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The Gastro-Entero-Pancreatic Hormone Response to Fasting in Obesity

W. J. Andrews[1]*, R. W. Henry[1], K. G. M. M. Alberti[2] and K. D. Buchanan[1]

[1] Department of Medicine, The Queen's University Belfast, Northern Ireland and [2]Department of Clinical Biochemistry, University of Newcastle-Upon-Tyne, UK

Summary. A comparison of the metabolic and gastro-entero-pancreatic hormonal responses of ten obese and eight lean subjects to 12 h and 36 h fasts has been made. Each subject was given a 50 g oral glucose tolerance test at the end of both 12 h and 36 h starvation. After the 12 h fast blood glucose and 3-hydroxybutyrate were similar in each group but blood glycerol was 30% higher in the obese subjects. Plasma insulin and vaso-active intestinal polypeptide were also higher in the obese subjects after 12 h starvation. After 36 h starvation in the lean subjects blood glucose was unchanged but on refeeding with 50 g oral glucose, glucose tolerance was impaired. In the same group blood glycerol and 3-hydroxybutyrate rose after 36 h starvation. Plasma glucagon, secretin and vaso-active intestinal polypeptide rose after 36 h starvation in the lean subjects but plasma insulin was unchanged. Refeeding with oral glucose suppressed the increased plasma glucagon, secretin and vaso-active intestinal polypeptide. After the 36 h fast in the obese subjects, blood glucose was unchanged, blood glycerol fell, but blood 3-hydroxybutyrate rose although to a reduced level in comparison with the lean subjects. In the obese group there was no change in plasma glucagon, secretin or vaso-active intestinal polypeptide after 36 h starvation, although plasma insulin fell. The results show different metabolic and gastro-entero-pancreatic hormonal responses to fasting in lean and obese human subjects and suggest an important metabolic role of glucagon, secretin and vaso-active intestinal polypeptide during starvation.

Key words: Insulin, glucagon, secretin, vaso-active intestinal polypeptide, glucose, glycerol and 3-hydroxybutyrate.

Obesity and its consequences are a major cause of disability in the Western World, and in many obese patients dietary therapy may be ineffective. Of major interest and possible therapeutic relevance are the metabolic and hormonal abnormalities previously described during fasting in obesity, which suggest impaired lipolysis [1–7, 28].

Within the gastro-entero-pancreatic hormones there is a group with structural homology (glucagon, secretin and vaso-active intestinal polypeptide – VIP) all of which exhibit lipolytic actions in vitro [8–11, 22, 23]. To examine whether these hormones have metabolic roles in vivo and to determine whether there is an abnormal response of this group to fasting in obesity, the plasma glucagon, secretin, VIP and insulin responses together with the blood glucose, glycerol and 3-hydroxybutyrate responses to 36 h starvation have been measured in lean and obese subjects.

Subjects and Methods

Ten obese subjects (two males and eight females, aged 21–51 years) all with a body mass index of 29 or greater [30] and eight lean controls (three males and five females, aged 17–41 years) were fasted overnight (12 h). A 50 g oral glucose tolerance test was then administered and peripheral venous blood samples were taken at 30 min intervals for 2 h by an indwelling catheter. After eating a 300 g carbohydrate diet for at least 3 days the same groups were fasted for 36 h and a 50 g oral glucose tolerance test was repeated with peripheral venous blood sampling as before.

Venous blood was withdrawn into ice-cold heparinized tubes and the plasma was separated by centrifugation at 1,000 *g* for 20 min at 4° C before division for hormone estimation. The samples for insulin assay were stored at −20°C.

The antibody used (GP 25) for the radioimmunoassay of plasma insulin, was raised in guinea-pigs against porcine insulin. This cross-reacts with porcine pro-insulin to approximately 50% on a weight basis, and shows identical cross-reactivity with porcine and human insulin. Human insulin was used for standards (Division of Biological Standards, National Institute for Medical Research,

