Comparative study of Fasting Proneurotensin levels in Obese and Non-Obese Individuals with special reference to BMI and Lipid Profile

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ABSTRACT

Obesity has become one of the most pressing health challenges of the twenty-first century, affecting individuals across all age groups and socioeconomic strata. It is no longer merely a lifestyle issue but a complex metabolic disorder with multifactorial origins and wide-ranging systemic effects. Neurotensin (NT), a tridecapeptide hormone synthesized by neuroendocrine cells in the small intestine and neurons in the central nervous system, has recently emerged as a potential biochemical link between lipid metabolism and obesity. The stable precursor fragment of this peptide, proneurotensin (Pro-NT), offers an analytically reliable measure of NT activity in circulation. The present study was designed to investigate fasting Pro-NT levels in obese and non-obese individuals and to assess its correlation with body mass index (BMI) and fasting lipid profile parameters, including total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG).

A hospital-based, case–control comparative study was conducted on ninety adults attending the master health check-up program at JSS Medical College and Hospital, Mysuru. Participants were classified into obese and non-obese groups based on Indian consensus BMI guidelines. Fasting blood samples were collected to estimate Pro-NT using an enzyme-linked immunosorbent assay (ELISA) and lipid profiles through standard enzymatic methods. Statistical analyses were performed using Student's *t*-test and Pearson's correlation, with a significance threshold of $p < 0.05$.

The findings revealed that obese individuals exhibited significantly higher mean Pro-NT levels (110.29 pg/mL) compared to non-obese controls (90.87 pg/mL, $p < 0.01$). Pro-NT levels demonstrated a strong positive correlation with BMI ($r = 0.41$, $p < 0.01$) and were significantly associated with TC ($p = 0.023$), LDL-C ($p = 0.043$), and TG ($p < 0.001$). No gender-related variations were observed. These results suggest that fasting serum Pro-NT is closely linked to adiposity and lipid dysregulation. Given its stability and reproducibility, Pro-NT may serve as a novel biochemical marker for early metabolic risk stratification. Routine estimation of Pro-NT could thus provide clinicians with an additional diagnostic tool to predict obesity-associated dyslipidemia and metabolic syndrome before the onset of overt disease.

KEYWORDS: Obesity, Proneurotensin, Dyslipidemia, Lipid profile, BMI, ELISA, Metabolic biomarker.

1. INTRODUCTION

Obesity is recognized globally as a chronic, relapsing, and multifactorial disease characterized by an imbalance between caloric intake and energy expenditure, leading to excessive fat accumulation in adipose tissue. It results from the complex interplay of genetic, behavioral, neuroendocrine, and environmental factors [1]. In India, the prevalence of obesity has been steadily increasing due to urbanization, sedentary lifestyles, and changes in dietary habits, making it a major contributor to the growing burden of non-communicable diseases. The health implications of obesity extend far beyond weight gain; it acts as a central determinant of insulin resistance, type 2 diabetes mellitus, hypertension, dyslipidemia, non-alcoholic fatty liver disease, and cardiovascular disorders [2].

From a pathophysiological standpoint, obesity is associated with dysregulated lipid metabolism and systemic inflammation. Under normal physiological conditions, excess fatty acids are stored safely in adipose tissue as triacylglycerols (TAGs). However, in obesity, increased lipolysis and sympathetic activation lead to elevated circulating free fatty acids (FFAs), resulting in oxidative stress, endothelial dysfunction, and lipotoxicity [3]. These alterations not only impair glucose and lipid metabolism but also contribute to insulin resistance and β-cell exhaustion, thereby amplifying metabolic derangements.

Emerging research has highlighted the role of neuropeptides in the regulation of energy balance and appetite. Among them, neurotensin (NT) has garnered particular attention. NT is a 13-amino-acid peptide secreted by N-cells of the small intestine and by neurons in the hypothalamus [4]. It exerts its biological effects through three receptors: NTR1 and NTR2, which are G-protein-coupled, and NTR3, a sortilin-related receptor involved in intracellular trafficking. These receptors are distributed widely in the gastrointestinal tract, pancreas, adipose tissue, heart, skeletal muscle, and the central nervous system [5].

