Cortisol increases gluconeogenesis in humans: its role in the metabolic syndrome

Samina KHANI and John A. TAYEK
Department of Internal Medicine, Harbor-UCLA Medical Center, 1000 W. Carson Street, Box 428, Torrance, CA 90509, U.S.A.

A B S T R A C T

Android obesity is associated with increased cortisol secretion. Direct effects of cortisol on gluconeogenesis and other parameters of insulin resistance were determined in normal subjects. Gluconeogenesis was determined using the reciprocal pool model of Haymond and Sunehag (HS method), and by the Cori cycle/lactate dilution method of Tayek and Katz (TK method). Glucose production (GP) and gluconeogenesis were measured after a 3 h baseline infusion and after a 4–8 h pituitary–pancreatic infusion of somatostatin, replacement insulin, growth hormone (GH), glucagon and a high dose of cortisol (hydrocortisone). The pituitary–pancreatic infusion maintains insulin, GH and glucagon concentrations within the fasting range, while increasing the concentration of only one hormone, cortisol. Two groups of five subjects were each given high-dose cortisol administration, and results were compared with those from a group of six 'fasting alone' subjects (no infusion) at 16 and 20 h of fasting. Fasting GP (12 h fasting) was similar in all groups, averaging 12.5 ± 0.2 μmol·min^{-1}·kg^{-1}. Gluconeogenesis, as a percentage of GP, was 35 ± 2% using the HS method and 40 ± 2% using the TK method. After 16 h of fasting, GP had fallen (11.5 ± 0.6 μmol·min^{-1}·kg^{-1}) and gluconeogenesis had increased (55 ± 5% and 57 ± 5% of GP by the HS and TK methods respectively; $P < 0.05$). High-dose cortisol infusion for 4 h increased serum cortisol (660 ± 30 nmol/l; $P < 0.05$), blood glucose (7.9 ± 0.5 mmol/l; $P < 0.05$) and GP (14.8 ± 0.8 μmol·min^{-1}·kg^{-1}; $P < 0.05$). The increase in GP was due entirely to an increase in gluconeogenesis, determined by either the HS or the TK method (66 ± 6% and 65 ± 5% of GP respectively; $P < 0.05$). Thus cortisol administration in humans increases GP by stimulating gluconeogenesis. Smaller increases in serum cortisol may contribute to the abnormal glucose metabolism known to occur in the metabolic syndrome.

INTRODUCTION

The metabolic syndrome is associated with increased cardiovascular mortality. Several studies have shown a significant association between android fat distribution and increased cortisol secretion [1]. This syndrome is associated with increases in serum and urinary free cortisol, and it has also been associated with increased blood glucose and glucose intolerance [2]. Recently, 90 patients with Type II diabetes were shown to have an elevated serum cortisol concentration compared with weight-matched controls [3]. Earlier work in humans demonstrated that cortisol administration can increase hepatic glucose production (GP) and the blood glucose concentration, but an inability to measure gluconeogenesis directly in the past has prevented the direct determination of the effect of cortisol on gluconeogenesis in humans [4].

We have shown previously in normal subjects and in cancer patients that, after an overnight fast, approx. 20%

Key words: cortisol, gluconeogenesis, glycogenolysis.

Abbreviations: GH, growth hormone; GP, glucose production; HS method, reciprocal pool method of Haymond and Sunehag; M_n, glucose molecule with n ^{13}C and $6-n$ ^{12}C atoms (in any position); MIDA, mass isotopomer distribution analysis; P–P clamp, pituitary–pancreatic clamp; TK method, Cori cycle/lactate dilution method of Tayek and Katz.

Correspondence: Dr John A. Tayek (e-mail tayek@humc.edu).

of GP is derived from non-glucose carbon sources, 20% by recycling of glucose (the Cori cycle) and 60% from hepatic glycogen [5]. We found in normal subjects that, during a period of 20–40 h of fasting, glycogenolysis is reduced, so that 90% of the GP is due to gluconeogenesis [6]. Theoretical arguments state that the current equations overestimate gluconeogenesis by a factor of two [7]; however, results obtained using the current method [5,6] for the measurement of gluconeogenesis are similar to those obtained using both the ^2H_2O and mass isotopomer distribution analysis (MIDA) methods, as demonstrated recently in children [8]. Use of the reciprocal pool model of Haymond and Sunehag (HS method) [9] eliminates the argument about the factor of 2, and provides results similar to those obtained using the Cori cycle/lactate dilution method of Tayek and Katz (TK method) [6]. In the present study, gluconeogenesis determined by both methods will be evaluated to determine the importance of the role of cortisol in the metabolic syndrome.