* Now Endocrinology Fellow, University of Texas at Dallas

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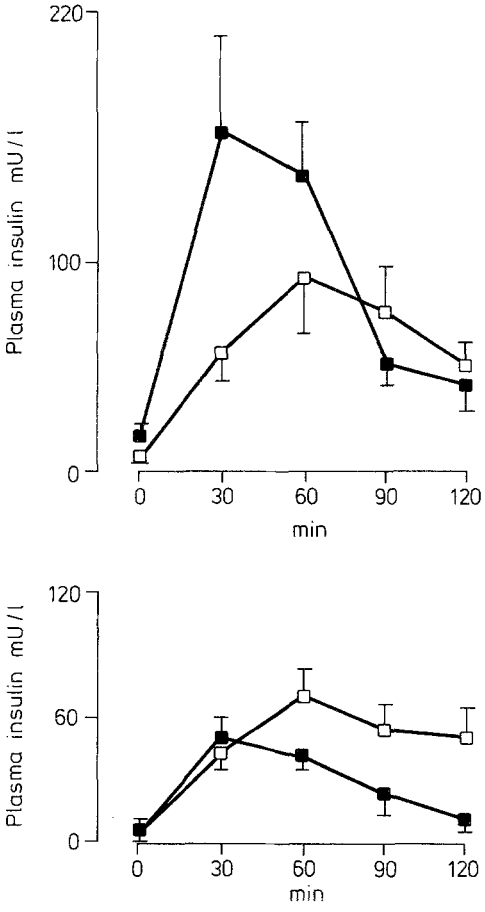

Fig. 1. A comparison of plasma insulin values (mean ± SEM) between obese (above) and lean (below) on refeeding with oral glucose after 12 h ■ and 36 h □ fasts

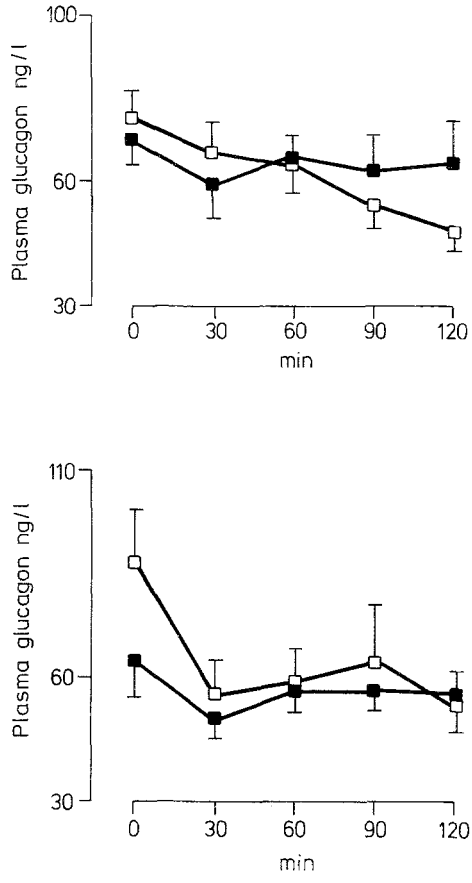

Fig. 2. A comparison of plasma glucagon values (mean ± SEM) between obese (above) and lean (below) on refeeding with oral glucose after 12 h ■ and 36 h □ fasts

London), I^{125} (Radiochemical Centre, Amersham, Bucks UK) was used for labelling and microfine silica was used for insulin purification [35]. Human charcoaled plasma was added to the standards to equilibrate the conditions between plasma samples and standards and dextran coated charcoal was used to separate free from bound hormone [17]. A sensitivity of 0.5 mU/l was achieved.

Plasma for glucagon, secretin and VIP assay was extracted with ethanol [14] and re-constituted before assay in 0.4 mol/l phosphate buffer, pH 7.4.

Antibodies for glucagon estimation were raised in New Zealand white rabbits to pork glucagon (Novo recrystallised) or beef-pork glucagon (Eli-Lilly recrystallised).

Glucagon I^{125} was prepared according to the method of Jorgenson and Larsen [36] and separation was achieved using serum and dextran coated charcoal [17]. The assay utilised two antibodies raised to pancreatic glucagon, the first named YY89 at a final dilution of 1:45,000 and the second YY57 at a final dilution of 1:22,500. YY89 reacts with the C-terminal region of glucagon and has been considered to be pancreatic glucagon specific although some species of gut glucagon react with YY89 [34] and some species of pancreatic glucagon do not react with YY89 (J. M. Conlon unpublished observations).

YY57 reacts with the N-terminal region of glucagon [34] and has been considered to be non-specific measuring total glucagon-like-immunoreactivity. Thus the material measured by YY89 is referred to a glucagon or C-terminal glucagon-like-immunoreactivity and that by YY57 is referred to as total glucagon or N-terminal glucagon-like-immunoreactivity. This system detects concentrations between 10 and 20 ng/l of glucagon. No cross reaction has been noted with other gut or islet hormones including insulin (Human MRC Standard, WHO Laboratory for Biological Standards, London), gastric inhibitory polypeptide and motilin (gifts from Professor J. Brown, Vancouver University, Canada), cholecystokinin-pancreozymin and VIP (both gifts from Professor V. Mutt, Karolinska Institute, Stockholm) and human synthetic gastrin.

