Reference intervals for glucose, β-cell polypeptides, and counterregulatory factors during prolonged fasting

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Højlund, Kurt, Mette Wildner-Christensen, Ole Eshøj, Christian Skjærbaek, Jens Juul Holst, Ole Koldkjær, Dorthe Møller Jensen, and Henning Beck-Nielsen. Reference intervals for glucose, β-cell polypeptides, and counterregulatory factors during prolonged fasting. Am J Physiol Endocrinol Metab 280: E50–E58, 2001.—To establish reference intervals for the pancreatic β-cell response and the counterregulatory hormone response to prolonged fasting, we studied 33 healthy subjects (16 males, 17 females) during a 72-h fast. Glucose, insulin, C-peptide, and proinsulin levels decreased (P < 0.001), and the levels of counterregulatory factors increased during the fast (P < 0.05; glucagon and free fatty acids (FFA) with a linear increase and epinephrine, norepinephrine, and cortisol with a clear underlying circadian rhythm). Growth hormone secretion increased from the first to third day of fasting (P < 0.05) but actually decreased from the second to third day of fasting (P = 0.03). Males had higher glucose and glucagon levels and lower FFA levels during the fast (P < 0.05), whereas no effect of gender on β-cell polypeptides was observed. A high body mass index resulted in higher insulin and C-peptide levels during the fast (P < 0.05). In conclusion, we have provided reference intervals for glucoregulatory factors during a 72-h fast. We observed a diminished β-cell response concomitant with an increased secretion of counterregulatory hormones. These results should be of clinical and scientific value in the investigation of hypoglycemic disorders.

catecholamines; glucagon; growth hormone; cortisol; free fatty acids

EPISODIC SYMPTOMS suggestive of hypoglycemia are a common clinical presentation in healthy-appearing adults seeking medical help. Often such patients cannot be studied during a spontaneous episode of hypoglycemia, and, if no obvious cause of hypoglycemia can be demonstrated, the supervised 72-h fast with continuous measurements of glucose, C-peptide, proinsulin, and insulin has traditionally been the diagnostic test of choice (36, 38). The 72-h fast is used to diagnose insulin-mediated hypoglycemia, where fasting fails to suppress the secretion of β-cell polypeptides. The ability of this test to diagnose abnormalities in the counterregulatory hormone response to prolonged fasting has never been examined fully.

The diagnosis of hyperinsulinemic hypoglycemia has previously been associated with some uncertainty, mainly because normal overnight fasting serum levels of the β-cell polypeptides have been used to discriminate between normal subjects and subjects with hypoglycemia. Recently, a diagnostic interpretation of data based on paired observations of plasma glucose and insulin, C-peptide and proinsulin obtained at the end of a 72-h fast has been suggested (36, 38). However, the diagnostic accuracy of these assays was <100% in the plasma glucose range of 2.8 to 3.3 mmol/l (27, 33, 40), where some persons are known to experience hypoglycemic symptoms (35, 36). Furthermore, the normal subjects used in these studies had been referred for evaluation of the possible presence of a hypoglycemic disorder and therefore did not represent an unselected healthy population (27, 33, 40). Thus, to our knowledge, well-defined reference intervals for insulin, C-peptide, and proinsulin during a 72-h fast in healthy humans have never been established. In addition to suppressed secretion of β-cell polypeptides, several counterregulatory factors are known to be activated in the prevention and correction of hypoglycemia (5, 8, 9, 21, 23). Increases in glucagon and catecholamines are involved in the first line defense of hypoglycemia (7, 11, 12, 17, 30), whereas increases in growth hormone (GH) and cortisol are supposed to play a role in the defense against prolonged hypoglycemia (6, 7, 13, 14, 30). Furthermore, increases in free fatty acids (FFA; mediated by epinephrine) have been reported to play a role in the counterregulation to hypoglycemia (16, 17, 28). Increases in all of these factors have been demonstrated in studies of prolonged fasting (4, 7, 19, 21, 23, 24, 28, 31, 34). However, reference intervals for the response...
of these glucose counterregulatory factors to a 72-h fast have never been published. Abnormalities in the secretion of these factors during prolonged fasting could previously have gone undetected as the actual cause of hypoglycemia, and this could explain why hormone deficiencies are so seldomly reported as the cause of hypoglycemia. Therefore, such reference intervals could be of large interest in cases where patients under prolonged fasting actually experience Whiplows triad but in whom insulin-mediated hypoglycemia can be excluded.