Several studies support a role for cortisol in the metabolic syndrome. A 5-day infusion of cortisol (hydrocortisone) in dogs increased GP and the conversion of alanine into glucose in the liver (a marker of gluconeogenesis) [10]. However, in the dog model, acute cortisol infusion has no effect on GP [11], except when insulin concentrations are reduced [12]. In humans, gluconeogenesis begins to increase at 2 h and reaches a maximum at 3 h during hypoglycaemia [13]. Whereas glucagon has been shown to increase gluconeogenesis as early as 3–4 h, there are no published data about the effects of adrenaline on gluconeogenesis in humans [14]. While the effects of adrenaline and glucagon are additive with regard to rates of GP and fasting glucose, the addition of cortisol to an adrenaline and glucagon infusion doubles the effect on GP and glucose concentration compared with that seen with the combined adrenaline and glucagon infusion alone [15].

The present study was performed to determine the ability of cortisol to increase gluconeogenesis in humans. Insulin concentrations were maintained at the fasting level in an attempt to minimize insulin's effect on liver gluconeogenesis. While increased serum insulin and cortisol levels can be seen in the metabolic syndrome, the insulin is less likely to be effective due to the endogenous state of insulin resistance. Two relatively new methods were used to estimate gluconeogenesis [6,9].

METHODS

Subjects

Ten normal subjects gave informed consent to the study, which was approved by the Harbor-UCLA Research and Education Institute IRB. The average age of the 10 subjects was 40 ± 4 years, and their body weight was 72 ± 3 kg (mean $\pm$ S.E.M.). On day 1, the subjects were placed on a balanced diet delivering calories at $1.25\times$ their estimated basal energy expenditure, 1 g of protein $\cdot$ day^{-1} $\cdot$ kg^{-1} and a minimum of 300 g of carbohydrate per day. Daily food intake was recorded. The last food (snack) was provided at 21.00 hours on day 2 of the study.

Infusion protocol

Infusions were started at 06.00 hours on day 3: five subjects (group 1) received a primed, continuous 7 h infusion, and five subjects (group 2) received an 11 h infusion, of 0.17–0.28 μmol of [U-$^{13}C_6$]glucose $\cdot$ min^{-1} $\cdot$ kg^{-1}. The isotope was 99% pure, and was obtained from Martek (Columbia, MD, U.S.A.). The priming dose was 16.8 μmol/kg. Data from group 1 (high cortisol; 16 h fast) and group 2 (high cortisol; 20 h fast) were compared with recently published data for six subjects who fasted for 16 and 20 h [14]. We selected the 7 and 11 h isotope infusions to also determine if the amount of label in the five carbon atoms of glucose (M_1–M_5; see Calculations section below) would alter the estimate of gluconeogenesis when using the reciprocal pool model (HS method) for the measurement of gluconeogenesis (see calculations below). The 11 h infusion will result in more ^{13}C label in M_1–M_5 of glucose (see below), which may influence the calculation of gluconeogenesis.

Baseline measurements of GP and gluconeogenesis were obtained after 3 h of infusion, at 09.00 hours. High-dose cortisol was administered to subjects in group 1 for 4 h between 09.00 and 13.00 hours, and to subjects in group 2 between 13.00 and 17.00 hours. (Group 2 was given a low-dose cortisol infusion between 09.00 and 13.00 hours, followed by a high-dose cortisol infusion between 13.00 and 17.00 hours; data from the low-dose cortisol period are not presented due to an error in the administration of insufficient insulin.) Gluconeogenesis was determined by sampling blood every 20 min between 3 and 4 h of cortisol infusion (between 12:00 and 13.00 hours for group 1, and between 16.00 and 17.00 hours for group 2).

We used a modified pituitary–pancreatic (P–P) clamp technique to control levels of critical hormones [16]. Metyrapone was not administered under the traditional P–P clamp protocol because of its potential ability to decrease glycogen stores [17] and hence alter the effect of cortisol on the liver. The P–P clamp in all subjects was started at 09.00 hours, and it consisted of a 4–8 h infusion of insulin (0.05 m-units $\cdot$ min^{-1} $\cdot$ kg^{-1}), somatostatin (0.1 μg $\cdot$ min^{-1} $\cdot$ kg^{-1}), glucagon (0.8 ng $\cdot$ min^{-1} $\cdot$ kg^{-1}), human growth hormone (GH; 7 ng $\cdot$ min^{-1} $\cdot$ kg^{-1}) and cortisol (1.8 μg $\cdot$ min^{-1} $\cdot$ kg^{-1}) (Figure 1). The goal of the hormone infusion was to maintain all hormone concen-

