Effect of dietary composition on the unconjugated hyperbilirubinaemia of Gilbert's syndrome¹

J. L. GOLLAN,² CAROL BATEMAN, AND BARBARA H. BILLING

From the Departments of Medicine and Dietetics, Royal Free Hospital, Hampstead, London

SUMMARY The influence of dietary composition on the unconjugated hyperbilirubinaemia of Gilbert's syndrome was studied in 29 patients. After a period on a normal diet (10 MJ) an intravenous infusion of 40% glucose (8.4 MJ) together with a 1.6 MJ oral diet for two days resulted in an increment in plasma bilirubin concentration of $127 \pm 18\%$ (mean $\pm$ SEM) above the basal level. Both the administration of intravenous Intralipid 20% and the return to a normal diet caused a prompt reversal of this glucose effect. An increment of $135 \pm 10\%$ in plasma bilirubin concentration was obtained when a standard 'fasting' diet (1.6 MJ) was given for two days. When the lipid content of this 'fasting' diet was increased from 33% to 85%, the rise in plasma bilirubin was only $49 \pm 19\%$. A 10 MJ oral diet for three days, which contained most of its energy content as carbohydrate and only 0.6% as lipid, produced a $76 \pm 12\%$ increase in plasma bilirubin concentration. When the lipid content of the diet was increased to 9% of the energy intake no significant change from the basal level was observed. These findings support the hypothesis that the hyperbilirubinaemia associated with both carbohydrate feeding and fasting is attributable, at least in part, to lipid withdrawal. Although a restricted dietary intake or the parenteral administration of lipid-free solutions has a marked effect on the hyperbilirubinaemia of patients with Gilbert's syndrome, normal daily variation in dietary composition is unlikely to cause a significant change. The influence of different feeding regimes on neonatal hyperbilirubinaemia requires investigation.

As early as 1906, Gilbert and Herscher noted that fasting produced a rise in plasma bilirubin concentration. This observation has been confirmed and the effect shown to be greater in patients with chronic non-haemolytic unconjugated hyperbilirubinaemia than in normal subjects (Felsher *et al.*, 1970; Barrett, 1971) and patients with liver disease or haemolytic anaemia (Owens and Sherlock, 1973; Felsher and Carpio, 1975). Recently, it has become apparent that dietary factors other than a reduced energy intake can increase the level of unconjugated hyperbilirubinaemia. In healthy male subjects an intravenous infusion of up to 6.3 MJ (1 500 Kcal) daily of glucose and amino acids for a period of 72 hours was shown to cause a twofold increase in plasma bilirubin concentration (Oyama, 1972).

Barrett (1975), in an attempt to identify the factors necessary for the reversal of fasting hyperbilirubinaemia, observed that intravenous glucose and amino acids increased plasma bilirubin concentration in two patients with Gilbert's syndrome. Similarly, in patients with the type 2 Crigler-Najjar syndrome, it has been demonstrated that fasting hyperbilirubinaemia cannot be reversed by intravenous glucose (Gollan *et al.*, 1975b). Furthermore, studies in Gunn rats, which have an unconjugated hyperbilirubinaemia due to a hereditary deficiency of hepatic bilirubin glucuronyl transferase, have shown that a diet composed predominantly of carbohydrate or protein doubled the plasma bilirubin concentration, whereas a high lipid diet had no such effect (Gollan *et al.*, 1975a).

The present study was designed to determine whether the unconjugated hyperbilirubinaemia in patients with Gilbert's syndrome was influenced by the nature of the diet or the route of administration and to establish the relationship of these findings to fasting hyperbilirubinaemia.

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²Address for communications: Department of Medicine, Royal Free Hospital, Pond Street, Hampstead, London, NW3 2QG.

Methods

PATIENTS

Twenty-nine patients with Gilbert's syndrome (21 males and eight females) whose ages ranged from 16 to 43 years (mean 28 years) were studied. The diagnosis was based on the finding of a mild unconjugated hyperbilirubinaemia in the presence of normal conventional tests of liver function. The haemoglobin concentration and reticulocyte count were normal and the presence of overt haemolysis was excluded. Hepatic histology was normal in all patients and the hepatic UDP-glucuronyl transferase activity, determined by the method of Black *et al.* (1970), was reduced in each of the 24 patients in whom the assay was performed. All drug therapy was stopped at least two weeks before admission. The investigations were performed after informed consent had been obtained from the patients and with the approval of the Royal Free Hospital Ethics Committee.

