Magnitude Of Rise In Proneurotensin Is Related To Amount Of Triglyceride Appearance In Blood After A Standardized Oral Intake Of Both Saturated And Unsaturated Fat

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Lipids in Health and Disease

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Abstract

Background: In rodents, neurotensin contributes to high fat diet induced obesity by facilitation of intestinal fat absorption. The effect of oral lipid load on plasma proneurotensin and relationship with plasma triglycerides in humans is unknown.

Aim: To investigate the acute effects of an oral lipid load (including both cream and olive oil) on proneurotensin and plasma triglycerides and their interrelationships in healthy individuals.

Setting/Methods: Twenty-two healthy subjects were given 150 ml of full milk cream (54g fat) and 59 ml of pure olive oil (54 g fat) in the fasted state at two different occasions separated by at least 1 week in random order. Venous blood samples were drawn at fasting, and hourly up to 4 hours after fat ingestion. We compared post-ingestion values of proneurotensin and plasma triglycerides with fasting levels. Further, post ingestion Area Under the Curve (AUC) of proneurotensin was correlated with the AUC of plasma triglycerides.

Results: Rise of plasma proneurotensin and plasma triglycerides were observed after ingestion of cream with maximum increase at 2 hours for proneurotensin [mean (95% confidence interval)] of 22 (12-31) pmol/L (P<0.001) and at 3 hours for triglycerides of 0.60 (0.43-0.78) mmol/L (P<0.001).

Similarly, plasma proneurotensin and plasma triglycerides increased after ingestion of olive oil with maximum increase of proneurotensin at 3 hours of 62 (46-78) pmol/L (P<0.001) and plasma triglycerides at 3 hours of 0.32 (0.18-0.45) mmol/L (P<0.001). The post lipid load AUC for proneurotensin correlated significantly with the AUC for plasma triglycerides both after cream (r=0.49, P=0.021) and olive oil (r=0.55, P=0.008), respectively.

Conclusion: Proneurotensin increases after an oral lipid load of both cream and olive oil and the degree of rise of post-ingestion proneurotensin is significantly related to the rise of post-ingestion plasma triglycerides. Our human data support recent results from animal studies suggesting that neurotensin contributes to intestinal absorption of lipids into the blood stream.

Background

The global increase in the incidence and prevalence of obesity is occurring in parallel with the burden of cardiovascular disease (CVD) and metabolic diseases such as type 2 diabetes, hypertension and
dyslipidemia. Adverse effects of obesity and dyslipidemia on CVD are confirmed in large prospective and observational studies [1]. However, prevention of significant numbers of CHD and residual risk factors remains unsettled. Moreover, as obesity is the key modifiable risk factor for diabetes and thus, for diabetes-related CVD, any novel mechanisms that highlight actions that can prevent obesity, would be of importance for prevention of cardiometabolic disease [2, 3].

Levels of plasma triglycerides, both in the fasted and the non-fasted state, are higher in diabetes patients with insulin resistance than in diabetes patients without insulin resistance [4], and this has been suggested to be an independent CVD risk factor [4, 5].

Neurotensin (NT) is a 13-amino acid peptide expressed primarily in the brain and neuroendocrine cells of the small intestine. It is assumed that the absolute majority of the NT that is measurable in blood comes from the periphery, i.e. from the small intestine, where it is stimulated by dietary fat intake [6].

In the brain, NT acts as a neurotransmitter and regulates different functions like temperature regulation, nociception, pituitary hormone secretion and dopaminergic transmission. [7]. In the small intestine, neuroendocrine cells secrete NT in response to fat ingestion, and in animal studies, neurotensin has shown to be instrumental to promote fat absorption from the small intestine into the blood stream.[8, 9].

Research in recent years have shown that elevated fasting level of proneurotensin, i.e. a peptide derived from the NT precursor hormone that is release in parallel with the mature peptide in equimolar amounts, [10] predicts future development of obesity, diabetes mellitus, cardiovascular disease and premature mortality. Moreover, high plasma level of proneurotensin among obese subjects is strongly linked to fat accumulation in the liver, and to both the presence and severity of Non-Alcoholic Fatty Liver Disease (NAFLD) [11]. Interestingly, rodent models genetically deficient of NT absorbed less fat from the small intestine and were protected from diet induced obesity, insulin resistance and hepatic steatosis when compared to animals with intact NT production [9]. In line with this finding, an identical phenotype was observed in rodents lacking one of the key receptors of NT, i.e. NT-receptor 3 (NTSR3)[12]. Thus, in animals, NT seems to promote intestinal fat absorption, high
fat diet induced obesity and liver steatosis. In humans, circulating NT rapidly rises several minutes after a meal enriched in fatty acid [10] and it was demonstrated that NT acts as a hormone released from the small intestine following fat ingestion and that it facilitates fat digestion by stimulating pancreatic secretion in rats[13]. Collectively these data suggest that NT (in humans commonly proxie-measured by the stable proneurotensin peptide) promotes intestinal uptake of fat and central storage in the liver, a nutrient saver mechanism that might be disadvantageous and contribute to obesity, NAFLD, diabetes and CVD at high levels of fat intake. Importantly, although it is well known that NT rises after fat intake in both animals and humans, no human study has examined whether post-lipid ingestion rise in NT actually contributes to intestinal uptake of lipids. As this would be a plausible mechanism linking high NT to obesity and its sequels, we here tested if the magnitude of the rise in proneurotensin, induced by ingestion of saturated fat (cream) or unsaturated fat (olive oil), is related to amount of triglycerides appearing in the circulation after such oral lipid challenge.