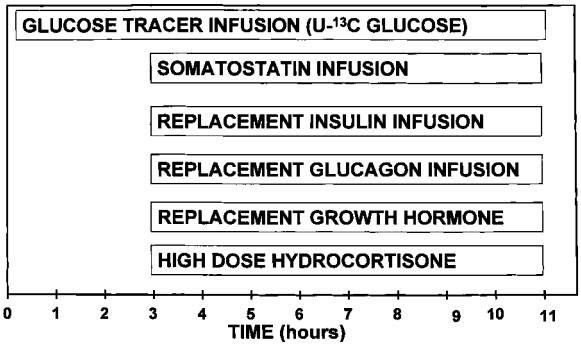

Figure 1 Experimental protocol

After 3 h, the subjects underwent a 4–8 h hormone infusion period. The P–P infusion study started after 3 h of a baseline stable glucose infusion or at 09.00 hours. In five subjects, cortisol (hydrocortisone) was infused at high dose (1.2 $\mu g \cdot min^{-1} \cdot kg^{-1}$) for a 4 h period (09.00 to 13.00 hours). In addition, five subjects received a low-dose cortisol infusion first (results not shown), and subsequently received a high-dose cortisol infusion between 7 and 11 h (13.00 to 17.00 hours). The doses of all the other hormones (insulin, GH, glucagon and somatostatin) were the same during the 4 h cortisol infusion periods. Six subjects were infused with glucose tracer only, and acted as 16 h and 20 h 'fasting alone' controls. There were five subjects in each of the two cortisol groups (16 h and 20 h).

trations in the fasting range, except for that of cortisol. Groups 1 and 2 were selected to test if the effects of time on liver glycogen content play a role in the ability of cortisol to increase GP. Clearly, liver glycogen decreases between 16 and 20 h of fasting, as does the rate of glycogenolysis [6,14]. In addition, gluconeogenesis increases between 16 and 20 h of fasting, so that comparisons with baseline rates of gluconeogenesis would not be the best way to demonstrate an effect.

Plasma glucose and lactate enrichment, as well as plasma insulin, C-peptide, glucose, glucagon, GH, cortisol, catecholamines, non-esterified ('free') fatty acids (NEFA) and amino acids, were determined every 20 min over the final 60 min of each infusion period (group 1, 12.00 to 13.00 hours; group 2, 16.00 to 17.00 hours). All hormones and substrates except for glucagon were measured as described previously [18]. Glucagon was assayed by RIA (Linco Research Inc., St. Charles, MO, U.S.A.) with an antibody that reports much lower glucagon concentrations than those reported previously using the Unger method [6,14]. The lower values are believed to reflect glucagon concentration more accurately. Amino acids were measured using a Beckman Gold amino acid analyser.

Calculations

The equations for the reciprocal pool model (HS method) for the measurement of gluconeogenesis and GP have been published recently [9]. *M* in the equations refers to the isotopomer fraction (enrichment) of glucose (i.e. glucose molecules with one ^{13}C and five ^{12}C atoms in any position are designated M_1; those with two ^{13}C and four ^{12}C atoms in any position are designated M_2, etc.). GP is determined as the infused dose of glucose divided by the enrichment of uniformly labelled glucose (M_6) in plasma:

$$\text{GP} = \text{infused dose } (\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}) / \text{plasma } M_6 \text{ enrichment} \quad (1)$$

Fractional gluconeogenesis is given by $\sum_1^5 E_{M_n}$ in M_1–M_5 divided by the $\sum_1^6 E_{M_n}$ of glucose in the entire molecule (M_1–M_6), where E_{M_n} is defined as the enrichment of glucose species M_n. This is then multiplied by the ratio of the entry rates of ^{12}C and ^{13}C. The ratio of the sums of the entry rates of ^{12}C and ^{13}C into M_1–M_5 is given as $\sum_1^5 {}^{12}C_{M_n}$ divided by $\sum_1^5 {}^{13}C_{M_n}$:

$$\text{Gluconeogenesis } (\%) = \frac{\sum_1^5 E_{M_n} \times \sum_1^5 {}^{12}C_{M_n}}{\sum_1^6 E_{M_n} \times \sum_1^5 {}^{13}C_{M_n}} \quad (2)$$

In the TK equation, the fractional rate of gluconeogenesis is the product of the Cori cycle and the dilution of hepatic lactate (eqn 3). Note that *m* is the enrichment in lactate, and *M* is the enrichment in glucose:

$$\text{Gluconeogenesis } (\%) = \frac{\sum_1^3 M}{\sum_1^6 M} \times \frac{\sum_1^6 M_n}{2 \times \sum_1^3 m_n} \quad (3)$$

Absolute gluconeogenesis is the product of percentage gluconeogenesis multiplied by GP. Non-gluconeogenic glucose release is obtained by subtraction of gluconeogenesis from GP.

Data analysis

Data from fasting subjects and from the two high-dose cortisol groups were compared by ANOVA. Simple linear and multiple-step linear regression analysis was carried out by the method of least squares. Significance was defined as $P < 0.05$. Data are represented for both methods for the calculation of gluconeogenesis as means $\pm$ S.E.M.