TECHNIQUES

Blood was obtained from all patients at 9 am and 4 pm for the determination of plasma bilirubin concentration (TBC), which was estimated by the method of Michaëlsson *et al.* (1965) using caffeine as the accelerator. Since the measurement of conjugated bilirubin at low concentrations is unreliable and the majority of the pigment in the plasma of patients with Gilbert's syndrome is in the unconjugated form, and in view of the observation that changes in response to the dietary regimes occurred in this fraction, the results have been expressed as total bilirubin. Values for normal subjects in this hospital were $\leq 15 \mu\text{mol/l}$. The differences between mean values in the various groups were assessed by Student's *t* test.

DIETARY REGIMENS

The administration of all diets was under the direct supervision of the dietitian. For two days before the

test period each patient was placed on a normal diet (I) of approximately 10 MJ (1 Kcal = 4.1868 KJ) which was selected according to taste and the baseline TBC determined (mean of four observations). At the completion of the test period all patients returned to the normal diet and measurement of TBC continued for a further one or two days. The composition of the various dietary regimens administered is shown in Table 1. In order to compare the effect of 'fasting' with that of different diets, eight patients received the standard low-energy diet (II) (1.6 MJ per day) for two days. This diet was used because a reduction in energy intake of this order has been shown to cause a similar effect to that of total energy withdrawal and is more acceptable to the patient. In five patients, after the baseline period, a central venous cannula was inserted through a peripheral vein and 1 500 ml 40% glucose (III) (8.4 MJ) administered by the intravenous (IV) route for two days with the standard low-energy diet. In two of these patients the IV cannulae were then withdrawn and the low-energy diet continued for two days before returning to a normal diet. In two other patients the IV glucose was replaced by 800 ml Intralipid 20% (IV) (KabiVitrum Ltd, Ealing, W5, U.K.) daily for a further two days, thus providing 6.7 MJ in the form of lipid in addition to the 1.6 MJ diet. The intravenous cannulae were then removed and the patients remained for two days on the standard low-energy diet (II) to determine whether the fasting response was influenced by the prior infusion of lipid.

The effect on TBC of a high-carbohydrate, low-lipid diet given orally was then compared with that of intravenous glucose. For three days after the baseline period a group of seven patients was given a 10 MJ fluid diet (V) which contained only 2 g lipid (0.6% of the total energy intake). The diet was composed predominantly of a glucose polymer, Gastro-caloreen (Scientific Hospital Supplies, Ltd) together with fruit juice and skimmed milk. This

Regimen		*Energy value/day	Composition (% total energy)			Lipid intake (g/day)
			Carbohydrate	Protein	Lipid	
Normal	I	10 MJ (2400 Kcal)	58	12	30	83
Low-energy, standard	II	1.6 MJ (400 Kcal)	45	22	33	15
Low-energy, standard + IV glucose	III	10 MJ (2400 Kcal)	91	4	5	15
Low-energy, standard + IV lipid	IV	8.4 MJ (2000 Kcal)	9	4	87	193
High-carbohydrate, low-lipid (fluid)	V	10 MJ	87.4	12	0.6	2
High-carbohydrate, reduced-lipid	VI	10 MJ	71	20	9	25
Low-energy, high-lipid	VII	1.6 MJ	5	10	85	37

Table 1 Composition of dietary regimens

*McCance and Widdowson (1969).

diet was selected as the most acceptable means of providing normal energy and protein requirements with only a trace of lipid. A further group of four patients was given a high-carbohydrate, reduced-lipid diet (VI) for three days, in which lipid provided 9% of total energy intake. This regimen was designed to provide a minimum of lipid in a diet composed of normal food-stuffs, in order to determine whether variation in the normal diet could influence TBC.

In order to determine whether fasting hyperbilirubinaemia could be explained by lipid deprivation, a modified baseline diet (10 MJ) which contained 37 g lipid (14% of the total energy intake) was given to five patients for two days, followed for a further two days by a 1.6 MJ diet (VII) with the same amount of lipid (low-energy, high lipid diet).

Results

The influence of the different diets on TBC in patients with Gilbert's syndrome is shown in Table 2. In order to eliminate the problem of comparison between patients with different baseline TBC values, the results of each group are expressed as the mean percentage increase in TBC. This was calculated from the maximum increment in TBC noted on the second test day divided by the mean value obtained for the baseline period.

The plasma bilirubin concentration in the 29 patients with Gilbert's syndrome when first estimated after admission ranged from 19 to 90 $\mu\text{mol/l}$. The mean value obtained during the two day baseline period in patients before the test period ($34.0 \pm 3.4 \mu\text{mol/l}$, mean $\pm$ SEM) was comparable with that observed after the test period ($31.1 \pm 3.2 \mu\text{mol/l}$). No diurnal variation of TBC was evident in patients during either the baseline or test periods.

