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Role of Ghrelin and Leptin on Skeletal Muscle Function of Metabolic Syndrome Patients

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Abstract: ***Background: Metabolic syndrome (MS) is a group of risk factors that raises risk of heart disease, diabetes, stroke, and other health problems. Aim and Objectives: The study aimed to find out role of ghrelin and leptin on skeletal muscle function of metabolic syndrome participants. Materials and Methods: This was a randomized cross - sectional study involved 150 MS patient. Fasting blood sugar, glycated hemoglobin, lipid profiles, ghrelin and leptin assays, and other anthropometrics measures were measured using standard protocols. Results: The results revealed that there was significant correlation between leptin level and nerve conduction study (NCS), and electromyograph (EMG). Conclusion: This study was showed that there was significant role of leptin level on skeletal muscle function.***

Keywords: Leptin; Metabolic syndrome; Ghrelin

1. Introduction

Ghrelin was discovered in 1999. Through its relation to specific hypothalamic receptors can express its orexigenic effect. The hormone is an acylated peptide, contains of 28 amino acids that is act as the endogenous ligand to the secretagogue receptors of growth hormone [1]. The hormone is secreted primarily by endocrine cells of the oxyntic glands in the fundal region and body of the stomach, the mucosa of the most part of small intestine, the lungs, the urinary tract organs, and the pituitary gland. The octanoylation of the inactive form has been done by enzyme ghrelin O - acyltransferase to change thehormone from inactive form to active one. An acyl side chain is critical both for the orexigenic and the gastric - emptying actions of ghrelin [2]. Its discovery has led to the modification of many physiological concepts and functions. There are many actions of this hormone in the human body, ranging from its role in the regulation of the immune and cardiovascular systems to the up - regulation of insulin - like growth factor, it has a pivotal role in the gastrointestinal system, with involvement in gastric emptying and intestinal motility [3] [4] [5]. Leptin, the adipocyte - derived hormone identified in 1994 for its main function in control of satiety and body weight regulation, is an adipokine secreted in a sex - specific manner. Leptin is hormone secreted by adipose tissues, normally send massages to the brain to tell it about the status of the energy store, the interest in leptin focused basically on appetite, metabolism, and adiposity. However, evidence was rapidly provided that this hormone exerts a number of extra - metabolic effects [6].

Metabolic syndrome is a cluster of risk factors that increase risk of cardiac problems, diabetes, cerebrovascular accident, and other health complications. When three of five following risk factors arepresent, metabolic syndrome has been diagnosed:

- Elevated blood glucose (sugar).
- Decreased concentration of HDL in the blood.
- Increased concentration of triglycerides in the blood.
- Large - scale waist circumference.
- Elevated arterial blood pressure.

Metabolic syndrome (MetS), also known as syndrome X, insulin resistance and other terms in the literature, is a constellation of cardiovascular disease risk factors that has been defined slightly differently by various organizations. The three most commonly used definitions for surveys and health care plansare:

WHO 1999 [7]:
Insulin resistance or glucose more than 6.1 mmol/L (110 mg/dl), 2 h glucose more than 7.8 mmol (140 mg/dl) (required) in addition to any two or more of the following:

1) High density lipoprotein (HDL) cholesterol less than 0.9 mmol/L (35 mg/dl) in male, less than 1.0 mmol/L (40 mg/dl) in female.
2) Triglycerides more than 1.7 mmol/L (150 mg/dl).
3) Waist/hip ratio more than 0.9 (male) or more than 0.85 (female) or BMI more than 30 kg/m2.
4) Arterial blood pressure more than 140/90 mmHg.

NCEP (National Cholesterol Education Program) 2005:

Existence of any three or more of the following:

1) Blood glucose more than 5.6 mmol/L (100 mg/dl) or drug treatment for raised blood glucose.
2) HDL cholesterol less than 1.0 mmol/L (40 mg/dl) in men, less than 1.3 mmol/L (50 mg/dl) in female or drug treatment for low HDL - C.
3) Blood triglycerides more than 1.7 mmol/L (150 mg/dl) or drug treatment for raised triglycerides.
4) Waist circumference more than 102 cm (male) or more than 88 cm (female).

