Circulating immunoreactive somatostatin in man.
Effect of age, pregnancy,
growth hormone deficiency and achlorhydria

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Abstract. Plasma immunoreactive somatostatin (IRS) levels were measured fasting at 09.00 h in groups of adult
individuals and children of different ages, as well as in pregnant women, in patients with pernicious anaemia
documented to be achlorhydric, and in children with growth hormone deficiency.

There was a gradual rise in the mean level of IRS from
the third decade (mean 35.8 ± 3.8 pg/ml), which reached
significance at the seventh (61.1 ± 8.4 pg/ml), eighth
(66.7 ± 5 pg/ml) and ninth decade (89.6 ± 13.8 pg/ml).
No change was observed in the second 28.3 ± 3.8 pg/ml
and third (31.1 ± 3.2 pg/ml) trimester of pregnancy
when compared with matched, non-pregnant controls
(29.7 ± 2.2 pg/ml); however, the children aged under
2 years (69.6 ± 11.2 pg/ml) had significantly higher values
than the eldest group (12 to 16 years old) (46.3 ± 7.2
pg/ml) (P < 0.05). In achlorhydric patients, basal (27.2
± 3.7 pg/ml; P < 0.01) and postprandial IRS (42.8 ±
7.7 pg/ml; P < 0.001) was significantly lower than in a
matched, normal control group (basal 59.4 ± 7.2; post-
prandial 132.1 ± 26.3 pg/ml). Growth hormone defi-
ciency was not associated with any differences in circu-
lation IRS, basally or after insulin hypoglycaemia, when
compared with values in normal children.

These results would suggest, 1) that age has a significant
effect on plasma IRS, and should be considered in the
interpretation of fasting plasma levels of IRS; 2) that
pregnancy and growth hormone deficiency is not accom-
panied by any changes in circulating IRS and presum-
ably, somatostatin binding proteins; 3) that gastric acid
is necessary for a normal release of IRS from the gastro-
intestinal tract to the circulation.

Somatostatin, initially isolated from ovine hypo-
thalamus has also been demonstrated in the gut,
pancreas, central and peripheral nervous system,
thyroid gland, amniotic fluid and placenta of dif-
ferent species; it was also found to circulate in
plasma of different species, including man (for
review see Reichlin 1983a,b).

Despite numerous publications aimed at dis-
covering its physiological role, its importance in
disease and the mechanisms involved in its secre-
tion and metabolism (Wass 1982; Reichlin 1983a,b),
little interest had been centered on the effect of age
on circulating immunoreactive somatostatin (IRS).
Therefore, the first aim of this study was to investi-
gate the levels of circulating IRS in healthy subjects
of ages ranging from a few weeks to the ninth
decade of life. Furthermore, since an arterio-
venous gradient has been demonstrated in the
umbilical vessels (Saito et al. 1983), and pregnancy
is associated with variations in the levels of plasma
binding proteins, the effect of pregnancy was also
investigated in a group of otherwise healthy wo-
men. Also, given the inhibitory effect of somatostatin on pituitary hormone release, it was decided to determine the concentration of circulating IRS in children with treated or untreated growth hormone deficiency, basally and in response to insulin hypoglycaemia. Finally, given that the main source of peripheral plasma IRS seems to be the gastrointestinal tract and that its release seems in part to be related to that of gastric acid (Schusdziarra et al. 1978; Colturi et al. 1984; Webb et al. 1984), it was decided to determine the concentration of circulating IRS in patients with documented pernicious anaemia and achlorhydria, basally and in response to a standard meal.

Materials and Methods

**Normal range of fasting basal IRS**

Fasting plasma samples were obtained from 76 adults, aged between 20 and 87 years. The 40 younger subjects (aged less than 50) were healthy hospital and laboratory personnel on no medication. The remaining 36 individuals were patients admitted to the hospital for different reasons, who were studied immediately prior to discharge. The diagnoses for these patients were 17 cardiovascular disorders, 8 respiratory infections, 7 venous ulcers and in 4 cases the admission was justified for social reasons in elderly patients. These patients were selected on the basis of the treatments received over the last month; these were various antibiotics, diuretics, non-steroidal analgesics, hypertensives, bronchodilators and diazepoxides; none had been on hormonal or antacid therapy. No patient suffered any known gastro-intestinal, renal, endocrine or hepatic disorder.