Physiologically, NT plays a crucial role in lipid digestion and absorption. It is released postprandially, particularly after ingestion of dietary fats, and promotes intestinal uptake of fatty acids through mechanisms that involve bile acid transport and enterohepatic circulation [6]. Animal experiments have shown that NT-deficient mice exhibit reduced intestinal fat absorption and are resistant to diet-induced obesity and insulin resistance. In the hypothalamus, NT modulates satiety by interacting with leptin and other appetite-regulating hormones, suggesting that it has dual peripheral and central roles in energy homeostasis [7].

The stable precursor fragment of NT, proneurotensin (Pro-NT), serves as a measurable biomarker for assessing NT activity in humans. Elevated circulating levels of Pro-NT have been linked to an increased risk of diabetes, cardiovascular disease, and certain cancers. However, the correlation of Pro-NT with BMI and lipid profiles has been inadequately studied, particularly in Indian populations, where obesity presents with distinct metabolic patterns compared to Western cohorts. Considering this gap, the present study was designed to evaluate fasting serum Pro-NT levels in obese and non-obese individuals and to explore its association with BMI and lipid parameters, thereby elucidating its potential as a surrogate marker for dyslipidemia and metabolic disorders [8].

2. MATERIALS AND METHODS

2.1. Study Design

The present study was designed as a hospital-based, case–control, comparative analysis conducted in the Department of General Medicine at JSS Medical College and Hospital, Mysuru, over a period of twenty months. Ethical approval for the study was obtained from the Institutional Ethics Committee prior to commencement, and written informed consent was secured from all participants in accordance

with the Declaration of Helsinki (2013 revision). The primary objective was to determine the difference in fasting serum proneurotensin (Pro-NT) levels between obese and non-obese individuals and to explore the relationship of these levels with body mass index (BMI) and fasting lipid profile parameters [9].

2.2. Inclusion and Exclusion criteria

A total of ninety adult subjects, aged between eighteen and sixty years, were recruited from individuals attending the Master Health Check-Up program at JSS Hospital. The study population was divided into two groups based on the Indian consensus guidelines for obesity: those with a BMI greater than 24.9 kg/m² were categorized as obese and served as the case group, while those with a BMI equal to or below 24.9 kg/m² were considered non-obese and served as the control group. The selection of participants was carried out using purposive sampling, ensuring representation across both sexes [10]. Individuals with secondary causes of obesity such as endocrine disorders, those diagnosed with diabetes mellitus or cardiovascular diseases, as well as pregnant or lactating women, chronic smokers, alcoholics, and participants on lipid-altering medications such as statins or fibrates were excluded from the study. These exclusion criteria were implemented to ensure that the observed changes in Pro-NT and lipid parameters were attributable primarily to obesity and not to other confounding metabolic or hormonal factors.

2.3. Data Collection and Handling Procedure

All participants underwent detailed clinical evaluation and anthropometric assessment. Height and weight were measured using calibrated stadiometers and digital weighing scales, and BMI was calculated using the standard formula of weight (in kilograms) divided by height squared (in meters). Each subject's medical history was recorded, and baseline demographic information was documented. Venous blood samples were collected in the early morning hours after a minimum of eight to ten hours of overnight fasting. Approximately five milliliters of blood was withdrawn under aseptic precautions, of which a portion was used for serum lipid estimation and another portion was processed for plasma Pro-NT measurement. Samples were centrifuged at 3000 rpm for fifteen minutes, and the separated serum and plasma were stored at –80 °C until further analysis to maintain analyte stability [11]. All samples were handled using standard biosafety and cold-chain procedures to prevent protein degradation.

2.4. Estimation of Lipid Profile

The fasting lipid profile, comprising total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C), was analyzed using enzymatic colorimetric assays on an automated biochemistry analyzer. The reagents and calibration standards used were of analytical grade and traceable to international reference standards. LDL-C values were calculated using Friedewald's equation in samples where triglyceride levels were below 400 mg/dL. Quality control measures were performed daily to ensure the precision and reproducibility of the lipid profile assays [12].

