

Original Research Article

COMPARATIVE STUDY OF THE LEVEL OF ATHROGENIC MARKERS; REMNANT CHOLESTEROL, NON-HDLc, TOTAL CHOLESTEROL-HDLc RATIOS AND LDLc-HDLc RATIOS, IN OVERWEIGHT AND OBESE SUBJECTS IN NNEWI SOUTH EAST NIGERIA.

ABSTRACT

BACKGROUND: Obesity elevates the risk of developing diabetes, coronary artery disease, fatty liver, gall stones, sleep apnea, arthritis and various types of cancer. Atherogenic indices have been suggested to be a link from obesity to coronary heart diseases, hence a predisposing factor in the development of cardiovascular disease by obese individuals. We looked at the level of these selected indices and their association with obesity.

METHOD: A total of 90 apparently healthy obese, overweight and normal weight participants who met the inclusion criteria were randomly enrolled into the study. They were grouped using their body mass index of 18.5 to 24.9 kg/m² (normal weight), 25 to 29.9 kg/m² (overweight) and ≥30 kg/m² (obese). The parameters were measured using standard laboratory methods and statistical analysis was performed using SPSS 20.0.

RESULTS: There was progressive increase of remnant cholesterol among the group but there was no statistical difference (p>0.05) in the mean calculated remnant cholesterol (mmol/l) among the obese (0.72±0.4), overweight (0.68±0.46), and normal weight (0.50±0.28) participants. Mean fasting very low density lipoprotein (VLDL) and triglyceride (TG) (mmol/l) were significantly higher (p<0.05) in overweight compared to normal weight participants (0.76±0.31 vs. 0.47±0.22) and (1.72±0.60 vs. 1.09±0.51) and obese participants (0.82±0.23 vs. 0.47±0.22) and (1.85±0.59 vs. 1.09±0.51) when compared to normal weight participants respectively, while plasma FBS levels (mmol/l) were significantly lower in normal weight participants when compared to overweight participants (3.45±0.79 vs. 4.46±1.82) and obese participants (3.46±0.79 vs. 4.94±1.26). RC correlated negatively with non-HDLc and was not significant (r=0.064 p 0.743), positively with TC/HDLc ratio but not significant (r 0.209, p 0.278) and negatively significant with LDLc/HDLc ratio (r=0.451, p 0.014*). Also in the overweight category, RC correlated negatively with non-HDLc, LDLc/HDLc but not significant (r=0.050, p 0.812; r=0.050, p 0.812) and positively with TC/HDLc ratio but not significant (r 0.021, p 0.920). Finally in the obese group it was observed that RC correlated negatively with non-HDLc (r=0.175, p 0.402), positively with LDLc/HDLc and TC/HDLc ratio but was not significant (r 0.313, p 0.127; r 0.012, p 0.953).

CONCLUSION: The association of calculated remnant cholesterol with BMI is weak. Non-HDLc is strongly associated with obesity as well as TC/HDLc and LDLc/HDLc ratios. There was a negative correlation between calculated RC and non-HDLc. This suggests that remnant cholesterol can be elevated in any individual without apparent increase in BMI.

KEYWORDS: Remnant Cholesterol, Lipid profile, Post-prandial dyslipidemia, Atherogenic Indices, Obesity.

Comment [FG1]: Evaluation through BMI is very restrictive. There are more current, reliable and useful evaluation criteria.

Comment [FG2]: Present here the objective(s) of the study. It is ultimately not understood what is specifically intended with this study.

Comment [FG3]: Do not put statistical data in the abstract. Display general results here and statistical data in the results.

INTRODUCTION

Obesity defined by the World Health Organization, as a Body Mass Index (BMI) $\geq 30\text{kg/m}^2$ has become an epidemic dimension in the world with Nigeria also having its own share of the burden as reported by Akarolo-Anthony et al., (1). This current trend of obesity has been attributed to the socio-economic and epidemiological transition of Nigeria from a lower income country to a middle income country, resulting in the change of lifestyle behaviors and dietary intake, as a result many Nigerians now live sedentary lifestyle while at the same time consuming diet with excess calories, as observed by Iloh et al., (2), thus resulting in high energy intake with low energy expenditure among most adult Nigerians. However, whatever is the causes of obesity, its long term impact on the life of adults across Nigeria are well documented. Obesity has been shown to be a predisposing factor in the rising prevalence of morbidity and mortality associated with non-communicable diseases like type-2 diabetes mellitus, hypertension, cancer, stroke among adults. (3). Cardiovascular diseases (CVDs), the leading cause of morbidity and mortality in the western World, are now emerging public health challenges in developing countries, (4). Accounting for 80% of deaths and 87% of related disability currently recorded in the low-and middle-income countries. In developing countries, mortality due to CVD is expected to rise to 19 million, with deaths among persons 15-59 years of age three to eight times as high in Tanzania and Nigeria as in England and Wales. (5).