RESULTS

Plasma cortisol concentrations were increased by at least 3-fold in the cortisol infusion groups (Table 1). Plasma insulin, glucagon and GH levels were not significantly different when compared with fasting alone (Table 1). NEFA concentrations were similar (mean 0.42 ± 0.03 mmol/l) in all three groups after 12 h of fasting (baseline). After 16 h of fasting (13.00 hours), the NEFA concentrations were significantly higher in the cortisol groups than in the fasting alone group (0.90 ± 0.13 and 0.82 ± 0.08 compared with 0.63 ± 0.09 mmol/l respectively; $P < 0.05$). NEFA concentrations were

Table 1 Hormone concentrations during a 16 h fast and at the end of a 4 h P–P clamp cortisol infusion study

Values are means ± S.E.M. (n); $^*P < 0.05$ compared with fasting alone. Results are shown at 16 h and 20 h of fasting. There were no differences in the 12 h values for all the groups (results not shown).

Group	Insulin (pmol/l)	Cortisol (nmol/l)	Glucagon (ng/l)	GH (μg/l)
16 h fasting alone (6)	40 ± 6	199 ± 27	42 ± 6	1.3 ± 0.3
16 h high cortisol (5)	31 ± 6	660 ± 30*	59 ± 6	3.1 ± 1.9
20 h fasting alone (6)	23 ± 4	124 ± 25	53 ± 10	2.0 ± 0.3
20 h high cortisol (5)	26 ± 7	728 ± 97*	68 ± 7	1.8 ± 0.3

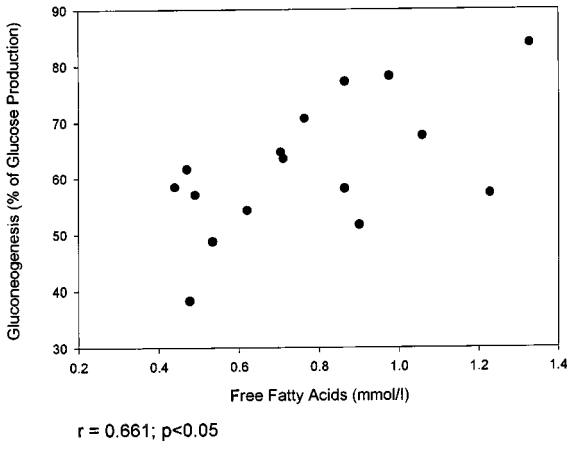

Figure 2 Correlation between percentage gluconeogenesis and NEFA concentration

The graph shows results from three groups at 16 h of fasting: six 'fasting alone' subjects, five subjects that had received high-dose cortisol, and five subjects just before receiving high-dose cortisol. All values were obtained at 13.00 hours. The figure demonstrates a modest correlation between gluconeogenesis and NEFA ('free fatty acid') concentration.

modestly correlated with percentage gluconeogenesis at 16 h ($r = 0.661$, $P < 0.05$; Figure 2).

Amino acid profiles were obtained after 16 h and 20 h of fasting to document the effects of cortisol infusion on plasma amino acid concentrations (Table 2). Plasma leucine, isoleucine and phenylalanine were significantly increased at the end of the 4 h cortisol infusion. Plasma amino acids were similar in all groups at baseline (12 h fasting; results not shown).

A 4 h administration of cortisol increased plasma glucose from 4.6 ± 0.2 to 7.9 ± 0.5 mmol/l at 16 h of fasting. There was a similar increase in the 20 h fasting, high cortisol group (Figure 3). In comparison, fasting glucose concentrations decreased from 5.1 ± 0.1 to 4.7 ± 0.1 mmol/l ($P < 0.05$) in the fasting alone (no cortisol) group between 12 and 16 h of fasting.

The isotope enrichment of plasma glucose is listed in Table 3 for the three groups. Very little enrichment was detected in M_4 and M_5, since the probability of recombination of two labelled trioses is very low (2% × 2% = 0.04%). Although the enrichments at M_4 and M_5 were very low, their values were used in the HS method (reciprocal pool method); however, they were not used in the TK method. The two methods provided rates of gluconeogenesis that were similar. As shown in Table 4, the average value was 61% by the HS method and 62% by the TK method. These values are expressed as a percentage of GP.

Table 2 Plasma concentrations of selected amino acids during a 16 h fast and during a P–P cortisol infusion study

Values are means ± S.E.M.; $^*P < 0.05$ compared with 16 h fasting alone group. The amino acids in **bold** are considered to be essential amino acids (needed in the diet) for adults; the ninth essential amino acid, methionine, was not measured on the Beckman Gold amino acid analyser.