2.5. Estimation of Fasting Proneurotensin Levels

Fasting plasma proneurotensin concentrations were determined using a quantitative sandwich enzyme-linked immunosorbent assay (ELISA) kit procured from Cloud-Clone Corporation, USA (Catalog No. CEA334Hu). The assay was carried out according to the manufacturer's protocol. The lyophilized standard supplied with the kit was reconstituted with one milliliter of standard diluent to obtain a 1000 pg/mL stock concentration, which was serially diluted in threefold increments to prepare working standards ranging from 12.35 to 333.33 pg/mL. Detection reagents A and B were prepared by diluting

the supplied 100× concentrates to 1× working concentrations. Frozen plasma samples were thawed gradually at 4 °C to prevent protein denaturation. To bring the concentrations within the calibration range, samples were diluted in a 1:4 ratio, and all assays were performed in duplicate [13].

The ELISA procedure involved pipetting 50 µL of standards, blanks, and diluted samples into designated wells of a pre-coated 96-well microplate, followed by the addition of an equal volume of Detection Reagent A. The plate was sealed and incubated at 37 °C for one hour to facilitate antigen–antibody binding. After washing three times with wash buffer to remove unbound reagents, 100 µL of Detection Reagent B was added to each well, and the plate was incubated again at 37 °C for thirty minutes. Subsequent washing was performed five times, after which 90 µL of substrate solution was added and incubated until a visible blue color developed, indicating enzymatic reaction [14]. The reaction was terminated by adding 50 µL of stop solution, leading to a color change from blue to yellow. Absorbance was immediately read at 450 nm using a Perkin Elmer multimode microplate reader. The concentration of Pro-NT in each sample was determined from a standard calibration curve generated by plotting the optical density values against the known concentrations of standards, and the values were multiplied by the dilution factor to obtain the final concentrations in pg/mL.

2.6. Statistical Analysis

Data obtained from all participants were compiled in Microsoft Excel and statistically analyzed using IBM SPSS Statistics software (Version 25). Continuous variables were expressed as mean ± standard deviation (SD). Comparisons between obese and non-obese groups were made using the independent Student's *t*-test to evaluate differences in mean values [15]. Pearson's correlation coefficient (*r*) was employed to assess the strength and direction of the relationship between fasting serum Pro-NT levels and lipid parameters. Statistical significance was set at $p < 0.05$ for all analyses.

3. RESULTS

3.1 Demographic and Anthropometric Characteristics

A total of ninety subjects participated in the study, equally divided into forty-five obese individuals and forty-five non-obese controls. The mean age of the study population was 42.6 ± 8.4 years, with comparable gender distribution across both groups. The mean BMI among the obese participants was 29.1 ± 1.6 kg/m², which was significantly higher than the 22.7 ± 2.1 kg/m² recorded in the non-obese group ($p < 0.001$). This distinct difference in BMI confirmed the adequacy of group stratification for subsequent biochemical comparisons.

3.2 Comparison of Fasting Serum Pro-NT Levels

When fasting serum proneurotensin levels were compared between the two groups, the mean concentration in obese individuals was found to be 110.29 pg/mL, whereas the mean in non-obese individuals was 90.87 pg/mL. The difference between these means was statistically significant with a *p*-value less than 0.01, demonstrating that obese individuals have markedly higher circulating levels of Pro-NT. This observation suggests that Pro-NT secretion or stability is enhanced in obesity, possibly reflecting a neuroendocrine adaptation to altered metabolic status. The corresponding graph depicting mean Pro-NT levels (Figure 1) shows a pronounced elevation in the obese group compared to the non-obese group, highlighting the association between increased adiposity and Pro-NT.

