.0 and linoleic acid (omega6) alpha linolenic acid (omega 3) ratio of 5-10 in the diet.<sup>2</sup> Saturated fats are fatty acids that conserve the elongated omega-3 fatty acids. Unsaturated fatty acids cause the tissues to lose these omega-3 fatty acids.<sup>4</sup>

Coconut oil is saturated fat unusually rich in medium chain fatty acids (lauric acid) and short chain fatty acids. Medium chain fatty acids are known to increase the metabolism and promote the weight loss. Coconut oil is very stable and the body is not burdened with oxidative stress as it is with other vegetable oils. Dietary coconut oil reduces our need for vitamin E.<sup>5</sup>

The usage of any one of the oils like safflower, sunflower and soyabean furnish very high PUFA/SFA ratio. They also provide high ratio of omega 6/omega 3. It has been observed that obesity is increasing directly in proportion to the ratio of unsaturated oil to the coconut oil in the diet.<sup>5</sup> Soyabean oil used by us has omega 6: omega 3 ratio of 10 and fatty acids percentage by weight for saturated is 16 %, 24% monounsaturated and 60% polyunsaturated fatty acids as compared to recommended below 33%, about 33% and above 33% respectively. Today our ratio of omega-6 to omega-3 averages from 20:1. Too much omega-6 is now recognized scientifically as being the cause or aggravator of many inflammatory conditions, weakening of immune system, cardiovascular diseases and many cancers. The most common offenders used in cooking and almost all manufactured foods are processed soyabean oil, corn oil, sunflower oil etc.

Dietary intake of the omega-6 polyunsaturated fatty acid linoleic acid was positively related to coronary artery disease.<sup>6</sup> PUFAs has adverse effect upon the thyroid gland causing hypothyroid like symptoms like weight gain, edema, and hypercholesterolemia.<sup>7</sup>

A slow rate of metabolism results from the unsaturated fats and refined carbohydrates whereas tissue response to thyroid hormone is actually enhanced by saturated fats especially short chain fatty acids.<sup>8</sup>

Soyabean oil is a polyunsaturated fat, consists mainly of linoleic and linolenic acid (long chain fatty acids). Unsaturated fats tend to convert carbohydrates into fat production, making stress and obesity more probable.

The deleterious effects are considered to be caused by the free radicals (ROO·, RO·, OH·) produced during peroxide formation from fatty acids containing methylene-interrupted double bonds, i.e., those found in naturally occurring PUFAs.

Lipids are very susceptible to free radical attack. Exposure of cell membrane to oxygen radicals stimulates the process of lipid peroxidation. The major fatty acids that undergo peroxidation in the cell membrane are linoleic acid (18:2), arachidonic acid (20:4), docosahexanoic acid (22:6) and other polyunsaturated fatty acids.<sup>9</sup> Aldehydes generated are biologically active. Moreover, these aldehydes are capable of forming protein cross-linkages that inactivate many cellular constituents, including membranes and enzymes.<sup>10</sup> In this process the fatty acid side chains of the membrane lipids are oxidized to hydroperoxides and peroxides.<sup>11</sup> These hydroperoxides are themselves reactive species and in the presence of oxygen cause subsequent reduction of further fatty acids resulting in extensive membrane, organellar and cellular damage.<sup>12</sup> In this way, the free radical damage is related to the amount of lipid peroxide level in the blood. Yagi, 1987 estimated serum lipid peroxide levels expressed in term of malondialdehyde of normal subjects and he observed that at middle age the lipid peroxide levels in male were significantly higher than those in females of corresponding age. He also reported that the lipid peroxide levels tend to increase with age, the physiological level in serum was less than 4 nmol/ml and the level above 4 nmol/ml serum was pathological.<sup>13</sup> It is formed mainly from the oxidation of arachidonic acid in the cell membrane.<sup>14</sup>

MDA is one of the end products of lipid peroxidation and extent of lipid peroxidation is measured by estimating MDA levels most frequently Malondialdehyde (MDA) is decomposition product of oxidized polyunsaturated fatty acids. This three-carbon dialdehyde has been proposed to arise from fatty acid hydroperoxides via several mechanisms.<sup>15</sup> Increased serum level of Urinary excretion of MDA was reported to be increased in response to the increase in the lipid peroxidation in vivo produced by vitamin E deficiency and administration of iron nitrilotriacetate.<sup>16</sup> Increased serum level of MDA has been reported in cardiovascular, neurological and other diseases.<sup>17</sup>

### **Material and methods**

**Animals:** The study will be conducted on 30 albino rabbits of either sex and weighing between 1-1.5 kg.

**Control group**-Blood sample will be collected from 10 rabbits and estimation of serum MDA levels and measurement of weight will be done at the start of study and then at the end of three months without feeding fat diet. They will be fed on standard pelleted (Gold Mohur) diet.