Non-HDL cholesterol (non-HDL-C) represents the cholesterol components carried by atherogenic lipoproteins such as LDL, very low-density lipoprotein (VLDL) and intermediate density lipoprotein (IDL). Higher non-HDL-C levels indicate increased risk of atherosclerosis (6). Remnant cholesterol also known as remnant lipoprotein is a very atherogenic lipoprotein composed primarily of very low density lipoprotein (VLDL), Intermediate density lipoprotein (IDL), and chylomicrons. It can also be stated that, remnant cholesterol is all plasma cholesterol that is not LDL or HDL cholesterol which are triglyceride rich lipoproteins (7). The total/high-density lipoprotein (HDL) cholesterol ratio, known as the atherogenic or Castelli index and the LDL/HDL cholesterol ratio, are two important components and indicators of vascular risk, the predictive value of which is greater than the isolated parameters. In this respect, an increase in total cholesterol concentration, and specifically LDL cholesterol, is an atherogenic lipid marker, whereas reduced HDL cholesterol concentration is correlated with numerous risk factors, including the components of the metabolic syndrome (8).

In this study we looked at the relationship of these atherogenic markers with BMI and the association of Remnant cholesterol with Non-HDLc, TC/HDLc and LDLc/HDLc ratios.

MATERIALS AND METHODS

This is a cross-sectional study, designed to compare the serum level of some lipid fractions, Remnant Cholesterol in normal, overweight and obese participants. Based on the calculated sample size, 90 consenting participants that fulfill the inclusion criteria were randomly recruited for the study. Informed consent of the participants was sought and obtained before enrollment into the study. Ethical approval was sought and obtained from the Ethics Committee (NAUTH/CS/66/VOL.11/154/2018/088) of Nnamdi Azikiwe University Teaching Hospital Nnewi. Questionnaires were administered and it served as a primary instrument for this study. The questionnaires were structured to reflect the health issues relevant to the objectives of the study. The study comprised of 90 obese and non-obese subjects, who are picked by non-probability sampling. Among them, 30 participants were known obese, 60 participants were non-

Comment [FG4]: Despite being an interesting topic, it would be important to support in the current literature its pertinence/importance for the scientific and professional community.

Comment [FG5]: The support references are generally very old. Important to update.

Comment [FG6]: The support references are generally very old. Important to update.

obese and between 20-50 years of age. The participants were labeled Normal weight (BMI 18.5-24.9 kg/m²), over-weight (BMI 25.0-29.9kg/m²) Obese ($\geq 30\text{kg/m}^2$). BMI was measured as weight in kilogram 0.01 divided by height squared (m²), WC was measured with a tape to the nearest 0.1cm at the end of a normal expiration at the midpoint between the subcostal plane and the iliac crest of an exposed abdomen. HC was also measured at the largest standing horizontal circumference of the buttock to determine the waist to hip ratio (WHR=WC/HC). Hypertension was defined to be systolic blood pressure $\geq 140\text{mmHg}$, diastolic blood pressure $\geq 90\text{mmHg}$. A fasting blood sample was obtained in the morning between 0800-0900 hours about 7ml, through venipuncture from each participant. Aliquots was drawn into fluoride oxalate container for plasma glucose estimation, lipid profile and later on a non-fasting blood sample was obtained from the participants and aliquots was drawn into plain tubes and allowed to clot at room temperature. The serum for remnant cholesterol was separated after collection by centrifugation at 5000g for 5min. Serum samples was aliquoted and frozen at -20°C for analysis later on. To ensure proper collection of fasting and non-fasting sample, the participants fast overnight and the blood sample collected in the morning between 0800-0900 hours and the participants had his/her breakfast after which the non-fasting samples was collected. Fasting lipid profile was assessed using commercially available kits (Randox) serum total cholesterol and high density lipoprotein HDLc, was determined by cholesterol oxidase method Allain *et al* (9) serum triglyceride by glycerol kinase method. Tindler (10) and LDLc was calculated using Friedwald formula. Friedwald, (11). And remnant cholesterol was calculated using the formula. $Rc = Tc - HDLc - LDLc$. (feldman, 2017). Non-HDL-C was calculated as the difference between TC and HDL-C. Atherogenic ratios were calculated as total/HDL cholesterol and LDL/HDL cholesterol. Fasting plasma glucose was determined using the enzymatic colorimetric method of Trinder (10). Statistical analysis was performed to compare the groups using ANOVA. The values were expressed as the Mean \pm S.D. Values were regarded as significant if p is ≤ 0.05 . Pearson correlation coefficient was used to correlate the parameters estimated.

