# Insulin, glucagon, Cortisol and growth hormone release in association with physiological decrements in the plasma glucose concentrations in fasting men

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*The release of potentially important glucose counter-regulatory factors (glucagon, Cortisol and growth hormone), and insulin were studied during Ramadan fasting. Twenty-two healthy volunteers participated in this study. The range of ages was 20 to 38 years (meantSD, 28+4.2), mean weight 73.9±6.1 kg, and mean body daily mass index (BMI) 25.5±2.7 kg/m$^2$. All subjects fasted the whole month and the average fasting time was about 16 hours. Venous blood samples were taken on four different days; one day before Ramadan (day zero), then on the first, 14$^{th}$ and 28$^{th}$ days of the month. In each of these four days, blood samples were taken at 4:00 PM (shortly before evening meal). At the end of the month, mean weight loss was 3.9 kg (p<0.05). Reduction in the mean plasma glucose concentration from 5.21'±0.37 mmol/L to 3.71 ±0.46 mmol/L were associated with increments in plasma glucagon (34.9±9.4 pmol/L; p<0.001) and Cortisol (378±154 nmol/L; p<0.05) at the end of fasting, and the increment in plasma growth hormone (GH) 169±39.5 pmol/L; p<0.05) only on day 14 of fasting. On the other hand, mean plasma insulin concentration was reduced (52.3±21.5 pmol/L; p<0.005) on day 28 of fasting. In view of their activation with physiological decrements in the plasma glucose concentration, one or more of these potentially important glucose counter-regulatory systems may play a physiological role in nonhypoglycemic glucose counter-regulation and the prevention of hypoglycaemia. [Turk J Med Res 1995, 13(5):180-183]*

Key Words: Fasting, Insulin, Glucagon, Cortisol, Growth hormone

Fasting was prescribed before Islam, by other religions and even atheists. In Islam, fasting in Ramadan is one of the five pillars of Islam. Total abstention from food, drink and sex from sunrise to sunset (averaging about 15 hours) during the month that lasts 29-30d is practised by muslims through the world. Food deprivation exerts one of today's most prevalent environmental influences on mankind. Disability and death from malnutrition usually occur before the energy reserves in the human body are exhausted (1). Most studies concerning endocrine effects of energy deprivation have been carried out in subjects suffering from malnutrition, or obesity (2-4). Fasting is sometime used to treat obesity (5). The effect of Ramadan fasting on various biochemical and hematological parameters have been extensively studied (6-8). However, comparatively less information pertaining to changes in hormone levels is available (9,10). Hypoglycemia stimulates the release of variety of hyperglycemic factors, including the pancreatic/ gastrointestinal hormone, glucagon; adrenocortical hormone, **Cortisol;** and the adenohypophysial hormone, GH (11).

The purpose of this study was to evaluate levels of plasma glucose counter-regulatory factors (glucagon, **Cortisol** and GH) and insulin hormone. We have measured insulin and counter-regulatory hormone responses during decrements in the plasma glucose concentration in normal human subjects during fasting.

## MATERIALS AND METHODS

Twenty-two healthy non-smoking male volunteers ranging between 20-38 years (28±4.2 years). Their weights ranged from 56 to 82 kg (73.9±6.1 kg), and their BMI from 22 to 26 (25.5±2.7 kg/m$^2$). All observed the Ramadan fast abstaining from food, drink and sex from 2:00 am till 5:45 pm daily (averages 16 hours) for 30 days. Daily working hours in the month are from 8:30 am to 4:00 pm. Sleeping hours, which were between 10:30 pm and 7:30 am are intrerrupted at 2:30 am by the intake of light meal "suhur". The subjects were housed in one place during the whole ex-

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**Table 1.** Anthropometric data for 22 healthy male subjects before Ramadan fasting

| n=20 | Range | MeanisD |
|---|---|---|
| Age (years) | 20-38 | 28±4.2 |
| Height (cm) | 162-78 | 170±1.82 |
| Weight (kg) | 56-82 | 73.9±6.1 |
| BMI (kg/m$^2$) | 22-26 | 25.5±2.7 |

BMI: Body mass index

perimental period. No subject received any medication for 2 months before or during the study. Each subject was informed both verbally and in writing as to the procedures involved in the experiment and signed informed consent. The protocol was approved by the Human Ethics Committee of the University of Dicle at Diyarbakır. Body weight was measured and venous blood samples were taken on four different days: One day before Ramadan fasting (day zero), then on the first, 14$^{th}$, and 28$^{th}$ day of Ramadan fasting. In each of these four days, blood samples were taken at 4:00 pm (shortly before evening meal).

**Anthropometry:** Body weight and height were measured in fasting state (before the study) with the subject in underwear and without shoes. BMI was calculated as weight (kg) divided by the square of body height (m).

**Plasma glucose:** Plasma glucose concentration was measured on the Astra-8 Auto-Analyser by the glucose oxidase method (12) with (Beckman Instruments Inc.) reagent kit.

