

## Mild riboflavin deficiency is highly prevalent in school-age children but does not increase risk for anaemia in Côte d'Ivoire

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There are few data on the prevalence of riboflavin deficiency in sub-Saharan Africa, and it remains unclear whether riboflavin status influences the risk for anaemia. The aims of this study were to: (1) measure the prevalence of riboflavin deficiency in children in south-central Côte d'Ivoire; (2) estimate the riboflavin content of the local diet; and (3) determine if riboflavin deficiency predicts anaemia and/or iron deficiency. In 5- to 15-year-old children ( $n$  281), height, weight, haemoglobin (Hb), whole blood zinc protoporphyrin (ZPP), erythrocyte glutathione reductase activity coefficient (EGRAC), serum retinol, C-reactive protein (CRP) and prevalence of *Plasmodium* spp. (asymptomatic malaria) and *Schistosoma haematobium* (bilharziosis) infections were measured. Three-day weighed food records were kept in twenty-four households. Prevalence of anaemia in the sample was 52%; 59% were iron-deficient based on an elevated ZPP concentration, and 36% suffered from iron deficiency anaemia. *Plasmodium* parasitaemia was found in 49% of the children. Nineteen percent of the children were infected with *S. haematobium*. Median riboflavin intake in 5- to 15-year-old children from the food records was 0.42 mg/d, ~47% of the estimated average requirement for this age group. Prevalence of riboflavin deficiency was 65%, as defined by an EGRAC value > 1.2. Age, elevated CRP and iron deficiency were significant predictors of Hb. Riboflavin-deficient children free of malaria were more likely to be iron deficient (odds ratio; 3.07; 95% CI 1.12, 8.41). In conclusion, nearly two-thirds of school-age children in south-central Côte d'Ivoire are mildly riboflavin deficient. Riboflavin deficiency did not predict Hb and/or anaemia, but did predict iron deficiency among children free of malaria.

### Riboflavin: Iron: Deficiency: Children: Anaemia: Côte d'Ivoire

Riboflavin (vitamin B<sub>2</sub>) is required for many metabolic pathways, usually as a precursor of the flavin coenzyme, NAD, in redox reactions (McCormick, 1997). Riboflavin deficiency is particularly common in regions where intakes of dairy products and meat are low (Neumann *et al.* 2003). Deficiency causes impaired growth, cheilosis, angular stomatitis, glossitis, dermatitis and impaired vision (Venkataswamy, 1967; Batey *et al.* 1992; FAO/WHO, 2001). Schoolchildren, in both developing and industrialised countries, are an age group at high risk for riboflavin deficiency (Powers, 2003).

In Western Africa, anaemia affects 50–80% of the children (ACC/SCN, 2000; WHO/UNICEF/UNU, 2001). There are many aetiologies of anaemia in African children. These include malaria, blood loss from parasitic infections, vitamin A deficiency and diets low in bioavailable iron (ACC/SCN, 2000). Riboflavin deficiency may also impair erythropoiesis and contribute to anaemia (Foy & Kondi, 1953; Foy *et al.* 1964). Suggested mechanisms for this effect of riboflavin deficiency are decreased mobilisation of iron from stores (Sirivech *et al.* 1974; Powers, 1995), decreased iron absorption

and increased iron losses (Adelekan & Thurnham, 1986; Powers *et al.* 1988; Butler & Topham, 1993). Whereas these mechanisms have been investigated in animals, there are few data from human studies. Riboflavin supplements given concurrently with iron improve the response to iron supplementation in pregnant and lactating women, adult males and schoolchildren (Buzina *et al.* 1979; Charoenlarp *et al.* 1980; Ajayi *et al.* 1990). A stable isotope study in adult Gambian men showed a greater increase in Hb values with riboflavin supplementation compared with controls (both groups received iron supplements), but failed to demonstrate an increase in iron absorption, suggesting that the improvement in Hb may have been due to iron mobilisation from stores (Fairweather-Tait *et al.* 1992).

The aims of the present study were to: (1) determine the prevalence of riboflavin deficiency in school-age children in southern Côte d'Ivoire; (2) estimate the riboflavin content of the local diet; and (3) investigate if riboflavin status contributes to anaemia or iron deficiency in this population.

