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EFFECT OF HIGH PROTEIN MEAL CONSUMED AT DIFFERENT FREQUENCY ON SERUM IGF-1 LEVELS IN PHYSICALLY ACTIVE INDIVIDUALS

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Abstract

Previous research studies suggest that consuming multiple small meals per day may increase insulin-like growth factor-1 (IGF-1), whereas less frequent meals may reduce circulating IGF-1 response. However, limited evidence exists regarding the effects of extremely low meal frequencies, such as one meal a day (OMAD) or two meals a day (2MAD), particularly in physically active individuals. Therefore, the present study aimed to investigate the impact of meal frequency on serum IGF-1 levels following high protein diet in physically active adults. In a cross-over study, 11 physically active participants consumed a high protein meal at two different frequencies in two testing sessions. In the first session, the participants consumed a single meal of 800 Kcal as OMAD, while in second session participants consumed 800 Kcal at two time points (i.e., 400 each). Anthropometric measurements were recorded using standard procedures. Fasting and post-prandial blood samples were collected for 12 hours in both sessions. Results show that from the Baseline (t = 0 min) to the time point 180-min, the mean serum IGF-1 levels for 2MAD were slightly higher compared to OMAD. However, mean serum IGF-1 area under the curve (AUC)(0-720min) was significantly higher (p = 0.046) for OMAD than for two meals a day. Furthermore, correlation analysis conducted during both sessions revealed a significant association between body weight and the serum IGF-1 response. In conclusion, meal frequency had a modest but statistically significant impact on IGF-1 levels postprandially in physically active individuals. These findings suggest that consuming high-protein meal as OMAD led to significant rise in IGF-1 levels compared to 2MAD in physically active individuals. However, the difference is minimal with limited statistical significance requiring further research.

Keywords: *2MAD, AUC, IGF-1, Meal frequency, OMAD, Physically active individuals*

INTRODUCTION

Physical activity, defined as voluntary skeletal muscle movement requiring energy expenditure, imposes greater demands on energy metabolism, fluid balance, and muscle repair (1). In order to facilitate these physiological functions, optimal nutrition is essential. Meal frequency defined as the total number of meals and snacks consumed in a day has become a growing area of interest due to evolving dietary patterns. In recent years individuals have adopted divers eating patterns such as grazing, intermittent fasting, or eating multiple small meals throughout the day. Although the importance of meal composition is well established, the optimal meal frequency for metabolic and hormonal health particularly in physically active individuals remains uncertain (2). The relationship between meal frequency and dietary requirements of physically active individuals is influenced by a variety of factors including the type and intensity of exercise, specifies training goals and general eating habits. Therefore, the meals frequency may need to be individualized based on individuals lifestyle, preferences and performance objectives (3).

Insulin-like growth factor-1 (IGF-1) is a peptide hormone produced in the liver in response to growth hormone to a larger extent and also influenced by nutrition. It is responsible for tissue growth, muscle regeneration and cardiovascular well-being (4 - 6). Dietary factors affecting modulation of IGF-1 includes total protein intake, calories availability and frequency of meals (7, 8).

Previous studies have shown that increased meal frequency might have enhanced IGF-1 levels, presumably through episodic sections of insulin (9). While lower meal frequency consumed as the one meal a day (OMAD) has been associated with reduced IGF-1 levels, lower caloric intake and possible advantage of longevity (10). However, much of the current data is derived from sedentary or mixed populations with limited data specifically focused on physically active individuals (11).

The effects of meal frequency on serum IGF-1 concentrations in active populations remain unclear, with inconsistent findings reported in the literature. Some key questions remain unsolved: What are the effects of high vs. low meal frequency on serum IGF-1 concentrations in exercise-trained humans? Does eating single meal daily (OMAD) vs multiple meals change IGF-1 differently? Is the impact of meal frequency on IGF-1 concentrations mediated via insulin responses or caloric equilibrium?

Based on current evidence, we hypothesized that serum IGF-1 levels would not differ significantly across various meal frequency patterns in physically active individuals. Therefore, the present study aimed to investigate the impact of low and moderate meal frequencies on serum IGF-1 concentrations in physically active individuals. Addressing this knowledge gap may help determine whether meal frequency can be strategically manipulated to optimize recovery, muscle growth, and metabolic health in physically active individuals.

MATERIALS AND METHODS

The current research utilized a cross-over design and was carried out at Institute of Basic Medical Sciences (IBMS), Khyber Medical University (KMU), Peshawar, Pakistan. The study was approved by the Advanced Study and Research Board (DIR/KMU-AS&RB/EO/IBMS/002322) of Khyber Medical University and ethical approval was obtained from the institutional Research Ethical Committee (KMU/IBMS/IRBE/8th Meeting/2024/1685-P) of Institute of Basis Medical Sciences, KMU.

