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Vitamin B_{12} status in kidney transplant recipients: association with dietary intake, body adiposity and immunosuppression

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Abstract

The aim of the present study was to evaluate the prevalence of vitamin B_{12} (B_{12}) deficiency in kidney transplant recipients (KTR) and its possible association with B_{12} dietary intake, body adiposity and immunosuppressive drugs. In this cross-sectional study, we included 225 KTR, aged 47·50 (SD 12·11) years, and 125 (56 %) were men. Serum levels of B_{12} were determined by chemiluminescent microparticle intrinsic factor assay and the cut-off of 200 pg/ml was used to stratify KTR into B_{12}-sufficient or B_{12}-deficient group. B_{12} dietary intake was evaluated by three 24 h dietary recalls and was considered adequate when ≥2·4 μg/d. Body adiposity was estimated after taking anthropometric measures and using the dual-energy X-ray absorptiometry (DXA) method. B_{12} deficiency was seen in 14 % of the individuals. B_{12}-deficient group, compared with the B_{12}-sufficient group, exhibited lower intake of B_{12} (median 2·42 (interquartile range (IQR) 1·41–3·23) *v.* 3·16 (IQR 1·94–4·55) μg/d, $P = 0·04$) and higher values of waist circumference (median 96·0 (IQR 88·0–102·5) *v.* 90·0 (IQR 82·0–100·0) cm, $P = 0·04$). When the analysis included only women, B_{12} deficiency was associated with higher total and central body adiposity measurements obtained with anthropometry (BMI, body adiposity index, waist and neck circumferences) and DXA (total and trunk body fat). Among individuals with adequate intake of B_{12}, the deficiency of this vitamin was more frequently seen in those using mycophenolate mofetil (MMF) (17 %) *v.* azathioprine (2 %), $P = 0·01$. In conclusion, the prevalence of B_{12} deficiency in KTR was estimated as 14 % and was associated with reduced intake of B_{12} as well as higher adiposity, especially in women, and with the use of MMF.

Key words: **Vitamin B_{12}: Kidney transplantation: Dietary intake: Body adiposity: Immunosuppression**

Vitamin B_{12} (B_{12}) is a generic term for all cobalamins biologically active in humans. It is a water-soluble vitamin[1,2], synthesised exclusively by micro-organisms[3] and its main sources are animal products[4]. Gastrointestinal absorption of B_{12} is complex[1] and requires intact function of stomach, pancreas and terminal ileum[2,4]. After absorption, B_{12} is converted into two coenzymes, adenosyl and methylcobalamin[5,6]. Adenosylcobalamin participates in the conversion of methylmalonyl-CoA to succinyl-CoA in mitochondria while methylcobalamin participates in the re-methylation of homocysteine to methionine[7] in the cytoplasm. The recommended intake of B_{12} is 2·4 μg daily[8] for adults and elders, and the British Committee of Hematology Standards suggests that serum levels of $B_{12} < 148$ pmol/l (200 pg/ml) would be sensitive enough to diagnose 97 % of individuals with B_{12} deficiency[9,10].

The main causes of B_{12} deficiency are vegan or vegetarian diet, diet poor in meat and dairy products, total or partial gastrectomy, ileum resection, use of metformin, drugs that block stomach acid and others[11]. B_{12} deficiency is characterised by haematological and neurological effects, and manifestations range from mild to severe, including glossitis, fatigue, macrocytic anaemia and peripheral neuropathy. Furthermore, B_{12} deficiency is strongly related to hyperhomocysteinaemia, a great risk factor for CVD[9,11–13].

An association between decreased serum levels of B_{12} and obesity has been observed in the general population by some

Abbreviations: AZA, azathioprine; B_{12}, vitamin B_{12}; DXA, dual-energy X-ray absorptiometry; KT, kidney transplantation; KTR, kidney transplant recipients; MMA, methylmalonic acid; MMF, mycophenolate mofetil; WC, waist circumference.

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authors[14–21]. However, to date, no study has evaluated the association of serum B_{12} levels with excessive body weight using a 'gold standard' method to evaluate body adiposity such as dual-energy X-ray absorptiometry (DXA).

