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Antioxidant Status before and after Dietary Intervention in Cardiovascular Disease (CVD) Patients

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ABSTRACT

There is some evidence showing an inverse correlation between dietary sources including natural antioxidant vitamins and the risk of cardiovascular disease (CVD). The aim of this study was to evaluate the effect of dietary antioxidants on oxidative stress in CVD patients. This study was carried out on 31 CVD patients and 63 healthy individuals. Nutritional status and dietary antioxidant vitamins were assessed by 48-hour recall. Superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities as well as the levels of vitamins A, E, C, total antioxidant capacity (TAC) and malondialdehyde (MDA) were determined before and after serving fresh fruits and vegetables for 3 months. Before intervention intake, levels of vitamins A, E and C were significantly lower in patients than in normal individuals ($P<0.001$). The serum levels of vitamins A, E and C were significantly lower in the cases than in the control subjects. After intervention, the serum levels of vitamins A, E and C were increased significantly ($P<0.0001$). Similarly, the levels of TAC as well as the activities of SOD and GPx were found to increase by end of 3 months. In addition, a significant increase of TAC and a decrease in MDA levels were observed. In conclusion, the findings show that dietary supplementation improves the antioxidant defense system in CVD patients.

Keywords: Cardiovascular disease (CVD), dietary antioxidants, interventional studies, oxidative stress

INTRODUCTION

A decreased antioxidant defense system and over-generation of free radicals play an important role in the pathogenesis of cardiovascular disease and several other diseases (Heistad, 2006). The resultant oxidative stress impairs activities of both enzymatic and non-enzymatic antioxidants (Serdar *et al.*, 2006). Numerous studies have reported that consumption of certain foods leads to a reduction in oxidative stress and myocardial infarction (MI) (Kaliora, Dedoussis & Sshmidt, 2006). Besides, a number of components in foods have been found to have antioxidant properties which include vitamins E and C, vitamin A and its precursor, beta-carotene, which is converted

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into vitamin A in the body (Mahan & Escott-stump, 2008). Each of these antioxidants has specific activities and usually works synergistically to improve the antioxidant ability of the body (Bagchi & Puri, 1998). Along with vitamins, enzymes also contribute to a body's defense systems against oxidative distress (Ruel *et al.*, 2005). Thus, the antioxidant status depends on the dietary supply of vitamins E, C and A, endogenous enzymatic levels of SOD, GPx and catalase (Papas, 1999; Serdar *et al*., 2006). Although vitamins C, E and beta-carotene are effective antioxidants *in vitro*, there is a limited number of studies on human beings that have addressed the efficacy of these vitamins as antioxidants *in vivo* (McCall & Balz, 1999). Similarly, Klipstein-Grobusch *et al.* (1999) have found an inverse association between high dietary beta-carotene intakes and risk of myocardial infarction (MI) in the elderly. Both *in vitro* and *in vivo* studies have demonstrated that tocopherol inhibits LDL oxidation and decreases the release of reactive oxygen species (ROS). Furthermore, it reduces the release of pro-inflammatory cytokines and inhibits monocyte endothelial cell adhesion (Dutta & Dutta, 2003). Antioxidants in foods decrease LDL-cholestrol oxidation and diminish arterial plaque formation (Padayatty *et al*., 2003). It has been supposed that antioxidant-rich foods prevent LDL-oxidation and prevent CVD (Ruel *et al*., 2005). The relationship between vitamin C supplementation, dietary intake of vitamin C, or its plasma concentration and the risk of coronary disease has been studied by a few, and their results have been inconsistent. However, the association between low plasma levels of vitamin C and increasing progression to atherosclerosis and MI has also been reported (Nyyssonen *et al*., 1997).

Although numerous studies have focused on individual nutrients and foods, it is well determined that multiple dietary factors influence the risk of developing CVD and its major risk factors (Lichtenstein *et al.*, 2006). According to several studies, CVD patients need to strengthen their anti-oxidant status. But there is rarely evidence regarding the effect of natural vitamin antioxidants in daily diet on oxidative stress among these patients. The present study was therefore undertaken to examine the potential beneficial impact of short-term dietary intake of natural antioxidants on oxidative stress and antioxidant status in CVD patients.