The majority of glucagon measured by C-terminal antibody after an overnight fast has a molecular weight approximating to pancreatic glucagon, and is referred to as glucagon [5] in this study. All molecular species of glucagon apart from the void volume species are recovered by extraction, thus molecular species less than 12,000 Daltons are recovered and although this data has been worked out only with regard to glucagon it would appear likely that the same criteria apply to secretin and VIP. Glucagon, secretin or VIP added to plasma give a recovery approximating to 100% after extraction.

Porcine natural secretin (Gastro-Intestinal Hormone Research Institute, Karolinska, Stockholm) and natural porcine VIP (donated by Professor V. Mutt, Karolinska Institute, Stockholm) were used for standards with pork synthetic secretin (donated by E. Wunsch, Max Planck Institute, Munich) and natural porcine VIP used for labelling.

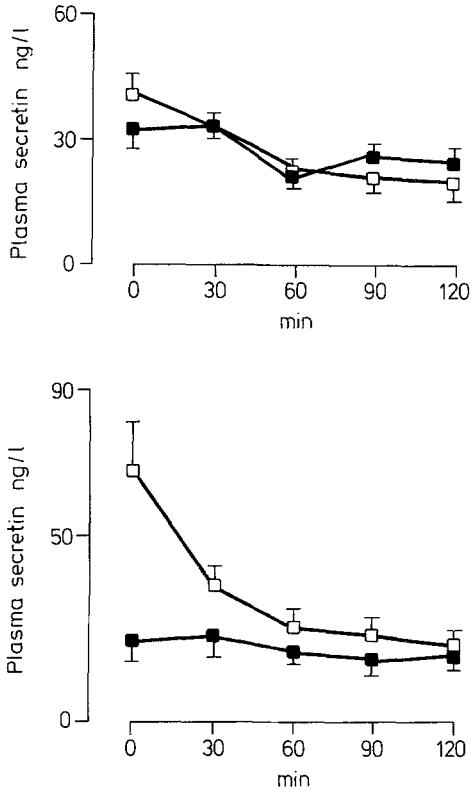

Fig. 3. A comparison of plasma secretin values (mean ± SEM) between obese (above) and lean (below) and refeeding with oral glucose after 12 h ■ and 36 h □ fasts

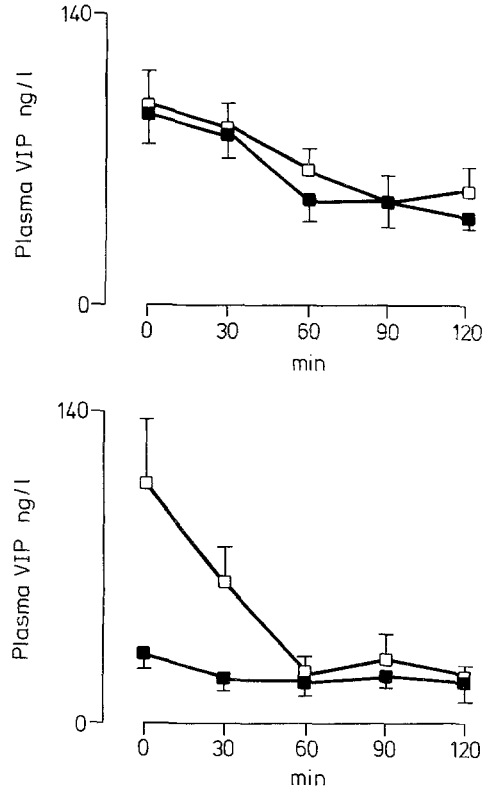

Fig. 4. A comparison of plasma vaso-active intestinal polypeptide (VIP) values (mean ± SEM) between obese (above) and lean (below) on refeeding with oral glucose after 12 h ■ and 36 h □ fasts

Secretin was labelled with I^{125} (Radiochemical Centre, Amersham, Bucks, UK) by the method of Holohan et al. [15] and antibodies were raised to porcine natural secretin [16]. This antibody (BB101) was used in a final titre of 1:36,000 and reacts with the C-terminal region of secretin. No cross-reaction was noted with glucagon, total glucagon, human insulin (Division of Biological Standards, National Institute for Medical Research, London) gastric inhibitory polypeptide and motilin (both donated by Professor J. Brown, Vancouver University, Canada) , cholecystokinin-pancreozymin (both donated by Professor V. Mutt, Karolinska Institute, Stockholm) or human synthetic gastrin. Extracts of human jejunum cross-reacted in the assay in an identical manner to the standards and using an ion exchange purified I^{125} secretin [16] a sensitivitiy of 6 ng/l was achieved, with 95% certainty over a range 0–300 ng/l.