RESULTS

Table 1 shows the mean anthropometric markers of all participants. There was no significant difference in the diastolic and systolic blood pressures. There were significant increase in the body mass index of all the participants (<0.001). There was also significance difference in the waist to hip ratio of the participants. **Table 2;** shows the mean of the various fasting biochemical parameters of obese, overweight and normal weight group. Significant change was observed in the fasting plasma Triglyceride (TG) and very low density lipoprotein (VLDL). ($p < 0.001$) in the obese subjects when compared with the corresponding control values. No significant change was observed in other lipid parameters. **Table 3;** show the mean of the various Non-fasting biochemical parameters of obese, overweight and normal weight group. Significant change was observed in the Non-fasting plasma Triglyceride (TG) ($p < 0.005$) in the obese subjects when compared with the corresponding control values. No significant change was observed in other lipid parameters. **Table 4;** Show the mean serum remnant cholesterol, total cholesterol-HDLc ratio, LDLc-HDLc ratio and fasting blood sugar, in the obese, overweight and normal weight participants. There was no significant difference in the mean RC between the obese and overweight when compared to the control. There was statistical significance in the fasting blood sugar level between the normal weight participants (3.46 ± 0.79) and overweight (4.46 ± 1.82) at ($P < 0.021$) Also between the normal weight and obese participants (3.46 ± 0.79 vs 4.94 ± 1.26) at ($P < 0.001$). No significant difference was observed in the level of the calculated remnant cholesterol among the groups. **Table 5;** shows the correlation of the measured parameters with BMI in obese participants. There was a positive correlation

Comment [FG7]: The sample is not clearly described. What's the average age? What about the standard deviation? The distribution by the three groups? Sex? It would also be interesting to know variables, identified by the literature, as strongly related, such as, do they engage in physical activity? How often? What is the diet?

Comment [FG8]: Presented objectively and clearly.

of the measured parameters in overweight and obese participants but not statistically significant. There was no significant correlation of BMI with other Parameters measured. **TABLE 6;** shows the correlation of remnant cholesterol with non-HDLc, total cholesterol/HDLc ratio, and LDLc/HDL ratio in normal weight, overweight, and obese participants.

Table 1 ANTHROPOMETRIC MAKER AND INDICES OF OBESITY. IT HIGHLIGHTS THE BMI, WHR, DIASTOLIC BLOOD AND SYSTOLIC BLOOD PRESSURES (MEAN±SD)

GROUPS	SBP (mmHg)	DBP (mmHg)	BMI (Kg/m ²)	WHR
NORMAL WEIGHT (A)	115.28±11.89	75.59±10.03	21.75±1.82	0.85±0.05
OVER WEIGHT (B)	118.92±25.02	78.40±9.55	27.11±1.02	0.86±0.07
OBESE (C)	127.00±10.86	85.64±12.25	33.85±4.51	0.96±0.10
f-test	3.310	6.246	123.387	18.038
p-value	0.042*	0.003*	<0.001*	<0.001*
A vs B	1.000	1.000	<0.001*	1.000
A vs C	0.040*	0.003*	<0.001*	<0.001*
B vs C	0.287	0.056	<0.001*	<0.001*

Table 2 LEVELS OF FASTING HDL-C, LDL-C, VLDL-C, TG, AND TC IN NORMAL WEIGHT, OVER WEIGHT AND OBESE GROUPS (MEAN±SD)

GROUPS	F-HDL-C (mmol/L)	F-LDL-C (mmol/L)	F-VLDL-C (mmol/L)	F-TG (mmol/L)	F-TC (mmol/L)
NORMAL WEIGHT (A)	1.00±0.21	1.77±0.67	0.47±0.22	1.09±0.51	3.39±0.66
OVER WEIGHT (B)	0.95±0.09	1.52±0.34	0.76±0.31	1.72±0.60	3.47±0.42
OBESE (C)	1.00±0.17	1.84±0.38	0.82±0.23	1.85±0.59	3.45±0.53
f-test	0.816	3.006	9.391	8.638	0.135
p-value	0.446	0.055	<0.001*	<0.001*	0.874
A vs B	0.732	0.201	<0.001*	<0.001*	1.000
A vs C	1.000	1.000	<0.001*	<0.001*	1.000
B vs C	0.906	0.067	0.019*	0.030*	1.000

