The effect of fasting on total serum bilirubin concentrations

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Thirty-seven healthy volunteers, 19 of whom had consistently elevated total serum bilirubin (TSB) concentrations, took part in an open, randomised cross-over study to determine the effect of fasting on TSB concentrations. The study comprised of two treatments. During one treatment period volunteers ate a standard supper but fasted for 24 h thereafter. During the other treatment period volunteers ate a standard supper, snacks, breakfast and lunch. TSB concentrations were measured at regular intervals. In both the normal and high bilirubin groups, minimum TSB values were recorded 4 h after the supper. A 24 h fast more than doubled TSB concentration from baseline values in both the normal and high bilirubin groups. A clinically relevant rise in TSB took place after 12 h into the fasting period (TSB of $17.3 \mu\text{mol l}^{-1}$ in the fasted group vs $14.0 \mu\text{mol l}^{-1}$ in the non-fasted group). When designing a clinical trial, selecting volunteers, or judging the tolerance of a new drug, the rise in TSB caused by fasting must therefore be taken into account, particularly in trials where volunteers or patients fast before entering the study.

Keywords fasting bilirubin

Introduction

Volunteers are often rejected for a clinical trial, or suspicion is cast on the drug tested in the clinical trial, when elevated total serum bilirubin (TSB) concentrations are recorded as part of the safety analysis of the trial.

Fasting, or a diet of less than 400 calories per day, can lead to an increase in TSB [1]. The effect of the duration of fast [2] and the mechanism responsible for the increase in TSB is unclear. Possible mechanisms, such as an increased bilirubin turnover and a diminished hepatic bilirubin clearance have been proposed [3]. A plausible hypothesis suggests that in the fasting state an increased hepatic uptake of non esterified fatty acids interferes with the hepatic clearance of bilirubin and thus contributes to the unconjugated hyperbilirubinaemia of fasting [4].

In Gilbert's Syndrome (i.e. benign, unconjugated, hyperbilirubinaemia), fasting has a more pronounced effect on TSB, which can be increased up to three times above pre-fasting values [1]. Abnormal values above the reference range, but not indicative of Gilbert's Syndrome (between 25 and $50 \mu\text{mol l}^{-1}$), may complicate the interpretation of screening and safety data.

Therefore, the aim of this study was to investigate the effect of fasting on total serum bilirubin (TSB) concentrations in volunteers in whom TSB was within the normal range and in those with consistently elevated TSB concentrations.

Methods

Study population

Thirty-seven healthy male volunteers aged between 19 and 29 years (mean age 21.65 years) and weighing between 66 and 105 kg (mean weight 80.78 kg) took part in the study; 19 had elevated TSB concentrations (above $25 \mu\text{mol l}^{-1}$) on at least three occasions and 18 had normal TSB concentrations (below $25 \mu\text{mol l}^{-1}$).

Written informed consent was obtained from all volunteers after they had received detailed instructions concerning the performance of the study, restrictions and possible adverse events. The study was performed in accordance with good clinical practice guidelines [5].

Study design

This was an open, randomised, cross-over study. According to the randomisation plan, the volunteers were allocated to fasting and non fasting groups. After a rest period of 1 week, the volunteers were crossed over to the alternate treatment.

Study protocol

The volunteers were not allowed any medication from 14 days prior to and during the study. Strenuous physical activities or ingestion of alcohol or stimulating beverages containing xanthine derivatives were not allowed for 24 h before and after each study day.

Volunteers were admitted to the clinic on the evening (19.00 h) of each study day, and an indwelling venous catheter was inserted into a forearm vein. The fasting group had a standard supper at 20.00 h, after which they fasted for 24 h. The non-fasting group also had supper at 20.00 h, followed by a light snack at 22.00 h, breakfast at 06.30 h, the next day, a light snack at 10.30 h, lunch at 12.30 and a light snack at 16.00 h. Supper was served at 20.00 h to the fasting and non-fasting groups. The fasting volunteers drank 125 ml tap water every hour, except between 23.00 h and 05.00 h and the non-fasting volunteers drank 125 ml tap water at 21.00 h, 24.00 h, 06.00 h, 10.00 h, 14.00 h and 18.00 h.

Venous blood samples (10 ml) were collected at the following times: 19.30 h, 22.00 h, 24.00 h, 06.00 h, 07.00 h, 08.00 h, 09.00 h, 10.00 h, 12.00 h, 14.00 h, 18.00 h, and 20.00 h.

Bilirubin assay

TSB concentrations were assayed by the method of Van den Bergh & Muller [6] which entails the measurement of azobilirubin after reaction with diazotized sulphanilic acid.

Statistical analysis

TSB concentrations at 24 h were compared with the baseline concentrations using ANOVA with the following effects: Group (normal volunteers vs volunteers with high bilirubin levels), subject within group, time (baseline vs 24 h), and time $\times$ group after logarithmic transformation of the data. Point estimates and 95% confidence intervals for the '24 h/baseline' mean ratios were calculated. The data were also analysed separately for each group of volunteers.