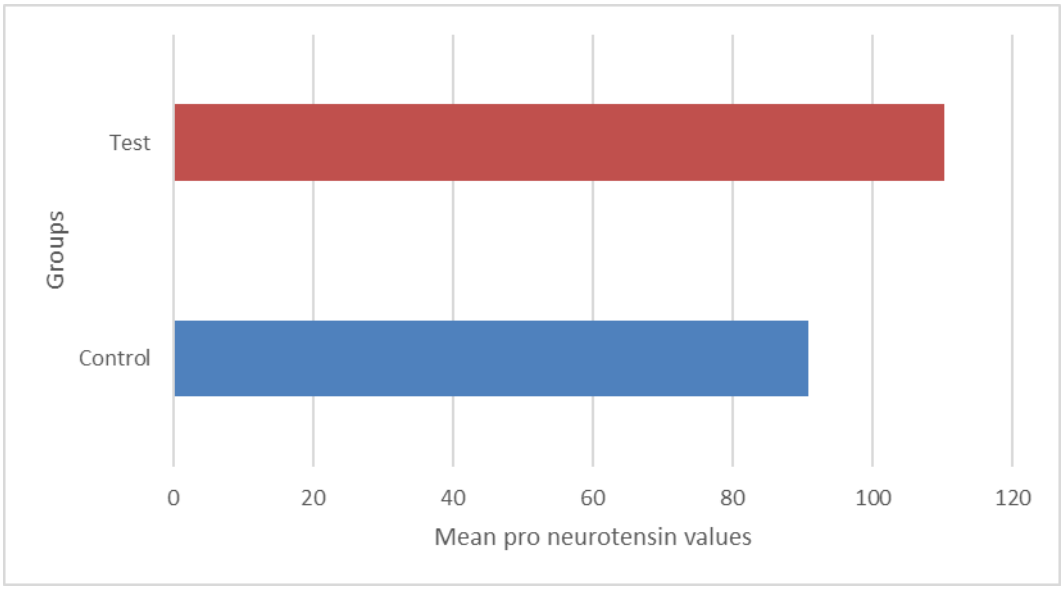

Figure 1: Mean values of fasting Pro-neurotensin in Cases and controls.

3.3 Correlation Between Pro-NT and BMI

A correlation analysis between BMI and Pro-NT revealed a significant positive relationship ($r = 0.41$, $p < 0.01$). The graphical representation (Figure 2) shows an upward linear trend, indicating that as BMI increases, fasting Pro-NT levels also rise proportionately. This correlation suggests that neurotensin activity, as reflected by its stable precursor, may be influenced by body fat content and lipid turnover. The observation aligns with existing evidence that neurotensin plays an important role in fat metabolism and storage, and its increased levels could either represent a compensatory regulatory mechanism or a pathophysiological contributor to obesity-related metabolic dysfunction.

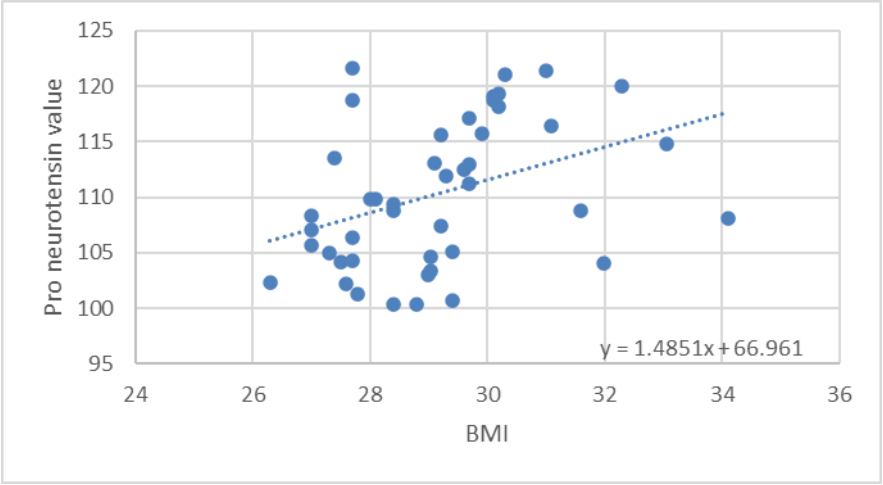

Figure 2: Correlation of Pro-NT and BMI in cases group.