METHODOLOGY

Subjects

The subjects included 31 CVD patients (14 male, 17 female; 55.3 years) admitted to the intensive cardiac care unit (CCU and post-CCU) wards at Khatam-Al-Anbia Hospital of Zahedan University of Medical Science (ZUMS), Iran. The clinical criteria included chest pain which lasted for up to 3 hours, echocardiographic changes and CPK, CKMB and LDH activities. The patients were receiving aspirin and beta-blockers for treatment. The control group included 63 age-matched healthy subjects (22 male, 41 female; 56.4 years) with normal electrocardiography and without any history of hypertension, heart disease, diabetes mellitus, renal or liver disease malignancy, endocrine disorders, pregnancy, operation or any acute medical condition.

Both case and control subjects were not on vitamin supplements either. The study protocol was approved by the local ethics committee of Zahedan University of Medical Science (ZUMS) and informed consent was obtained from all patients and healthy individuals.

Dietary assessment

Dietary intake data on the kind and amount of daily food intake was recorded by 48-hour recall questionnaire. Mean levels of energy, macronutrients and antioxidant vitamins were analysed by food processor software (Nutritionist III software). Briefly, a 48-hour

food recall questionnaire was used as a method of data collection. The patients answered the questionnaire in the presence of a research team member. It consisted of two parts. The first section of the questionnaire included the items related to personal characteristics [age, sex, height and weight, chest pain, supplement use (vitamins or minerals), receiving medicine for treatment and history of chronic diseases].

The second part of the questionnaire included 48-hour recall of foods based on a 24-hour recall from a sample of foods for every day (Form 1). According to this method, all of the subjects recalled the foods and amounts of foods which they consumed in the past 24 hours and the day before with standard food models to evaluate intake energy and nutrients being analysed by Nutritionist III software. Before recording data about food intake, all of the patients had been advised by a nutritionist and followed up regularly.

The total level of energy, macronutrients (protein, fat and carbohydrate) and antioxidant vitamins (A, E and C) intake was determined by using the locally developed Food Processor Software. This procedure (24-hour recall method by the patients) was repeated over two days. The mean of daily consumed energy, macronutrients and antioxidant vitamins was calculated before and after nutritional intervention separately.

Standard reference tables were used to convert household portions to grams for computerisation. After coding the diaries, the dietary recall form was linked to a nutrient database (Nutritionist III) and nutrient intakes calculated using the Mosby Nutritract Software for conversion of quantity to serving of consumed food. For mixed dishes, food groups were calculated according to their ingredients. The data related to Nutritionist III was modified according to the Iranian Food Composition Table. This software is based on Iranian food habits and was used for the assessment of macro- and micro-nutrients intake by the 48-hour recall food questionnaire.

Interventional studies

Initially, the CVD patients were exposed to information on the importance of diet and natural antioxidant vitamins in physiological processes, healthy living and prevention of oxidative stress. During the study, all the patients were visited and followed-up by the nutritionist and cardiologist. The process of diet therapy and trend of the educational programme only in the CVD patients was followed up by the nutritionist and nursing colleagues regularly.

The duration of the interventional study was three months during which time the patients consumed 5 servings of fresh fruits and vegetables (orange, date, dark grape, plum, peach, nectarine, lemon, cherries, lettuce, capsicum, tomato, cabbage, pumpkin, squash) rich in vitamins C, E, and A or provitamins. Also, a variety of vegetable oils, nuts, seeds and their oil, animal and dairy products which are rich sources of vitamin E along with the regular CVD dietary regimen were recommended. They were free to consume more servings of the food groups. A regular follow-up was carried out during the period of study. On completion of the 3 months, another 48-hour dietary recall questionnaire was again administered and the effectiveness of nutritional intervention was assessed by drawing blood and measuring the oxidant and antioxidant markers.

Blood sampling and analysis

Following a 12-hour fast period, venous blood samples were collected in different tubes, before and after intervention and once for control group. Heparinsed tubes were used for measurement of MDA, TAC, SOD, GPx and vitamin C, and tubes without anticoagulant were used for the measurement of vitamins A and E. Plasma and serum were obtained by centrifugation at 3000 rpm for 10 minutes and stored at -80 ^{o}C until analysis.

Total antioxidant capacity (TAC)

Total antioxidant capacity of plasma was measured by the ferric reducing / antioxidant power (FRAP) assay (Hashemi *et al*., 2010).