5) Blood pressure more than 130/85 mmHg or drug treatment for Hypertension.

IDF (International Diabetes Federation) 2006:

Waist circumference more than 94 cm (male) or more than 80 cm (female) in addition to existence of two or more of the following:

1) Blood glucose more than 5.6 mmol/L (100 mg/dl) or diagnosed diabetes.
2) HDL cholesterol less than 1.0 mmol/L (40 mg/dl) in male, less than 1.3 mmol/L (50 mg/dl) in female or drug treatment for low HDL - C.
3) Blood triglycerides more than 1.7 mmol/L (150 mg/dl) or drug treatment for elevated triglycerides.
4) Arterial blood pressure more than 130/85 mmHg or drug treatment for hypertension.

Other organizations like the American Association of Clinical Endocrinologist (AACE) 2003 and the European Group for the Study of Insulin Resistance (EGIR) used little bit different definitions but they are not as commonly used [7].

This study was conducted to emphasize the role of serum ghrelin and leptin level on skeletal muscle function of metabolic syndrome participants.

Materials and methods

The study was conducted between December 2017 and December 2020, it included 150 MS participants aged between 19 and 65 years old in Khartoum state (Sudan). Data on Personal information and anthropometric measures variables were collected through questionnaire, and personal interviews, physical examinations and blood samples collection were performed during two clinic visits.

Inclusion and Exclusion Criteria:
Selected participants should be meet followings criteria; diabetic type 2, obese, and dyslipidemic. While exclusion criteria were, type 1 diabetic patient, overweight or normal weight subjects, subjects with normal lipid profile, patient treated by exogenous insulin, age less than 19 and more than 65 years old.

Study Variables:
A questionnaire was completed for each patient including age, gender, and other variables. Weight, height, and waist circumferencefor each individual have been measured using electronic scales (digital personal scale for orbit, up to 180 kg - DLS 0.004, precision, 0.1 kg), portable extendable stadiometer (B07CBKDM9T Seca), and non - elastic flexible measuring tapes with buckles, respectively. Body mass index (BMI) was calculated as weight in kilos divided by the square of height in meters. Blood pressure was measured using validated automatic devices (omron M3 blood pressure monitor). Blood samples were obtained from each participant at refer clinic after 12 h fasting. We measured fasting blood glucose (FBG), glycosylated hemoglobin (HbA1c), insulin, total cholesterol, triglycerides (TGs), high - density lipoprotein (HDL) cholesterol, and low - density lipoprotein (LDL) cholesterol. Serum ghrelin were measured by enzyme immunoassay using two monoclonal antibodies (26G Keewaydin Drive, Alpco Diagnostics). These analyses were carried out using standardized methods at a central laboratory of general hospital.

Metabolic syndrome:
The National Cholesterol Education Program–Adult Treatment Panel III (NCEP ATP III) criteria were used to diagnose the MS. According to the NCEP ATP III definition, MS is present if three or more of the following five criteria are met: Waist circumference (WC) over 40 inches (men) or 35 inches (women), blood pressure over 130/85 mmHg or are taking blood pressure medication, fasting TG level over 150 mg/dl, fasting HDL< 45 mg/dl in men, and HDL < 55 in women A FBG level greater than 100 mg/dl or are taking glucose - lowering medications.

Ethical Clearance:
Written informed consent was obtained from all participants. Ethical approval was obtained from research committee of the department of physiology International Africa University. (Reference No.1/12/2017).

Statistical Analysis:
Analysis was performed using SPSS V 25.0 and Excel for 150 participants. All variables were expressed as mean (standard deviation), in addition to normal distribution of gender to their percentage. Leptin level was analyzed in correlation with age, gender, income, times of exercise/week, arterial blood pressure, FBG, HbA1c, insulin level, BMI, WC, T - cholesterol, and TG, correlation was significant when $P < 0.05$.