Physically active men aged 18-25 years, with body mass index (BMI) values ranging from 18 to 25 kg/m^2 and percentage fat mass ranging from 12% to 22%, were recruited. Individuals with history of smoking, diagnosed illness, recent weight loss or gain of ~10% over three months and using drugs such as corticosteroids regularly were excluded from the study. Participants who met the eligibility criteria were fully informed about the study protocol and provided written informed consents. All the participants received meals of 800 Kcal at two different frequencies, as One-Meal-A-Day (OMAD) and Two-Meals-A-Day (2MAD) during two testing sessions. Participant recruitment and flow through the study are shown in Fig. 1.

The sample size for the present study is calculated based on a study conducted by Messina, Joan Sanchez-Gurmaches, et al. on meal frequency, using IGF-1 as the outcome variable. This study found that 11 individuals are required to detect a significant difference in IGF-1 levels, with the power of 80% and $\alpha \leq 0.05$ (12). In terms of statistical analysis, effect sizes were estimated, and comparisons were made on a within-subject basis to optimize power. Fifteen participants were screened for eligibility, four of whom were excluded due to not meeting the inclusion criteria or refusing to participate. Eleven eligible participants were randomized to receive either the OMAD intervention first and then 2MAD, or vice versa. Participants completed both sessions and were included in the final analysis (Fig. 1).

On every study visit, the participants were given a standardized meal that consisted of 800 calories. The macronutrient composition of the meal was as follows: 26.5% carbohydrates (about 212 kcal), 40.5% proteins (about 324 kcal), and 33% fats (about 261 kcal).

Meal frequency protocols were as follows:

- At the first visit (One-Meal-A-Day; OMAD protocol), the whole 800-calorie meal was taken in one sitting in the morning (Table I).
- On the second visit (Two-Meals-A-Day; 2MAD protocol), the 800-calories were separated into two meals of 400 calories each, eaten in the morning and after six hours after the first meal.

- All protocol sessions lasted 12 hours, during which the participants were kept under observation for blood collection and outcome evaluation.
- Tables I and II shows details of the standardized meal used in this study.
- The standardized 800-calorie meal was composed of grilled chicken, egg, and milk (as the major protein source), bread (roti: as a source of carbohydrates), served with a portion of mixed vegetables.
- This food combination was chosen to provide high-quality protein, complex carbohydrates, and healthful unsaturated fats. The participants consumed water *ad libitum*.

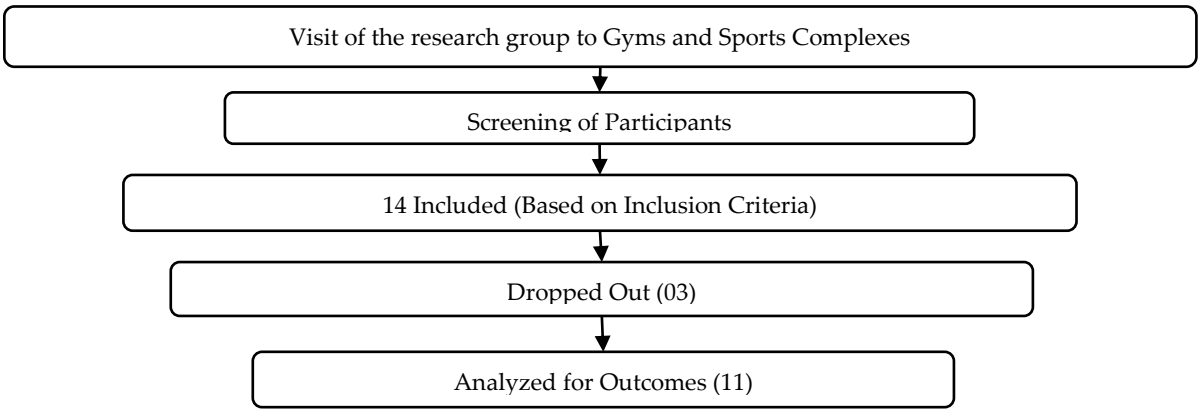

Fig. 1. Participants recruitment flowchart

Table I. Session-1 (One meal a day)