Superoxidase Dismutase (SOD) and Glutathione Peroxidase (GPx) activities

SOD and Gpx activities were determined using commercial assay kit (Randox, UK) as described previously (Karajibani *et al*., 2009).

Vitamins A, E and C assay

Serum levels of vitamins A and E were measured using high pressure liquid chromatography (HPLC) and plasma vitamin C concentration was measured specterphotometrically as described previously (Karajibani *et al.*, 2009)

Malondialdehyde (MDA) assay

Plasma MDA level was measured based on the reaction between MDA and thiobarbituric acid (TBA) using the method described by Satoh(1978). The absorbance was read at 530 nm and expressed as micromoles of malondialdehyde per litre (Karajibani *et al*., 2009).

Statistical analysis

Results are expressed as means ± S.D. Statistical analysis was performed using SPSS 11.5 software**.** Unpaired sample *t*-test and independent sample *t*-test were used to compare the groups. A P-value less than 0.05 was considered statistically significant.

RESULTS

Of the 31 CVD patients, 2 patients had to be withdrawn, as they were not able to keep to the dietary recommendation of CVD for the duration of the study. The remaining 29 patients completed the study without any problems. Of the total, 59.2% of patients were female and 40.8% male. Education level (years of schooling) demonstrated an indirect relationship between literacy and the occurrence of the disease. The majority (47% and 40 %) of the subjects were found to be illiterate, 26% and 21% had completed primary school, while around 13% and 16% had bachelor degrees and higher qualifications in patients and controls, respectively.

Clinical parameters, blood pressure (systolic and diastolic), CPK, CK-MB and LDH in CVD patients showed significantly ($P<0.001$) higher values than healthy subjects at baseline. The levels gradually decreased after intervention ($P<0.01$) (Table 1).

The results showed that after intervention, an improvement in the consumption pattern of vegetables and fruits (75.9% vs. 53.5%), white meat (75.9% vs. 57.7%), and dairy products (88.7% vs. 96.5%). These results showed that there was a modification in food pattern in these patients.

Dietary analysis showed no significant variation in the intake of macronutrients between the patients and control groups (Table 2). Only, carbohydrate amounts showed insignificant higher intakes while protein and fat intakes were in lower quantities before intervention. The intake further decreased except for protein after a 3-month intervention. The overall calorie values were accordingly affected. Similarly, the dietary intake of vitamins A, E and C was found to be low in the subjects before the start of intervention and the control group ($P<0.001$) which significantly improved on intervention ($P<0.001$) as shown in Table 2.

The levels of TAC, SOD activity and non-enzymatic antioxidant content, vitamins A, E and C of blood showed a significant increase after a 3-month intervention ($P<0.001$), while GPx activity showed an insignificant increase ($P>0.05$). Except for serum vitamin A ($P<0.01$), the increase in vitamins E and C, and SOD activity still remained lower than the values of the control group. Though, MDA levels were found to

Table 1. Clinical characteristics of CVD patients and controls before and after intervention

Groups	*CVD patients*		*Control group n=63*	*P- value*		
Parameters	*Month 0 (n=31)* **a**	*Month 3 (n=29)* **b**	**c**	**a,b**	**a,c**	**b,c**
Age (yr)	55.3±11.8	-	56.4±11.3		0.91	
BMI (kg / m²)	6.1±4.4	26.0± 3.7	26.5±4.6	0.77	0.81	0.59
Blood pressure (mm / Hg):						
Systolic	144.7±15.4	131.2±16.1	114.1±11.0	0.001	0.002	0.001
Diastolic	92.9±14.2	82.8±9.9	73.4±8.6	0.001	0.002	0.001
CPK (U / L)	368.2±364.4	202.5±271.1	101.6±38.8	0.006	0.005	0.005
Range	(49.0 – 1075.0)	(46.0-1027.0)	(34.0 – 189.0)			
LDH (U / L)	704.2± 220.5	616.0 ±197.4	336.4± 76.8	0.005	0.001	0.001
CKMB (U / L)	70.4 ± 51.6	42.9 ±31.0	14.4 ± 4.8	0.004	0.001	0.001

Values are mean ± SD.