Table 3 LEVELS OF NON-FASTING HDL-C, LDL-C, VLDL-C, TG AND TC IN NORMAL WEIGHT, OVER WEIGHT AND OBESE GROUPS (MEAN±SD)

GROUPS	NF-HDL-C (mmol/L)	NF-LDL-C (mmol/L)	NF-VLDL-C (mmol/L)	NF-TG (mmol/L)	NF-TC (mmol/L)
NORMAL WEIGHT (A)	1.05±0.27	1.87±0.68	0.44±0.23	1.02±0.52	3.42±0.80
OVER WEIGHT (B)	1.08±0.30	1.56±0.47	0.66±0.35	1.67±0.93	3.32±0.47
OBESE (C)	1.07±0.23	1.95±0.58	0.62±0.22	1.37±0.53	3.66±0.75
f-test	0.088	3.167	5.031	6.023	1.625
p-value	0.916	0.048*	0.009*	0.004*	0.204
A vs B	1.000	0.176	0.014*	0.003*	1.000
A vs C	1.000	1.000	0.049*	0.182	0.253
B vs C	1.000	0.058	1.000	0.418	0.614

Table 4 LEVELS OF RC, NON-HDLc, TOTAL/HDLc RATIO, LDLc/HDLc RATIO AND FBS IN NORMAL WEIGHT, OVER WEIGHT AND OBESE GROUPS (MEAN±SD)

GROUPS	RC(mmol/l)	FBS (mmol/L)	N-HDL-C (mmol/l)	TC/HDL-C	LDL/HDL-C
NORMAL WEIGHT (A)	0.50±0.28	3.46±0.79	2.39± 0.45	3.08±0.67	1.61±0.70
OVER WEIGHT (B)	0.69±0.46	4.46±1.82	2.52±0.33	3.65±0.42	1.60±0.42
OBESE (C)	0.72±0.40	4.94±1.26	2.45±0.36	3.45±0.32	1.84±0.26
f-test	2.853	8.740	0.501	1.357	1.558
p-value	0.064	<0.001*	0.608	0.264	0.217
A vs B	0.199	0.021*	0.960	0.413	0.457
A vs C	0.095	<0.001*	1.000	1.000	0.072
B vs C	1.000	0.647	1.000	0.534	0.331

RC = Remnant Cholesterol, FBS = Fasting Blood Sugar, N-HDL-C = Non High Density Lipoprotein Cholesterol, * Significance.

Table 5 CORRELATION OF BMI WITH LIPID PROFILE, AND RC IN NORMAL WEIGHT, OVERWEIGHT AND OBESE INDIVIDUALS

PARAMETRS	NORNAL WEIGHT		OVERWEIGHT		OBESE	
	r	p-value	r	p-value	r	p-value
BMI vs F-HDL	-0.202	0.293	0.083	0.693	0.217	0.298
BMI vs F-LDL	-0.041	0.831	-0.108	0.607	0.089	0.672
BMI vs F-VLDL	-0.087	0.654	0.223	0.285	0.085	0.685
BMI vs F-TG	-0.134	0.490	0.024	0.909	0.031	0.882
BMI vs F-TC	-0.181	0.348	-0.141	0.502	0.141	0.503
BMI vs FBS	-0.103	0.593	0.010	0.962	0.021	0.919
BMI vs RC	-0.002	0.993	-0.312	0.129	-0.074	0.726
BMI vs non-HDLc	-0.131	0.498	-0.172	0.411	0.097	0.645

TABLE 6 THE CORRELATION OF REMNANT CHOLESTEROL WITH NON-HDL CHOLESTEROL, TOTAL CHOLESTEROL/HDLc RATIO, AND LDL/HDL CHOLESTEROL RATIO IN NORMAL WEIGHT, OVERWEIGHT, AND OBESE PARTICIPANTS.