## Subjects and methods

### Study site

The study sites were two villages in Côte d'Ivoire from geographically distinct regions. Orbaff is a rural village in the Dabou district of Côte d'Ivoire, 10 km from the southern coast. The climate is tropical, with a temperature of  $\geq 27^{\circ}\text{C}$  and a relative humidity of  $\geq 80\%$  much of the year. Plantain, rice, yam and dried, smoked fish are eaten regularly. Bringakro is further north (District Toumodi), about 180 km northwest of Abidjan. The region is situated in the transition zone from rainforest to savannah, with a mean temperature of  $27^{\circ}\text{C}$  and a relative humidity of 70%. Staple foods in this area are yam, plantain, cassava and red palm oil. The surveys were conducted in May 2004 in Orbaff (Wegmuller *et al.* 2006), and in May 2005 in Bringakro.

### Subjects and enrolment

In both villages, a random sample was taken from children attending grades 1–5 in primary schools, with 50 and 28% of children in these grades enrolled in Bringakro and Orbaff, respectively. Informed written consent was obtained from the school directors and the parents of the children. The Ethical Committee of the Swiss Federal Institute of Technology in Zürich, Switzerland, and the Scientific Council of the CSRS in Abidjan, Côte d'Ivoire gave ethical approval for the study. A total sample size of 280 children was calculated assuming a change in Hb of 3 g/l as clinically relevant, a standard deviation (SD) of Hb of 12 g/l, a significance level of 95%, and a power of 75% for a two-sided outcome.

### Screening

Weight and height were measured upon registration (surname, first name, sex, date of birth) of the child. For these measurements, children removed their shoes and wore light clothing. Weight was recorded to the nearest 0.1 kg (Skyline, Soehnle-Waagen GmbH & Co. KG, Murrhardt, Germany), and height to the nearest 0.5 cm (Person check<sup>®</sup>, KaWe, Asperg, Germany) by the local nurse and an experienced technician. Age was obtained from the school registers, where age is taken from birth certificates. Venous blood samples (7.5 ml) were drawn in the morning into EDTA-treated evacuated tubes, and blood samples were kept in the dark on ice during transport to a nearby laboratory. A morning casual spot urine sample was collected for measurement of microhaematuria (as an indicator for bilharziosis). After the screening, children with iron deficiency were given supplemental oral iron for 12 weeks (daily 60 mg of iron as  $\text{FeSO}_4$ ), and children with bilharziosis were given a single dose of praziquantel (40 mg/kg).

### Dietary assessment

Three-day weighed food records were collected in Orbaff in 24 randomly selected households. The records were done on three consecutive days (including two weekdays and one weekend day). In the village, family meals are traditionally eaten from shared bowls, but to estimate individual food intake during the 3 days of recording, families ate from individual bowls. Edible

portions of all food and beverages were weighed using a Soehnle scale (Vera 67 002, Soehnle-Waagen GmbH & Co. KG, Murrhardt, Germany) accurate to  $\pm 1$  g during preparation and consumption. Meals typically consisted of a cooked staple and a sauce. When the meal was ready, the total weight of the cooked staple and sauce was weighed. After the meal, the food that remained uneaten was weighed and the proportion of staple to sauce was estimated by a team of experienced investigators. Subjects absent during the day were asked about their food intake and amounts eaten outside the home; these estimations were then added to the food records. Riboflavin intake was calculated using food composition tables (Tourey *et al.* 1965; FAO, 1968; Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin, 1996) and food analysis software EBISpro (University of Hohenheim/Stuttgart, Germany).