Ingredients	Quantity	CHO (g)	Protein (g)	Fats (g)	Calories (K cal)
Grilled Chicken	220g	0g	58g	22.5g	435
Large boiled egg	1 (50g)	1g	5.5g	6g	80
Mixed vegetables	1 Cup	7.5g	2.5g	0.5g	45
Skimmed milk	250ml	12g	10g	0g	90
Roti	1 (100g)	32.5g	5g	0g	150
Total Calories		53g (26.5%)	81g (40.5%)	29g (33%)	800

Table II. Session-2 meal (Two meals a day - morning & evening)

Ingredients	Quantity	CHO (g)	Protein (g)	Fats (g)	Calories (K cal)
Grilled chicken	110 g	0	14.5	11.25	217.5
Boiled egg	½	0.5	2.75	3	40
Mixed vegetables	½ Cup	3.75	1.25	0.25	22.5
Skimmed milk	125 ml	6	5	0	45
Roti	½	16.25	2.5	0	70
Total Calories		26.5 (26.5%)	40.5 (40.5%)	14.5 (33%)	400

Blood samples from all participants were collected into serum separator (gel) tubes. Blood was allowed to clot at room temperature for 10-15 minutes, after which it was centrifuged at 1000 rpm for 15 minutes at 4°C. The serum, separated after centrifugation, was handled gently and moved to sterile 0.5 mL cryovials and then placed at −80°C until analysed for biochemical assessment.

Serum levels of IGF-1 were determined by Human IGF-1 ELISA kits (Bioassay Technology Laboratory, Shanghai, China; Catalog No: YX-090707H, Batch No: 202311). All the assays were performed in the Physiology Laboratory of the Institute of Basic Medical Sciences (IBMS), Khyber Medical University (KMU), Peshawar, Pakistan. The assay was conducted as per the manufacturer's protocol. Optical density (OD) was read at 450 nm on a microplate reader (Model: ELx800, BioTek Instruments, USA). Results were determined from the standard curve and reported in ng/mL. All the serum samples were tested in duplicate to provide confidence in measurements. Intra-assay and inter-assay coefficient of variation (CV) were kept at <10%. Each assay run was calibrated by plotting calibration curves, and control samples supplied by the manufacturer were incorporated to assess assay performance. Duplicate samples with a coefficient of variation >15% were retested.

STATISTICAL ANALYSIS

Data were entered and processed by the Statistical Package for the Social Sciences (SPSS) software, version 26. Descriptive statistics, such as mean and standard deviation (SD), were computed for baseline characteristics like age, weight, height, BMI, muscle mass, waist circumference, and serum IGF-1 levels. Paired sample t-tests were utilized to contrast serum IGF-1 concentrations between the OMAD and 2MAD protocols. Shapiro-Wilk's test was applied to check data distribution. Pearson's correlation analysis was employed to investigate the correlation between meal frequency and IGF-1 concentrations.

Spearman's correlation coefficients were employed to determine the correlations between the non-normally distributed variables, such as body composition parameters, waist circumference, and age. The serum IGF-1 area under the curve (AUC) at pre-specified time points was computed by the trapezoidal rule. The trapezoidal rule approximates the integral by breaking the curve into several trapezoids, computing the area of each trapezoid separately, and adding them to obtain the overall AUC. A p-value of < 0.05 was used as statistically significant.

RESULTS

Table III shows the baseline characteristics of study participants (n = 11). The mean age, weight, height, and body mass index (BMI) was 21.91 ± 1.64 years, 74.73 ± 4.45 kg, 1.88 ± 0.10 meters and 21.27 ± 2.08 kg/m^2, respectively. Body composition measurement shows that the mean muscle mass was 30 ± 2.32 kg and muscle mass percentage was 40 ± 2.39%, while the waist circumference was 39 ± 1.58 inches.

Table III. Baseline characteristics of study participants (n = 11)

Baseline characteristics	Mean ± SD
Age (Years)	21.91 ± 1.64
Weight (Kg)	74.73 ± 4.45
Height (m)	1.88 ± 0.10
BMI (kg/m^2)	21.27 ± 2.08
Muscle Mass (kg)	30 ± 2.32
Muscle Mass (%)	40 ± 2.39
Waist Circumference (inches)	39 ± 1.58

*BMI: Body Mass Index

Comparison of mean serum IGF-1 levels for both meal frequencies at different time intervals is shown in Fig. 2. From Baseline (t = 0 min) to 180 min, the mean serum IGF-1 levels tend to have a slight difference in both sessions (OMAD & 2MAD). However, serum IGF-1 levels for 2MAD were slightly higher compared to OMAD. This suggests that the 2MAD pattern leads to slightly higher IGF-1 levels shortly after meal consumption, as compared to OMAD. At 360 min time point (t = 360 min), there was a major difference between mean IGF-1 levels on both days. The major difference in IGF-1 levels in both sessions was also seen between 180 mins (t = 180 mins) and 360 mins (t = 360 mins). However, at every other time point in both sessions (OMAD & 2MAD), there was a very slight difference in IGF-1 levels. This suggests that IGF-1 levels tend to remain almost similar in both meal frequencies i.e., OMAD and 2MAD. These findings indicate that although 2MAD showed a short-term rise, OMAD had higher overall IGF 1 across the day (Fig. 2).