a,b Significant difference between month 0 and month 3 of CVD patients

a,c Significant difference between months 0 of CVD patients and controls

b,c Significant difference between months 3 of CVD patients and controls

Table 2. Intake of energy, macro nutrients and dietary antioxidant vitamins in CVD patients and controls before and after intervention

Groups	*CVD patients*		*Control group n=63*	*P -value*		
Parameters	*Month 0 (n=31)* **a**	*Month 3 (n=29)* **b**	**c**	**a,b**	**a,c**	**b,c**
Energy (kcal / d)	2183.2±441.3	2158.3±414.5	2175.0±458.0	0.12	0.93	0.87
Carbohydrate (g / d)	350.7 ±82.1	346.7 ±79.0	20.3 ±89.6	0.13	0.12	0.18
Protein (g / d)	66.2 ±16.2	69.0 ±15.0	85.3 ±20.3	0.19	0.0001	0.0001
Fat (g / d)	57.3 ± 14.6	55.1 ± 13.5	61.4 ± 17.9	0.08	0.27	0.09
* Vitamin. A (µg RE / d)	440.7 ± 141.1	664.2 ± 230.2	638.3 ±337.3	0.0001	0.002	0.71
**Vitamin. E (mg alpha-TE / d)	6.1 ± 1.2	8.4 ± 3.0	8.1 ± 1.6	0.0001	0.001	0.58
Vitamin. C (mg / dl)	46.2 ± 19.5	64.8 ± 18.7	79.8 ± 20.8	0.0001	0.001	0.001

Values are mean ± SD

a,b Significant difference between month 0 and month 3 of CVD patients

a,c Significant difference between months 0 of CVD patients and controls

b,c Significant difference between months 3 of CVD patients and controls

***** Vitamin A intake was expressed as Retinol Equivalent (RE). One RE is equal to 1 µg retinol or 6 µg beta-carotene or 12 µg of other provitamin A carotenoids (Ribaya-Mercado *et al.*, 2004)

****** The new recommendations for vitamin E are expressed as mg alpha-tocopherol equivalents (mg alpha TE). 1 mg of alpha–tocopherol equals 1.5 IU vitamin E (Hathcock *et al.*, 2005).

Table 3. Antioxidant and oxidant status in CVD patients and control before and after intervention

Groups	*CVD patients*		*Control group n=63*	*P value*		
Parameters	*Month 0 (n=40)* **a**	*Month 3 (n=31)* **b**	**c**	**a,b**	**a,c**	**b,c**
TAC (μmol/L)	547.0 ± 116.2	671.9 ± 125.8	789.4 ±158.5	0.001	0.0001	0.001
SOD (U/gHb)	911.6 ± 251.1	1153.2 ± 262.0	2273.5±552.2	0.0001	0.0001	0.0001
GPx (U/gHb)	14.0 ± 3.6	17.5 ± 3.8	30.7 ±7.7	0.056	0.0001	0.0001
Vitamin A (μg/dL)	52.0 ± 13.3	69.4 ± 16.1	60.4 ± 12.8	0.0001	0.0001	0.005
Vitamin E (μg/dL)	495.8 ±103.8	590.0 ±170.5	696.2 ± 169.5	0.001	0.001	0.006
Vitamin C (mg/dL)	0.56 ±0.23	0.98 ± 0.31	1.2 ±0.42	0.0001	0.0001	0.08
MDA (μmol/L)	0.21 ±0.03	0.17 ± 0.03	0.12 ± 0.02	0.0001	0.001	0.001

Values are mean ± SD
a,b Significant difference between month 0 and month 3 of CVD patients
a,c Significant difference between months 0 of CVD patients and control
b,c Significant difference between months 3 of CVD patients and control

decrease significantly (P<0.0001) in the patients, they remained higher than in the control subjects (P<0.001) (Table 3).

DISCUSSION

When the critical balance between generation of reactive oxygen/nitrogen species (ROS, RNS) and body antioxidant defense system is impaired, oxidative damage may affect cardiovascular function (Bagchi & Puri, 1998; Duthie, 1999). Although, there are several dietary factors which can influence the risk of developing CVD (Lichtenstein *et al*., 2006), the emphasis on whole diet is appropriate to ensure nutrient adequacy and energy balance (Kris-Etherton *et al.*, 2004; Lichtenstein *et al*., 2006). It was observed that most of the patients were illiterate which in parallel to other factors such as profession, sedentary activity, less awareness, poor diet, overweight, and oxidative stress, have been assumed to be important in the CVD complications. Many CVD risk factors interact physiologically in its etiology. During nutritional intervention, 2 patients were omitted from the sample because they were not able to keep to the dietary recommendations.