The two treatments (fasting and non-fasting) were compared by subjecting the 10 h, 12 h and 24 h TSB concentrations to an analysis of variance with the following effects: Group, subject within group, treatment, period, and treatment $\times$ group, after logarithmic transformation of the data. Point estimates and 95%

Table 1 Summary of data for total serum bilirubin: comparison of fasting and non-fasting state for concentrations at 10 h, 12 h and 24 h, and comparison of 24 h and baseline values

Variable	n	Bilirubin concentrations non-fasting ($\mu\text{mol l}^{-1}$)			Bilirubin concentrations fasting ($\mu\text{mol l}^{-1}$)			Mean ratio (%)*	95% CI (%)**
		Geometric mean	s.d.	Range	Geometric mean	s.d.	Range		
<i>Baseline</i>									
All volunteers	37	17.5	2.11	7.00–63.0	16.6	1.98	6.00–75.0		
High values ¹	19	31.4	1.75	10.0–63.0	26.2	1.80	7.00–75.0		
Normal values ²	18	9.43	1.28	7.00–16.0	10.3	1.47	6.00–26.0		
<i>10 h</i>									
All volunteers	37	14.0	1.92	5.00–49.0	15.2	2.16	3.00–80.0	108	96.0–121
High values ¹	19	23.2	1.61	9.00–49.0	26.9	1.59	10.0–80.0	115	96.3–137
Normal values ²	18	8.27	1.34	5.00–16.0	8.34	1.71	3.00–28.0	101	84.3–121
<i>12 h</i>									
All volunteers	37	14.0	2.02	4.00–51.0	17.3	2.00	5.00–77.0	123	109–138
High values ¹	19	23.4	1.66	9.00–51.0	28.1	1.66	9.00–77.0	119	99.6–142
Normal values ²	18	8.13	1.49	4.00–18.0	10.3	1.53	5.00–26.0	127	106–151
<i>24 h</i>									
All volunteers	37	13.7	2.13	5.00–57.0	35.2	1.85	12.0–120	258	231–287
High values ¹	19	23.9	1.83	6.00–57.0	57.0	1.50	27.0–120	238	196–290
Normal values ²	18	7.56	1.38	5.00–14.0	21.2	1.36	12.0–35.0	280	251–312
<i>Comparison of 24 h and baseline values</i>									
		Mean ratio (%) ³		95% CI (%) ⁴	Mean ratio (%) ³		95% CI (%) ⁴		
All volunteers		78		72.0–85.0	212		190–236		
High values ¹		76.1		65.9–87.8	218		184–259		
Normal values ²		80.1		72.1–89.1	205		175–240		

^{*}Geometric mean of individual 'fasting/non-fasting' ratios.

^{**}95% conventional confidence interval for the 'fasting/non-fasting' mean ratio after logarithmic transformation of the data.

¹Volunteers with elevated TSB concentrations (above 25 $\mu\text{mol l}^{-1}$) on at least three occasions.

²Volunteers with normal TSB concentrations (below 25 $\mu\text{mol l}^{-1}$).

³Geometric mean of individual '24 h/baseline' ratios.

⁴95% conventional confidence interval for the '24 h/baseline' mean ratio after logarithmic transformation of the data.

confidence intervals for the 'fasting/non-fasting' mean ratios of those variables were calculated. The data of each group of volunteers were also analysed separately.

Results and Discussion

Comparisons of 24 h and baseline TSB concentrations, and of fasting and non-fasting concentrations at 10 h, 12 h and 24 h are shown in Table 1. The geometric mean TSB concentrations during the fasting and non-fasting treatment periods are shown in Figure 1, for both the normal and high bilirubin groups of volunteers.

During both the fasting and non-fasting treatment periods, and in both groups of volunteers (normal and high TSB values, respectively), minimum TSB concentrations were obtained 4 h after the supper (Figure 1). Following the non-fasting treatment, the 24 h TSB concentrations were somewhat lower (about 20%) than baseline concentrations in both groups of volunteers (Figure 1, Table 1). This indicates that the controlled and regular intake of food can lower TSB concentrations.

For both groups, a slight increase in TSB concentrations was recorded when the fasting and non-fasting states were compared after 10 h. A clinically relevant rise in TSB concentration took place after 12 h, and TSB concentrations at 24 h were about

2.5 times higher after a fast compared with the non-fasting treatment (Figure 1, Table 1). Thus the increase in TSB concentrations following a 24 h fast is considerable; while the absolute increases for the normal and high bilirubin groups of volunteers differ, the percentage increases for the two groups are similar, namely about 20% after a 12 h fast, and about 150% after a 24 h fast.

Conclusion

The rise in TSB caused by fasting should be taken into account when designing a clinical trial, selecting volunteers, or judging the tolerance of a new drug. Elevated TSB concentrations measured after a prolonged fast (more than 10 h) may not be drug or disease induced. TSB concentrations for safety profiles should not be taken after a prolonged fast, but between 2 and 6 h following the most recent meal.

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Figure 1 Geometric mean TSB values in the volunteers with normal TSB concentrations ($n = 18$); fasting (---○---) and non-fasting (—○—) and in volunteers with consistently elevated TSB concentrations ($n = 19$); fasting (---■---) and non-fasting (—■—).

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