3.4 Relationship Between Pro-NT and Total Cholesterol (TC)

In addition to its relationship with BMI, fasting Pro-NT levels demonstrated significant correlations with various lipid parameters. Individuals with higher total cholesterol (TC) values exhibited higher Pro-NT levels, with a statistically significant p-value of 0.023. The corresponding graph (Figure 3) illustrates this positive trend, suggesting that Pro-NT might reflect lipid metabolic activity. Since

neurotensin is known to facilitate intestinal absorption of dietary fats, elevated levels of this peptide may be indicative of increased lipid uptake, resulting in higher circulating cholesterol levels.

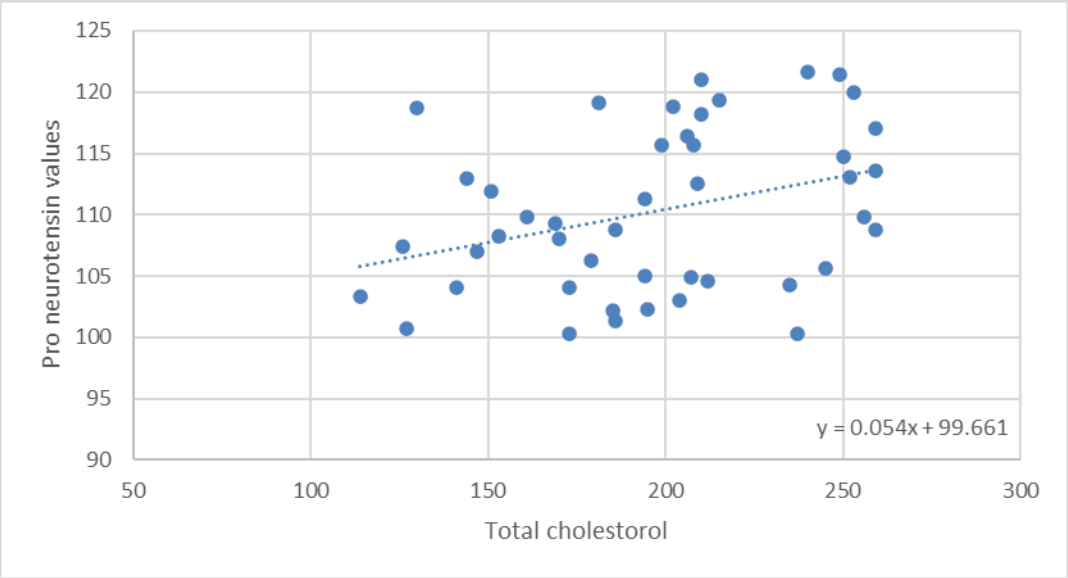

Figure 3: Correlation of TC and Pro-NT in cases group.

3.5 Relationship Between Pro-NT and LDL-Cholesterol (LDL-C)

Similarly, Pro-NT showed a positive correlation with low-density lipoprotein cholesterol (LDL-C), the atherogenic component of the lipid profile. The association, though modest (p = 0.043), implies that neurotensin-related mechanisms could contribute to cholesterol transport and LDL metabolism. Figure 4 displays this relationship, where rising LDL-C levels correspond with gradual increases in Pro-NT. This finding suggests that neurotensin pathways might influence lipid redistribution and hepatic lipoprotein processing.