### Laboratory analysis

In Orbaff, Hb measurement was performed on the day of blood sampling on an AcT8 Counter (Beckman Coulter, Krefeld, Germany) using three-level controls provided by the manufacturer. In Bringakro, because the AcT8 Counter was not available, the Hemocue system was used (Hemocue, Angelsborg, Sweden) using two-level controls provided by the manufacturer. Venous blood was used for both methods; values obtained by these two Hb methods from venous blood are highly correlated (Morris *et al.* 2001). Zinc protoporphyrin (ZPP) was measured on washed red blood cells within 7 d of blood sampling in duplicate with a haematofluorometer (Aviv Biomedical, Lakewood, NJ, USA) (Hastka *et al.* 1992), with standards provided by the manufacturer. C-reactive protein (CRP) in samples from Bringakro was measured using an automated chemiluminescent high sensitivity immunoassay system (IMMULITE<sup>®</sup>, Diagnostic Products Corporation, Los Angeles, CA, USA), and Orbaff CRP samples were measured using nephelometry (TURBOX<sup>®</sup>, Orion Diagnostics, Espoo, Finland). Anaemia was defined as Hb  $< 120$  g/l in children aged  $\geq 12$  years, and Hb  $< 115$  g/l in children aged 5–12 years (WHO/UNICEF/UNU, 2001). Iron deficiency was defined by a ZPP value  $> 40$   $\mu\text{mol/mol}$  haem (Hastka *et al.* 1992). CRP values  $< 10$  mg/l were defined as normal (Pepys, 1981). Serum retinol (SR) was measured by HPLC (Merck-Hitachi, Tokyo, Japan) according to Tanumihardjo *et al.* (2004) with reference material from the National Institute of Standards and Technology (Gaithersburg, MD, USA); vitamin A deficiency was defined as an SR  $< 0.70$   $\mu\text{mol/l}$ , and low vitamin A status as an SR  $< 1.05$   $\mu\text{mol/l}$  (WHO, 1996). Riboflavin was measured by the erythrocyte glutathione reductase activity coefficient (EGRAC) assay using a modification of the method of Dror *et al.* (1994). In our laboratory, the inter- and intra-assay CV of the EGRAC method is 3 and 4%, respectively. Although there is no consensus for EGRAC cut-off values for riboflavin deficiency (Tillotson & Baker, 1972; Prasad *et al.* 1992; Sauberlich, 1999), we used cut-offs specified for this EGRAC assay (Dror *et al.* 1994), i.e. values  $> 1.2$  indicated marginal deficiency and values  $> 1.4$  indicated clear deficiency (Sauberlich, 1999). For statistical analysis, EGRAC values  $> 1.2$  were used. On the day of blood sampling, whole blood was used to prepare a thick and a thin smear for malaria parasites by the

Giemsa coloration technique (WHO, 1990). Parasites were counted against leucocytes; fields containing >200 leucocytes were counted and if <10 parasites were identified, the counting continued up to 500 leucocytes. These counts were converted to the number of parasites per  $\mu\text{l}$  of blood, assuming a leucocyte count of 8000/ $\mu\text{l}$  (WHO, 1990). On the day of blood sampling, haematuria, as an indirect marker for infection with *Schistosoma haematobium*, was assessed semiquantitatively by dipstick (Combur 7, Roche Diagnostics GmbH, Mannheim, Germany).

#### Statistical analysis

Data processing and statistical analysis were done using Excel (XP 2002; Microsoft, Redmond, CA, USA) and SPSS 13.0 (SPSS Inc., Chicago, IL, USA). Height-for-age and weight-for-age Z-scores were calculated using Epi-Info (Epi-Info Version 3.3.2, Centers for Disease Control and Prevention, Atlanta, GA, USA). All data except ZPP were normally distributed and are presented as means and their SD. ZPP data were skewed and are presented as medians (ranges); for statistical analysis, these values were log-transformed. One-way ANOVA was used to test for differences for all scaled variables among the different geographic areas; the Mann-Whitney test was applied for ordinal data. Two-tailed Pearson's correlation coefficients were used to examine the relationship between iron status or anaemia and riboflavin status, malaria, inflammation, SR and haematuria. Stepwise linear regression on Hb and logZPP was performed with age, sex, EGRAC, SR, CRP, malaria and Hb or logZPP as independent variables. Odds ratios for the risk of anaemia or iron deficiency in riboflavin deficiency were calculated. *P* values <0.05 were considered statistically significant.

## Results

#### Three-day weighed food records

The 3 d weighed food records were kept in 24 families comprising 207 subjects (median age: 16 years; range: 2–81 years); the results for children 6–15 years of age (*n* 71) are reported here. Daily intakes of riboflavin ranged from 0.17 to 1.16 mg, with a median of 0.42 mg, representing 70 and 47% of the estimated average requirement for children 6–8 years of age and those aged 9–13, respectively (Institute of Medicine, 2001). Riboflavin intake was low due to infrequent consumption of animal foods, with the exception of smoked fish, and the negligible riboflavin content of cassava, the dietary staple. Median (range) iron intake was 9.8 mg (3.6–19.8) mg/d, well above the estimated average requirement of 6.3 mg/d for 6- to 15-year-old children. Only 21% of children had iron intakes below the estimated average requirement. However, the bioavailability of the dietary iron was probably low, as 90% of iron was non-haem iron.