A comparison of the Area under Curve (AUC) for OMAD and 2MAD is presented in Table IV. The findings reveal that the area under the IGF-1 curve was elevated for OMAD with a mean of 28908.04 ± 5149.94 ng/ml. In contrast, 2MAD displayed a slightly lower mean of 27703.30 ± 4360.70 ng/ml. However, it is worth mentioning that the difference was statistically significant though biological relevance may be limited.

These findings indicate a statistically significant difference in serum IGF-1 levels between two meals, with OMAD showing higher levels of IGF-1. These findings highlight the importance of understanding the impact of meal frequency on IGF-levels in physically active individuals.

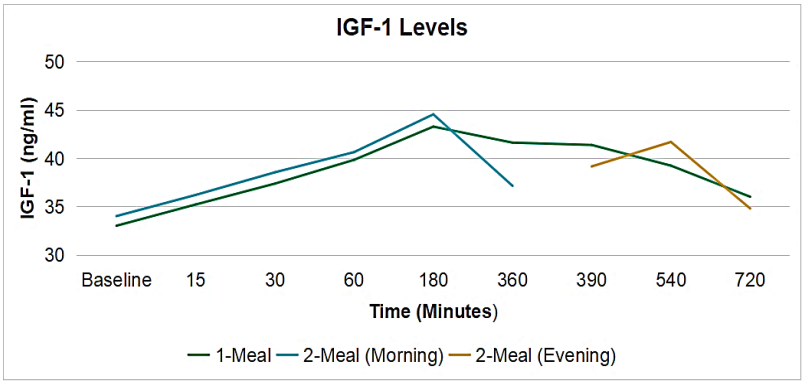

Fig. 2. Comparison of serum IGF-1 levels at different time points for one meal a day (OMAD) and two meals a day (2MAD) eating pattern

Table IV. Comparison of IFG-1 area under the curve (AUC) between one meal a day (OMAD) and two meals a day (2MAD) eating patterns (n = 11)

	Mean ± SD	P-Value
IGF-1 (ng/ml) AUC OMAD	28908.04 ± 5149.94	0.046
IGF-1 (ng/ml) AUC 2MAD	27703.30 ± 4360.70	

Table V shows the correlation among age, body composition, waist circumference and serum IGF-1 levels in physically active individuals. A significant positive correlation was reported in participants' weight and muscle mass (r = 0.635, p = 0.036), indicating that individuals with higher body weight tend to have greater muscle mass, which implies connection between body weight and muscle development. Muscle mass and muscle percentage were positively correlated (r = 0.673, p = 0.023), indicating that individuals with higher muscle mass tend to have greater percentage of muscle. This emphasizes the link between overall muscle mass and the proportion of muscle in the body. Additionally, weight shows a significant positive correlation with IFG-1 AUC (OMAD & 2MAD) (r = 0.649, p = 0.31 & r = 608, p = 0.048), implying that individuals with higher weight tend to have higher values for AUC 1 & 2 combined. Overall, these findings highlight the relationship between IGF-1 levels and factors influencing muscle mass such as body composition, weight and age.

Table V. Correlation between anthropometric measures, body composition and serum IGF-1 AUC for OMAD and 2MAD (n = 11)

	Age (Years)	Weight (Kg)	Height (m)	BMI (kg/m^2)	Muscle mss (Kg)	Muscle mass (%)	Waist circumference (Inches)	Serum IGF-1 AUC (OMAD)
Weight (Kg)	-0.223							
Height (m)	-0.052	0.585						
BMI (Kg/m^2)	-0.013	0.003	**-0.795***					
Muscle Mass (kg)	-0.558	**0.635***	0.067	0.300				
Muscle Mass (%)	-0.381	0.059	-0.160	0.138	**0.673***			
Waist Circumference (Inches)	-0.184	-0.054	0.054	-0.123	0.026	-0.013		
Serum IGF-1 AUC (OMAD)	0.187	**0.649***	0.334	0.049	0.317	-0.245	0.156	
Serum IGF-1 AUC (2MAD)	0.266	**0.608***	0.258	0.098	0.268	-0.212	0.042	0.946