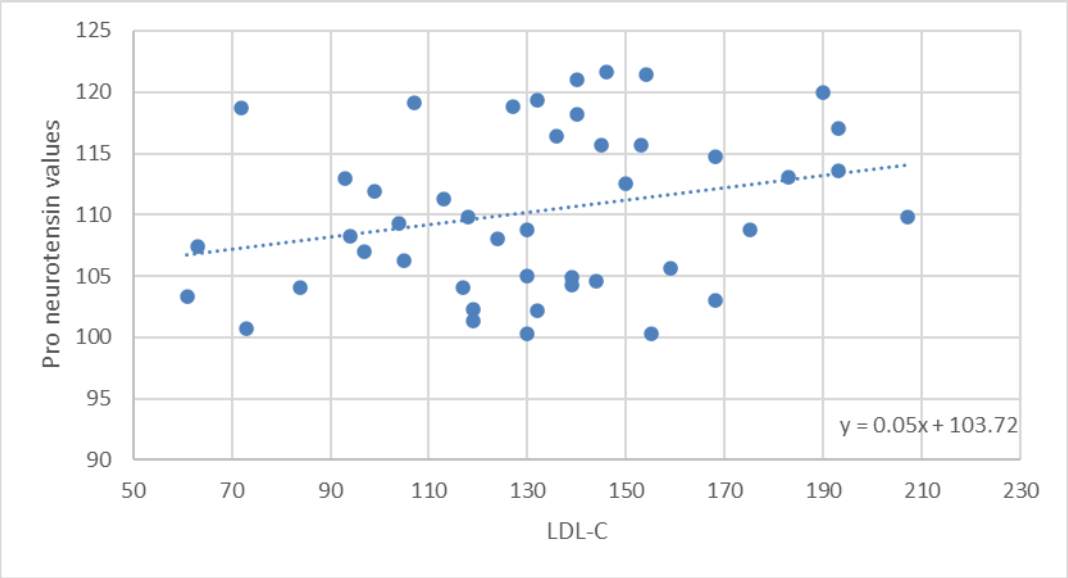

Figure 4: Correlation of LDL-C and Pro-NT in cases group.

3.6 Relationship Between Pro-NT and Triglycerides (TG)

The strongest correlation was observed between fasting Pro-NT levels and serum triglycerides (TG), with a highly significant *p*-value less than 0.001. Figure 5 presents a steep linear relationship between these two variables, demonstrating that individuals with higher TG levels consistently exhibited elevated Pro-NT concentrations. This finding is biologically plausible, as neurotensin has been shown to enhance intestinal fat absorption and facilitate chylomicron formation, processes that directly affect serum triglyceride concentrations. In the context of obesity, this relationship could reflect a positive feedback loop in which increased dietary fat absorption stimulates neurotensin release, which in turn further enhances lipid uptake, perpetuating hypertriglyceridemia and adipose accumulation.

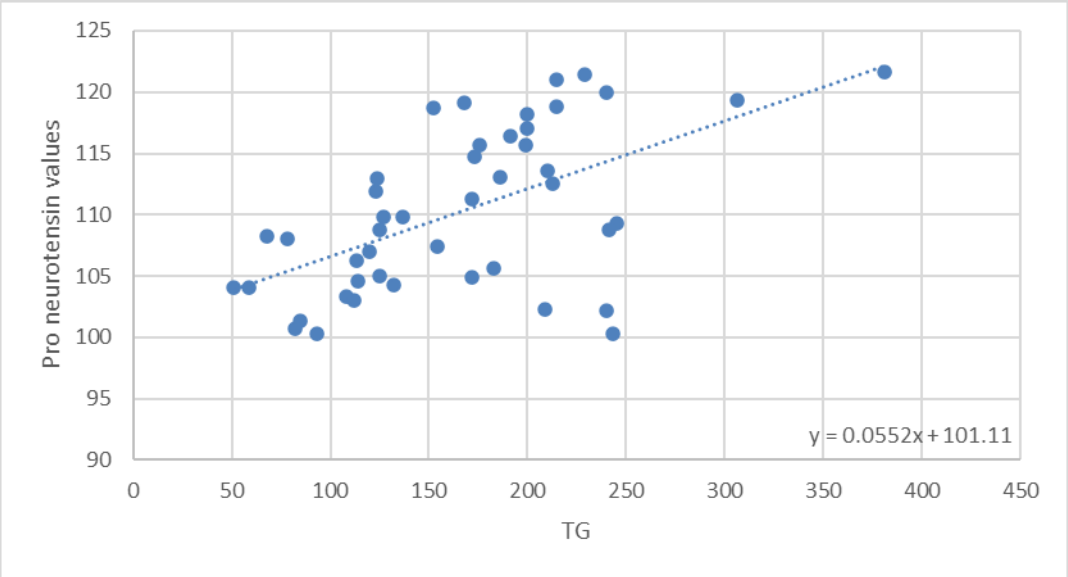

Figure 5: Correlation of TG and Pro-NT in cases group.