Kidney transplantation (KT) is the treatment of choice for most end-stage renal disease patients[22]. The goal in the management of kidney transplant recipients (KTR) is to avoid the risk of adverse events and comorbidities that may lead to graft failure or death, in particular obesity, infections, cancer and CVD[23–25]. Diarrhoea is a common finding in KTR and can be related to the use of immunosuppressive drugs[26,27], as the gastrointestinal tract is involved in the metabolism of several of these drugs, and adverse gastrointestinal events, occurring in more than 80 % of patients after KT, are attributed to its use[28]. Among the immunosuppressive drugs, mycophenolate mofetil (MMF) is extensively hydrolysed to mycophenolic acid by esterases in the stomach, small intestine, blood, liver and other tissues. There is evidence that patients using MMF frequently experience diarrhoea (from 15·6 to 32·5 %)[29], which is associated with villous atrophy in the duodenum and erosive inflammation in the ileum[30,31], conditions that could favour B_{12} malabsorption.

Although B_{12} deficiency may favour CVD[13], an important cause of mortality in KTR[32], at the present moment data about the prevalence of B_{12} deficiency in these patients are scarce. We can hypothesise that these patients may be at increased risk of B_{12} deficiency due to the recommendation to decrease the intake of animal protein before KT (during non-dialysis treatment)[33]; the excessive weight gain that is common in KTR[25]; and the use of MMF.

Therefore, the aim of the present study was to evaluate the prevalence of B_{12} deficiency in KTR and its association with B_{12} dietary intake, body adiposity and immunosuppressive regimen based on MMF.

Methods

This cross-sectional study followed the guidelines laid down in the Declaration of Helsinki, and all procedures were approved by the Committee on Ethics and Research of Pedro Ernesto University Hospital (CAAE: 38268714.0.0000.5259). Written informed consent was obtained from all participants.

We enrolled, prospectively, 368 unselected adults (18–70 years) with functional kidney grafts >180 d after transplantation. Exclusion criteria were actual or former use of B_{12} supplements, AIDS, cancer, autoimmune diseases, acute illness, amputation, liver failure, mental disorders and pregnant or lactating women.

Individuals who met the eligibility criteria and agreed to take part in the study were submitted to clinical, laboratory and nutritional evaluations. Data collected in the medical record included date of KT, type of graft donor and current use of immunosuppressive drugs. Blood sampling as well as the anthropometric measurements were taken from 07.00 to 09.00 hours after 12 h fasting and were performed within the period of 30 d in which dietary intake was evaluated. KTR were asked about lifestyle habits and the occurrence of diarrhoea on appropriate interviews. Patients who smoked at least one cigarette daily or those that stopped smoking within the previous 6 months were considered smokers. KTR who reported consumption of alcoholic beverages one or more times in the week were considered alcohol consumers. The habitual physical activity was evaluated by the Baecke questionnaire which assesses the physical activity in three subscales: at work, sports during leisure time and other physical activities during leisure time[34,35].

Dietary intake

B_{12} dietary intake was assessed by three interviewer-administered 24 h dietary recalls (two weekdays and one weekend day). The first two 24 h recalls were obtained face-to-face and the last one through a telephone call. The 24 h recalls interval between the first and the last was 30 d.

The 24 h recalls were obtained by two dietitians trained to ask the patients to enumerate all the information about the food and drink they had consumed from midnight to midnight in the previous day, including the quantity.

Nutrient analysis of the 24 h recalls was performed using the software Food Processor Plus® (ESHA Research) and two Brazilian Food Composition Tables[36,37]. An average of the three recalls was used in the analysis. Adequate intake of B_{12} was considered when ≥2·4 μg/d[8].

Laboratory parameters

The serum level of B_{12} was determined by chemiluminescent microparticle intrinsic factor assay using a commercial kit (Abbott) at the Laboratory of Nuclear Medicine at Pedro Ernesto University Hospital. This assay is designed to have a total CV ≤ 11 % for concentrations in the range of the low, medium and high controls.

Blood samples were also analysed to measure creatinine, urea, Hb, haematocrit, mean corpuscular volume, mean corpuscular Hb and mean corpuscular Hb concentration. These analyses were performed at the Pedro Ernesto University Hospital's central laboratory. Serum urea and creatinine were determined by kinetic method. Creatinine was calibrated to IDMS: COBAS 6000 (Roche/Hitachi). The estimated glomerular filtration rate was estimated using the Chronic Kidney Disease Epidemiology Collaboration equation[38].