The present study was designed to establish reference intervals for the β-cell polypeptides and the most important glucose counterregulatory factors during a classic 72-h fast and to investigate the impact of gender and body mass index (BMI) on these factors to improve the diagnosis of hyperinsulinemic hypoglycemia and other hypoglycemic disorders.

SUBJECTS AND METHODS

Subjects. Thirty-three healthy Caucasian volunteers with no previous history of hypoglycemic symptoms were studied in the Department of Endocrinology at Odense University Hospital, Denmark. To study the effects of gender and BMI, an equal number of males and females and subjects with normal and high BMI were included. A high BMI was a priori defined as BMI ≥ 27 kg/m² for males and BMI ≥ 25 kg/m² for females. Age, BMI, and the proportion of subjects with high BMI were similar in males and females (Table 1). Two to three weeks before the prolonged fast, every subject underwent a routine medical examination, including clinical history, medication, blood pressure, electrocardiogram, and blood analysis including, hemoglobin, liver enzymes, creatinine, and a lipid profile. An oral glucose tolerance test according to the World Health Organization criteria was performed to ensure a glucose tolerance in the normal range. None of the participants was taking medications except oral contraceptives or suffered from hyperlipidemia, hypertension, diabetes, ischemic heart disease, or liver disease. In addition, all subjects had normal serum creatinine precluding reduced renal clearance as a cause of high levels of C-peptide or proinsulin. All participants underwent a 72-h fast according to a standard protocol on patients admitted to our department. The subjects were told to fast at home from 10:00 PM until they presented at the department at 7:30 AM the next day. At 8:30 AM the 72-h fast was started. A cannula was inserted in a forearm vein, and blood samples were drawn every 3 h for determinations of plasma levels of glucose and pancreatic glucagon and serum levels of insulin, proinsulin, C-peptide, plasma, cortisol, and GH. Serum levels of albumin and FFA were measured every 6 h. Serum epinephrine and norepinephrine were measured in six volunteers only (3 males, 3 females, mean BMI 25.8 (19.9–36.5), mean age 25.5 (19.9–43.1)). During the study, the participants were permitted to drink water ad libitum and to walk around the hospital area while under surveillance. One-half hour before sampling and between 10:00 PM and 7:00 AM, subjects were required to rest. All participants completed the 72-h fast without developing hypoglycemic symptoms or biochemical hypoglycemia (plasma glucose <2.5 mmol/l). Compliance with the fast was assessed by determination of FFA and hydration by determination of albumin concentration. For unknown reasons, glucose, insulin, C-peptide, and proinsulin levels increased just before termination of the fast, and we have therefore decided that “the end of the fast” refers to the values of these variables measured after 69 h of fast, where nadir in almost every subject was obtained.

The study was approved by the Local Ethics Committee and was performed in accordance with the Helsinki Declaration. Informed written consent was obtained from all participants.