**Study group** –Rabbits will be divided into 2 groups of 10 each –

**Group 1-** Consist of 10 rabbits and will be fed on 10 ml coconut oil /day for 3 months in the diet.

**Group 2-** Consist of 10 rabbits and will be fed on 10 ml soyabean oil /day for 3 months in the diet.

**Exclusion Criteria**-The Rabbits who will die or fall sick or don't take fat diet will be excluded from our study.

### **Collection of blood samples**

Rabbit was placed in restraining cage and xylene was applied over the ear. Blood sample of 5 ml from each rabbit was collected from marginal vein in the plain test tube under aseptic conditions using disposable needle. Blood sample was allowed to clot at room temperature and serum was separated by centrifugation at 3000 rpm for 10 mins.

**Weight measurement:** was done by using single pan weighing machine for small animals.

**Estimation of malondialdehyde** was done by thiobarbituric assay (TBA) method using ELICA Spectrophotometer (Buege et al., 1978).

## Results

**TABLE 1 SHOWING THE COMPARISON OF DIFFERENCE OF SERUM MDA LEVELS (MEAN  $\pm$ SD) (AT 0 WEEKS AND AFTER 3 MONTHS) BETWEEN CONTROL AND COCONUT OIL GROUP**

Group	Mean $\pm$ SD
Control	0.0003 $\pm$ 0.000019
Coconut	0.0002 $\pm$ 0.00002

**TABLE 2 SHOWING THE ANALYSIS OF VARIANCE AND P VALUE COMPARING MDA LEVELS (AT 0 WEEKS AND AFTER 3 MONTHS) BETWEEN CONTROL AND COCONUT OIL GROUP**

	Degree of freedom	Mean Square	P-value
Between	1	3.01 $\times 10^{-8}$	0.2499, not significant
Within	18	2.13 $\times 10^{-8}$	

Table 1 &2 shows that serum MDA levels were decreased in rabbits fed on coconut oil as compared to control group but the decrease was not significant. (P=0.2499)

**TABLE 3 SHOWING THE COMPARISON OF DIFFERENCE OF SERUM MDA LEVELS (MEAN  $\pm$ SD) (AT 0 WEEKS AND AFTER 3 MONTHS) BETWEEN CONTROL AND SOYABEAN OIL GROUP**

Group	Mean $\pm$ SD
Control	0.000283 $\pm$ 0.000019
Soyabean	0.0005 $\pm$ 0.000061

**TABLE 4 SHOWING THE ANALYSIS OF VARIANCE AND P VALUE COMPARING MDA LEVELS (AT 0 WEEKS AND AFTER 3 MONTHS) BETWEEN CONTROL AND SOYABEAN OIL GROUP**

	Degree of freedom	Mean Square	P-value
Between	1	2.54 $\times 10^{-7}$	0.0318, Significant
Within	18	4.69 $\times 10^{-8}$	

Table 3&4 shows serum MDA levels were increased significantly (P=0.0318) in rabbits fed on soyabean oil for 3 months as compared to control group.

**TABLE 5 SHOWING THE COMPARISON OF DIFFERENCE OF MEAN SERUM MDA LEVELS (MEAN $\pm$ SD) (AT 0 WEEKS AND AFTER 3 MONTHS) BETWEEN COCONUT OIL AND SOYABEAN OIL GROUP**

Group	Mean $\pm$ SD
Coconut	0.000196 $\pm$ 0.00002
Soyabean	0.0005 $\pm$ 0.000061

**TABLE 6 SHOWING THE ANALYSIS OF VARIANCE AND P VALUE COMPARING MDA LEVELS (AT 0 WEEKS AND AFTER 3 MONTHS) BETWEEN COCONUT OIL AND SOYABEAN OIL GROUP**

	Degree of freedom	Mean Square	P-value
Between	1	$4.59 \times 10^{-7}$	0.0012, Significant
Within	18	$3.12 \times 10^{-8}$	

Table 5&6 shows that MDA levels were significantly higher ( $P=0.0012$ ) in case of soyabean oil group as compared to coconut oil group.

**TABLE 7 SHOWING THE COMPARISON OF DIFFERENCE OF WEIGHT GAIN (MEAN  $\pm$ SD) (AT 0 WEEKS AND AFTER 3 MONTHS) BETWEEN CONTROL AND COCONUT OIL GROUP**

Group	Mean $\pm$ SD
Control	$0.25 \pm 0.0500$
Coconut	$0.22 \pm 0.0553$

**TABLE 8 SHOWING THE ANALYSIS OF VARIANCE AND P VALUE COMPARING WEIGHT GAIN (AT 0 WEEKS AND AFTER 3 MONTHS) BETWEEN CONTROL AND COCONUT OIL GROUP**

	Degree of freedom	Mean Square	P-value
Between	1	0.0022	0.7641, not significant
Within	18	0.0237	

Table 7&8 shows that there was less gain in weight in case of coconut oil group as compared to control group but this is not significant ( $P=0.7641$ ).

