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Effect of Milk or Milk Derived Food Supplementation on Body Composition of Young Indian Women

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ABSTRACT

Background: Lysine intake in India is low mainly due to consumption of cereals as staple diet and processing loss of lysine. The present study was aimed to assess the impact of supplementation either in the form of dietary supplements of lysine, calcium and vitamin D in order to identify an appropriate approach for the improvement of body composition.

Methods: One hundred and twenty volunteer subjects in the age group of 20-30 years residing in Punjab Agricultural University hostels were selected for supplementary trial. Supplementation of milk and its products in amounts to fill the gap of dietary intake and adequacy of protein as well as limiting amino acid lysine in their diets for a period of 12 weeks was done to determine its effect on body composition.

Result: The diets were also poor in protein quality as the limiting amino acid lysine was only 55% of the recommended level. Supplementation of milk and its products resulted in a significant reduction in body fat (6.62%) and visceral fat rating (9.47%) and a significant increase in skeletal muscle mass (3.98%) and bone mass (2.72%). The study recommends that improving the quality of diets through dietary supplementation of lysine, calcium and vitamin D is an effective approach to achieve optimum body composition.

Key words: Body composition, Lysine, Milk derived supplements, Muscle mass, Vitamin D.

INTRODUCTION

Protein intake in the diet directly affects muscle mass as higher amounts of dietary protein leads to greater availability of plasma amino acids resulting in an increased protein synthesis (Motil *et al*. 1981). The larger protein intake than recommendations improve the metabolic function of muscles as more amino acids enhance mitochondrial protein synthesis required for substrate metabolism as well as myofibrillar proteins synthesis (Bohe *et al*. 2003).

Lysine the first limiting amino acid of Indian diets and is involved in many metabolic functions of the body such as production of energy, biosynthesis of collagen and elastin and maintenance of healthy bones. To fulfill the minimum amount of lysine from cereals, the required total protein intake should be higher than the daily recommended intake. So, taking cereals as the main source of lysine will lead to excess of daily energy intake and deficiency of lysine (Leinonen *et al*., 2019). Therefore, in countries with very high percentage of cereal-based protein contribution, the average per person lysine intake is proceeding towards the limit of lysine deficiency. In these countries, there is a great possibility that a greater part of the population especially the growing children are taking diet that does not supply enough lysine (Moya, 2016).

Dairy is an essential part of the food culture in India and particularly in Punjab. Of the total global protein consumption by humans, the share of plant-based protein is currently about 60% and animal protein about 40% (Boland *et al*., 2013). The amino acids and lysine present in milk proteins are extremely digestible (Rutherfourd and Moughan, 2005). Fat-free fluid milk increases muscle mass and decreases fat mass (Josse *et al*., 2010). Furthermore, reduction in serum PTH and an increase in serum 25 hydroxy vitamin D levels in milk were significantly related to increased dietary calcium intakes and may possess positive effect on bone turnover. It was found to be associated with weight loss, reduced risk of type 2 diabetes and cardiovascular diseases. (Thorning *et al*., 2016).

Milk and its derived foods are the best source of animal based lysine especially for vegetarians. The bioactive components such as branched chain amino acids in whey protein might be responsible for the positive effects on muscles (Etzel, 2004). The epidemiological evidence also shows that with the consumption of dairy foods decreases prevalence of metabolic disorders. Also, that greater intake of dairy protein, moderate restriction in energy intake and combined resistance and aerobic training can result in favorable body composition, strength and fitness changes (Josse *et al*., 2010).

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Keeping in view, the lower intakes of lysine, calcium and vitamin D in Indian population due to demographic, economic, cultural and religious factors, the present study was taken to study the impact of dietary supplementation of lysine, calcium and vitamin D in order to identify an appropriate approach for the improvement of body composition of young Indian women.

MATERIALS AND METHODS

Selection of subjects

One hundred and twenty volunteer subjects in the age group of 20-30 years residing in Punjab Agricultural University hostels were selected from a group of 473 girls who were part of a nutritional survey. The formal consent of the subjects and approval of Institution Ethical Committee of Punjab Agricultural University, Ludhiana (No. DR-8323-32-19-4-19) was obtained. The study was conducted between September-December 2019.

