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bncdoc.id	HWT
bncdoc.title	Gut: Journal of Gastroenterology and Hepatology.
bncdoc.info	Gut: Journal of Gastroenterology and Hepatology. Sample containing about 59868 words from a periodical (domain: applied science)
Text availability	Worldwide rights cleared
Publication date	1985-1993
Text type	Written books and periodicals
David Lee's classification	W_ac_medicine

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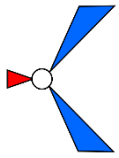

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five minutes at 10000 g and the supernatant labelled and stored at -20°C until assayed. The stored aliquots of urine and serum were later thawed at room temperature, thoroughly whirlmixed, centrifuged for five minutes at 10000 g, and the supernatant assayed until the APGPR ELISA for free APGPR that we have previously described. All samples were assayed at a 50% dilution in immunoassay buffer and corrected for dilution after subtraction of the assay detection limit. Urinary APGPR excretion was calculated as a product of the urinary concentration for a given sample and the volume/ hour of urine output over that sampling period. Two 7.5 ml urine samples from one male subject at 75 and 90 minutes after the onset of the meal, collected and stored as previously described, were thawed at room temperature, pooled, lyophilised, and then reconstituted by mixing with 6 ml distilled water. This pooled concentrated urine was then centrifuged at 10000 g for five minutes and 1 ml of supernatant chromatographed on a Sephadex G-25 column (0.9×43 cm) in TRIS buffered saline (TBS) containing 50 mM TRIS/HCl, 0.15 MNaCl, 3.1 mM NaN 3 at pH 7.3 at a flow rate of 39.5 ml/ hour and 1.056 ml fractions collected and assayed using the APGPR ELISA. Similarly, three 8 ml urine samples collected at 240, 300, and 360 minutes after the onset of the meal from the third morbidly obese subject were pooled, lyophilised, and reconstituted as before with 6 ml distilled water. The resultant pooled concentrated urine was centrifuged and 1 ml chromatographed and assayed as before. The chromatography column was calibrated using synthetic APGRP in 50 mM TRIS buffered saline and the fractions assayed for APGPR. STATISTICAL ANALYSIS The results of sequential APGPR propeptide assay of urine and serum were analysed using the Wilcoxon matched pairs signed ranks test and the urinary APGPR concentrations and excretion from normal and morbidly obese groups analysed using the Kruskal-Wallis one way analysis of variance (ANOVA). Results APGPR CONCENTRATION IN SERUM In the normal subjects the fasted mean serum APGPR concentration was at the detection limit of the assay but postprandially two peaks of APGPR immunoreactivity were seen in the first and second hours after the start of the meal (Fig 1). Only the peak at 90 minutes, however, achieved a significant difference ($p<0.05$) from the premeal value. No APGPR was detected in any of the serum samples from the three morbidly obese patients while fasted or after the test meal. APGPR CONCENTRATION AND EXCRETION IN URINE In the normal subjects (Fig 2A), the APGPR concentrations in urine increased after **the test meal** and again two peak mean values of APGPR immunoreactivity were seen in the first and second hours at 45 and 75 minutes after

the start of the meal

corresponding in time with the peaks of serum APGPR found in the normal subjects. These peaks were both significantly different from the premeal values ($p<0.05$). In the obese subjects (Fig 2B) there was no significant overall increase in urinary APGPR concentration after **the meal** and there was no significant difference between the urinary APGPR concentration at any given time postprandially between the morbidly obese and normal groups. In the first and third obese subjects (MO1

	and MO3), however, the total urinary APGPR concentration slowly increased towards the end of the sampling period, four to six hours after the onset of the meal. Urinary APGPR excretion (Fig 3A) in the normal group increased to a peak mean value at 82.5 minutes after the start of the meal and was significantly increased at 7.5, 67.5, and 82.5 minutes compared with the fasted values. In the obese subjects the total urinary APGPR excretion showed no significant overall increase (Fig 3B) although in the second obese subject (MO2) the APGPR excretion in urine was similar to that seen in normal subjects - that is, an early peak within the first two hours after the onset of the meal. Again there was no significant difference between the morbidly obese and normal group's urinary APGPR excretion at any given time postprandially. Figure 4 shows a comparison of the APGPR urine concentrations between the normal subjects and MO1 and MO3 emphasising the differing appearance of the immunoreactive signal between these two groups. The significant difference at 45 and 360 minutes postprandially between the results in the normal and these two morbidly obese subjects can not be accounted for by a difference in the urine output (one way ANOVA, $p>0.05$). A similar difference in urinary APGPR excretion postprandially was seen between the normal subjects and MO1 and MO3 and was significant at 15, 30, and 45 minutes after the start of the meal (data not shown). CHROMATOGRAPHY OF URINARY APGPR Assay of fractions of pooled and lyophilised urine from the second APGPR immunoreactivity peak from one of the normal male subjects clearly showed that the immunoreactive signal coeluted with synthetic APGPR suggesting that the immunoreactive peak was principally a result of the native peptide (Fig 5A). Finally chromatography and assay of the lyophilised late pooled urine APGPR peak in the third morbidly obese subject again confirmed that the main immunoreactive signal comigrated with APGPR (Fig 5B). Discussion The N terminal pentapeptide of pancreatic procolipase is highly conserved in nature with only three forms found to occur in the higher vertebrates, VPDPR, VPGPR, and the APGPR form found in humans and chickens. This homology is not confined only to the kinking proline residues and the long basic side chain of arginine but also