Methods

22 healthy young subjects (10 men and 12 women) without any medication, volunteered to participate in this study. Participants received milk cream and olive oil at two separate occasions and all routine plasma laboratory analyses were performed using certified methods at the University Hospital’s central clinical lab. Blood pressure was measured in the seated position after 5 minutes rest.

After an over-night fast (from 10 PM the evening before the test day) all 22 subjects underwent ingestion of 150 ml of milk cream (54g fat) and 59 ml of pure olive oil (54 g fat) at two different occasions in random order, with the two oral lipid loads separated by one week. Blood samples were taken for measurement of plasma triglycerides, proneurotensin and blood glucose in the fasted state and at every hour for 4 hours. Plasma proneurotensin was measured from stored plasma, frozen at –80°C and stored immediately after sampling. Proneurotensin assays were performed blinded to clinical data at an independent laboratory (ICI immunochemical Intelligence GmbH, Berlin, Germany) by using a one-step sandwich immunoassay based on a chemiluminescence label and coated-tube technique (SphingoTec©, Hennigsdorf, Germany). The limit of detection of proneurotensin precursor
fragment was 1.9 pmol/L. [14] [15]

**STATISTICAL ANALYSIS:**

Clinical characteristics at the first visit are presented as means ± standard deviations (SD) for continuous variables and as numbers and percent (%) for dichotomous variables. The individual hourly plasma concentration values of proneurotensin, triglycerides and glucose after the respective oral lipid load was compared to the fasted value (post lipid load value minus fasted value) using paired t-test and the delta values are presented as means and 95% confidence intervals. We also calculated the change at the maximal post-lipid ingestion concentration of proneurotensin and triglycerides versus the baseline concentration. Further, areas under the Curve (AUC) of change in plasma proneurotensin and triglycerides post lipid ingestion were assessed using Pearson correlation analysis. Differences were considered statistically significant when two-tailed p-values were less than 0.05.

**Results**

The baseline (first visit) clinical characteristics of the study subjects are shown in Table 1, indicating healthy status. Dynamic changes of proneurotensin and triglycerides after ingestion of cream and olive oil, respectively, are shown in Figure 1. After ingestion of cream, proneurotensin in plasma rose significantly after one 1 hour and reached its maximum at 2 hours mean (95% confidence interval) of 22 (12–31) pmol/L (P<0.001) after which it declined. Triglycerides also rose significantly after 1 hour and reached its maximum after 3 hours 0.60 (0.43–0.78) mmol/L (P<0.001) before declining.

Similarly, after ingestion of olive oil, proneurotensin rose significantly after 1 hour and reached its maximum values after 3 hours 62 (46–78) pmol/L (P<0.001). Triglycerides rose significantly after 2 hours and reached its maximum after 3 hours 0.32 (0.18–0.45) mmol/L (P<0.001).

The post lipid load AUC for proneurotensin correlated significantly with the AUC for plasma triglycerides both after cream (r = 0.49, P = 0.021) and olive oil (r = 0.55, P = 0.008) (Figure 2).

In contrast to proneurotensin and triglycerides, plasma glucose concentrations significantly decreased at all-time points after lipid ingestion (both after cream as well as after olive oil) as compared to the fasted plasma glucose levels. (Supplementary Table), however, the AUC of glucose was not
significantly correlated to the AUC of proneurotensin neither after cream ($r = -0.20$, $P = 0.36$) nor after olive oil ($r = -0.25$, $P = 0.25$).

**Discussion**

We here show for the first time that there is a relationship between the degree of post-lipid ingestion rise in plasma concentration of proneurotensin and triglycerides in humans, as demonstrated by significant correlation between the post lipid load AUC of proneurotensin and the corresponding AUC of triglycerides after two different forms of lipid loads, i.e. cream and olive oil. This finding extends evidence of a pivotal role of NT in intestinal fat absorption from prior animal studies [9], to suggest a similar role of NT in humans.