Collection of data before and after the trial

The trial was conducted for a period of three months. The data of the subjects of two groups *i.e*. Group I: Control group (Without supplementation) and Group II: Experimental Group (diet supplemented with milk or milk derived foods) before and after the supplementation trial was collected for anthropometric parameters (Jellife, 1966), body composition parameters, biochemical profile and bone mineral density. Bioelectrical impedance was used to measure body composition of the subjects using TANITA Body composition analyzer BC-420MA.The blood was analyzed for 25(OH) vitamin D on Cobase 601 analyzer (Roche Diagnostics). Albumin was analyzed on a Cobas c 501 (Roche Diagnostics). Hemoglobin level of the subjects was determined by HemocueHb301 system.

Supplementation plan

A supplementation trial of 12 weeks was planned to assess the efficacy of supplementation of milk and its products to improve the muscle mass and bone health. Two matched supplementation groups were:

Group I: Control

Group II: Experimental

The level of supplementation for dietary supplements was determined to eliminate the gap between daily intake and requirements of lysine, calcium and vitamin D (ICMR, 2010). The supplementation plan is given in Table 1.

As majority (94.16%) of the subjects was diagnosed with vitamin D deficiency, the experimental group was also given a sachet of vitamin D (60000IU) once a week. The supplementation trial was followed for five days a week by the subjects.

As per calculations, the daily lysine supplementation was 700, 560 and 560 mg in Group II. The supplementation plan met the daily requirements of the subjects for total protein in Group II *i.e.* 53.99 g (Table 2). The supplemented diets were offering lysine/g of protein more than the 45 g as daily recommended level (FAO, 2013).

Statistical analysis

The data was analyzed using SPSS software. Mean and standard deviations were computed. Students t-test (un-paired) was used to compare the subjects in different age groups and paired t-test was used to compare the data before and after the supplementation trial. Analysis of Variance (One-way ANOVA) and post hoc test was employed to assess the difference of nutrients eaten in different meals. Correlation coefficients (r) were computed to determine the relationships between various parameters of the study.

RESULTS AND DISCUSSION

Anthropometric parameters

Anthropometry is an important tool in screening the changes that are considered major risk factors for developing obesity related chronic diseases which may lead to increased mortality in adults. Table 3 depicts comparison of anthropometric measurements of the subjects before and after the supplementation trial. A significant ($p \leq 0.01$) increase in weight and a non- significant increase in waist circumference were found among the subject in Group I. However, after the trial, subjects of the Group II had higher weights and lower waist circumference when compared with the reference value of 55 kg and 80 cm, respectively.

The supplementation trial resulted in a non-significant decrease in BMI of the subjects belonging to Group II, while,

Table 1: Supplementation plan for meeting the daily gap of lysine, calcium and vitamin D (N=60).

Food item	Meal		
	Breakfast	Lunch	Dinner
Group I (n=30)			
-	-	-	-
Group II (n=30)			
Milk* and its products	250 ml milk	200 ml milk/200 g curd/70 g paneer	200 ml milk/200 g curd/70 g paneer
Vt. D sachet	60,000 IU/week		
Tablet Calcium	-	-	-

Group I: Control.

Group II: Dietary supplementation of milk and its products; *Composition of milk/100 g: Energy: 70 kcal, protein: 3.18 g, lysine: 279.11 mg, fat: 4.4 g, calcium: 117.4 mg.

Table 2: Supplementation plan for young adult women to meet their intake gap of lysine, protein and calcium through milk and its products during a 12-weeks trial (N=60).

Meal	Daily intake	RDA	Gap	Group II (n=30)	
				Supple.level	Level after suppl.
Lysine, mg	910.48	2475	1565	1820	2730
Protein, g	32.8	55	22.2	21.9	54.7
Calcium, mg	280.91	600	319.09	767	1047.91

Group I: Control (No supplementation).

Group II: Dietary supplementation of milk and its products.

Table 3: Basic and derived anthropometric measurements of selected young adult women before and after supplementation trial (N=60).