2. Result Findings

The baseline characteristics of the study participants are shown in Table 1. The mean age of the participants was 45.4 ± 13.5. The mean BMI of participants was 35.9 ± 3.7. The mean of WC/inches was 42.8 ± 3.6. The mean of total cholesterol mg/dl was 309.0 ± 69.1. The mean of total HDL mg/dl was 43.2 ± 12.1. The mean of total LDL mg/dl was 112.9 ± 28.1. The mean of TG mg/dl was 303.6 ± 103.2. The mean of FBG mg/dl was 141.4 ± 19.9. The mean of glycosylated hemoglobin (HbA1c%) was 6.8 ± 650. The mean of insulin level mμ/ml was 18.9 ± 9.8. The mean of leptin level ng/ml was 44.0 ± 21.8. The mean of active ghrelin level pg/ml was 18.6. Group 1, 48 participants (23 men, 25 women) which represent 32%, their active ghrelin level was ranged 16.9 - 17.9, group 2, 102 participants (61 men, 41 women) which represent 68%, their active ghrelin level was >17.9. The mean of total ghrelin level pg/ml was 655.1. Group 1, 4 participants (2 men, 2 women) which represent 2.7%, their total ghrelin level was ranged 600 - 639.9, group 2, 146 participants (82 men, 64 women) which represent 97.3%, their total ghrelin level was > 639.9.

Results presented in Table 2 show correlation of leptin level with nerve conduction study (NCS), and electromyograph (EMG) which are used for skeletal muscle function evaluation. There was significant correlation between leptin level and NCS $P < 0.05$. There was significant correlation between leptin level and EMG $P < 0.05$. Results presented in Table 3 show correlation of ghrelin level with NCS, and

EMG. There was no correlation between ghrelin level and NCS, and EMG P > 0.05.

Results presented in Table 4 show 15 EMG features and comparison between normal and abnormal EMG participants for tibialis anterior (TA) muscle, there was 46 normal participants compared with 104 abnormal (NCS), the results revealed that elevated figures of EMG features for abnormal rather than normal mostly.

Table 1: participants general characteristics

Variable	Total (150) mean ± (SD)	Men 84 (56%)	Women 66 (44%)	Percent (100%)
Age (year)	45.4 (13.5)			
19 - 35	38	23	15	25.30%
36 - 51	55	24	31	36.70%
52 - 65	57	37	20	38%
BMI	35.9 (3.7)			
30 - 34.9	70	48	22	46.70%
35 - 39.9	56	30	26	37.30%
≥ 40	24	6	18	16%
Waist circumference (WC) /inches	42.8 (3.6)			
≤40	42	12	30	28%
≥40.5	108	72	36	72%
Total cholesterol mg/dl	309.0 (69.1)			
192 - 300	77	52	25	51.30%
302 - 400	61	27	34	40.70%
415 - 560	12	5	7	8%
HDL mg/dl	43.2 (12.1)			
15 - 35	36	17	19	24%
36 - 56	95	56	39	63.30%
58 - 70	19	11	8	12.70%
LDL mg/dl	112.9 (28.1)			
70 - 120	118	70	48	78.70%
125 - 170	23	11	12	15.30%
180 - 220	9	3	6	6%
Triglyceride mg/dl	303.6 (103.2)			
150 - 300	82	52	30	54.70%
310 - 450	53	27	26	35.30%
460 - 600	15	5	10	10%
Fasting blood glucose mg/dl	141.4 (19.9)			
110 - 140	102	56	46	68%
145 - 170	35	24	11	23.30%
177 - 200	13	4	9	8.70%
HbA_1C %	6.8 (.650)			
6.5 - 7	138	80	58	92%
7.2 - 7.9	2	1	1	1.30%
08-11	10	3	7	6.70%
insulin level mμ/ml	18.9 (9.8)			
1 - 24.9	104	60	44	69.30%
≥25	46	24	22	30.70%
leptin level ng/ml	44.0 (21.8)			
2.5 - 21.8	11	10	1	7.30%
≥21.9	139	74	65	92.70%
Active ghrelin level pg/ml	18.6 (.922)			
16.9 - 17.9	48	23	25	32%
> 17.9	102	61	41	68%
Total ghrelin level pg/ml	655.1 (5.8)			
600 - 639.9	4	2	2	2.7
>639.9	146	82	64	97.3
Nerve conduction study	.653 (.477)			
Normal	52	37	15	34.70%
Abnormal	98	47	51	65.30%
Electromyogram	.693 (.462)			
Normal	46	33	13	30.7
Abnormal	104	51	53	69.3