	Concentration (μmol/l)	
Amino acid	High cortisol	Fasting alone
Leucine	171 ± 16*	137 ± 13
Isoleucine	78 ± 4*	65 ± 7
Valine	224 ± 8	210 ± 9
Aspartic acid	33 ± 11	31 ± 7
Threonine	145 ± 16	126 ± 7
Serine	156 ± 20	146 ± 18
Glutamine + glutamate	392 ± 62	377 ± 65
Glycine	211 ± 11	226 ± 16
Alanine	359 ± 26	340 ± 36
Tyrosine	67 ± 8	57 ± 5
Phenylalanine	77 ± 6*	66 ± 5
Tryptophan	30 ± 2	27 ± 2
Ornithine	59 ± 8	57 ± 7
Lysine	229 ± 25	260 ± 26
Histidine	89 ± 3	89 ± 6
Arginine	191 ± 42	192 ± 35
Total	2511 ± 117	2406 ± 207

Baseline (09.00 hours) rates of gluconeogenesis and GP were similar in the two cortisol groups and the fasting alone group at 12 h of fasting (results not shown). Gluconeogenesis as a percentage of total GP was 35 ± 2% using the HS equation and 40 ± 2% using the TK equation ($P < 0.05$). Between 12 and 16 h of fasting, the glucose concentration decreased by 9% (see above) and GP decreased by 10% ($P < 0.05$). During

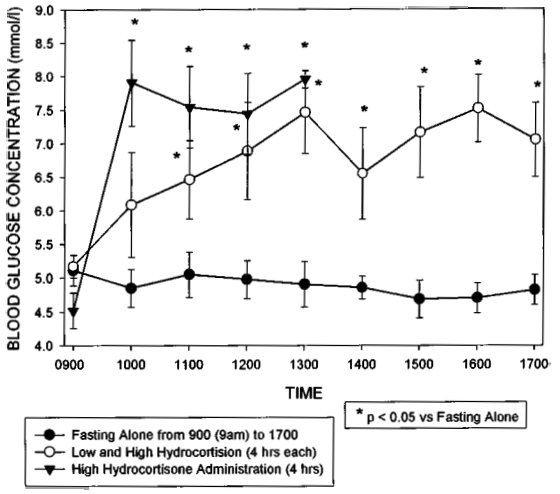

Figure 3 Effects of cortisol administration on blood glucose

Shown are changes in blood glucose over the 7–11 h study period. Both group 1 and group 2, who underwent high-dose cortisol (hydrocortisone) infusion under P–P conditions, showed greater increases in blood glucose than in the fasting alone group. Maximum glucose concentrations were similar in groups 1 and 2.

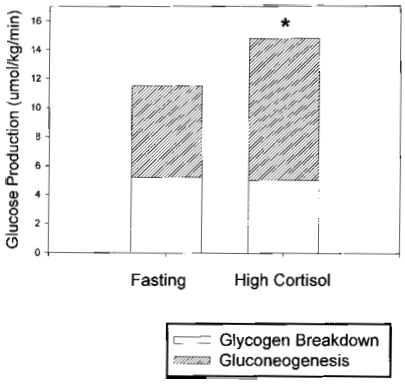

Figure 4 Effects of cortisol on gluconeogenesis and GP

The bars represents net GP; the hatched bar denotes gluconeogenesis and the empty bar denotes glycogen breakdown (GP minus gluconeogenesis, as an estimate of glycogen breakdown). Data are from the 16 h 'fasting alone' group and from the 16 h high-cortisol fasting group. High-dose cortisol infusion increased GP and gluconeogenesis compared with fasting alone. A similar effect was seen at 20 h of fasting (results not shown).

these 4 h of fasting, gluconeogenesis increased significantly to $55 \pm 5\%$ and $57 \pm 5\%$ (or 6.3 ± 0.7 and 6.5 ± 0.6 $\mu mol \cdot min^{-1} \cdot kg^{-1}$), as calculated using the HS and TK methods respectively. This was probably due to mobilization of glycogen in the liver and a slow increase in gluconeogenesis to maintain euglycaemia.

In the 16 h high-dose cortisol group, GP was increased compared with that in the fasting alone group (14.8 ± 0.8 and 11.5 ± 0.6 $\mu mol \cdot min^{-1} \cdot kg^{-1}$ respectively; $P < 0.05$) (Figure 4). Gluconeogenesis as a percentage of GP increased from 55–57% to 65–66% at 16 h of fasting ($P < 0.05$) (Table 4). The increase in GP of 3.3 $\mu mol \cdot min^{-1} \cdot kg^{-1}$ was due mostly to the increase in gluconeogenesis, calculated as 3.5 and

Table 3 Plasma glucose isotope enrichment at 16 h of fasting with and without a high-dose cortisol infusion

Values are means ± S.E.M. Similar data were obtained at 20 h of fasting (results not shown).