Additionally, fasting plasma samples from 81 healthy children selected from the patients bled in the laboratory of a paediatric hospital were investigated. The reason for bleeding was a pre-operative evaluation for adenoidtonsilslectomy or a normal analytical control after a respiratory or urinary infection in otherwise healthy children. The ages of these children ranged from 3 months to 16 years. They were divided into 4 groups: infants under 2 years, pre-adrenarchal children (aged 2 to 7 years), adrenarchal (aged 8 to 11 years) and pubertal (aged 12 to 16 years).

**Fasting IRS in pregnancy**

Samples for IRS were obtained from 7 women in the second trimester, and 13 women in the third trimester of a normal pregnancy, coinciding with routine blood tests to monitor the normal course of their pregnancy. Their values were compared with those of 15 healthy, non-pregnant, age-matched women.

**IRS in growth hormone deficient (GHD) children**

Twenty-two patients aged between 6 and 19 years, with GHD formed this group. The diagnosis of GHD was made after evaluating the child’s growth curves and GH response to t-Dopa, insulin hypoglycaemia and/or clonidine (maximal GH response less than 7 ng/ml). Nine of these patients were studied prior to initiating replacement therapy, while the remaining 13 were evaluated during GH treatment (0.1 IU/kg body weight im twice weekly for 9 to 21 months). In all, IRS was measured basally, and in 8, IRS was also measured after a diagnostic iv insulin tolerance test (0.1 IU/kg body weight). Basal IRS values were compared to those of a group of 9 age-matched children with no endocrine abnormality. The response to hypoglycaemia was compared to that of 7 children who had undergone a similar diagnostic insulin hypoglycaemia, which demonstrated no endocrine abnormality, so that the diagnosis of constitutional delayed growth was made. Samples for IRS were obtained basally, and at 60 and 90 min after insulin. These time points were chosen, since we knew from previous studies that they represent those, where a maximal response can be expected (Wass et al. 1981; Webb et al. 1984).

**IRS in pernicious anaemia (PA) and achlorhydria**

Nine patients (mean age 59 years, range 32 to 70 years) with documented PA and achlorhydria were studied on 2 different occasions in random order. The diagnosis was established by Schilling tests, positive parietal cell antibodies, megaloblastic anaemia, low levels of vitamin B12, hypergastrinaemia and a lack of response of gastric acid to pentagastrin administration. On one occasion, a standard breakfast containing 67 g of carbohydrates, 20 g of fat and 17 g of protein was administered and blood samples obtained basally and intermittently for 3 h; for the control study, blood was drawn at the same time points but patients were maintained fasting. Their results were compared with those of an age matched (mean age 66 years, range 57 to 86 years) control group of 5 healthy volunteers, who were studied before and after a standard breakfast.

Plasma IRS was measured by radioimmunoassay as previously described (Penman et al. 1979). Prior to assay, somatostatin was extracted using Vycor glass; [125I]tyrosine11 somatostatin was used as a tracer together with a highly specific rabbit antisomatostatin serum (initial dilution 1:30 000) which gives a lower limit of detection of 10 pg/ml. This antibody is directed against the central portion of somatostatin-14 and cross-reacts on an equimolar basis with somatostatin-14 and somatostatin-28 (Penman et al. 1979). The intra-assay coefficient of variation was 12% for values around 70 pg/ml and 13% for concentrations around 200 pg/ml, while the coefficient of variation between assays was 15% for values around 70 pg/ml. All samples diluted in parallel to the cyclic somatostatin standard.
All patients included in this study gave their informed consent to the study, which was approved by the hospital ethical committees.