In the patients given the standard low-energy diet (II) for two days the mean increase in TBC was 135%. A comparable rise (127%) was observed in the patients who received an intravenous infusion of 40% glucose (III) in addition to the standard low-energy diet for two days. The degree of hyperbilirubinaemia was unchanged in the two patients who continued with the 1.6 MJ diet for a further two days after cessation of the glucose infusion

Fig. 1 Changes in plasma bilirubin concentration after (a) substitution of IV glucose for the normal diet and (b) a standard low-energy diet in a patient with Gilbert's syndrome. Oral diet: small dots. IV glucose: large dots

(Fig. 1), but reverted to baseline values on returning to a normal oral diet. In the two patients who received 20% Intralipid for two days after the glucose infusion there was a prompt reversal of the hyperbilirubinaemia (Fig. 2) which could not be accounted for by interference of the lipid in the analytical method (Gollan *et al.*, 1975a). Subsequent restriction of energy intake with the standard 1.6 MJ diet (Fig. 2), produced a smaller increase (14% and 32%) than that observed in the group of patients who received a low-energy intake after a normal diet (Table 2).

Administration of the oral high-carbohydrate, low-lipid diet (V) for three days in seven patients produced a mean increase in TBC of 76%, which was maximal on the second day (Fig. 3). The level of hyperbilirubinaemia achieved by oral carbohydrate feeding was significantly less than that

Regimen	No. of patients		Plasma bilirubin concentration (mean $\pm$ SEM)	
			Baseline ($\mu\text{mol/l}$)	Increase (%)
Low-energy, standard	II	8	33.0 ± 7.9	135 ± 10
Low-energy, standard + IV glucose	III	5	32.7 ± 8.7	127 ± 18
High-carbohydrate, low-lipid (fluid)	V	7	28.9 ± 3.6	76 ± 12
High-carbohydrate, reduced-lipid	VI	4	51.9 ± 11.1	8 ± 5
Low-energy, high-lipid	VII	5	46.2 ± 8.4	49 ± 19

Table 2 Influence of dietary composition on plasma bilirubin concentration in patients with Gilbert's syndrome

Fig. 2 Changes in plasma bilirubin concentration (9 am and 4 pm daily) in two patients associated with IV glucose and IV lipid administration, followed by a standard low-energy diet. Oral diet: small dots. IV glucose: large dots. IV Lipid: flecks.

Fig. 3 Influence of a high-carbohydrate, low-lipid (0.6% of total energy) diet on plasma bilirubin concentration in a patient with Gilbert's syndrome. Normal diet: dots. High-carbohydrate, low-lipid diet: hatching.

which resulted from intravenous glucose administration ($P < 0.025$) or the standard low-energy diet ($P < 0.001$). In two additional patients fed with the high-carbohydrate, low-lipid diet (V) the increase in TBC was less marked (34% and 41%) and, as the response to energy restriction (1.6 MJ) was also reduced (49% and 17% respectively), these patients have not been included in the results presented in Table 2. In the four patients who received a high-carbohydrate diet (VI) containing 9% of the total energy intake as lipid, there was no significant change in TBC when compared with the mean baseline value.

When the low-energy, high-lipid diet (VII) was given to five patients to assess whether fasting hyperbilirubinaemia could be attributed to the withdrawal of dietary lipid, the mean increase in TBC (49%) was considerably less than that obtained with the standard 1.6 MJ diet ($P < 0.001$). In one of the patients included in this group, the increase in TBC (124%) was, however, similar to that observed with the standard low-energy regimen.

Discussion

These studies have shown that different dietary constituents can influence the level of unconjugated hyperbilirubinaemia in patients with Gilbert's syndrome, in a manner similar to that previously observed in Gunn rats (Gollan *et al.*, 1975a). A comparable degree of hyperbilirubinaemia was obtained whether or not intravenous glucose (8.4 MJ) was given with the standard low-energy diet. This is in agreement with our previous observation in patients with Crigler-Najjar syndrome (Gollan *et al.*, 1975b) and indicates that the hyperbilirubinaemia of fasting cannot be entirely explained by the reduction in energy intake. It is not, however, possible to conclude that glucose *per se* enhances the hyperbilirubinaemia unless one accepts that it stimulates a mechanism similar to fasting and that the effect which is obtained on the standard low-energy diet is maximal and cannot be further enhanced. It is of interest that the two patients with a diminished fasting response also had a reduced response to the high-carbohydrate, low-lipid diet (V); this observation appears to provide another example of the apparent heterogeneity of Gilbert's syndrome (Powell *et al.*, 1967; Berk *et al.*, 1972).