Assays. All blood samples were centrifuged immediately at 4°C and stored at −20°C until analysis. Care was taken to analyze samples from each patient within the same run. All samples were analyzed in duplicate. The only exception was GH, which due to a very low intra-assay coefficient of variation (CV) was analyzed in single determinations only. Plasma glucose was measured by the glucose dehydrogenase oxidation method. Intra- and interassay CVs were lower than 2%. Serum FFA was measured using an enzymatic colorimetric procedure with a “NEFA C” kit (Wako Chemicals, Neuss, Germany). Serum insulin, proinsulin, and C-peptide were measured by a noncompetitive time-resolved immunofluoroassay (TR-IFMA; Wallac Oy, Turku, Finland) as described previously (41). Detection limits for serum insulin, C-peptide, and proinsulin were 9, 18, and 2 pmol/l, respectively. Intra- and interassay CVs were 5 and 7% for insulin, 9 and 8% for proinsulin, and 10 and 8% for C-peptide. In the insulin assay, cross-reactivities with proinsulin, C-peptide, and 32-33 split proinsulin were all <0.4%. Serum GH was measured using a commercial noncompetitive TR-IFMA (Wallac Oy; see Ref. 18). Intra- and interassay CVs were <5%. Pancreatic glucagon concentrations were measured in ethanol-extracted plasma (final concentration 70% vol/vol) by an RIA using antisera 4305,125I-labeled porcine glucagon (a generous gift from Novo Nordisk, Bagsvaerd, Denmark), and synthetic human glucagon (Peninsula, Merseyside, St. Helens, UK) as standards. The detection limit was ~1 pmol/l, the intra-assay CV was <5% at a level of 15 pmol/l (25), and the interassay CV was below 11%. Serum cortisol was measured by RIA (Orion Diagnostics, Espoo, Finland). The detection limit was 5 nmol/l, and the intra-assay CV was <3%. Plasma epinephrine and norepinephrine were measured by a radioenzymatic assay with some modifications as described previously (29). Intra-assay CVs for norepinephrine and epinephrine in samples containing basal values were 6 and 8%, respectively. Corresponding values of interassay CVs for norepinephrine and epinephrine were 7 and 11%, respectively.

Statistical analysis. All statistical analyses were performed using SPSS/PC + 5.0. Nonparametric methods were used, as almost all variables were nonnormally distributed when tested by normal plot and the Shapiro-Wilk test. Mann-Whitney rank sum test was used to compare unpaired data, whereas the Wilcoxon matched-pairs signed-ranks test was used in evaluating differences between paired observations. Chi square test was used to compare categorical data. The Sign test was used to test the proportions of subjects having at least a 10% increase in each of the β-cell polypeptides from 69 to 72 h of fast. To reduce type 1 errors, 24-h mean

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Values are medians and ranges. BMI, body mass index. No significant differences were observed when comparing males and females.
concentrations of all variables for every day (1st day, 2nd day, and 3rd day) were calculated. The 24-h mean levels (from 8:30 AM to 5:30 AM every day) were used to examine variables with a circadian variation and effects of gender and BMI. Data are presented as median and 5th and 95th percentiles defining 90% reference intervals. Data are displayed graphically by plots of median values with their reference intervals as error bars. This will of course hide any variation in the shape of the curves for different individuals. Data for median curves that are not good indicators of the typical curve are discussed in the text. The impact of gender and BMI is displayed as median curves in cases where a significant effect was observed. The catecholamines are presented as means ± SE, but data are tested by nonparametric tests. P values <0.05 were considered statistically significant.

RESULTS

Plasma glucose. Plasma glucose decreased steadily from 5.2 (4.3–6.2) (median and 5th and 95th percentiles) mmol/l and reached a plateau after ~2 days of fasting from which it remained constant at 3.7 (3.0–4.4) mmol/l throughout the fast (P < 0.001; Fig. 1). During the entire fast, the median level of plasma glucose was higher in males than in females (Fig. 1), but differences in 24-h mean levels were only significant during the last 48 h (day 2, P = 0.02 and day 3, P = 0.01). No significant differences in 24-h mean levels of plasma glucose between subjects with normal or high BMI were observed. The individual curves showed only minor variations compared with the median curve. The lowest plasma glucose value measured was 2.7 mmol/l. The lowest individual values were observed between 18 and 72 h of fast (3.3–4.4 mmol/l for males, 2.7–4.6 mmol/l for females, 2.7–4.2 mmol/l for subjects with normal BMI, and 3.0–4.6 mmol/l for subjects with high BMI). Age correlated positively with plasma glucose at the start (r = 0.41, P < 0.05) and at the end (r = 0.36, P < 0.05) of the fast but with none of the other variables measured.