Values are correlation coefficients. *p < 0.05. BMI: Body Mass Index; AUC: Area Under the Curve; OMAD: One Meal a Day; 2MAD: Two meals a Day

DISCUSSION

In the current study the impact of high protein diet consumed at two different frequencies as OMAD and 2MAD on serum IGF-1 response was evaluated in physically active individuals. Results show

that both meal frequencies produced comparable pos-prandial IGF-1 response during the initial 6 hours, indicating that immediate hormonal responses are primarily influenced by meal composition rather than frequency of consumption. However, over the 12 hours period, a slight statistically significant increase in IGF-1 AUC is observed following OMAD compared to 2MAD. These implied that short-term rise in IGF-1 dynamics remain similar, meal frequency may influence cumulative hormonal response.

These results agree with those of Longland *et al.,* who observed significance difference in IGF-1 AUC across two meal patterns (3MAD & 6MAD) (13). Similarly, animal studies have also reported that variation in meal frequencies including intermittent fasting can influence IGF-1 levels in mice and rats (14), supporting the role of meal frequency in hormonal regulation.

In contrast to the study of Fontana *et al.,* who reported reduced IGF-1 levels following long-term low-protein, low-calorie diet combined with endurance exercise, the present study utilizes high protein dietary intervention (15). This may explain the maintenance of IGF-1 levels across both meal frequencies, highlighting the vital role of protein intake in regulating the IGF-1 axis and potentially attenuating the suppressive effects of reduced meal frequency.

The biological processes underlying the reported effects of meal frequency on IGF-1 concentrations in physically active humans may include several interrelated processes including nutrient signaling, insulin sensitivity, hormonal control, and cell growth (16). Fasting or lower meal frequency (as OMAD and 2MAD) has been shown to enhance insulin sensitivity. In fasting state insulin secretion is decreased, potentially making insulin more effective at stimulating glucose entry when food is finally consumed (17). Meal frequency can influence insulin dynamics, which share overlapping signaling pathways with IGF-1 (18). The consumption of a single meal as OMAD may induce a quick insulin response compared to 2MAD which may produce a more sustained insulin response, thereby influencing hepatic IGF-1 production. In addition, protein intake, particularly amino acid such as leucine promotes IGF-1 release through nutrient-sensing pathways, such as mechanistic Target of Rapamycin (mTOR) signaling pathway (19). Thus, distribution of protein intake into various meals may differentially regulate this response.

Hormonal regulation may also explain the finding of this study. Growth hormone (GH) also plays role in regulation of IGF-1 response, which is secreted in pulsatile manner and may be elevated in fasting state or with lower meal frequency. This explains the slightly higher IGF-1 response with OMAD in the present study. In addition, fasting related metabolic alterations (20), including enhanced insulin sensitivity (17) and increased fat oxidation (21), may indirectly modulate IGF-1 responses.

The results of the current study may have practical implications for physically active individuals and athletics due to the influence of meal frequency on energy availability, recovery and metabolic responses during training (22, 23). Although lower meal frequency and fasting may enhance fat oxidation during activity such exercise and training, distribution of protein intake across meals may better support muscle protein synthesis and recovery. Therefore, meal frequency plays an important role in achieving certain goals during training and exercise besides meal composition and caloric intake.

Regardless of the findings of this study, there are certain potential limitations. The inclusion of only male, relatively small sample size and acute nature of the study may limit the generalizability of these findings to other diverse population and to assess other long-term metabolic adaptions. In addition, only one type of meal composition, i.e., high protein was tested, which further limits the application across other types of meal compositions.

In summary, while OMAD resulted in a slightly higher overall IGF-1 response compared to 2MAD, the difference was modest. This suggests that meal frequency alone may have a limited impact on IGF-1 regulation when protein intake is adequate. Future studies should include larger and more diverse populations, particularly women, and explore the effects of different meal compositions and longer intervention periods to better explain the relationship between meal frequency and IGF-1 responses.

CONCLUSION

In conclusion, meal frequency had a modest but statistically significant impact on IGF-1 response postprandial in physically active individuals. These findings suggest that consuming high-protein meal as

OMAD led to significant rise in IGF-1 response compared to 2MAD in physically active individuals. However, the difference is minimal with limited statistical significance requiring further research. In addition, IGF-1 and body weight are positively correlated.

Conflict of interest:

There is no conflict of interest in this study.

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Authors' contribution:

MOM Conceptualization, formal analysis, writing–original draft; BH & Eh Investigation: DEM BH & IA critical analysis, review and editing.

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