VIP was iodinated by the Chloramine-T method [18] and the labelled hormone was purified by adsorption to silica with elution into acidified ethanol before storage at –20 °C in acidified ethanol. Natural porcine VIP was conjugated to ov-albumin for immunisation into New Zealand white rabbits, and the antibody raised (BK154) was used in a final dilution of 1:40,000. The assay detects 5 ng/l with 95% certainty over the range 0–300 ng/l. There is no cross-reactivity in the assay with glucagon, secretin or gastric inhibitory polypeptide and the antibody is predominantly C-terminal reactive. Separation of free from bound hormone in the radioimmunoassay was accomplished using serum and dextran coated charcoal [17] and standards were prepared in an alcohol extract of charcoaled horse serum to mimic the unknown sample. Further aspects of the assay have been reported elsewhere [19].

Blood for metabolites was mixed with 5 ml ice cold 0.8 mol/l perchloric acid for estimation of blood glucose, glycerol and 3-hydroxybutyrate by enzymic fluorimetric continuous flow assay [20].

Statistical Analysis

Results are expressed as mean ± SEM with comparison of results assessed by analysis of variance ('areas under the curve' were measured assuming a straight line between points). The two tailed Student 't' test was applied to the results and the level of significance was set at $p < 0.05$.

Results

Blood Glucose and Intermediary Metabolites

Blood glucose after 12 h fast was similar in each group (lean subjects 4.2 ± 0.1 mmol/l versus obese subjects 4.4 ± 0.2 mmol/l) and after 36 h fast no change in blood glucose occurred in either group. In the lean subjects glucose tolerance worsened after 36 h fast ($p < 0.001$) but there was no change in the obese subjects.

Blood glycerol in the lean subjects (0.18 ± 0.035 mmol/l) was 30% lower than in the obese sub-

jects (0.26 ± 0.04 mmol/l) after the 12 h fast. Suppression with oral glucose was only achieved in the obese subjects ($p < 0.001$). After the 36 h fast blood glycerol rose in the lean subjects to 0.23 ± 0.043 mmol/l ($p < 0.001$) and suppressed on refeeding with oral glucose ($p < 0.001$) at 30 min. In the obese subjects, blood glycerol fell after the 36 h fast to 0.16 ± 0.02 mmol/l ($p < 0.001$) and suppressed on refeeding with oral glucose ($p < 0.05$) at 30 min.

Blood 3-hydroxybutyrate was similar in lean and obese subjects after the 12 h fast (0.19 ± 0.12 versus 0.11 ± 0.07 mmol/l). Suppression was not achieved with oral glucose. After the 36 h fast 3-hydroxybutyrate rose to 1.68 ± 0.47 mmol/l in the lean subjects ($p < 0.001$) and in the obese subjects to 0.65 ± 0.16 mmol/l ($p < 0.001$). Concentrations after the 36 h fast were higher in the lean group ($p < 0.025$), but suppression on refeeding with oral glucose was achieved in both the lean subjects ($p < 0.05$) and the obese subjects ($p < 0.05$) at 60 min.

Hormones

Insulin. After the 12 h fast plasma insulin was higher in the obese subjects (15.0 ± 2.8 versus 7.2 ± 3.1 mU/l; $p < 0.05$). Starvation (36 h) suppressed plasma insulin to 8.6 ± 1.2 mU/l ($p < 0.05$) in the obese. On refeeding with oral glucose the insulin response (calculated by comparison of areas under the insulin response curves) increased only in the lean subjects ($p < 0.05$; Fig. 1).

Glucagon. Glucagon concentrations after the 12 h fast were similar in lean and obese subjects (64.4 ± 9.8 versus 71.5 ± 7.2 ng/l). No change was observed in either group on feeding with oral glucose. After the 36 h fast glucagon rose in the lean subjects to 91.9 ± 15.6 ng/l ($p < 0.025$). No change was observed in the obese. On refeeding with oral glucose suppression was achieved at 30 min in the lean subjects ($p < 0.005$) and at 90 min in the obese subjects ($p < 0.01$; Fig. 2).

Secretin. After the 12 h fast plasma secretin was similar in lean and obese subjects (30.6 ± 4.8 versus 32.5 ± 5.2 ng/l) but oral glucose feeding suppressed plasma secretin at 60 min, only in the obese subjects ($p < 0.05$). The 36 h fast raised plasma secretin in the lean subjects to 70.6 ± 12.6 ng/l ($p < 0.001$) but no change was observed in the obese subjects. Refeeding with oral glucose suppressed plasma secretin in the lean subjects after 30 min ($p < 0.001$) and in the obese subjects after 60 min ($p < 0.001$) (Fig. 3).