When an oral diet composed predominantly of carbohydrate (V) was given, a significant rise in the hyperbilirubinaemia of patients was observed, although this was less dramatic than that seen with intravenous glucose. This discrepancy between oral and intravenous feeding may reflect the relative plasma concentrations of nutrients achieved by the

different routes of administration. In this study glucose was used as the major source of carbohydrate; however, there is some evidence that the increase in TBC is independent of the type of carbohydrate administered, as a similar effect has been observed during an intravenous infusion of mannitol (Barrett, 1975) and in Gunn rats fed on a diet composed predominantly of fructose (Gollan *et al.*, 1975a). The absence of an effect on TBC of hypertonic saline administered intravenously indicates that more than a simple osmotic or volume change is involved (Barrett, 1975).

Results similar to those observed in Gilbert's syndrome were obtained with the high-carbohydrate, low-lipid diet (V) in two patients with the Crigler-Najjar type 2 syndrome. The baseline bilirubin concentrations of 136.8 and 292.4 $\mu\text{mol/l}$ increased by 103 $\mu\text{mol/l}$ in response to the diet (Gollan, unpublished data). Since both the Crigler-Najjar type 2 and Gilbert's syndromes are generally considered to have a similar pathogenesis, these findings suggest that the magnitude of the response to a high carbohydrate, low-lipid intake, whether administered orally or parenterally (Gollan *et al.*, 1975b) is related to the severity of the defect in plasma bilirubin clearance.

The infusion of Intralipid after intravenous glucose resulted in a prompt reversal of the hyperbilirubinaemia to the baseline level, despite the fact that the total energy intake from lipid was less than that of glucose. The fact that a normal fasting response followed a glucose infusion, but was not evident after intravenous lipid was probably due to incomplete clearance of the large amounts of lipid administered. These studies suggest that lipid inhibits the mechanism whereby carbohydrate feeding elevates TBC and, indeed, only a relatively small amount appears to be necessary, since TBC was unchanged in the patients who received the high-carbohydrate diet (VI) containing 9% of the total energy intake as lipid.

The relative absence of lipid was a consistent feature in all the 10 MJ regimens which produced an increase in TBC. This observation supports the hypothesis that the hyperbilirubinaemia associated with high carbohydrate and protein feeding is related to the withdrawal of lipid from the diet. Our findings with the standard low-energy diet suggested that fasting hyperbilirubinaemia might also be due to the withdrawal of lipid and, indeed, when the lipid content of the 1.6 MJ diet was increased from 33% to 85% (37 g), the degree of hyperbilirubinaemia attained was significantly reduced. The finding that the TBC did not remain at the baseline level does, however, suggest that other factors may be involved in the fasting response.

Further studies are required to determine the balance that is necessary between energy intake and dietary lipid to maintain the baseline TBC. The absolute amount of dietary lipid does not appear to be the rate limiting factor as the high-carbohydrate, reduced-lipid diet (VI) provided less lipid, but a higher energy intake than the low-energy, high-lipid diet (VII). The mechanisms put forward to explain fasting hyperbilirubinaemia include the enhanced catabolism of haem (Bakken *et al.*, 1972; Lundh *et al.*, 1972) and the impaired hepatic clearance of bilirubin (Bloomer *et al.*, 1971; Bensinger *et al.*, 1973). Whether similar mechanisms apply to the hyperbilirubinaemia which follows the withdrawal of dietary lipid has not yet been established.

The increase in TBC in response to reduced energy intake has been advocated as a useful diagnostic test to distinguish Gilbert's syndrome from other causes of unconjugated hyperbilirubinaemia (Owens and Sherlock, 1973). The results of our studies suggest that the increase will be optimal with a low-lipid regimen.

It is unlikely that the degree of hyperbilirubinaemia in patients with Gilbert's or the Crigler-Najjar syndromes will be greatly influenced by the normal daily variation in dietary composition, unless lipid is virtually excluded. However, the intravenous administration of lipid-free solutions containing carbohydrate or protein is likely to have a significant effect. Since the incidence of Gilbert's syndrome may be as high as 6% of the population (Owens and Evans, 1975), it is possible that unconjugated hyperbilirubinaemia may be recognized for the first time in some patients receiving parenteral feeding; which could also account for some undiagnosed cases of postoperative jaundice. Whether other types of unconjugated hyperbilirubinaemia are affected by the dietary constituents, in a similar manner to Gilbert's syndrome, remains to be investigated. Further studies are warranted in neonatal hyperbilirubinaemia, as lipid deprivation, which accompanies some of the initial feeding regimes used in premature infants, might be expected to aggravate their hyperbilirubinaemia. On the basis of these studies it is recommended that the administration of a small amount of lipid should be included in all diets given to jaundiced neonates.

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