Taken together, the results from this study clearly indicate that fasting serum Pro-NT levels are significantly elevated in obese individuals compared to non-obese controls, and these levels exhibit positive correlations with BMI, total cholesterol, LDL-C, and triglycerides. The correlation with triglycerides was the most robust, underscoring the close physiological link between neurotensin activity and lipid metabolism. Interestingly, even within the non-obese group, individuals with relatively higher Pro-NT levels tended to have higher lipid values, suggesting that elevated Pro-NT may serve as an early biochemical marker of dyslipidemia, preceding the onset of overt obesity. The consistency and strength of these findings support the hypothesis that proneurotensin plays a key neuroendocrine role in modulating lipid absorption and energy storage, and its measurement could provide valuable insight into metabolic health status in both obese and non-obese populations.

4. DISCUSSION

The present study sought to investigate the relationship between fasting proneurotensin levels, obesity, and lipid parameters among Indian adults. The findings demonstrate a clear and statistically significant elevation of Pro-NT in obese individuals compared to non-obese controls, with consistent correlations observed across BMI and lipid indices. These results align with earlier studies conducted by Melander and colleagues (2012), who reported that elevated Pro-NT levels were predictive of diabetes, cardiovascular disease, and increased mortality in longitudinal cohorts. Similarly, Li et al. (2016) demonstrated in animal models that NT is indispensable for diet-induced obesity and fat absorption, reinforcing the physiological role of this peptide in energy homeostasis.

The positive correlation between Pro-NT and triglycerides observed in this study mirrors findings from Barchetta et al. (2018), who identified Pro-NT as a lipid-induced gastrointestinal peptide linked with visceral adipose inflammation. Elevated Pro-NT likely reflects enhanced NT signaling in response to high dietary fat intake, facilitating lipid absorption through increased bile acid transport and micellar formation. In turn, these processes contribute to elevated serum triglycerides and cholesterol, linking NT activity to dyslipidemia. Interestingly, our study did not reveal significant gender differences in Pro-NT levels, contrary to earlier reports by Melander et al. (2012), who found higher levels among women. The discrepancy may be due to differences in sample size, population demographics, and gender distribution.

Another key finding of our investigation is the observation that even among non-obese individuals, those with higher Pro-NT levels exhibited unfavorable lipid profiles. This suggests that elevated Pro-NT could serve as an early indicator of metabolic derangement preceding the clinical manifestation of obesity. Such insight opens the possibility of using Pro-NT as a screening biomarker for pre-metabolic syndrome states, offering clinicians a valuable tool for early lifestyle or pharmacological interventions. The biological plausibility of our findings can be explained through NT's physiological roles. NT is co-secreted with cholecystokinin (CCK) following fat ingestion. While CCK promotes pancreatic enzyme secretion and gallbladder contraction, NT enhances intestinal lipid uptake. NT also influences central energy regulation by acting on hypothalamic nuclei, thereby modulating appetite and energy expenditure. Consequently, persistently elevated NT signaling could contribute to increased lipid storage, systemic dyslipidemia, and adiposity, consistent with our observations.

In summary, the results of this study strengthen the evidence linking neurotensin pathways to obesity and lipid metabolism. They suggest that Pro-NT, due to its stability and ease of quantification, could be utilized as a potential biomarker for identifying individuals at risk of developing metabolic complications.

5. CONCLUSION

The current study demonstrates a statistically significant elevation of fasting proneurotensin levels among obese individuals and establishes a positive correlation between Pro-NT, BMI, total cholesterol, LDL-C, and triglycerides. These findings support the hypothesis that Pro-NT plays a regulatory role in lipid metabolism and adiposity and that its elevated levels may signal early metabolic imbalance. Routine evaluation of fasting Pro-NT levels could thus serve as an adjunct biomarker for screening individuals at risk for obesity-related metabolic disorders and dyslipidemia. Further large-scale, longitudinal studies are warranted to validate these associations and to determine whether therapeutic modulation of the neurotensin pathway could contribute to future anti-obesity strategies.

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