β-Cell polypeptides in subjects with plasma glucose ≤3.3 mmol/l. In the period from 18 to 69 h of fast (excluding 72-h samples), we observed 69 measurements of plasma glucose ≤3.3 mmol/l in 10 subjects (30%). The corresponding values of insulin, C-peptide, and proinsulin were ≤26, 249, and 10 pmol/l, respectively (Fig. 2). From 63 to 69 h of fast, the corresponding β-cell polypeptides were all suppressed below the recently published diagnostic criteria for hyperinsulinemia: insulin ≥18 pmol/l; C-peptide ≥200 pmol/l; and proinsulin ≥5 pmol/l (38). From 18 to 60 h of fast, we observed 52 measurements of plasma glucose ≤3.3 mmol/l in nine subjects (27%; all females). In 20 out of these 52 cases (38%; 6 out of 9 females), the diagnostic criteria for hyperinsulinemia were exceeded by at least one of the corresponding β-cell polypeptides. During the entire fast, 35% (6/17) of the healthy females had plasma glucose ≤3.3 mmol/l concomitant with β-cell polypeptides, exceeding the diagnostic criteria for hyperinsulinemia.

Serum insulin, proinsulin, and C-peptide. For all three hormones, there was a marked decline during the first 30 h of fast, after which a more modest fall during the rest of the fast was observed. The concentration of serum insulin decreased from 33 (16–69) to 9 (<9–19) pmol/l at the end of the fast (P < 0.001; Fig. 3). The levels of serum proinsulin fell from 6 (3–17) to 3 (2–5) pmol/l (P < 0.001; Fig. 3), and serum C-peptide decreased from 421 (260–785) to 127 (74–295) pmol/l (P < 0.001; Fig. 3). After comparison of 24-h mean levels, no significant gender differences in circulating levels of serum insulin, proinsulin, and C-peptide were observed, although the median levels of C-peptide almost throughout the fast were higher in males than in females (Fig. 4). Twenty-four hour mean levels of C-peptide were significantly higher in subjects with high BMI every day during the fast (P < 0.05). Insulin levels were also significantly higher in subjects with high BMI on the first day (P = 0.003) and the second day (P = 0.03) and tended to be higher on the third day (P = 0.06). Although the median values of proinsulin at separate points of time were higher in subjects with high BMI (Fig. 4), statistical significance comparing 24-h mean levels was not reached on any day.

Plasma glucagon, serum cortisol, and serum GH. Plasma glucagon increased gradually throughout the fast from 10 (5–17) to 23 (10–38) pmol/l after 72 h of fast (P < 0.001; Fig. 5). The 24-h mean levels of plasma glucagon were higher in males on the first day (P = 0.04), second day (P = 0.06), and third day (P =
Glucagon median levels were higher in subjects with normal BMI during the last half of the fast (Fig. 5), but when comparing 24-h mean levels no significant difference was observed on any day.

As indicated by Fig. 6, serum cortisol fluctuated in a circadian rhythm with peaks at 8:30 AM and troughs at 8:30 PM. As indicated by the narrow reference intervals, this was also the typical curve for each individual. After comparison of 24-h mean levels, a significant increase from the first to the second day [1.09 (0.66–4.05) vs. 1.92 (0.22–5.85) µg/l, \( P < 0.001 \)], but a significant decrease from the second to the third day was observed [1.92 (0.22–5.85) vs. 1.50 (0.26–4.74) µg/l, \( P = 0.03 \)]. Still, on the third day, levels of GH were significantly higher than on the first day (\( P < 0.05 \)). Twenty-four hour mean levels of GH were significantly higher in subjects with normal BMI in the last 24-h period (\( P = 0.006 \)), whereas no differences were seen in GH levels with respect to gender.