Results are expressed as mean ± standard error of the mean (SE). Comparisons between the different groups of individuals were tested for significance by analysis of variance and by the Newman-Keuls multiple range test. If only 2 groups were compared, a Mann-Whitney U test was used. When the IRS response to a stimulus was analyzed, comparisons between the groups at the different time points, and between basal and stimulated values were made.

**Results**

The fasting IRS levels in adults and children are seen in Fig. 1. In adults the circulating IRS value were grouped according to age decades; a statistically significant increase in mean circulating IRS was observed after the seventh decade of life. Thus, IRS was significantly higher in the seventh (61.1 ± 8.4 pg/ml) than in the third decade (35.8 ± 3.8 pg/ml) \((P < 0.05)\); in the eighth decade (66.7 ± 5 pg/ml) than in the third \((P < 0.01)\) and fourth decades (34.7 ± 4.7 pg/ml) \((P < 0.01)\); and in the ninth decade (82.6 ± 13.8 pg/ml) than in the third \((P < 0.01)\) and fourth decades \((P < 0.01)\).

When the 4 groups of children were compared, a tendency with increasing age for plasma IRS concentration to fall was observed, which although just escaped statistical significance when all 4 groups were analyzed, was statistically different when the youngest infants aged under 2 years (69.6 ± 11.2 pg/ml) were compared with the adolescent 12 to 16 year olds (46.3 ± 7.2 pg/ml) \((P < 0.05)\). The 2 to 7 year olds (52.3 ± 3.1 pg/ml) and 8 to 11 year olds (51.7 ± 3.7 pg/ml) had intermediate values.

In pregnancy, no changes in basal plasma IRS were found in women in the second (28.3 ± 3.8 pg/ml) and third trimester (31.1 ± 3.2 pg/ml) when compared with the non-pregnant controls (29.7 ± 2.2 pg/ml).

Basal IRS concentrations in children with and without GHD did not differ significantly (no GHD 51 ± 6.6 pg/ml; untreated GHD 67.1 ± 9.1 pg/ml; treated GHD 58.4 ± 9.4 pg/ml). Independently of the status of their GH secretion, after insulin hypoglycaemia there was a slight, albeit statistically non-significant mean rise in circulating IRS in normal and GHD children, which were not significantly different (Fig. 2). However, taken individually one out of 7 normals, and 4 out of 8 GHD children did not show a significant rise in circulating IRS. This is in sharp contrast with the adult

### Fig. 1.

Basal circulating immunoreactive somatostatin (IRS) in healthy adults and children.

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data, where all subjects demonstrated a significant rise from basal at 60 and 90 min (Webb et al. 1984). In all cases, blood glucose fell 50% or more.

Basal IRS in achlorhydric patients was significantly lower (27.2 ± 3.7 pg/ml) than in the control group (59.4 ± 7.2 pg/ml) (P < 0.01). Furthermore, the postprandial rise in these patients (42.8 ± 7.7 pg/ml) was also significantly lower than in the normal subjects (132.1 ± 26.3 pg/ml) (P < 0.001) (Fig. 3). No change was observed during the saline control study in the achlorhydric patients (mean values between 28 and 16 pg/ml).

**Discussion**

This study demonstrates for the first time that basal circulating somatostatin levels fall throughout childhood, but in adults rise with age, and that achlorhydric patients with pernicious anaemia have significantly lower circulating IRS, both basally and postprandially, when compared with the levels attained in a group of healthy, age-matched volunteers. This differs from other hormones which increase with age, such as human pancreatic polypeptide, which is normal basally and in response to food in patients with pernicious anaemia (Floyd et al. 1977).

A rise in circulating IRS has been demonstrated after the seventh decade of life. The mechanisms
responsible for this increase remain uncertain. A possible explanation for this rise in circulating IRS might be related to the decline in renal function which occurs with aging (Marcus et al. 1984). Somatostatin has been shown to undergo metabolization in the kidney, and an impaired metabolic clearance rate of somatostatin has been observed in uraemic patients (Sheppard et al. 1979); however, in another study, no rise in plasma somatostatin-like immunoreactivity was observed in patients with end-stage renal failure (Lundquist et al. 1979). Thus, it is unlikely that a slight impairment of renal function, not capable of increasing circulating urea and creatinine levels above the normal range, could be the main responsible factor for the rise in IRS observed in elderly patients.