VIP. After the 12 h fast plasma VIP was higher in the obese subjects than in the lean subjects (91.0 ± 12.6 versus 33.1 ± 7.1 ng/l; $p < 0.005$). Oral glucose suppressed plasma VIP at 60 min only in the obese subjects ($p < 0.001$). Plasma VIP rose after 36 h fast only in the lean group (117.5 ± 28.3 versus 96.5 ± 14.5 ng/l; $p < 0.001$) but oral glucose refeeding suppressed plasma VIP in the lean subjects at 30 min ($p < 0.005$) and in the obese subjects at 60 min ($p < 0.01$; Fig. 4).

Discussion

The enhancement or depression of lipolysis may be described as the ratio of 'insulin and other anti-lipolytic hormones to glucagon and other lipolytic hormones' [39]. During fasting, which is a condition of fuel need, when the sympathetic nervous system output falls [40], insulin must fall or glucagon and other lipolytic hormones must rise if lipolysis is to occur. A rise in glucagon has been demonstrated during fasting in normal weight subjects [24], but it has been suggested that the lipolytic effect of glucagon may only be exerted when the trophic effect of insulin on adipose tissue can be circumvented [21].

Our results have demonstrated a rise in glucagon without a concomitant fall in insulin during 36 h starvation in lean subjects. In contrast a fall in insulin to levels comparable with the lean subjects, was observed in the obese group after 36 h starvation but no change was observed in plasma glucagon. Thus 36 h fasting induces a different hormonal response in lean and obese subjects which may result in the observed differences in metabolite responses between the two groups. In the lean subjects the rise in glucagon during the 36 h fast may be sufficient to overcome the insulin effect and stimulate lipolysis whereas the fall in plasma insulin without a concomitant rise in glucagon in the obese group appears not to have stimulated a comparable lipolytic response. However, a state of impaired fat mobilisation in obesity has been previously suggested [1–3, 26, 27] but this is a controversial concept [28], and the metabolic results of fasting in the obese subjects may result from changes in gluconeogenesis [38]. Rises in plasma secretin during fasting in lean volunteers have previously been noted [12] and Stout et al. [13] found correlations between non-esterified free fatty acid concentrations and plasma secretin during fasting in lean subjects. Greenberg and Bloom [33] could not confirm these findings and suggested that interference in the secretin assay by products of lipolysis [41] might explain these early results. However Henry et al. [12] enlarged their original study and highly significant rises in plasma secretin were recorded in 50 lean subjects during 36 h fasts [37].

The results of our study do not suggest assay interference by lipolytic products. However it appears that

plasma secretin behaves similarly to plasma glucagon during fasting. These findings might only reflect the different gastro-intestinal responses to fasting in lean and obese subjects or might even be permissive, but from our results it can be speculated that secretin has metabolic importance in vivo during starvation.

Increases in plasma VIP have also been previously recorded during fasting in normal healthy men [25] and the lipolytic effects of this hormone have been demonstrated in vitro [10, 31, 32]. Our results suggest that plasma VIP behaves similarly to plasma glucagon and secretin during 36 h fasting. Our inability to observe a rise in VIP in the obese subjects indicates that this hormone may also have metabolic influence during starvation. However the increased plasma VIP observed after a 12 h fast in the obese group is of interest. This may reflect the gastro-entero-pancreatic hormonal heterogeneity of obese human subjects (Andrews WJ, Henry RW and Buchanan KD, unpublished observations); although suppression was achieved with a 50 g oral glucose tolerance test.

The ability of 50 g oral glucose to suppress plasma glucagon, secretin and VIP in both groups after 36 h starvation lends further support to the concept that these peptides have physiological metabolic importance during fasting.

The plasma insulin and blood glucose responses to refeeding after 36 h starvation suggest that the replacement of stored energy substrate and the inhibition of further substrate breakdown is of prime importance in lean subjects whilst the responses in the obese subjects may be the result of increased gluconeogenesis in this group during starvation.

Although this study did not allow for differentiation between secretion and clearance of the measured hormones, the results emphasize the different metabolic responses of obese and lean human subjects during starvation and suggest that these responses may in part anyway, be related to gastro-entero-pancreatic hormones.

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Dr. W. J. Andrews
Human Diabetes Study Center
c/o NIH-NIADDK
4212 North 16th Street
Phoenix, AZ 85016, USA