PARTICIPANTS	PARAMETERS	r	p-value
NORMAL WEIGHT	RC vs nonHDL	-0.064	0.743
	RC vs TC/HDL-C	0.209	0.278
	RC vs LDL-C/HDL-C	-0.451	0.014*
OVERWEIGHT	RC vs nonHDL	-0.050	0.812
	RC vs TC/HDL-C	0.021	0.920
	RC vs LDL-C/HDL-C	-0.050	0.812
OBESE	RC vs nonHDL	-0.175	0.402
	RC vs TC/HDL-C	0.012	0.953
	RC vs LDL-C/HDL-C	0.313	0.127

DISCUSSION

In this study, a total of 90 participants were recruited, and classified according to groups using BMI. BMI was used as a yardstick for general obesity. WHR were used as predictors of cardiovascular risk factor and measure of central obesity. The systolic blood pressure and diastolic blood pressure make up the total blood pressure in the body. This study demonstrated a significant difference in the blood pressure among the groups. The systolic and diastolic blood pressure may be related to risk of cardiovascular diseases in a linear fashion, which might be due to obesity. The higher the level of BMI and blood pressure the greater the incidence of cardiovascular accidents, suggesting that those with higher blood pressure and increased BMI tend to have higher serum cholesterol level. (12), this might be caused by the increase in the level of fatty materials such as cholesterol and other lipid fraction leading to the thickening of the arterial walls affecting arterial blood pressure and causing an increase in the blood pressure. This agrees with a study carried out in Omani in 2006 which shows that obese individuals has significantly elevated blood pressure when compared with the controls. (13). Obesity is associated with many deleterious changes in lipid biosynthesis or metabolism, the significant of this finding in the elevation of TG is usually associated with reduced levels of HDL-c suggesting a possible metabolic interaction between these two lipid fractions. The mechanism to this relation may be due to the increase in fat deposition in obese subjects which is associated to leptin resistance in the peripheral tissues and which might also involve insulin resistance, as stipulated by Howard et al, leading to varied alterations in intracellular signaling and

Comment [FG9]: It is important to articulate with the literature and the significance for the intervention.

changes in substrate handling resulting to increase in the synthesis and secretion of TG enriched VLDL particles (14).

From our results, there was a progressive increased of remnant cholesterol level in the groups in normal weight, in overweight and in obese participants above the projected normal range and percentile by Varbo et al, this show that race might have role to play in the pathophysiology of atherosclerosis induced by remnant cholesterol, and there was no significant statistical difference in the level of the remnant cholesterol among the groups. This suggest that the association of remnant cholesterol and cardiovascular disease is not caused only by adiposity, mechanistically it can be explained by remnant cholesterol ability to cause atherosclerosis in the arterial wall which is a process that has been assumed to be driven by remnant cholesterol concentration in the blood stream, independent of it reasons for elevations in overweight and obesity, this finding is in correspondence with Varbo et al., (15).

In the correlation analysis of RC with other atherogenic markers, we observed in normal weight participants, that RC correlated negatively with non-HDLc but was not significant ($r=0.064$, $p=0.743$), positively with TC/HDLc ratio but not significant ($r=0.209$, $p=0.278$) and negatively significant with LDLc/HDLc ratio ($r=0.451$, $p=0.014^*$). Also in the overweight category RC correlated negatively with non-HDLc, LDLc/HDLc but not significant ($r=0.050$, $p=0.812$; $r=0.050$, $p=0.812$) and positively with TC/HDLc ratio but not significant ($r=0.021$, $p=0.920$). Finally in the obese group it was observed that RC correlated negatively with non-HDLc ($r=0.175$, $p=0.402$), positively with LDLc/HDLc and TC/HDLc ratio but was not significant ($r=0.313$, $p=0.127$; $r=0.012$, $p=0.953$).

Atherogenic indices (RC) calculated in this study was increased 1.5 fold above the reference range but was not statistically significant, (n-HDL-C, TC/HDL-C and LDL/HDL-C ratios) calculated in this study were within the reference range, also RC was not significantly correlated with BMI across the groups, but non-HDLc correlated positively BMI in the obese group, this observation is in accordance with the study of Denke, M.A *et al.*, in which they observed that in the analysis of men enrolled in NHANES II, increasing obesity (assessed by BMI) was associated with higher triglyceride, total and non-HDL cholesterol levels, and lower HDL-C.(16) In middle-aged and older men, LDL-C did not vary with BMI and the elevation in total cholesterol was due mainly to an increased non-HDL-C level (i.e. an increase in cholesterol carried by VLDL and IDL particles). Our study also showed that non-HDL-C is more strongly correlated with obesity than RC. Similarly, accumulation of visceral (abdominal) adipose tissue which drives the metabolic syndrome and diabetes, is associated with the lipoprotein profile of obesity with a normal LDL-C despite elevated levels of atherogenic lipoproteins and cardiovascular risk, this observation was in accordance with Yusuf, S *et al.*, (17) and this indicates that even though the obese participants appear healthy, there is tendency for the development of metabolic syndrome, chronic diseases or cardiovascular disease apparently.