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As indicated by Fig. 6, serum cortisol fluctuated in a circadian rhythm with peaks at 8:30 AM and troughs at 8:30 PM. As indicated by the narrow reference intervals, this was also the typical curve for each individual. After comparison of 24-h mean levels, a significant increase from the first to the second day [228 (132–368) vs. 269 (196–588) nmol/l, \( P < 0.001 \)] was seen, whereas a slight increase from the second to the third day was insignificant [269 (196–588) vs. 293 (223–479) nmol/l, \( P = 0.4 \)]. The 24-h mean levels of serum cortisol were independent of gender and BMI, and the median curves were completely superimposable. Serum GH fluctuated in a much more irregular manner than cortisol, and the median curve was in no way representative for the typical or individual curve (Fig. 6). However, levels of GH peaked at about 11:30 PM every day. As with cortisol, the 24-h mean levels increased significantly from the first to the second day [1.09 (0.66–4.05) vs. 1.92 (0.22–5.85) µg/l, \( P < 0.001 \)], but a significant decrease from the second to the third day was observed [1.92 (0.22–5.85) vs. 1.50 (0.26–4.74) µg/l, \( P = 0.03 \)]. Still, on the third day, levels of GH were significantly higher than on the first day (\( P < 0.05 \)). Twenty-four hour mean levels of GH were significantly higher in subjects with normal BMI in the last 24-h period (\( P = 0.006 \)), whereas no differences were seen in GH levels with respect to gender.
Plasma epinephrine and norepinephrine. As for cortisol, both plasma epinephrine and norepinephrine concentrations fluctuated in a circadian rhythm with increases at about 5:30–8:30 PM and decreases at about 2:30–5:30 AM every day (Fig. 7). The 24-h mean levels of plasma epinephrine increased significantly from 8.4 ± 3.7 (SE) ng/l the first day to 15.2 ± 6.5 ng/l the second day ($P = 0.005$) and to 20.9 ± 6.7 ng/l the third day ($P < 0.001$). Likewise, the 24-h mean levels of norepinephrine rose significantly from 115.6 ± 30.6 ng/l the first day to 130.6 ± 32.0 ng/l the second day ($P = 0.03$) and to 178.3 ± 37.6 ng/l the third day ($P = 0.004$).

Serum FFA and albumin. Serum FFA increased gradually from 0.47 (0.20–0.81) mmol/l to a final level of 1.46 (0.78–2.39) mmol/l after 72 h of fasting ($P < 0.001$; Fig. 8), indicating that no food was consumed. FFA decreased markedly between 8:30 PM and 8:30 AM every night but returned to the expected increasing levels in the mornings. Compared with the values at 8:30 PM every day, 69, 91, and 71% of the subjects experienced a decrease in FFA levels at 2:30 AM on the first, second, and third day, respectively (all $P < 0.05$).

After correcting for changes in albumin levels, 63, 88, and 67% of the subjects experienced a decrease in FFA levels on the first, second, and third day, respectively, which was significant on the second day only ($P < 0.05$). The median levels of serum FFA were higher in females than in males during the entire fast (Fig. 8), but, when comparing 24-h mean levels, statistical significance was observed on the first and the third day ($P = 0.004$ and $P = 0.01$). We observed no significant differences in the 24-h mean levels of FFA between subjects with normal or high BMI, although the median levels were higher in subjects with normal BMI during the last two-thirds of the fast.