#### Anthropometry

Mean age of the screened children was 10.2 (SD 2.3) years, mean weight and height were 31.8 (SD 10.1) kg and 1.38 (SD 0.15) m, respectively. Forty-four percent of the children were female. As shown in Table 1, the children from the

two villages differed significantly in sex ratio, age, weight and height. However, height-for-age as well as weight-for-age Z-scores were not significantly different. Eight percent of the children were stunted (height-for-age Z-score less than  $-2$  SD) and 11% were undernourished (weight-for-age Z-score less than  $-2$  SD).

#### Prevalence of anaemia, iron deficiency and iron deficiency anaemia

A high prevalence of anaemia (52%) was found in the studied population (Table 1). Nearly 60% of all children had iron deficiency, and iron deficiency was present in 69% of the anaemic children. Iron deficiency anaemia was present in 36% of all children. As expected, ZPP and Hb were significantly correlated (Table 2), but no other parameter alone influenced these two variables. Vitamin A deficiency is thought to contribute to anaemia (Reddy, 1998), but only 2% of the children in the sample were vitamin A deficient. Comparing riboflavin-deficient (*n* 182) and riboflavin-sufficient (*n* 99) children, ZPP concentrations were 51 (17–296) and 46 (20–49)  $\mu\text{mol/mol}$  haem, the prevalence of iron deficiency was 63.2 and 52.5%, and the prevalence of iron deficiency anaemia was 35.7 and 36.4%, respectively.

#### Prevalence of malaria parasites and microhaematuria

Despite a high prevalence of malaria parasites (49%), the severity of infestation was low, with only 8% of the children having a parasite load >1000/ $\mu\text{l}$  blood, and only two children having clinical malaria, as defined as a parasite load >5000/ $\mu\text{l}$  blood (Trape, 1985). *Plasmodium* load was positively correlated with SR, but with no other variable (Table 2). Microhaematuria, an indicator for *S. haematobium* infection, was present in 19% of the children.

#### Prevalence of inflammation

The prevalence of inflammation or infection, as indicated by elevated CRP concentrations, was moderate in both villages. Only about 15% of the children had an elevated CRP despite a considerably higher prevalence of *Plasmodium* parasitaemia. This discrepancy may at least partly be explained by the generally low malaria severity.

#### Prevalence of riboflavin deficiency

Two-thirds of the children suffered from riboflavin deficiency, but the degree of deficiency was mild. Only 2% of children had EGRAC values >1.4, suggesting clear deficiency. Of the riboflavin-deficient children, 7.5% showed EGRAC values >1.4. EGRAC was not correlated with any other measured variable (Table 2).

#### Regression analysis and odds ratios

In the stepwise linear regression on Hb, age (*P*<0.001), log ZPP (*P*<0.001) and CRP (*P*<0.05) were significant predictors. With logZPP as the dependent variable, Hb (*P*<0.001) and EGRAC (*P*<0.05) were significant predictors. Overall, correlations were modest.

**Table 1.** Anthropometric, nutritional and health characteristics of Ivorian children in two villages of Côte d'Ivoire: Orbaff, in the southern tropical zone, and Bringakro, in the savannah transition zone