Anthropometric measurements

The anthropometric measurements were taken twice by two experienced dietitians, and mean values were used. The height was measured using a stadiometer, accurate to ±0·5 cm and the weight was obtained with a digital scale, accurate to ±0·1 kg (Filizola S.A.), with the patients wearing light clothing with no shoes and after emptying the bladder. The measure of the waist circumference (WC) was taken in the standing position midway between the lowest rib and the iliac crest, at mid-exhalation while the hip circumference was measured at the widest point over the hip/buttocks area with the tape parallel to the floor[39]. The neck circumference was measured in the midway of the neck between mid-cervical spine

Table 1. Demographic and clinical characteristics and laboratory parameters of kidney transplant recipients according to vitamin B_{12} status (Absolute values and percentages; medians and interquartile ranges (IQR); mean values and standard deviations)

	Vitamin B_{12}-sufficient group (*n* 194)		Vitamin B_{12}-deficient group (*n* 31)		
Characteristics	*n*	%	*n*	%	*P**
Sex					0·49
Men	106	55	19	61	
Women	88	45	12	39	
Age (years)					0·44
Median	49·0		50·0		
IQR	41·0–56·0		44·0–58·0		
Type of graft donor: deceased	92	47	19	61	0·15
Time of transplantation (months)					0·97
Median	114·0		61·0		
IQR	27·0–167·0		26·0–182·0		
Alcohol consumers	15	8	3	10	0·72
Smoking habits	4	2	0	0	0·42
Laboratory parameters					
Estimated glomerular filtration rate (ml/min per 1·73 m^2)					0·22
Mean	52·86		47·94		
SD	21·52		14·59		
Hb (g/dl)†					0·18
Median	12·7		12·5		
IQR	11·7–14·1		10·3–13·6		
Haematocrit (%)					0·16
Median	38·65		37·5		
IQR	35·3–42·4		31·7–40·8		
Mean corpuscular volume (fl)					0·02
Mean	86·36		90·30		
SD	7·48		7·78		
Mean corpuscular Hb (pg)					0·03
Mean	28·36		29·67		
SD	2·77		2·63		
Mean corpuscular Hb concentration (g/dl)†					0·85
Mean	32·82		32·87		
SD	1·08		1·05		
Physical activity					
Work index					0·50
Mean	2·80		2·89		
SD	0·60		0·56		
Sport index					0·54
Median	2·25		2·0		
IQR	1·75–2·75		1·75–2·50		
Leisure time index					0·78
Mean	2·65		2·60		
SD	0·68		0·59		
Total Baecke score					0·80
Mean	7·73		7·66		
SD	1·34		1·26		
Co-morbidities					
Hypertension	157	81	26	84	0·70
Diabetes	39	20	8	26	0·47
Dyslipidaemia	153	79	24	77	0·87
Diarrhoea	25	13	6	23	0·17
Immunosuppressive regimens					
Mycophenolate based	134	69	25	80	0·23
Azathioprine based	52	27	5	17	0·24
Other drugs					
Antihypertensive drugs	157	81	26	84	0·70
Lipid-lowering drugs	83	43	14	45	0·79
Oral antidiabetic drugs	23	12	4	13	0·84
Metformin	21	11	4	13	0·77
Insulin	12	6	3	10	0·47
Proton pump inhibitors	35	18	7	23	0·50

* Vitamin B_{12}-sufficient group *v.* vitamin B_{12}-deficient group (Student's *t* test or Mann–Whitney test).
† To convert Hb and mean corpuscular Hb concentration in g/dl to g/l, multiply by 10.