Increase in C-peptide, insulin, proinsulin, and glucose in the last sample. As seen in Fig. 3, most subjects experienced an increase in serum C-peptide, insulin, and proinsulin from 69 to 72 h of fast. Demanding an increase of >10% (to ensure increases higher than the CVs for these assays), the proportions of subjects with increasing levels of C-peptide, insulin, and proinsulin were 97, 87, and 81%, respectively, and, for all three β-cell polypeptides, this increase was significant ($P < 0.001$).
DISCUSSION

The physiological mechanisms that prevent insulin-induced hypoglycemia and hypoglycemia during prolonged fasting have been studied and reviewed intensively (6, 7, 11, 13, 14, 16, 17, 28, 30, 31). Recently, a diagnostic interpretation of data obtained at the end of a 72-h fast has been suggested to distinguish between normal subjects and patients with hyperinsulinemic hypoglycemic disorders (27, 33, 36, 40). However, to our knowledge, well-defined reference values for most of the known glucose regulatory factors in healthy subjects have never been established. The present study was carried out to provide reference intervals for the most important factors contributing to glucose homeostasis during a classic 72-h fast and to study the impact of gender and BMI.

In the current study, plasma glucose, serum insulin, C-peptide, and proinsulin all decreased as reported in other studies (4, 7, 27, 31, 33, 40), and, characteristically for all, a pronounced initial decline during the first day of fast was followed by a more modest fall during the rest of the fast, which is in agreement with a previous study (31). The reference intervals of C-peptide, insulin, and proinsulin narrowed markedly during the last 9 h of the fast, and in most subjects serum levels of insulin and proinsulin reached the detection limit of the assays. This implies that the secretion of β-cell polypeptides essentially ceased in most subjects. In addition, we found glucose levels to be significantly lower in females than in males during the last 48 h of the fast, also reported previously (31, 40). We therefore expected higher levels of β-cell polypeptides in males during the last part of the fast (40). However, we found no effect of gender on the levels of β-cell polypeptides, as reported earlier for insulin (31). β-Cell polypeptides rapidly reached baseline levels in both sexes, probably eliminating any difference in the last part of the fast. Contrary to a recently published study (40), we observed significantly higher levels of C-peptide and insulin throughout the fast in subjects with high BMI. Our data further establish that the well-described insulin resistance in obese individuals is preserved even after prolonged fasting.

The main purpose of a 72-h fast is to demonstrate endogenous hyperinsulinism. In a series of studies, it was demonstrated that diagnostic problems primarily exist in the plasma glucose range of 2.8–3.3 mmol/l (27, 33, 40), in which some subjects experience hypoglycemic symptoms and stop the fast (35, 36). In this glucose range, the diagnostic accuracy of insulin, C-peptide, and proinsulin to discriminate between normal subjects and patients with insulinoma was found to be >100% for all hormones. In the period of 18–60 h of fast, as many as 35% of the healthy females with plasma glucose ≥3.3 mmol/l in our study had β-cell polypeptides exceeding the recently published diagnostic criteria for hyperinsulinemia (36, 38), whereas from
63 to 69 h of fast none out of the 10 subjects with plasma glucose ≤3.3 mmol/l exceeded these diagnostic criteria. Our data suggest that, in females who terminate the fast before 63 h have passed, the recently published diagnostic criteria for hyperinsulinemic hypoglycemia should be used with some caution (27, 33, 36, 38, 40). Among 178 patients with confirmed insulinoma, only one completed the 72-h fast without fulfilling the diagnostic criteria for hyperinsulinemia (37). This observation together with our data indicates that the greatest problem must be not to overdiagnose hyperinsulinemic hypoglycemia in females stopping the fast before 72 h had passed. Contrary to the healthy subjects investigated in the studies from which the criteria for hyperinsulinemia are derived (27, 33, 40), the healthy subjects in our study were not referred for evaluation of the possible presence of a hypoglycemic disorder, which to some extent renders our data less biased. The reference intervals for insulin, C-peptide, and proinsulin could be useful not only in the diagnosis of hyperinsulinemic hypoglycemia but also in the investigation of other abnormalities in glucose homeostasis in which the secretion of β-cell polypeptides could be of interest.