Table 2: Correlations of leptin level with nerve conduction study, and electromyogram

		Leptin level	Nerve conduction study	Electromyogram
Leptin level	Pearson Correlation	1	.637**	.538**
	Sig. (2 - tailed)		0	0
	N	150	150	150

Table 3: Correlations of active ghrelin level with nerve conduction study, and electromyogram

		Active ghrelin level	Nerve conduction study	Electromyogram
Active ghrelin level	Pearson Correlation	1	-0.058	0.058
	Sig. (2 - tailed)		0.484	0.484
	N	150	150	150

Table 4: EMG features and comparison for tibialis anterior (TA) of diabetic polyneuropathy (DPN)

No.	EMG Features	Normal (n 52)	Abnormal (n 98)
1	Enhanced Mean Absolute Value (EMAV)	8.9	17.9
2	Enhanced Wavelength (EWL)	2.5+e03	6.1+e03
3	Mean Absolute Value (MAV)	25.6	73.3
4	Wavelength (WL)	2e+03	5e+03
5	Zero Crossing (ZC)	0	0
6	Slope Sign Change (SSC)	1807	1570
7	Root Mean Square (RMS)	33.6	94.8
8	Average Amplitude Change (AAC)	0.1735	0.5068
9	Difference Absolute Standard Deviation Value (DASDV)	0.3079	0.8788
10	Log Detector (LD)	21.3	44.1
11	Modified Mean Absolute Value (MMAV)	20.6	57.9
12	Myopulse Percentage Rate (MYOP)	1	1
13	Simple Square Integral (SSI)	1.7e+05	1.2e+09
14	Variance (VAR)	1.35e+03	9.67e+03
15	Maximum Fractal Length (MFL)	4.76	5.67

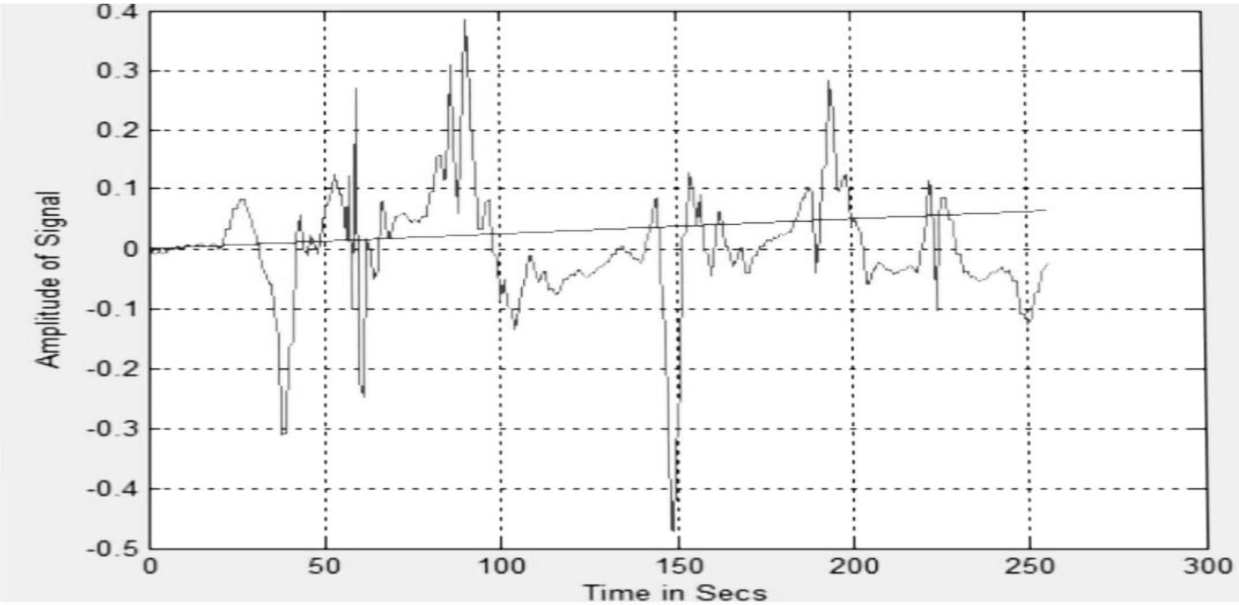

Figure 1: show myopathic participant pattern of signals taken for approximately 250 ms. shows the motor unit action potential MUAP have shorter duration, smaller in amplitude and increased polyphasicity