Somatostatin has also been found to be metabolized in the liver (Sacks & Terry 1981; Webb et al. 1983), pancreas (Taborsky & Ensink 1983) and the splanchnic area (Webb et al. 1983). Given that many of these organs undergo changes in the normal aging process, it is feasible that a combination of interacting factors may be causally related to the increase in circulating IRS in the older individuals. Furthermore, the potentially different molecular nature of IRS in these various situations, which might explain some of the variations observed by different investigators, has not been adequately evaluated.

Furthermore, gastric mucosa atrophy and subsequent decrease in gastric acid are known to occur in over 90% of patients, by the seventh decade (Kimura 1972); it has been postulated that a change in the ratio of cells secreting gastrin to cells secreting somatostatin may be related to this atrophy (Vierling & Reichen 1982). Indeed, a decrease in the ratio of gastrin-secreting/somatostatin-secreting cells has been found to occur with age in the stomach of the rat (Lehy et al. 1979). However, a decrease in gastric acid secretion, together with a rise in circulating plasma gastrin are known to occur both in achlorhydria and in elderly patients, deposite opposite changes in circulating IRS.

IRS has been found to exist in the human placenta and amniotic fluid (Fitzpatrick & Patel 1979). However, we found no difference in circulating peripheral IRS during pregnancy when compared with matched non-pregnant women, confirming the earlier results of Saito et al. (1983). These investigators also found IRS to be higher in umbilical cord blood, than in simultaneous maternal peripheral venous samples, and an arteriovenous gradient, suggesting a fetal origin of this somatostatin-like immunoreactivity. We have found higher IRS levels in infants aged under 2 years when compared with older children aged 12 to 16 years. These results are in agreement with the preliminary report of Koshimizu et al. (1984), where the infants less than 9 months old also had higher levels than the older children. As with elderly patients, these differences may reflect varying degrees of functional maturation of the gastro-intestinal tract, or increased availability of somatostatin when compared with other gastrointestinal or pancreatic hormones (Adelman & Sartin 1984).

Circulating IRS concentrations probably do not reflect alterations in the hypothalamic-pituitary control of GH secretion, since children with documented GHD did not differ from others with no GH abnormality. The more heterogeneous response to insulin observed in children as compared to that seen in adults (Webb et al. 1984) may be related to the different degree of hypoglycaemia attained in both groups. Children received too thirds of the insulin dose administered to the adults, and even though the hypoglycaemic nadir was not significantly different, glucose recovery after hypoglycaemia was much more prompt in the children (at 90 vs 150 min).

Acid administered intragastrically to dogs has been shown to stimulate IRS release into the circulation (Schusdziarra et al. 1978). Further, the iv administration of cimetidine, an H2 blocker which inhibits gastric acid secretion, has been found to partially decrease circulating IRS after food (Wass et al. 1981) and insulin hypoglycaemia (Webb et al. 1984). Truncal vagotomy in patients with peptic ulcer disease (Glaser et al. 1981) and autonomic neuropathy in diabetics (Hilsted et al. 1982; Fernández-Castañer et al. 1985) are also known to abolish the IRS response to insulin hypoglycaemia, pointing towards a possible role of gastric acid in the IRS release. This hypothesis is strengthened by the recent finding of Colturi et al. (1984), that physiological concentrations of circulating somatostatin are capable of inhibiting gastric acid release; however, gastrin inhibition was only attained when several-fold higher circulating levels of the infused tetradecapeptide were reached. Nevertheless, gastric acid may not be the only factor, since with cimetidine there is not complete abolition of the postprandial IRS response, as seen after atro-
pine (Lucey et al. 1985). Thus, it is possible that other factors present in these achlorhydric patients with pernicious anemia, may contribute to their deficient circulating IRS levels.

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