Anthropometric	Group I (n=30)			Group II (n=30)		
measurements	Before	After	t-value	Before	After	t-value
Basic						
Height, cm	157.53±4.67	-	-	156.40±5.02	-	-
Weight, Kg	54.72±9.26	55.42±9.10	3.76**	58.32±10.10	58.17±10.08	0.02 NS
Waist circumference, cm	71.80±8.08	72.47±8.27	2.01NS	75.34±9.34	74.80±9.55	0.68 NS
Hip circumference, cm	94.91±6.74	95.59±6.90	1.25 NS	98.08±8.06	97.11±7.40	0.96 NS
Derived						
BMI, Kg/m^2	22.09±3.45	22.32±3.37	2.91**	23.85±4.24	23.81±4.46	0.18 NS
Waist-hip ratio	0.76±0.06	0.76±0.06	0.43 NS	0.77±0.05	0.77±0.05	0.29 NS
Waist-height ratio	0.46±0.05	0.46±0.05	0.40 NS	0.48±0.06	0.48±0.07	0.62 NS

Group I: Control (No supplementation).

Group II: Dietary supplementation of milk and its products.

Values are Mean±SD; *Significant at 5%** Significant at 1%, NS: Non-Significant.

Table 4: Per cent changes in anthropometric measurements of selected young adult women after 12-weeks supplementation trial (N=60).

Anthropometric measurements	Group I	Group II
Height, cm	-	-
Weight, Kg	1.28	-0.26
Waist circumference, cm	0.93	-0.72
Hip circumference, cm	0.72	-0.99
BMI, Kg/m^2	1.04	-0.17
Waist-hip ratio	-	-
Waist-height ratio	-	-

Group I: Control (No supplementation).

Group II: Dietary supplementation of milk and its products.

a significant (p≤0.01) increase in BMI of control group was observed which could be due to increase in the weights of this group.

On the other hand, WHR remained same in both the groups. The values were found to be lower in comparison to reference value of 0.8. Similarly, the waist height ratio of the subjects in the experimental group was also lower than the reference value of 0.5 both before and after the supplementation trial.

Table 4 shows the percent change in anthropometric measurements in the control and experimental group. A slight non-significant decrease in the weight, waist, hip circumference and BMI of the experimental group was observed after the intervention trial. A study reported that children consuming highest dairy intake group were 38% less likely to be overweight or obese compared to those in the lowest dairy intake group (Lu *et al*., 2016). An increase in dairy intake of one serving per day was associated with 0.65% lower body fat a 13% lower risk of overweight or obesity.

Body composition parameters

As individuals age, percent body fat increases and lean mass and bone mineral density decrease. Furthermore, the increase in fat mass (FM) is distributed more specifically in the abdominal region, an area associated with cardiovascular disease and diabetes (Gallagher *et al*., 2010). Therefore, it is pertinent to maintain good body composition with more of muscle mass and less of fat mass. The body composition parameters of the subjects in control and experimental group before and after the supplementation trial have been presented in Table 5.

The fat mass% and Fat Mass Index decreased significantly (p≤0.01) in Group II after the supplementation trial however, a significant (p≤0.05) increase in Fat Mass Index (p≤0.05) was observed in Group I with no supplementation. On the other hand, the visceral fat also decreased significantly (p≤0.05) in Group II. The Muscle Mass, Muscle Mass% and Muscle Mass Index increased significantly (p≤0.05 and 0.01) in Group II as contrary to Group I.

Fat-free mass index and fat mass index eliminate the differences of the body fat percentage associated with one's height and can independently evaluate body fatness from changes in FFM and therefore, can be a useful measure of obesity. The FMI and FFMI of the subjects in experimental group in the supplementation plan was higher when compared with the reference value of FMI and FFMI as 5.5 and15.4 kg/m^2, respectively (Schutz *et al*., 2002).

Increase in FFMI showed a positive effect of milk and its products. These findings are in accordance with another study reporting that consumption of fat-free fluid milk can help in gaining greater muscle mass and considerable loss in fat mass (Thorning *et al*., 2016). Similarly, Iglay *et al*. (2009) found that consumption of omnivorous diets with a higher protein intake by consuming more eggs and dairy foods were effective in significantly increasing the lean body mass.

