Letters to the editor

Plasma human amylin levels

Dear Sir,

Eriksson et al. [1] report that amylin (which has also been called islet amyloid polypeptide) is unlikely to be involved in the development of Type 2 (non-insulin-dependent) diabetes mellitus since people at risk of developing diabetes exhibit "normal" plasma amylin concentrations. At present no standardised values are available to determine what constitutes "normal" or "abnormal" plasma amylin levels in man. Insulin levels are measured in a single-step procedure. Plasma human amylin levels are not [1, 2] and, thus, the reported amylin values may be underestimated. Amylin and its homologue, calcitonin gene-related peptide, readily adsorb onto plastic. Special procedures must be adopted for handling these peptides [3]. For human amylin measurements the ideal storage conditions of plasma need to be established and reported (e.g. what is the utility of frozen plasma; the length of time plasma can be stored frozen before amylin measurements are made; which protease and peptidase inhibitors need to be included with the plasma). Also, the steps undertaken to prevent spontaneous fibril formation by human amylin should be described. The loss of native human amylin (not synthetic human amylin) in the convoluted plasma extraction procedure needs to be reported. Furthermore, the kinetics of amylin and insulin release from the pancreas are different. In the rat it is possible that independent mechanisms exist for the secretion of amylin and insulin [4]. Plasma amylin secretion shows a progressive rise during perfusion of isolated rat pancreases with glucose [4]. Consequently, the time course for measurement of amylin and insulin should be long enough to determine the area under the curve for both hormones. Clearly this has not been done in the study reported by Eriksson et al. [1]. Indeed the values for the area under the amylin curve in people at increased risk of developing diabetes ("at risk" group) is, in reality, the area under a half-curve. Indeed, if it is possible to extrapolate the data reported by Eriksson et al. [1] then we can determine roughly the time when insulin and amylin levels would reach basal values in the control and "at risk" groups. Thus, the plasma insulin levels would reach basal values for the control and for the "at risk" groups after 150 and 180 min respectively. In marked contrast, at the end of the experimental procedure the plasma amylin levels are still falling in the control group and have only reached a plateau in the "at risk" group. Basal plasma values for amylin would be reached after 240 min in the control subjects and by 450 min in the "at risk" group. Therefore, people at risk of developing diabetes probably exhibit abnormally high rates of amylin secretion, possibly exposing peripheral tissues to higher than normal levels of amylin during a period ranging from 2–7 h after a rise in blood glucose concentration. This is most likely to impair the response of skeletal muscle to insulin after the second and third phases of insulin secretion in a day. Clearly, to investigate this possibility studies examining the plasma levels of amylin in humans need to be repeated over a longer time course. Importantly it now seems likely that the molar ratios of amylin to insulin vary greatly depending on the nutritional status of the animal and how long after feeding the blood is sampled.

We originally demonstrated that calcitonin gene-related peptide inhibits insulin-mediated glucose metabolism in skeletal muscle in vitro at concentrations as low as 100 pmol/l [5]. Recent studies have

also demonstrated that amylin modulates insulin-mediated glucose metabolism in skeletal muscle at these low concentrations (200–400 pmol/l) both in vitro and in vivo [6–8]. Therefore, the effects of amylin on insulin-mediated glucose metabolism in skeletal muscle occurs at concentrations 5–10-fold (not 100–1000-fold as stated by Eriksson et al. [1]) greater than the glucose-induced circulating amylin concentrations reported by Eriksson et al. [1]. Of course this concentration for the effect/plasma concentration ratio changes dramatically if human plasma amylin levels are underestimated and in conditions associated with higher-than-normal concentrations of amylin, such as glucose-intolerance [1] and Type 2 (non-insulin-dependent) diabetes mellitus.