	The two villages ( <i>n</i> 281)		Orbaff ( <i>n</i> 171)		Bringakro ( <i>n</i> 110)	
Age* (years)†	10.2	2.3	9.9	2.3	10.8	2.2
Height* (m)†	1.38	0.15	1.37	0.14	1.41	0.17
Weight* (kg)†	31.8	10.1	30.4	8.7	33.9	11.6
Height-for-age Z-scores	-0.3		-0.2		-0.4	
Prevalence of stunted children, less than - SD ( <i>n</i> (%))	23 (8)		14 (8)		9 (9)	
Weight-for-age Z-scores	-0.6		-0.6		-0.7	
Prevalence of undernourished children, less than - SD ( <i>n</i> (%))	27 (10)		15 (9)		12 (11)	
Sex ratio (female: male)†	0.8		0.7		1.0	
Hb (g/l)*	116	12	117	12	115	12
Zinc protoporphyrin (μmol/mol haem)‡	49.5 (17-296)		53.0 (24-296)		45.2 (17-288.5)	
Prevalence of anaemia ( <i>n</i> (%))		145 (52)	83 (48)		62 (56)	
Prevalence of iron deficiency anaemia ( <i>n</i> (%))	101 (36)		61 (36)		40 (36)	
Prevalence of iron deficiency without anaemia ( <i>n</i> (%))	167 (59)		112 (66)		55 (50)	
Serum retinol (μmol/l)*	1.25§	0.32	1.30	0.2	1.22	0.31¶
Prevalence of vitamin A deficiency ( <i>n</i> (%))	2 (1)		0 (0)		2 (2)	
Riboflavin EGRAC*	1.25	0.12	1.26	0.12	1.24	0.11
Prevalence of EGRAC > 1.2 ( <i>n</i> (%))	182 (65)		109 (64)		73 (66)	
Prevalence of <i>Plasmodium</i> parasitaemia ( <i>n</i> (%))	78 (49)**	27 (55)††	51 (46)			
Prevalence of microhaematuria ( <i>n</i> (%))†††	31 (19)‡‡		2 (3)§§		29 (26)	
Prevalence of elevated CRP ( <i>n</i> (%))	43 (15)		23 (13)		20 (18)	

EGRAC, erythrocyte glutathione reductase activity coefficient; CRP, C-reactive protein.

\* Mean and standard deviation.

† Schools differ significantly by ANOVA ( $P < 0.05$ ).

‡ Median (range).

§ *n* 162.

|| *n* 55.

¶ *n* 107.

\*\* *n* 159.

†† *n* 49.

‡‡ *n* 161.

§§ *n* 51.

In riboflavin-deficient subjects, the odds ratios (95% CI) for anaemia and iron deficiency were 0.69 (0.42, 1.13) and 1.55 (0.94, 2.55), respectively. Because EGRAC values may be influenced by malaria (see below), odds ratios were also calculated after excluding subjects with malaria. In riboflavin-deficient subjects (EGRAC > 1.2) without *Plasmodium* infection (*n* 80), the odds ratio for iron deficiency was significant (3.07; 1.12, 8.41).

## Discussion

Mild riboflavin deficiency was very common in the study children, with nearly two-thirds of the children affected. A previous

Ivorian study reported EGRAC values > 1.68, but no information on the age of the subjects or the prevalence of riboflavin deficiency was provided (Arnaud *et al.* 2001). A food survey in central Côte d'Ivoire found low consumption of dairy products and meat, the usual major dietary sources of riboflavin, but riboflavin intakes were not reported (Staubli-Asobayire, 2000). Although there are few data on the riboflavin status of children in sub-Saharan Africa, the few published studies suggest that riboflavin deficiency may be common. A study in Botswana reported that 33-40% of the examined children had an EGRAC  $\geq$  1.4 (Abrams *et al.* 2003). In Kenya, approximately one-third of the children were riboflavin deficient, measured as red blood cell riboflavin (Siekmann *et al.* 2003). In 2- to

**Table 2.** Pearson's correlation coefficients between nutrition and health-related variables in school-aged children in two villages of Côte d'Ivoire

	ZPP	CRP	EGRAC	Malaria	<i>Schistosoma</i>	SR
Hb	-0.175*	-0.062	0.112	-0.010	0.060	0.093
ZPP	-	0.037	0.054	0.041	-0.117	0.055
CRP	-	-	-0.018	-0.041	0.066	-0.164†
EGRAC	-	-	-	0.043	-0.110	0.022
Malaria	-	-	-	-	-0.045	0.214†
<i>Schistosoma</i>	-	-	-	-	-	-0.003

ZPP, zinc protoporphyrin; CRP, C-reactive protein; EGRAC, erythrocyte glutathione reductase activity coefficient; SR, serum retinol.

\* Correlation is significant at the 0.05 level.

† Correlation is significant at the 0.01 level.

5-year-old South African children, intakes of riboflavin were only 49 % of the RDA (Faber *et al.* 2001).