The body composition parameters were compared after the supplementation trial (Fig 1-3). The subjects of Group II reported loss in percent body fat and visceral fat rating after the supplementation. Increase in percent muscle mass (3.37%), skeletal muscle mass (3.98%) and percent bone mass (2.72%) was also observed in Group II as compared to decrease in the same parameters of Group I subjects.

The supplementation plan resulted in a decrease of 6-8% in percent body fat, 3-9% in visceral fat rating and an increase of 3-4% in muscle mass, 3-5% in skeletal muscle mass and 2-4% in bone mass. The positive change observed in body composition parameters in experimental group was attributed to balancing of the meals of young women for lysine, calcium and vitamin D. Hence, usefulness of diets balanced for indispensable amino acid lysine, along with calcium and vitamin D through the inclusion of milk and its products cannot be undermined where the regular diets are grossly inadequate in these vital nutrients.

Blood parameters

Vitamin D has important role in cardiovascular and musculoskeletal function. Adequate serum 25 (OH) D has been correlated with muscle strength and the ability to improve strength. 25(OH)D has many important roles in the human body so it is essential to get it assessed in routine.

The mean serum 25 (OH) D levels of the subjects before supplementation were very low (9.20 to 12.75 ng/ml) in control and experimental group. The experimental group was given a sachet of 60000 IU of vitamin D once a week for 12

Table 5: Body composition of selected young adult women before and after the supplementation trial (N=60).

Body composition	Group I (n=30)			Group II (n=30)		
parameters	Before	After	t-value	Before	After	t-value
Fat mass (Kg)	13.82±6.41	14.38±6.31	2.51*	16.59±7.03	15.53±7.12	4.77**
Fat mass%	24.13±7.56	24.55±7.73	1.08NS	27.47±6.72	25.65±7.07	5.27**
Fat mass index	5.55±2.49	5.77±2.45	2.49*	6.80±2.91	6.37±2.96	4.79**
Visceral fat rating	1.77±1.17	1.80±1.27	0.87NS	2.43±1.85	2.20±1.75	2.54*
Muscle mass (Kg)	38.82±3.10	38.42±2.98	1.52 NS	39.42±3.23	40.65±3.38	6.56**
Muscle mass, %	72.00±7.15	70.34±7.08	3.68**	68.76±6.50	71.08±6.71	5.75**
Skeletal muscle mass	21.85±2.14	18.76±2.51	13.23**	19.84±3.19	20.63±2.83	3.20**
Muscle mass index	8.81±0.79	7.56±0.95	13.29**	8.11±1.19	8.43±1.09	3.30**
Fat free mass (Kg)	40.90±3.26	41.19±3.34	1.36 NS	41.53±3.39	42.74±3.57	5.59**
Fat free mass,%	75.87±7.54	79.48±6.87	5.98**	72.45±6.85	74.74±7.08	5.38**
Fat free mass index	16.49±1.14	16.60±1.15	1.35 NS	17.01±1.58	17.51±1.67	5.57**
Bone mass (Kg)	2.09±0.17	2.07±0.16	0.94 NS	2.11±0.16	2.16±0.17	5.11**
Bone mass%	3.87±0.40	3.80±0.42	3.12**	3.68±0.36	3.78±0.36	4.62**

Group I: Control (No supplementation); Group II: Dietary supplementation of milk and its products.
Values are Mean±SD; *Significant at 5%;**Significant at 1%, NS: Non-Significant.

Table 6: Blood parameters, Bone mineral density (BMD) and physical fitness of selected young adult women before and after the supplementation trial (N=60).

Blood parameters	Group I (n=30)			Group II (n=30)		
	Before	After	t-value	Before	After	t-value
Vitamin D (ng/ml)	9.62±4.87	11.33±7.80	1.33 NS	9.20±3.70	31.82±13.02	8.87**
Calcium (mg/dl)	9.38±0.25	9.41±0.24	0.74 NS	9.38±0.25	9.34±0.27	0.47 NS
Albumin (g/dl)	4.63±0.23	4.52±0.26	0.64 NS	4.61± 0.22	4.69±0.19	0.52 NS
BMD T-scores	-1.26±0.71	-1.37±0.86	0.59NS	-1.07±0.25	-1.01±0.65	0.61 NS
VO_{2max}	35.25±2.05	33.46±1.74	4.78**	34.46±2.49	35.53±3.20	2.40*

Group I: Control (No supplementation).
Group II: Dietary supplementation of milk and its products.
Values are Mean±SD; *Significant at 5%,**Significant at 1%, NS: Non-Significant.