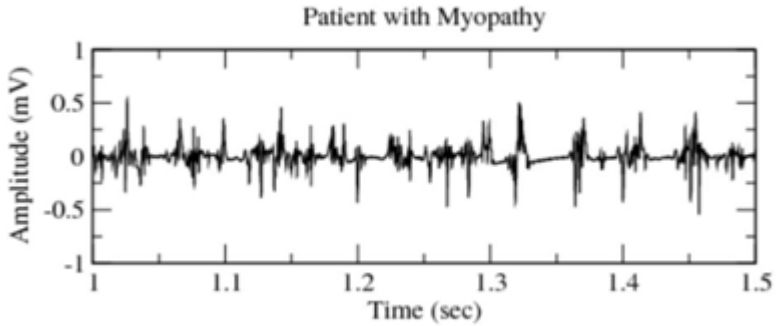

Figure 2: show myopathic participant pattern of signals taken for approximately 250 ms. shows the motor unit action potential MUAP have shorter duration, smaller in amplitude and increased polyphasicity. This figure is taken from previous study for comparison purpose [8].

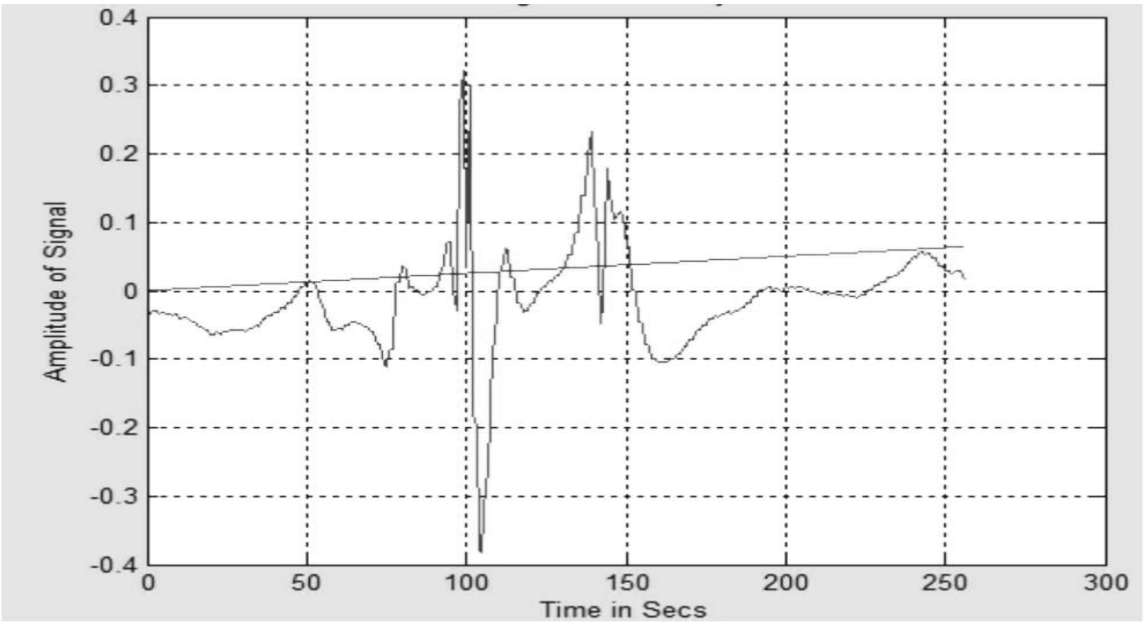

Figure 3: show healthy participant pattern of signals taken for approximately 250 ms. The signals are sharp at the middle and with less frequency spikes on both sides. The amplitude of the signal varies approximately between 0.3 and - 0.4.