In the present study, dietary macro-nutrient composition of CVD patients showed an adequate intake of carbohydrate, protein and fat, providing for proportionate daily energy of 64.3%, 12.8%, and 23% respectively, well within the range of general recommendations (Mahan & Escott- stump, 2008). A diet rich in vegetables and fruits is a strategy for lowering the energy density of the diet to control energy intake and also provides natural antioxidant vitamins (Lichtenstein *et al.*, 2006). Diet has a profound effect on body antioxidant status which can be strengthened and maintained by supplying exogenous antioxidants and precursors of endogenous antioxidants (Papas, 1999). Hence, in the present intervention study, the intake of seasonal fruits and vegetables for vitamins C and A and vegetable oils, animal and dairy products for vitamin E as a natural source of antioxidant were recommended.

According to WHO, a daily intake of about 450 grams of special fruits and vegetables can provide an appropriate proportion of antioxidant vitamins which help in maintaining cardiovascular health (Naidoo *et al*., 1998; Polidori *et al*., 2002). The

recommended daily allowance (RDA) of vitamins E and C is 10-15 IU (8-10 mg á-TE / day) (Hathcock *et al.*, 2005; Papas, 1999) and 60 mg / day (Mahan & Escott-stump, 2008; Papas, 1999), respectively. The FAO / WHO recommendations for safe intake of vitamin A for men and women aged 19-65 y are 600 and 500 µg retinol (RE) / d; that for all subjects aged>65 y is 600 µg / RE / d (FAO / WHO, 2004; Ribaya-Mercado *et al*., 2004).

In the present study, CVD subjects before the start of the intervention had low endogenous vitamin antioxidant values in comparison with normal subjects which may be related to the low dietary intake as per recommended dietary allowance (RDA) (Hathcock *et al*., 2005; Mahan & Escott-stump, 2008).

Adherence to a recommended diet for a period of 3 months increases the intake of vitamins A, E, and C levels in parallel with the antioxidants status of the body. This suggests that exogenous antioxidant availability influences the endogenous antioxidant defense system.

The low dietary intake of vitamins might be due to several factors; non-availability of vitamin-rich foods, lack of variety and awareness, process of preparation and preservation, and above all absorption and bioavailability. Though dietary fats are essential for proper absorption of fat soluble vitamins, yet a restricted intake is suggested only to subjects with cardiovascular problems (Papas, 1999). Maintenance of body antioxidant status through dietary management is recommended over the supplements (Jha *et al*., 1995; Kris-Etherton *et al*., 2004). Proper diet composition can provide all the exogenous antioxidant substances which have synergistic co-operative effect, unlike individual intake as supplements.

In the present study, despite the dietary improvement in vitamin C intake after intervention, the increased serum levels remained still lower than in the control. This could be a result of its synergistic action towards vitamin E; the increase in the intake amount and serum levels not being adequate to prevent exhaustion of vitamin E and at the same time maintain the normal level. Gale *et al.* have reported that both dietary amounts of vitamin C and plasma ascorbate value are related to risk of death from stroke in old people (Gale *et al*., 1995). Besides, changes in dietary vitamin E content is likely to depend on whether the consumed foods are cooked or raw, as it is known to be degraded by exposure to heat, light and air (Dutta & Dutta, 2003).

After intervention, unlike vitamins E and C, serum level of vitamin A was significantly higher than in normal individuals**.** Vitamin A and carotenoids can autoxidise when O^2 tension increases, and thus are most effective antioxidants at low oxygen tensions that are typical of physiological levels found in tissues (Palace *et al*., 1999). It has been documented that eating foods high in carotenoids can prevent atherosclerosis, although clinical trials have not been able to provide such evidence (Kohlmeier & Hastings, 1995). The beta-carotene in the diet always comes along with other naturally occurring carotenes. It is quite likely that other carotenoids in the diet are equally or more important than beta-carotene alone (Kohlmeier & Hastings, 1995). On the other hand, supplements of beta-carotene may actually promote deficiencies of the other natural carotenes and overall that may hurt more than it helps (White *et al*., 1994).