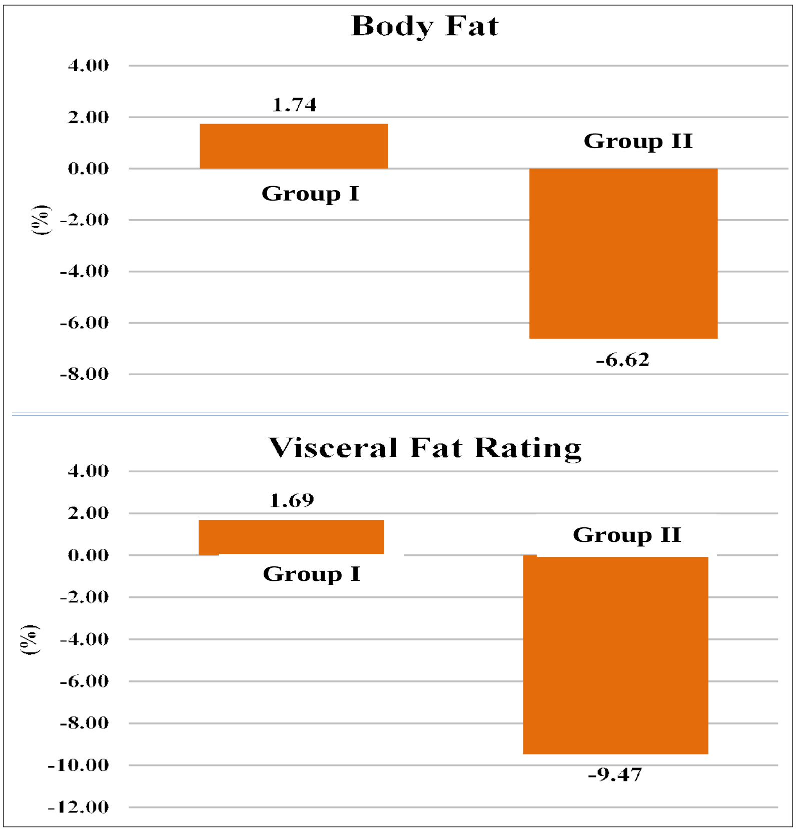

Fig 1: Per cent changes in body fat and visceral fat rating of the selected young women after 12 weeks supplementation trial.