**TABLE 9 SHOWING THE COMPARISON OF DIFFERENCE OF WEIGHT GAIN (MEAN  $\pm$ SD) (AT 0 WEEKS AND AFTER 3 MONTHS) BETWEEN CONTROL AND SOYABEAN OIL GROUP**

Group	Mean $\pm$ SD
Control	$0.25 \pm 0.0500$
Soyabean	$0.45 \pm 0.0561$

**TABLE 10 SHOWING THE ANALYSIS OF VARIANCE AND P VALUE COMPARING WEIGHT GAIN (AT 0 WEEKS AND AFTER 3 MONTHS) BETWEEN CONTROL AND SOYABEAN OIL GROUP**

	Degree of freedom	Mean Square	P-value
Between	1	0.2691	0.0021, Significant
Within	18	0.0209	

Table 9&10 shows that weight gain was more in rabbits that have consumed soyabean oil as compared to standard gold mohur diet (control) and this was significant ( $P=0.0021$ ).

**TABLE 11 SHOWING THE COMPARISON OF DIFFERENCE OF WEIGHT GAIN (MEAN $\pm$ SD) (AT 0 WEEKS AND AFTER 3 MONTHS) BETWEEN) COCONUT OIL AND SOYABEAN OIL GROUP**

Group	Mean $\pm$ SD
Coconut	$0.22 \pm 0.0553$
Soyabean	$0.45 \pm 0.0561$

**TABLE 12 SHOWING THE ANALYSIS OF VARIANCE AND P VALUE COMPARING WEIGHT GAIN (AT 0 WEEKS AND AFTER 3 MONTHS) BETWEEN COCONUT OIL AND SOYABEAN OIL GROUP**

	Degree of freedom	Mean Square	P-value
Between	1	0.3200	0.0021, Significant
Within	18	0.0249	

Table 11&12 shows that there was significantly more weight gain in case of soyabean oil group as compared to coconut oil group.(P=0.0021)

### Discussion

This study was planned to find out the effect of saturated & unsaturated fat on lipid per oxidation which was estimated by MDA levels & weight gain in rabbits.

The findings of our study revealed decrease in serum MDA levels after feeding coconut oil for 12 weeks as compared to control group but this decrease is not significant (P=0.2499) (Table 2). This effect may be attributed to the inhibitory effect of coconut oil on microsomal lipid per oxidation and superior antioxidant action.<sup>18</sup>

Whereas, serum MDA levels were significantly increased in rabbits fed on soyabean oil as compared to control group (P =0.0318)(Table 4). The high level of serum MDA(i.e. index of lipid per oxidation) may be due to unsaturation or the presence of many double bonds in PUFAS, which are very labile and easily peroxidised.

Rabbits in soyabean oil group when compared with coconut oil group showed significantly higher levels of MDA i.e., more lipid per oxidation. (P=0.001203)(Table 6) The results are consistent with the concept that saturated fatty acids are not under normal conditions easily subject to cell-damaging lipid peroxidation, whereas PUFAs that are commercially sold often undergo lipid per oxidation that damages their molecular structure. Lipid per oxidation starts as soon as they are extracted from its source.<sup>19</sup>

There was less gain in weight in coconut oil group as compared to those in control group though this is not significant(P=0.7641)(Table 8). Rabbits in soyabean oil group showed more gain in weight when compared to those in control group and this gain was significant(P=0.0021)(Table 10)

Rabbits in soyabean oil group showed more gain in weight when compared to those in coconut oil (P=0.0021) (Table 12)

Hence, results of our study showed that weight gain was maximum in rabbits who have consumed soyabean oil & least in coconut oil group.

This can be due to reason that obesity increased directly in proportion to ratio of unsaturated oil to saturated oil in the diet.

Increased weight gain in soyabean oil group when compared to coconut oil group could be due to the fact that shorter chain fatty acids (in saturated) have fewer calories per gram than longer chain fatty acids (in unsaturated).

### Conclusion

The results of our studied showed that unsaturated fats cause more lipid peroxidation and more gain in weight as compared to saturated fats. Thus, the concept of using polyunsaturated fat in the diet and avoiding saturated fat completely from the diet should be changed to use of both saturated and unsaturated fats to keep a balance.

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