Glucagon and catecholamines play a primary role in the first line defense against hypoglycemia (5, 8, 11, 12, 17, 30). As expected, prolonged fasting induced a marked and gradual increase in the levels of pancreatic glucagon as reported earlier (4, 7, 31). Plasma glucagon levels were lower in females throughout the fast, and this finding contrasts to previous observations (31). This discrepancy must be due to methodological differences. In the study of Merimee and Fineberg (31), glucagon was measured using the 30K assay, in which “big plasma glucagon” is unidentified in contrast to the assay we used (42). Elevations of FFA within the physiological range have been shown to have a significant inhibitory effect on glucagon secretion in humans even during arginine-induced hypoglycemia (20), although the latter are more controversial (2, 22). Therefore, the higher FFA levels in women in our study can explain the lower but still increasing secretion of glucagon during fasting. Comparing the 24-h mean levels, we demonstrated a statistically significant increase of epinephrine and norepinephrine during the fast, as expected from earlier studies on the effect of fasting and on insulin-induced hypoglycemia (4, 7). The measurements of catecholamines were done to verify an increasing secretion and a circadian rhythm with nocturnal troughs. Because of the small number of subjects, reference intervals have not been calculated. In a previous fasting study, the circadian variation of norepinephrine and epinephrine was not demonstrated, probably because blood samples were taken only two times a day, at 8:30 AM and 8:30 PM (4). The blunted response of catecholamines at sleep is an important observation because it will significantly increase the risk of hypoglycemia in subjects in whom the glucagon response to hypoglycemia is absent. This phenomenon is in fact believed to be responsible for the greater risk of hypoglycemic episodes at night in subjects with insulin-treated diabetes (26). In our study, the circadian variation and increase of both catecholamines were similar, suggesting a role for both hormones in the glucose counterregulation. Defects in catecholamine and glucagon release as a cause of hypoglycemia are very seldomly reported (9, 36), except in long-term diabetes. The presented reference intervals for glucagon are very broad, and therefore the applicability is limited to some extent. However, in cases of spontaneous hypoglycemia where hyperinsulinism can be excluded, a low glucagon response may point out an abnormality in the counterregulatory response of this hormone, which can then be further investigated by other means.

Cortisol and GH play a demonstrable role as counterregulatory hormones only in the defense against prolonged hypoglycemia (6, 13, 14, 30). In agreement with these studies and with observations during prolonged fasting (4, 7, 34), we found the 24-h mean values of cortisol to increase during the fast. In addition to this increase, there was an underlying circadian variation with peaks in the morning and nocturnal falls. As reported earlier, the cortisol levels did not increase significantly from the second to the third day of fasting (4), suggesting that the counterregulatory response of cortisol might not increase further beyond 72 h of fast. In contrast to the findings of Galvao et al. (19), high
BMI did not diminish the day-to-night variation of cortisol levels in the present study. For cortisol, no effects of gender or BMI were found. In studies of the effect of prolonged fasting, significant increases in the 24-h mean levels of GH and in the amplitude and frequency of GH secretory bursts have been demonstrated (21, 23, 24). This is consistent with the GH data from our study, of which the GH levels were highly individual and highly episodic but with increasing 24-h mean levels from the first to the second day and from the first to the third day but actually decreasing from the second to the third day, exactly as reported in previous studies (4, 34). Apparently, the counterregulatory response of GH is attenuated with fasting beyond 2 days. In contrast to cortisol and catecholamines, the pattern of GH secretion was highly episodic, and, as stated before (21), it would be necessary to sample at least every 5 min to catch the pulses of this hormone. As reported earlier, we found no gender effect on the GH levels (4), whereas high BMI was accompanied by statistically significant lower levels of GH in the last day of the fast as previously reported (18, 19). Hypopituitarism and adrenal insufficiency are the common hormonal causes of hypoglycemia (10, 36). Especially in Addison’s disease, other characteristic clinical features may be entirely absent. The applicability of the reference intervals for cortisol seems obvious, whereas that for GH seems to be of rather limited value in the light of the large individual variation.