The results showed that vitamin C, a water soluble antioxidant, was the only one to show a positive direct and indirect relationship with the activity of enzymatic antioxidant (GPx), and lipid peroxide product (MDA) respectively. This is supported by the study of Block *et al.* on different factors associated with oxidative stress; he found that plasma ascorbic acid level was the only factor that had a significant inverse association with MDA (Block *et al*., 2002). Sood *et al*. has suggested that the decline in the ascorbic acid level in AMI subjects could be due to the increased oxidative stress caused by free radicals

(Sood *et al*., 2007). Along with synergism with vitamin E, ascorbic acid enhances synthesis of NO, leading to improved endothelial function and inhibits the buildup of oxidised LDL in arteries, a major contributing factor to atherosclerosis, and which plays an important role in cardio-protective action (Gale *et al*., 1995; Serdar *et al*., 2006).

Several studies support dietary intervention for lowering the risk of CVD and being a source of vitamin antioxidants (Krauss *et al*., 2000; Kris-Etherton *et al*., 2004). Fruits and vegetables in the diet are known to be rich in nutrient and non-nutrient components which have greater effect on the markers of oxidative damage and increase the antioxidant capacity; hence their intake in the diet of CVD subjects should be improved (Dragsted *et al*., 2004; Manios *et al*., 2005). According to the American heart association (AHA), five to nine servings of fruits and vegetables per day along with low fat dairy products are recommended for these patients (Krauss *et al*., 2000; Kris-Etherton *et al*., 2004). Similarly, consumption of five servings of fruits and vegetables is reported to provide sufficient amounts of antioxidants (Padayatty *et al*., 2003). The dietary recommendations made in our study are in accordance with these studies.

Similar to our study (Serdar *et al*., 2006; Sozmen *et al*., 1998), the activities of SOD and GPx were found to be lower than in the controls and before the start of dietary interventions ($P<0.01$). The SOD ($P<0.0001$) and GPx ($P>0.05$) activities, though increased after intervention, yet remained lower than in the control ($P<0.0001$). This is in disagreement with the findings of Dragsted *et al*. where SOD activity was not significantly affected by dietary interventions, but GPx activity was significantly higher in the fruit and vegetable groups than in the placebo and supplement groups (Dragsted *et a*l., 2004).

The improved status of antioxidants on intervention is confirmed by their direct relationship with total antioxidant capacity (TAC) and negative correlation with oxidant products, MDA. Only vitamin C and GPx have been the antioxidants which showed a significant positive correlation with each other. This indicates the co-operative effect of enzymatic antioxidants with other types in increasing the body defense or offering protection from oxidative cellular damage. Although consumption of vitamin supplements provide health benefits, the evidence currently does not support the routine use of antioxidant vitamin supplements as prevention against CVD (Jha *et al*., 1995; Kris-Etherton *et al*., 2004). High serum antioxidant vitamins are increasingly being associated with reduced risk of CHD and diet is a key factor which modifies blood antioxidant vitamin levels (Bolton-Smith *et al*., 1992). Plant foods are naturally rich in nutrients and non- nutrients which lead to a decrease in oxidative damage and increase the antioxidant capacity offering a protective effect on CVD risk factors (Dragsted *et al*., 2004; Manios *et al*., 2005).

The limitation of our study is that the 48-hour recall method (questionnaire) is susceptible to recall bias, such as identification of food intake and portion sizes. However, collection of dietary data by training the patients in this study decreased this type of error. Patients were questioned as to whether the day of recall was a usual day and therefore food intake recall for these two days reflects the usual intake of subjects.

In conclusion, our findings indicate that nutritional intervention as per recommended dietary intake improves the antioxidant status in CVD patients. However, investigation on non-nutrient antioxidants in plant foods and a study over a longer duration are necessary to ascertain the preventive effect of diet for CVD patients.

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Form 1. Questionnaire of food consumption (Recall: first ☐day / second ☐day)

1-Name: **2-No** ☐☐ **date:**

List of food consumed (Meal) / 24h	Ingredients (g)	Amount (g) net or number
Breakfast		
Snack		
Lunch		
Snack		
Dinner		
Snack (before sleep)		

Interviewer Signature