	Enrichment (% above baseline)					
Conditions	M_1	M_2	M_3	M_4	M_5	M_6
Fasting alone ($n = 6$)	0.268 ± 0.032	0.255 ± 0.026	0.350 ± 0.029	0.061 ± 0.003	0.011 ± 0.004	2.00 ± 0.14
Cortisol ($n = 5$)	0.392 ± 0.091	0.312 ± 0.062	0.425 ± 0.076	0.062 ± 0.003	0.010 ± 0.003	2.10 ± 0.39

Table 4 Comparison of the use of the TK and HS methods for calculation of gluconeogenesis

Values are means ± S.E.M. Significance of differences: $^*P < 0.05$ compared with 16 h fasting alone; †$P < 0.05$ compared with 20 h fasting alone. At 12 h fasting the HS method calculated a lower percentage of GP compared with the TK method (‡$P < 0.05$). At all other times there were no significant differences between the two methods in estimates of percentage gluconeogenesis.

		Gluconeogenesis (% of GP)	
Group	GP ($\mu mol \cdot min^{-1} \cdot kg^{-1}$)	HS method	TK method
12 h fasting alone ($n = 16$) (09.00 hours)	12.5 ± 0.2	35 ± 2‡	40 ± 2
16 h fasting alone ($n = 6$) (13.00 hours)	11.5 ± 0.6	55 ± 5	57 ± 5
16 h fasting + high cortisol ($n = 5$)	$14.8 \pm 0.8^*$	$66 \pm 6^*$	$65 \pm 5^*$
20 h fasting alone ($n = 6$) (17.00 hours)	10.1 ± 0.6	70 ± 4	71 ± 4
20 h fasting + high cortisol ($n = 5$)	12.5 ± 0.9†	80 ± 3†	76 ± 1†
Overall mean ± S.E.M.		61 ± 8	62 ± 6

3.1 μmol·min^{-1}·kg^{-1} by the HS and TK methods respectively. To express this as a percentage, gluconeogenesis accounted for 95–106 % of the observed increase in GP. These values suggest that all of the increase in GP is due to an increase in gluconeogenesis. The plasma cortisol concentration was significantly correlated with fasting blood glucose ($r = 0.650$; $P < 0.01$) and with gluconeogenesis ($r = 0.430$, $P < 0.05$).

After 20 h of fasting, cortisol infusion had again increased GP to a value greater than that seen with fasting alone (12.5 ± 0.9 compared with 10.1 ± 0.6 μmol·min^{-1}·kg^{-1}; $P < 0.05$). After 20 h of fasting alone, gluconeogenesis accounted for 70–71 % of GP, whereas when cortisol was administered this percentage increased to 76–80 % of GP (Table 4). High-dose cortisol increased the absolute rate of gluconeogenesis, calculated using the TK equation, when compared with 20 h of fasting alone (9.5 ± 0.7 compared with 7.2 ± 0.7 μmol·min^{-1}·kg^{-1}; $P < 0.05$). Using the HS equation, the absolute rate of gluconeogenesis was also increased significantly by cortisol (10.0 ± 0.7 compared with 7.1 ± 0.7 μmol·min^{-1}·kg^{-1}; $P < 0.05$). The calculated 2.3 (TK equation) or 2.9 (HS equation) μmol·min^{-1}·kg^{-1} increase in gluconeogenesis accounted for the observed increase in GP of 2.4 μmol·min^{-1}·kg^{-1}. The 7 and 11 h [U-$^{13}C_6$]glucose infusions provided similar estimates of gluconeogenesis using the HS and TK equations (Table 4).

DISCUSSION

Although cortisol administration is known to increase GP, its effects in humans on gluconeogenesis have been difficult to verify. Only recently have several methods become available for the estimation of gluconeogenesis in humans. The present study involved the acute administration of cortisol over a 4 h period under P–P clamp conditions and determination of the effects on gluconeogenesis and GP. Administering high-dose cortisol increased GP, mostly by increasing gluconeogenesis. While recent data suggested that cortisol can increase glycogen breakdown in the liver by mobilization of lysosomes containing glycogen-hydrolysing glucosidase and glycogen breakdown activity [19], we did not observe this effect.

Rats which undergo adrenalectomy show reduced gluconeogenesis and glycogen breakdown [20]. Patients with adrenal insufficiency frequently have hypoglycaemia, which is probably due to reduced gluconeogenesis, glycogenolysis or both. As early as 1 h into the 4 h high-dose cortisol infusion the blood glucose level was increased. While we did not measure gluconeogenesis earlier than 3 h, the observed increase in blood glucose may have been due to a combination of effects, including an increase in gluconeogenesis, an increase in glycogen breakdown and/or a decrease in glucose utilization. Earlier work in chickens demonstrated that cortisol increases glycogen breakdown as early as 45 min [21], so that some of the observed increase in blood glucose may have been due to liver glycogen mobilization [19,21]. Unfortunately, blood samples were not obtained at 1 or 2 h to determine the early effects of cortisol on glycogenolysis or gluconeogenesis. However, blood samples were obtained between 3 and 4 h. Gluconeogenesis accounted for nearly all of the observed increase in GP.