Yours sincerely,
B. Leighton

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Response from the authors

Dear Sir

The problem of defining normal laboratory values in scientific research is certainly universal and we agree that there is no consensus regarding "normal" plasma islet amyloid polypeptide (IAPP) concentrations in humans. We are aware of the problems with the handling and storage of IAPP and agree that the optimal conditions still need to be established. The methods used for sampling and handling of the IAPP samples have been previously described. Briefly, blood was drawn into chilled polypropylene tubes containing EDTA·Na₂ (1 mg/ml) and Trasylol (500 U/ml). The samples were immediately centrifuged, stored at -40°C, and assayed within 2 weeks. The plasma was centrifuged again, and diluted to one-half with 0.9% NaCl. The sample was applied to a Sep-Pak C-18 cartridge (Waters Chromatography Div., Milford, Mass., USA), which was pre-equilibrated with 0.9% NaCl. The cartridge was washed with 0.9% NaCl and 0.1% trifluoroacetic acid solution, then absorbed materials were eluted into polypropylene tubes with 60% acetonitrile (CH₃CN) solution containing 0.1% trifluoroacetic acid. Triton X-100 (0.1%, 10 µl) and bacitracin (10 µg) were added to column eluates to avoid adsorbance onto tubes during lyophilization. The sample was dissolved in RIA buffer prior to testing by RIA. In this procedure, recovery of "cold" and radiolabelled human native IAPP was 83.4 ± 3.5% (SEM) and 87.6 ± 1.5% (SEM), respectively. Inter- and intra-assay variations were less than 13% and 8%, respectively [1, 2].

We agree that sampling for IAPP could have been extended beyond the 2 h oral glucose tolerance test (OGTT) to obtain a more reliable estimate of plasma IAPP concentrations. It is, however, unlikely that the lack of these prolonged measurements would influence the conclusions drawn. We have, however, compared IAPP responses in 10 insulin-resistant (M-value during euglycaemic, hyperinsulinaemic clamp 3.9 ± 4 mg·kg⁻¹·min) and 10 insulin-sensitive (M-value 6.4 ± 6 mg·kg⁻¹·min) first-degree relatives of Type 2 (non-insulin-dependent) diabetic patients. In these subjects we found similar IAPP responses to the OGTT (sum of IAPP-concentrations during the OGTT; 97 ± 11 pmol/l vs 92 ± 12 pmol/l; *p* = NS). It is difficult to envisage the insulin-resistant subjects having the same IAPP response during the OGTT as the insulin-sensitive subjects, but increasing later on.

The circulating IAPP levels in humans have been reported to be around 10⁻¹¹ mol/l, i.e. similar to the basal levels we found. Leighton refers to the study by Frontoni et al. [3] as an example of a study where low concentrations of IAPP modulate insulin-mediated glucose disposal in skeletal muscle. However, the authors state: "the infusion of 10 pmol·kg⁻¹·min⁻¹ amylin (plasma concentration 5 × 10⁻¹¹ mol/l) did not affect insulin-mediated glucose metabolism, whereas the infusion of 500 pmol·kg⁻¹·min⁻¹ amylin (plasma concentration 10⁻⁸ mol/l during the euglycaemic clamp studies determined the onset of severe insulin resistance through a 32% reduction in insulin-mediated glucose uptake..." [3]. This means that the IAPP concentrations that affect glucose metabolism in skeletal muscle were 100–1000-fold higher than the circulating concentrations measured in our study.

In the study by Koopmans et al. [4] performed in rats, basal plasma IAPP levels were less than pmol/l. IAPP levels of 220 ± 75 pmol/l antagonized insulin action only in the liver. IAPP levels of 4750 ± 750 pmol/l were required to induce a 30% reduction of insulin-stimulated peripheral glucose uptake. These concentrations were 60-fold higher than the basal circulating levels measured. Koopmans and co-workers concluded that IAPP causes in vivo insulin resistance predominately in the liver [4]. Two recent studies also challenge the view that physiological elevations in plasma IAPP

concentrations will induce insulin resistance in skeletal muscle [5, 6]. Infusion of human HIAPP raised circulating IAPP levels 12- to 50-fold above basal, but no effect was observed on glucose or insulin homeostasis [6].

Given these points, we do not think that underestimation of IAPP levels due to technical reasons will significantly influence the conclusions as IAPP values in all groups would be influenced to the same degree. Of importance, peripheral IAPP levels did not differ between insulin-resistant or insulin-sensitive relatives, and the response to an oral glucose load was similar, with no evidence of hypersecretion of IAPP. We already pointed out in our report, that our data do not conclusively exclude IAPP as a possible contributor to insulin resistance in subjects at increased risk of developing Type 2 diabetes, but they certainly do not support such an association [7]. On the other hand, IAPP levels were increased in subjects with impaired glucose tolerance who also started to demonstrate defects in their insulin secretion [8]. From the data shown it seems more likely that elevated IAPP levels are involved in the pathogenesis of insulin deficiency than in the pathogenesis of insulin resistance.

Yours sincerely,

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