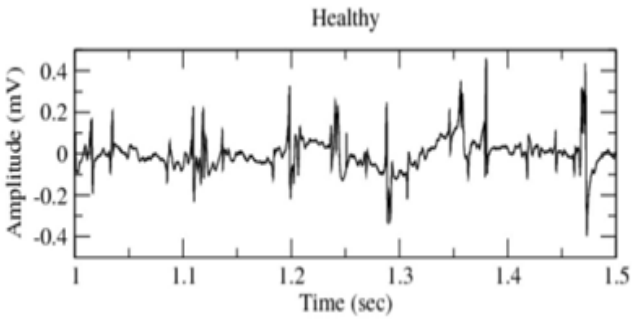

Figure 4: show healthy participant pattern. This figure is taken from previous study for comparison purpose [9].

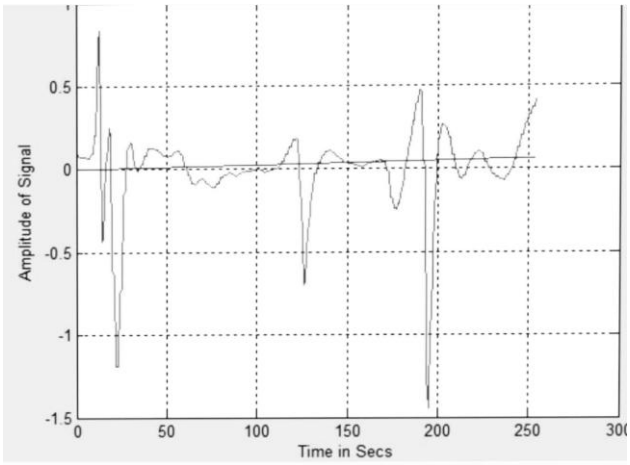

Figure 5: show neuropathic participant pattern of signals taken for approximately 250 ms. Neuropathy normally have high peak to peak but both are polyphasic which means they have around four phases in short durations.

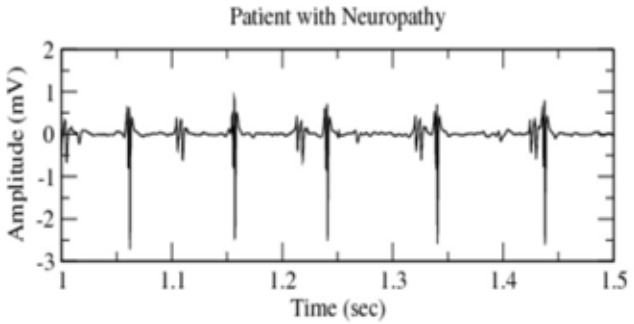

Figure 6: show neuropathic participant pattern of signals taken for approximately 250 ms. This figure is taken from previous study for comparison purpose [9]. Same characteristics of waves can be seen.

3. Discussion

In this study, we used two parameters for skeletal muscle function assessment, nerve conduction study (NCS), and electromyograph (EMG) which are widely used in clinical practice investigations.

There was significant correlation between leptin level and nerve conduction study, and electromyogram. P value < 0.05. all participants were metabolic syndrome and this will ensure the high level of plasma leptin and the muscle and nerve impairments. This study showed significantly higher levels of leptin in most patients 11 participants (8 men, 3 women) which represent 7.3%, their leptin level was ranged 2.5–21.8, within normal range, while 139 participants (74 men, 65 women) which represent 92.7%, their leptin level was ≥21.9, that above normal. Other studies reported similar findings that increased BMI was associated with increased leptin levels. These levels were directly proportional to subcutaneous fat and were inversely proportional to abdominal fat index and/ or waist - hip ratio. [10] This is logic explanation that might explain why leptin levels were higher in females rather than in males. As females have fat deposition in subcutaneous depot and males have more visceral fat. [11] Although it has clearly been established that females secrete 3–4 times more leptin than males and this sexual dimorphism in leptin secretion is exacerbated with overweight and obesity, the origin and the physiological consequences of this sexual dimorphism remain not will - defined [12] [13].