The long-term effects of cortisol on gluconeogenesis in humans have not been reported. However, patients with malaria have persistent elevations in serum cortisol and glucagon and significantly increased gluconeogenesis [22]. We have shown that a similar increase in glucagon concentration in normal subjects increases the rate of gluconeogenesis to 8.5 μmol·min^{-1}·kg^{-1} [14]; this is not as high as seen in patients with malaria (14.4 μmol·min^{-1}·kg^{-1} [22]). In our present study, cortisol alone increased gluconeogenesis by 10.0 (HS equation) or 9.6 (TK equation) μmol·min^{-1}·kg^{-1}, depending on the method used. However, the rate of gluconeogenesis of 14.4 μmol·min^{-1}·kg^{-1} observed in patients with malaria suggests that there is a synergistic effect of glucagon and cortisol. Earlier work has demonstrated that the addition of a cortisol infusion can double the effects of a combined adrenaline and glucagon infusion on GP [15]. Unfortunately, in the present study, the combination of high cortisol and high glucagon was not tested.

Effects of cortisol on glucose metabolism

In the present study, high-dose cortisol increased GP by increasing gluconeogenesis alone. As expected, the serum glucose concentration also increased during the 4 h cortisol infusion (from 4.8 to 8.7 mmol/l). This increase in blood glucose occurred after 60 min (Figure 3), which may have been due to a rapid effect of cortisol on gluconeogenesis [13]. While we did not determine gluconeogenesis before 3–4 h, earlier work suggests that, during recovery from hypoglycaemia, gluconeogenesis starts to increase within 120 min and reaches a maximum by 3 h [13]. While cortisol concentrations were doubled during hypoglycaemia, other hormones were also increased (glucagon, adrenaline, GH), which may have also contributed to the early onset of gluconeogenesis [13]. Synergistic glycaemic actions of counter-regulatory hormones have been demonstrated with glucagon and adrenaline when cortisol is added [15]. While synergism was not investigated in the present study, cortisol did increase gluconeogenesis and blood glucose.

Limitations of the study

Clearly the increase in serum cortisol of 460–600 nmol/ml (16–22 μg/dl) is greater than one would expect to see in patients with the metabolic syndrome.

The rationale for the high dose was to determine if the effect on gluconeogenesis was measurable within a 4–8 h period of a cortisol infusion. The similar results after 4 h and 8 h suggest that the 4 h infusion period is sufficient to evaluate effects on gluconeogenesis. Lower concentrations of cortisol, such as are seen in the metabolic syndrome, need to be studied to determine the role of cortisol in the pathophysiology of the abnormal glucose metabolism observed in patients with the metabolic syndrome.

In addition, caution should be used when comparing the cortisol groups with a separate group of normal subjects who fasted for the 16–20 h period. During the study period (09.00 to 17.00 hours), the serum cortisol concentration decreased. This may have accentuated the observed effect of cortisol. In this type of experiment, when gluconeogenesis increases between 12 and 20 h of fasting [6], the use of a baseline comparison of gluconeogenesis at 12 h of fasting would not be appropriate. Four subjects were administered replacement cortisol, glucagon and insulin concentrations [14]. The rate of GP was similar to that seen at 16 h of fasting [14], suggesting that the use of 'fasting alone' subjects in the present study may not have overestimated the observed effects. Clearly, prospective studies of small increases in serum cortisol concentrations are needed in order to understand the influence of cortisol in the metabolic syndrome.

Effects of cortisol on NEFA concentrations

Plasma cortisol decreases in the early morning as part of a normal diurnal fall. While some authors believe that this fall may be responsible for the parallel fall in GP, Boden et al. [23] have suggested that changes in GP may instead be due to changes in NEFA concentrations. In the present study, serum NEFA concentrations increased over time in all groups. Both of the cortisol groups demonstrated a significant increase in NEFA which was greater than that seen with fasting alone. It is important to point out that cortisol increases lipolysis and NEFA concentrations. Of note is the modest correlation of NEFA concentration with percentage gluconeogenesis ($r = 0.661, P < 0.05$; Figure 2). While this does not prove a cause and effect, the association between NEFA and rate of gluconeogenesis suggests that a relationship exists. Earlier observations have demonstrated a correlation between NEFA and gluconeogenesis in normal subjects ($r = 0.665$, $P < 0.05$, $n = 14$) [5,6], subjects with Type 2 diabetes ($r = 0.616$, $P < 0.05$, $n = 9$) [24] and cancer patients ($r = 0.599$, $P < 0.05$, $n = 13$) (data from [5]).