CONCLUSION

This study found out that the association of calculated remnant cholesterol with BMI is weak. Non-HDLc is strongly associated with obesity. There was a negative correlation between calculated RC and non-HDLc. This implies that remnant cholesterol can be elevated in any individual without apparent increase in BMI. Suggesting, it to be predictive of cardiovascular incidents irrespective of BMI.

Comment [FG10]: Very poor. There is important data that should be addressed here in general.

REFERENCES

1. Akarolo-Anthony SN, Willett WC, Spiegelman D, Adebamowo CA. (2014). Obesity epidemic has emerged among Nigerians. *BioMedical Central Public Health*. 14:455.
2. Iloh GUP, Amadi AN, Nwankwo BO, Ugwu VC. (2011). Obesity in adult Nigerians: a study of its pattern and common primary co-morbidities in a rural Mission General Hospital in Imo state, south-eastern Nigeria. *Nigerian Journal of Clinical Practice*. 14(2).
3. Weiss R, Dziura J, Burgert TS, et al. (2004). Obesity and the metabolic syndrome in children and adolescents. *New England Journal of Medicine*. 350(23):2362–2374.
4. World Health Organization. (2002). *The world health report: Reducing risks, promoting healthy lifestyles*. Geneva.
5. Reddy KS. Cardiovascular disease in developing countries: (2002). Dimensions, determinants, dynamics and directions for public health action. *Public Health Nutrition*. 5:231-7.
6. Virani, S.S., et al., Barriers to non-HDL cholesterol goal attainment by providers. *Am J Med*, 2011. 124(9): p. 876-80 e2.
7. Jorgensen AB, Frikke-schmidt R, West AS, Grand P, Nordestgaard BG, Tybarg-Hansen A (2013). "Genetically elevated non-fasting triglyceride and calculated cholesterol as a casual risk factor for myocardial infarction". *European Heart journal*. 34(24):1826-1833.
8. Ascaso J, González Santos P, Hernández Mijares A, et al. (2007). Management of dyslipidemia in the metabolic syndrome, recommendations of the Spanish HDL Forum. *American Journal of Cardiovascular Drugs*. 7:39–58.
9. Allen CC, Poon LS, Chan CS, Richmond W, Fu PC. (1974). Enzymatic determination of total Serum Cholesterol. *Clinical chemistry*. 20:470-475.
10. Trinder P (1969). Determination of Glucose in Blood Using Glucose Oxidase with an Alternative Oxygen Acceptor. *Annals of clinical biochemistry*. 6-24
11. Friedwald WT, Levy RI, Fredrickson DS (1972). Estimation of the concentration of low density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. *Clinical Chemistry*. 18 (6):499-502.
12. Keaven M, Anderson, Patricia M. Odell, Peter W.F. Wilson, William B. Kannel, Cardiovascular disease risk profiles. (1991). *American Heart Journal*, Volume 121, Issue 1, Part 2, Pages 293-298, ISSN 0002-8703, [https://doi.org/10.1016/0002-8703\(91\)90861-B](https://doi.org/10.1016/0002-8703(91)90861-B).
13. Masoud Y Al-Maskari, Adel A Al-naqdy (2006). Correlation between serum leptin levels, body mass index and obesity in Omanis. *Sultan Qaboos University Medical Journal*. 6:27-31.
14. Howard BV, Ruotolo G, Robbins DC (2003). Obesity and dyslipidemia. *Endocrinology of Metabolic Clinic North America*. 1(1):27-32.
15. Varbo A, Freiberg JJ, Nordestgaard BG (2018). "Remnant cholesterol and myocardial infarction in normal weight, overweight, and obese individuals from Copenhagen General Population Study". *Clinical Chemistry*. 64(1):219-230.

Comment [FG11]: For the purpose of the study, the references should be more current. The most recent is one from 2018.

16. Denke, M.A., C.T. Sempos, and S.M. Grundy, (1993). Excess body weight. An underrecognized contributor to high blood cholesterol levels in white American men. *Arch International Medicine*. 153(9): p. 1093-103.
17. Yusuf, S., et al., (2005) Obesity and the risk of myocardial infarction in 27,000 participants from 52 countries: a case-control study. *Lancet*. 366(9497): p. 1640-9.

UNDER PEER REVIEW