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Table 2. Dietary intake according to vitamin B_{12} status in kidney transplant recipients (Medians and interquartile ranges (IQR))

	Vitamin B_{12}-sufficient group (*n* 194)		Vitamin B_{12}-deficient group (*n* 31)		
Variables	Median	IQR	Median	IQR	*P**
Energy (kcal/d)†	1768·0	1398·3–2179·0	1764·7	1459·8–2230·3	0·81
Energy (kcal/kg per d)†	25·2	19·4–33·4	23·1	19·8–33·7	0·79
Protein (g/d)	89·53	68·61–105·0	81·6	57·0–106·3	0·66
Protein (g/kg per d)	1·28	0·90–1·65	1·06	0·80–1·41	0·33
Carbohydrates (g/d)	245·8	188·6–318·2	246·9	197·1–306·4	0·97
Lipids (g/d)	46·3	35·6–61·2	55·1	44·2–75·4	0·25
Fibre (g/d)	22·2	16·0–30·3	22·2	15·6–31·3	0·93
Fe (mg/d)	13·1	9·4–16·8	13·0	11·9–14·3	0·80
Vitamin B_1 (mg/d)	1·50	1·09–1·84	1·60	1·09–1·95	0·63
Vitamin B_2 (mg/d)	1·55	1·13–1·96	1·60	1·10–2·25	0·62
Vitamin B_3 (mg/d)	20·4	14·1–28·6	18·5	12·4–23·1	0·37
Vitamin B_6 (mg/d)	1·54	1·14–1·91	1·49	1·23–2·00	0·74
Biotin (μg/d)	4·94	2·92–8·30	4·05	2·79–6·69	0·53
Vitamin B_{12} (μg/d)	3·16	1·94–4·55	2·42	1·41–3·23	0·04
Vitamin C (mg/d)	51·9	24·1–104·4	33·0	20·4–67·2	0·10
Vitamin K (μg/d)	21·2	11·4–39·7	21·6	8·1–76·2	0·91
Vitamin D (μg/d)	1·51	0·68–3·55	2·02	0·99–3·86	0·62
Vitamin E (mg αTE/d)	1·67	0·98–2·26	1·23	0·92–1·81	0·40
Folate (DFE/d)	475·2	326·2–670·3	511·7	377·4–601·9	0·94

αTE, α-tocopherol equivalents; DFE, dietary folate equivalents.
* Vitamin B_{12}-sufficient group *v.* vitamin B_{12}-deficient group (Student's *t* test or Mann–Whitney test).
† To convert energy in kcal to kJ, multiply by 4·184.

and mid-anterior neck, if palpable, just below the laryngeal prominence[40].

BMI was calculated using the standard equation (kg/m^2)[41]. Body adiposity index estimates body fat (%) using two anthropometric measurements (hip circumference and height) and was determined as described by Bergman *et al.*[42].

Dual-energy X-ray absorptiometry

The DXA procedure was performed by a trained technician using a GE Medical SystemsLunar® (Madison) with the patient in the supine position. The DXA system performs rectilinear scans over the length of the body. The scan begins at the top of the patient's head and moves downward towards the feet. The program allows scanning up to 205 lines. During the scan, the source shutter opens to emit an X-ray beam. The software calculates fat mass, lean tissue and bone mineral mass. Fat-free mass is calculated as the sum of lean tissue plus bone mineral mass. Body composition was evaluated in total body and different sites, such as trunk. Visceral adipose tissue was estimated with the software CoreScan VAT [43].

Immunosuppressive treatments

At the renal transplant outpatient clinic at Pedro Ernesto University Hospital, the most frequently used immunosuppressive regimens for long-term follow-up include a calcineurin inhibitor (cyclosporine or tacrolimus) or a mammalian target of rapamycin inhibitor (everolimus or sirolimus) in addition to an antimetabolite (MMF or azathioprine: AZA). All treatment regimens are administered in combination with steroids (prednisone 5 mg/d).

Statistical analysis

Sample size was determined based on a pilot study conducted by our group in which B_{12} deficiency was registered in 20 % of KTR[44]. Then, considering that the number of KT outpatients in our service is 450, and a 95 % CI, the minimum sample size should be 160 patients.

A standard statistical package (STATA software, version 12.0; StataCorp) was used to perform statistics analysis. Normality was tested by the Shapiro–Wilk normality test. Continuous variables with normal distribution were expressed as mean values and standard deviations. Medians and interquartile ranges (IQR) were used to summarise variables with non-normal distribution. The individuals were stratified into two groups according to the levels of B_{12}. KTR with values <200 pg/ml were allocated to the B_{12}-deficient group and those with values ≥200 pg/ml to the B_{12}-sufficient group[1]. The two groups were compared with the use of Student's *t* test, Mann–Whitney test or χ^2 exact test, as appropriate. The multiple logistic regression analysis was performed to assess the association of B_{12} deficiency with body adiposity. The accepted level of statistical significance was 5 %.