Amino acids and cortisol

Essential amino acids are those amino acids that can only be derived in the plasma compartment by the process of proteolysis (muscle protein breakdown). Three of the essential amino acids, leucine, isoleucine and phenylalanine, were increased by cortisol infusion. Since the subjects were fasting during the study, the only sources of these three amino acids were intracellular, which suggests that the elevated concentrations of leucine, isoleucine and phenylalanine were derived from muscle proteolysis.

Previous evidence suggests that cortisol increases the concentrations of hepatic gluconeogenic precursors, as well as improving gluconeogenic efficiency [25]. High-dose cortisol administration increases leucine and phenylalanine levels after 9–12 h [26]. These plasma levels are increased due to an increase in the plasma rate of appearance, which is an index of proteolysis [26]. While the rate of appearance of leucine and phenylalanine was not measured in the present study, the observed increase in the plasma concentrations of these amino acids is probably due to the proteolytic effect of cortisol.

Comparison of the HS and TK methods for calculation of gluconeogenesis

The data in Table 4 demonstrate that, at 12 h of fasting, the HS method underestimated the TK method by five absolute percentage points ($P < 0.05$). A recent publication by Haymond and Sunehag [9] demonstrated that their method gave a value that was three absolute percentage points below that obtained using the TK method ($50 \pm 7\%$ compared with $53 \pm 7\%$). The present paper describes results in a total of three groups (Table 4), with a mean average (when including results from all conditions) that is one percentage point lower with the HS method compared with the TK method ($61 \pm 8\%$ compared with $62 \pm 6\%$). Thus use of the reciprocal model (HS method) provides similar estimates of gluconeogenesis as the TK method, and eliminates the need to determine lactate enrichment.

The lower values obtained in the 12-h fasted subjects by the HS method may have been due to the low enrichment. A low enrichment in M_1–M_5 may underestimate the rate of gluconeogenesis when one uses the reciprocal pool model (HS method). However, the difference was modest (5%) and within the expected range determined by other methods. The low plasma enrichment of glucose in M_1–M_5 was due in part to the high costs of using the [$^{13}C_6$]glucose isotope.

Criticism of the method

Landau [7] argued that the determination of gluconeogenesis has an error of a factor of 2, due to the number 2 used in the estimate of the dilution of hepatic lactate/pyruvate. A rebuttal to these arguments has been published [27]. Measurement of overall GP is agreed to be accurate. It is the absolute rate of gluconeogenesis that has been criticized to be overestimated by a factor of 2 when we use the TK equation. To adjust for this, readers can divide the gluconeogenesis value from the TK method by 2. However, this argument does not apply to the assumptions in the newer reciprocal pool model (HS

method) [9]. Even with the correction factor of 2 in the TK equation, the increase in gluconeogenesis in the high-dose cortisol group in the present study would still be significantly greater than that seen in the 16 h fasting alone group (4.8 ± 0.1 compared with 3.2 ± 0.1 $\mu mol \cdot min^{-1} \cdot kg^{-1}$; $P < 0.05$). However, we believe that this adjustment is wrong, because Landau et al. [7,28] based their claim upon a graphic model in which the glucose pool was divided into two arbitrary subpools: a large one with as much as 80% 'in brain', etc. 'that is oxidized to CO_2 and not recycled', and a small one (20%) in which recycling occurs. However, recycling of the glucose molecule has no borders, and recycling affects the whole-body glucose pool, not just 20% of the glucose pool. The isotopomer pattern in glucose taken from different parts of the systemic blood will be the same, irrespective of the site from which the blood is sampled. The models of Landau et al. [28] are untenable, and provide no support for their criticism of our equation to estimate gluconeogenesis. Furthermore, the reciprocal pool model provides similar results to our method and to the MIDA method in infants and in many other conditions, as reported recently [8]. Finally, the present study demonstrates that the reciprocal pool model provides realistic estimates of gluconeogenesis in humans, and that the values obtained are very similar to those obtained by the TK method.

Conclusion

Using a P–P clamp infusion protocol, high-dose cortisol infusion increased blood glucose and GP by stimulating gluconeogenesis. The effect of cortisol on gluconeogenesis was similar if the subject had fasted for 16 or 20 h. This is the first study in humans that demonstrates the ability of cortisol to increase gluconeogenesis. These data demonstrate that cortisol increases GP by stimulating gluconeogenesis within 3–4 h. Thus the elevation of cortisol noted to occur in the metabolic syndrome may contribute to hepatic gluconeogenesis and abnormal glucose metabolism.

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