FFA increased gradually during the entire fast as reported in other studies (7, 21, 23, 24, 31, 33), confirming that the subjects abided by the rules of the fast. However, we found unexpected reductions in FFA levels at 2:30 AM. These decrements were unexpected because single GH pulses have been reported to increase FFA levels typically after 2–3 h and typically after the nocturnal mean peak of GH (32). In the current study, we observed a nocturnal fall in catecholamine levels, and reduced catecholamine-mediated lipolysis may explain the concomitant nocturnal decline in FFA levels (16, 17). However, this hypothesis needs to be verified. The median levels of FFA were significantly higher in females on the first and the third day, in agreement with earlier reports (31), and there was no significant effect of high BMI on FFA levels. Patients with insulinomas have been reported to have significantly lower fasting levels of FFA than healthy subjects (33), but, from our data, it seems likely that this difference was obtained mainly because the FFA levels in healthy subjects after 72 h of fast were compared with levels of FFA in patients with insulinoma measured at the time they stopped the fast. Future studies may show whether the presented reference intervals for FFA can uncover diseases of glucose homeostasis.

Although the volunteers in our study were kept under continuous surveillance during the entire fast, rested in their beds from 10:00 PM to 8:30 AM the last day, and experienced a continuing increase in FFA and glucagon levels, the levels of insulin, C-peptide, and proinsulin increased significantly from 69 to 72 h of fasting. In another study of prolonged fasting, this phenomenon was not observed (4). This discrepancy could be explained by the fact that their last samples were drawn 6 h before termination of the fast. In studies of Service et al. (27, 33, 40), this was not observed either. For insulin, this could be ascribed to the higher detection limit of 35 pmol/l (22, 28, 31) vs. 9 pmol/l in our study, whereas this is not the case with C-peptide. However, it is well known that the anticipation of food intake stimulates gastric secretion by stimulating vagal efferents (cephalic phase), and accompanying increases in certain gastrointestinal hormones together with stimulation of the vagal efferents to the pancreatic β-cells may explain this phenomenon (3). However, this warrants further investigations. Prolonged fasting in most cases is conducted to establish the mechanisms for hypoglycemia. In this respect, β-cell polypeptides are expected to be maximally suppressed at the end of a 72-h fast. However, our data suggest that the levels of β-cell polypeptides at the terminal sampling time point should be interpreted with some caution.

The strength of this study derives from the fact that the presented reference intervals are derived from healthy subjects without any previous hypoglycemic events, and, to our knowledge, this is the largest study simultaneously measuring the pancreatic β-cell response and the counterregulatory hormone response to prolonged fasting. However, the sample size is small with respect to the limits of the reference intervals, and the generalization of these data to the population at large should be done with caution. In the clinical settings, the reference intervals should therefore be used with common sense, and type 1 error risk could be reduced by sampling every 6 h instead of every 3 h. The relevance of measuring counterregulatory factors comes in question when hormone deficiencies are suspected. If blood is drawn for these analyses, the levels can be determined afterward in subjects who terminate the fast before 72 h of fast and have plasma glucose in the hypoglycemic range (≤3.3 mmol/l). Furthermore, it could be of notable scientific value to compare the counterregulatory response of healthy subjects with the response in patients with verified hypoglycemic diseases and subjects with postprandial hypoglycemic symptoms, first-degree relatives of diabetic subjects, etc.

In summary, the present study has provided reference intervals for the β-cell polypeptides and the most important glucose counterregulatory factors during a classic 72-h fast. The secretion of β-cell polypeptides essentially ceased in most subjects. Gender had no effect on the secretion of β-cell polypeptides during prolonged fasting but resulted in higher glucose levels, higher glucagon levels, and lower FFA levels in males. High BMI resulted in higher insulin and C-peptide levels and lower GH levels during the last day of fasting. These results might be helpful in the future discrimination between different hypoglycemic disorders, such as insulinoma, adult nesidioblastosis, hormone deficiencies, and noninsulinoma pancreatico-nous hypoglycemia (39), and may be in yet unknown hypoglycemic disorders.
REFERENCES