**Radio immunoassay of hormones:** Blood samples were collected at 4:30 pm during non-fasting and fasting days. Plasma was separated in a refrigerated (4-8°C) centrifuge, and was stored at -20°C until analysed. Hormone assays were conducted in the radio immunoassay laboratory of Dicle Medical Centre, where insulin (13), glucagon (14) and GH (15) were assayed using RIA kits, supplied by Diagnostic Products Corp. (Los Angels, C A, USA) and Cortisol was assayed by kits supplied by Amersham (Oakville, Ontario, Canada). All measurements were conducted in duplicate with appropriate quality control samples.

**Statistical analysis:** Data were analysed by the Student's t test. A minimum level of significance was set at $p<0.05$.

## RESULTS

All the twenty-two male subjects completed the fasting for the whole month of Ramadan. Subjects characteristics are shown in Table 1. In observing the effect of fasting on body weights, it was evident that there was a significant fall in body weight. The mean weight at the beginning of the month was 73.9±6.1 kg and at the end of the month was 70.0±6.2 kg. The mean weight loss for the four-week period was 5.2% ($p<0.05$) (Table 2).

Significant increments in the mean plasma glucagon, **Cortisol** and GH, and decrements in the mean plasma insulin concentration were associated with the 5.27±0.37 to 3.71 ±0.46 mmol/L ($p<0.001$) decrements in the plasma glucose concentration during Ramadan fasting. Mean plasma glucagon concentration rose from 25.1 ±6.1 to 34.9±9.4 pmol/L ($p<0.001$) and mean plasma **Cortisol** concentration rose from 292±102 to 378±154 nmol/L ($p<0.05$) at the end of the month. The GH concentration showed some increase only at the middle of the month (day 14), but at the end of Ramadan, GH level was in the prefasting level. On the other hand, mean plasma insulin level decreased from 76.0±24.4 to 52.3±21.5 pmol/L ($p<0.005$), compared with the base-line values at the end of Ramadan (Table 2).

## DISCUSSION

Food intake is therefore, expected to be reduced because generally two meals are consumed between the sunset and down hours and the appetite is generally reduced because of the altered timings of the meals (16). In studies conducted by Shoukry (17), Muazzam and Khaleque (18), no significant difference in body weight was observed with Ramadan fasting. In this study, a significant reduction in the body weight during the month, particularly during the fourth week, is indicative of the effect of reduced food intake (Table 2). The mean body weight at the beginning of the month was 73.9±6.1 kg and at the end of the month 70.0±6.2 kg. An average weight loss of 3.9 kg per subject

**Table 2.** Effect of Ramadan fasting on body weight and levels of some hormones in healthy male subjects

| n-20 | Day 0 (base-line) | Day 1 | Day 14 | Day 28 |
|---|---|---|---|---|
| Body weight (kg) | 73.9±6.1 | 73.9±6.3 | 71.8±6.7 | 70.0±6.2*** |
| Glucose (mmol/L) | 5.27±0.37 | 3.98±0.39* | 3.60±0.61* | 3.71 ±0.46* |
| Insulin (pmol/L) | 76.0±24.4 | 70.3±34.4 | 48.8±20.1* | 52.3±21.5" |
| Glucagon (pmol/L) | 25.1 ±6.1 | 28.4±5.2 | 33.7±6.6* | 34.9±9.4* |
| Cortisol (nmol/L) | 292±102 | 322±125 | 413±113** | 378+154*** |
| GH (pmol/L) | 144+37.1 | 146±38.6 | 169+39.5"* | 158±40.8 |

Means±SD, GH: Growth hormone, *$p<0.001$, **$p<0.005$, * **$p<0.05$

during four weeks. This loss in body weight is consistent with other studies (19-22).

Jenson et al (23) found a significant decrease in plasma glucose level of 13 healthy subjects during Ramadan fasting. Similarly, Nomani and Hallak (24) noted that in 16 healthy volunteers, the mean glucose level was significant lower during fasting. In the present study, intermittent 16-hour-a day abstinence form food and drinks resulted in significant decline of plasma glucose levels on the first, 14$^{th}$ and 28$^{th}$ days of Ramadan ($p<0.001$). Present data are consisted with the results of Nomani and Hallak, and Jenson et al.