In this study, although malaria was endemic, with 49 % of the children infected, the parasite load was generally low. This prevalence is similar to that reported in a previous Ivorian study, but the severity of infection was higher in that study (Staubli Asobayire *et al.* 2001). A study conducted in eastern Côte d'Ivoire found that malaria affected 74–86 % of 7- to 11-year-old children and 68 % of children >12 years old (Girardin *et al.* 2004). Microhaematuria as an indicator of *S. haematobium* infection varied markedly between villages, with nearly one-third of the children affected in Bringakro. This high prevalence of *S. haematobium* infection is consistent with a previous report from this region of Côte d'Ivoire, where 24.6 % of the children had microhaematuria, although data varied markedly depending on the nearby presence of standing water (N'Goran *et al.* 1998). Anaemia prevalence in the present study (52 %) was similar to that found in an earlier report in Ivorian children (46 %) (Staubli Asobayire *et al.* 2001). However, in that study, the prevalence of elevated ZPP levels was higher (83 %) than in the present study (59 %).

There are several limitations to this study. First, the use of a single measure of iron status (ZPP) probably reduced our ability to define iron deficiency accurately (Zimmermann *et al.* 2005). Previous studies have suggested that ZPP may be elevated by infection and/or inflammation, and thus iron deficiency using ZPP may be overestimated in areas of endemic infection (Staubli Asobayire *et al.* 2001). However, in the present study, there was no correlation between ZPP and CRP, presence of malaria or suspected schistosomiasis. It has been suggested that acute malaria may spuriously elevate EGRAC (Anderson *et al.* 1994; Traummüller *et al.* 2003). However, acute malaria was rare in our study population, and there was no relationship between EGRAC and malaria, so our estimate of riboflavin deficiency based on EGRAC is likely to be valid.

The public health impact of widespread riboflavin deficiency in schoolchildren is unclear; but it may influence iron metabolism (Sirivech *et al.* 1974; Adelekan & Thurnham, 1986; Powers *et al.* 1988; Butler & Topham, 1993; Powers, 1995). In our sample, anaemia and iron deficiency were common, with prevalences >50 %, and two-thirds of the anaemic children were iron deficient. Neither linear regression nor calculation of relative risk identified riboflavin deficiency as a significant predictor of Hb or anaemia. Thus, our data do not support a detrimental effect of riboflavin deficiency on anaemia, as suggested by earlier studies (Foy & Kondi, 1953, 1958), a finding that might be explained by the low severity of the riboflavin deficiency in our sample. However, the risk of iron deficiency was increased 3-fold in children with riboflavin deficiency and without malaria. There are two likely explanations for this association. First, poor overall quality of the diet may have led to multiple micronutrient deficiencies; however, there was a very low prevalence of vitamin A deficiency, and iodine status appeared to be adequate. Secondly, the results could reflect impairment of iron absorption and/or metabolism by riboflavin deficiency, as suggested by earlier studies (Powers *et al.* 1988; Butler & Topham, 1993; Powers, 1995). In three trials in children and pregnant women (Charoenlarp *et al.* 1980; Powers *et al.* 1983; Suprpto *et al.* 2002), riboflavin and iron supplementation produced a greater increase in Hb compared with iron supplements

given alone, although the results may have been confounded by concomitant folic acid supplementation in two (Charoenlarp *et al.* 1980; Suprpto *et al.* 2002). In contrast, another trial reported no additional benefit of riboflavin plus iron compared with iron supplementation alone in young adults (Powers & Bates, 1987). A study in a small number of pregnant women reported that riboflavin supplementation alone or in combination with iron supplementation did not prevent a decrease in Hb, although the decrease was less in the treated group than in the control group (Powers *et al.* 1985). Similarly, no effect could be found in a riboflavin supplementation trial in Croatian schoolchildren with adequate Hb levels (Suboticanec *et al.* 1990). Taken together, these data suggest that the effect of riboflavin status on Hb is variable, and may be confounded by the multifactorial aetiology of anaemia, particularly in countries in sub-Saharan Africa.

In conclusion, there is a high prevalence of mild riboflavin deficiency in Ivorian schoolchildren, but severe deficiency was rare. Riboflavin deficiency was associated with iron deficiency in children free of malaria parasites. However, there was no significant association between riboflavin status and anaemia.

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