Previously, high levels of proneurotensin have been shown to predict obesity, diabetes mellitus and cardiovascular disease, as well as show a relationship with NAFLD [11, 14, 16]. It is essential to understand mechanisms behind such relationships in order to understand if it might be meaningful to target the neurotensin system pharmacologically. Although we did not directly measure lipid translocation from the intestinal lumen to the blood stream, one can assume that rise in plasma concentration of triglycerides after standardized oral lipid loads more or less exclusively comes from the orally added lipids. Moreover, given the fact that the amount of rise in plasma concentration of proneurotensin after two different oral lipid loads significantly correlated with the rise in plasma triglycerides, it is reasonable to assume that post-lipid ingestion rise of proneurotensin contributes to translocation of the orally added fat from intestinal lumen to the blood stream.

Of note, most of the previous epidemiological studies have examined the associations between fasting level of proneurotensin and cardiometabolic diseases [14, 16, 17], and whether proneurotensin triggered by fat containing meals has a different relationship with risk of cardiometabolic disease is not known but should be further studied. We already know that high postprandial triglycerides is not only a characteristic feature of diabetes mellitus and insulin resistance but also is an independent risk factor of future cardiovascular events, and might be stronger than the fasting level of triglycerides. [18] Given the link between post-prandial triglyceridemia and cardiovascular risk, and our current findings of relationship between post-lipid
ingestion plasma concentration of proneurotensin and triglycerides, one can speculate that
neurotensin contributes to the cardiovascular risk associated with high post-prandial triglycerides.
This certainly needs further studies but necessitates discussion on whether proneurotensin levels are
modifiable.
The most obvious intervention to reduce postprandial proneurotensin levels is to reduce its trigger,
i.e. fat intake. From a pharmacological point of view, the intestinal lipase inhibitor orlistat is of
particular interest. Orlistat inhibits both the suggested key effect of neurotensin, i.e. intestinal fat
absorption, and intestinal release of neurotensin into the blood stream, the latter being explained by
absence of the trigger for neurotensin production and secretion in the enterocyte following orlistat
administration, i.e. intracellular fatty acids. Thus, we suggest that in future studies of subjects with
high post-prandial proneurotensin and an energy conserving phenotype due to enhanced intestinal fat
absorption, we might benefit from low fat intake and possibly orlistat therapy in order to both reduce
intestinal fat absorption and to block neurotensin secretion in order to prevent other potentially
harmful effects of high neurotensin (e.g. hepatic fat accumulation)[9].
We acknowledge some of the limitations in our study. We cannot explain the mechanisms by which
post-lipid ingestion rise in proneurotensin affects plasma postprandial triglyceride concentrations,
however, the suggested mechanism emanates from previous animal experimental studies. The study
included a rather small number of normal subjects. As we were interested in the physiological
interplay between fat, proneurotensin and triglycerides, we selected to study normal healthy subjects.
It will be important to test the pathophysiological relationships in human diseases associated with
high proneurotensin, i.e. obesity, type 2 diabetes, NASH and cardiovascular disease.

Conclusion
In conclusion, proneurotensin increases sharply after an oral lipid load of both cream and olive oil and
the degree of rise of post-ingestion proneurotensin is significantly related to the rise of post-ingestion
plasma triglycerides. Our human data support recent results from animal studies suggesting that
proneurotensin contributes to intestinal absorption of lipids into the blood stream.

List Of Abbreviations
Declarations

Ethics approval and consent to participate

All participants have provided written informed consent to participate.

Consent for publication

The data does not include any individual personal data including individual details, images or videos and hence consent for publication is not applicable in this study.

Availability of data and materials

Data analysis are included in the published article and is available on request.

Competing interests

JS is employed by Sphingotec GmbH, a company having patent rights in the proneurotensin assay and commercializing it. AB is CEO of Sphingotec GmbH and holds shares in this company. The other authors declare that they have neither disclosures nor financial or non-financial competing interests.

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Authors Contribution

OM and AF carried out the study, participated in the design of the study and performed the statistical analysis. All authors have participated in design and coordination and helped to draft the manuscript.
All authors have read and approved the final manuscript.

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**Table**

Due to technical limitations, Table 1 is only available for download from the Supplementary Files section.

**Figures**
Figure 1

Changes of proneurotensin and triglycerides after ingestion of cream and correlation of post lipid load AUC for proneurotensin with the AUC for plasma triglycerides after cream.
Figure 2
Changes of proneurotensin and triglycerides after ingestion of olive oil and correlation of post lipid load AUC for proneurotensin with the AUC for plasma triglycerides after olive oil.

Supplementary Files
This is a list of supplementary files associated with this preprint. Click to download.
SUPP TABLE 1.docx
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