These data document significant increments in plasma glucagon, Cortisol and GH, and decrements in plasma insulin after reduction of the plasma glucose concentration from the high physiological level of 5.27 mmol/L to the low physiological level of 3.71 mmol/L in healthy male subjects during fasting. These findings have clear implications with respect to the physiology of activation of glucose counter-regulatory systems. Small changes in blood glucose may decrease the secretion of insulin and increase the secretion of glucagon (25,26). Thus, a synergism of the decrease in circulating insulin and increase in glucagon may be viewed as the cause of stimulated hepatic gluconeogenic mechanisms early in fasting (27). On the other hands, excessive insulin destruction induced by starvation reduces the effectiveness of insulin (28); this, too, seems possible, since Mirsky and Perisutti (29) have shown that insulinase activity in rat liver slices rises during starvation. Elegant studies by Chill (30) and Felig (31) summarised in two review articles have established the homeostatic response to continuous starvation. The liver begins glycogenolysis four to five hours after a meal. This process may maintain blood glucose for 12 to 15 hours along with gluconeogenesis which accounts for 25% of glucose released in the circulation. In the metabolic responses to fasting, insulin and glucagon play major roles. However, changes in plasma concentrations of Cortisol, GH and catecholamines may have permissive roles. Lower plasma insulin (32) and glucose (33) concentrations, coupled with higher plasma glucagon (34), Cortisol (11) and GH (35) might enhance lipolysis during fasting. The first goal of the metabolic events in fasting is maintenance of glucose homeostasis. Glucose homeostasis is supported early in fasting by rapid rates of hepatic gluconeogenesis. The primary regulators of this initial response are the reduction in plasma insulin and the increase in glucagon. There are precedents for increments in circulating levels of potentially important glucose counter-regulatory factors associated with plasma glucose counter-regulatory factors associated with plasma glucose decrements to levels not generally considered to represent hypoglycaemia. For example, Glick (36) concluded that plasma glucose decrements of only 20-30 mg/dl were sufficient to trigger GH secretion in most normal human subjects. The changes in Cortisol release may be a result of increased pituitary adrenocorticotropic hormone (ACTH) release (and, by inference, increased hypothalamic corticotropin releasing hormone release), altered adrenal responsiveness to unchanged hypothalamic-pituitary activity, or both. Since plasma ACTH concentrations were not measured in this study, we cannot state unequivocally that the increased plasma Cortisol concentrations were a result of fasting-induced hypothalamic-pituitary hormone release. However, the changes in both pulsatile and periodic release, which are probably centrally effected, suggest that hypothalamic pituitary activity was altered during fasting. Alterations in both plasma ACTH and Cortisol have been demonstrated in patients with eating disorders. Thus, there appears to be a quantitative spectrum of counter-regulatory responses to decrements in the plasma glucose concentration such that the magnitude of the counter-regulatory response is inversely related to the absolute plasma glucose concentration.

In conclusion, it is apparent that Ramadan fasting induces a number of endocrine manifestations, which seem to be biologically adequate, ie to maintain sufficient glucose levels for normal brain function by mobilising glucose from various sources and to reduce metabolic rate in peripheral tissues in healthy males. The causal interrelations of these endocrine changes require further exploration.

**Açlıkta plasma glukoz konsantrasyonunda fizyolojik düşmeyle birlikte insulin, glukagon, kortizol ve büyüme hormonu salınımı**

*Potansiyal olarak önemli glukoz karşıt ayarlayıcı faktörlerin (glukagon, kortizol ve büyüme hormonu) ve insulin salınımı ramazan açlığı süresince çalışıldı. Bu çalışmaya 22 sağlıklı gönüllü katıldı. Yaş aralığı 20 ile 38 arasında (meantSD, 28±4.2) değişmekte ortalama ağırlık 73.9±6.11 grve ortalama günlük vücut kitle indeksi (VKI) 25.5±2.7 kgr/m$^2$ idi. Bütün bireyler tüm ay boyunca ortalama açlık süresi 16 saat olacak şekilde aç kaldılar. Dört farklı günde venöz kan örnekleri alındı. Ramazandan 1 gün önce (0. gün), ramazanın 1., 14. ve 28. gününde kan örnekleri alındı. Bu dört günde kan örnekleri iftarı açmadan hemen önce (16.°° de) alındı. Ayın sonunda ortalama kilo kaybı 3.9 kgr idi ($p<0.05$). Açlığın sonunda plazma glukoz konsantrasyonunun 5.27±0.37 mmol/L'den 3.71 ±0.96 mmol/L'ye düşmesine plazma glukagon (34.9±9.4 pmol/L; $p<0.001$) ve kortizol (378+154 nmol/L; $p<0.05$) düzeylerinde artma ve açlığın 14. gününde plazma büyüme hormon düzeyinde (169±39.5 pmol/L; $p<0.05$) artma eşlik ediyordu. Diğer taraftan plazma insulin konsantrasyonu açlığın 28. gününde düştü (52.3 ±21.5 pmol/L; $p<0.005$). Plazma glukoz konsantrasyonundaki fizyolojik düşmeye paralel olarak, potansiyel olarak önemli bu karşıt glukoz ayarlayıcı bir veya birden fazla faktörün artması nonhypoglisemik glukoz karşıt ayarlanmasında ve hipoglisemin önlenmesinde rol oynayabilir. [Turk J Med Res 1995, 13(5): 180-183]*

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