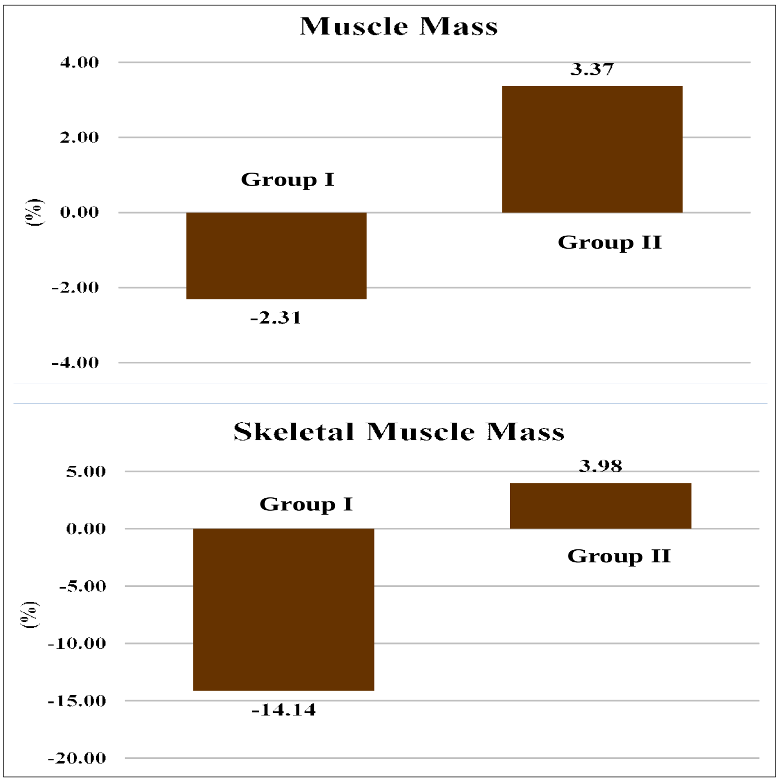

Fig 2: Per cent changes in muscle mass and Skeletal muscle mass of the selected young women after 12 weeks supplementation trial.

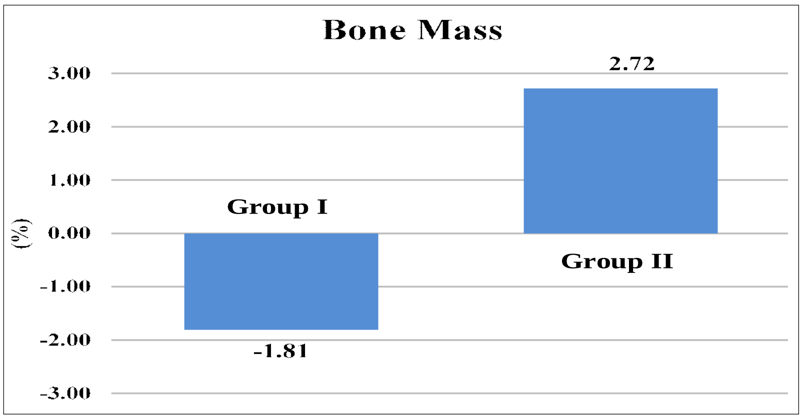

Fig 3: Per cent changes in bone mass of the selected young women after 12 weeks supplementation trial.

weeks. The levels of serum 25 (OH) D increased significantly ($p \leq 0.01$) in Group II from 9.20±3.70 to 31.82±13.02ng/ml after the supplementation.

The vitamin D levels in control group were low after the supplementation trial (Table 6). A study reported that levels above 30 ng/ml are optimal for overall health and may be required to support exercise regimens sufficient to maintain a healthy body composition (Holick, 2007). On deriving the correlations of vitamin D and other parameters, it was found that vitamin D levels were positively and significantly associated with fat free mass%, muscle mass% and $VO_{2\ max}$, the value of r being 0.540**, 0.821** and0.232* while it was negatively but significantly correlated with weight, BMI, fat mass, fat mass index and visceral fat with r values as- 0.782**, -0.831**, -0.208*, -0.199* and -0.285**, respectively. Overweight individuals that lose weight, have a subsequent increase in serum 25 (OH) D levels (Funderback *et al*., 2018). An inverse association was also observed between serum 25 (OH) D and waist circumference, a measure of visceral fat (Cheng *et al*., 2010).

Calcium works in conjunction with vitamin D to support bone health with adequate intakes of vitamin D promoting increased intestinal absorption of calcium. The calcium and albumin levels of the subjects in the experimental group were found to be normal both before and after the supplementation period which could be attributed to the calcium homeostasis in the body.

CONCLUSION

Protein quality and quantity is vital to maintain optimum muscle mass which is responsible for balancing various metabolic activities for sustainability of healthy human life. The Indian vegetarian diets being deficient in lysine, calcium and vitamin D have negative effect on muscle mass and bone health. The daily diets of the selected subjects met only 55% of the recommended level of lysine. Twelve weeks of supplementation of milk and milk derived foods along with weekly supplementation of vitamin D resulted in a significant reduction in body fat and visceral fat and a significant increase in skeletal muscle mass and bone mass. It is concluded that improving the quality of diets through dietary supplementation can be an effective approach to achieve optimum body composition. Food fortification and/ or targeted nutrition supplementation policies must be implemented to reduce the burden of nutrient deficiency-related conditions in the vulnerable populations.

Conflict of interest: None.

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