In this study, there was no significant correlation between active ghrelin level and NCS and EMG, however, there may be effect on muscle function generally. It had been reported that there are many arguments about exact ghrelin action on muscles. Muscle atrophy, characterized by reduced muscle mass and impaired muscle function, accompanies many diseases, such as cachexia from cancer, and muscle denervation. Ghrelin can indirectly increase muscle mass by increasing food intake and activating the GH/Insulin - like growth factor - 1 (IGF - 1) axis in cachexic mice [14]. There is also evidence of direct cellular effects. It has been shown that ghrelin promotes myocyte differentiation and fusion in C2C12 myoblasts [15]. A recent study further demonstrates that the protective effect of ghrelin in fasting - and denervation - induced muscle atrophy is mediated by mTOR and Akt signaling [16]. Overall, the results from many but not all ghrelin system loss - of - function mouse models suggest that the feeding response and body weight changes mediated by ghrelin are likely dispensable when food availability is plentiful. Contrary to the results from many of these loss - of - function mouse models, though, pharmacological intervention to inhibit the ghrelin system by reduction of bioavailable ghrelin or by daily administration of GHSR or GOAT antagonists to HFD fed mice causes lower body weights and/or reduced food intake [17] [18]. Thus, we believe it is still premature to write the definitive biography on the relationship of the endogenous ghrelin system to our overall body weight and feeding control systems. The role of the ghrelin system to body weight and feeding phenotypes observed under different nutritional environments and situations, in particular, remains enigmatic. Ghrelin had no impact on the mass of individual leg muscles, even when correcting for body mass. The ratio of wet to dry muscle was similar between groups (data not shown), suggesting that changes in fluid content were not responsible for changes in muscle mass. In the sub - group used for body composition analysis, body fat and protein were lower in both ghrelin - and saline - treated zymosan rats compared to naïve rats. While no difference was seen in body protein composition between ghrelin and saline - treated zymosan groups, the percentage of body fat was higher in those given ghrelin ($p<0.05$) [19].

In this study, fifteen EMG features presented are the ones extensively used for EMG signal classification in literature. The RMS value of the EMG signal for healthy subjects came out to be 33.6, whereas the RMS value for patients with DPN disorder was more than twice, i. e.94.8. Similarly, EMAV, EWL, MAV, WL, SSC, AAC, DASDV, LD, MMAV, MYOP, SSI, VAR and MFL demonstrated large differences in values, which could be a possible indication of abnormal muscular activations in patients with DPN disorder. The ZC for both the EMG signals was detected to be zero because of the physiological constraint, i. e. muscular activations can either be positive or zero [20] [21].

In this study 46 of 150 participants were normal EMG, pattern of signals taken for approximately 250 ms. The signals are sharp at the middle and with less frequency spikes on both sides. The amplitude of the signal varies approximately between 0.3 and - 0.4. The result was in agreement with other study [Swaroop R, Kaur M, Suresh P, Sadhu PK], while remaining 104 of 150 participants were abnormal EMG (myopathic), pattern of signals taken for approximately 250 ms. shows the motor unit action potential MUAP have shorter duration, smaller in amplitude and increased polyphasicity, the result was in agreement with other study [22].

In this study 52 of 150 participants were normal NCS, The RMS value of the EMG signal was 33.6, whereas the RMS value for remaining 98 of 150 participants with DPN disorder was more than twice, i. e.94.8. Similarly, EMAV, EWL, MAV, WL, SSC, AAC, DASDV, LD, MMAV, MYOP, SSI, VAR and MFL demonstrated large differences in values, in addition, pattern of signals taken for approximately 250 ms. Neuropathy normally have high peak to peak but both are polyphasic which means they have around four phases in short durations.

4. Strength and Limitation of the Study:

This study carried out on MS patients; they were (DM II, obese, and dyslipidemic). We examined the different anthropometric, and ghrelin, and leptin level were measured to investigate their role on skeletal muscle function. The study was carried out in specific region most of the participant share same lifestyle variables.

5. Conclusion

There was significant correlation between leptin level and nerve conduction study, and electromyogram, P value < 0.05. there was no significant correlation between ghrelin and NCS and EMG P value > 0.05. Most participants with abnormal EMG and NCS, which indicate the crucial role of leptin and to lesser extend ghrelin hormones in this abnormality.

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