Results

A total of 368 KTR were interviewed, of which 227 met the eligibility criteria and agreed to participate in the study. Of these, 225 completed all evaluations and were included in statistical analyses. Mean age was 47·50 (SD 12·11) years (range 18–70 years), and 125 (56 %) were men. Mean transplant duration was 110·24 (SD 89·83) months (range 6–331 months), and mean B_{12} levels were 362·57 (SD 169·25) pg/ml (range 83–1042 pg/ml). The prevalence of B_{12} deficiency was 14 %.

Table 3. Parameters of body adiposity according to vitamin B_{12} status in kidney transplant recipients (Medians and interquartile ranges (IQR); mean values and standard deviations)

	Vitamin B_{12}-sufficient group (*n* 194)		Vitamin B_{12}-deficient group (*n* 31)		
Parameter	Median	IQR	Median	IQR	*P**
Anthropometry					
BMI (kg/m^2)	25·5	22·8–29·2	26·0	23·9–30·1	0·23
Men	25·3	25·8–29·2	24·1	23·5–26·2	0·77
Women	25·6	22·7–29·1	29·6	26·3–33·2	0·01
Body adiposity index (%)	29·3	25·7–33·6	29·2	26·0–36·4	0·48
Men	26·1	24·4–29·1	26·5	24·9–29·9	0·62
Women	33·6	30·1–36·9	39·1	35·9–41·0	0·01
Waist circumference (cm)	90·0	82·0–100·0	96·0	88·0–102·5	0·04
Men	91·5	84·0–103·0	91·0	84·2–98·0	0·57
Women	86·0	80·0–97·7	102·8	100·0–114·0	0·0003
Neck circumference (cm)					0·26
Mean	36·82		37·59		
SD	3·47		2·80		
Men					0·64
Mean	38·74		38·41		
SD	2·85		3·00		
Women					0·04
Mean	34·36		36·04		
SD	2·51		1·50		
Dual-energy X-ray absorptiometry					
Total body fat (%)					0·27
Mean	33·87		36·46		
SD	9·50		11·71		
Men					0·96
Mean	28·94		28·83		
SD	7·80		7·25		
Women					0·01
Mean	40·90		47·89		
SD	6·98		6·40		
Total body fat (kg)	21·7	16·4–29·3	23·6	17·3–30·7	0·43
Men	20·4	15·6–26·1	17·8	14·9–22·9	0·46
Women	24·8	19·0–31·0	32·6	29·4–46·8	0·01
Trunk body fat (%)					0·22
Mean	36·83		40·23		
SD	11·23		12·71		
Men					0·10
Mean	32·83		32·84		
SD	10·65		9·67		
Women					
Mean	42·52		51·3		0·02
SD	9·51		7·59		
Trunk body fat (kg)	12·2	8·6–16·6	14·4	9·2–18·4	0·23
Men	11·7	8·6–16·1	9·68	8·56–14·36	0·63
Women	12·7	8·5–17·1	19·7	15·9–25·2	0·01
Visceral fat (kg)	0·95	0·52–1·66	1·12	0·70–1·99	0·32
Men	1·21	0·62–1·86	0·99	0·63–2·08	0·93
Women	0·71	0·34–1·30	1·27	0·72–1·72	0·08

* Vitamin B_{12}-sufficient group *v.* vitamin B_{12}-deficient group (Student's *t* test or Mann–Whitney test).

The characteristics of the participants are shown in Table 1 according to B_{12} status. Mean corpuscular volume and mean corpuscular Hb were significantly higher among patients with B_{12} deficiency. The frequency of diarrhoea and MMF use was higher (without reaching statistical significance) in B_{12}-deficient when compared with the B_{12}-sufficient KTR (Table 1).

The intake of B_{12} was significantly lower in participants with B_{12} deficiency (Table 2). B_{12} intake was considered adequate in 143 KTR (64 %) and sixteen patients out of them exhibited B_{12} deficiency.

Considering participants of both sexes, individuals who had B_{12} deficiency exhibited higher values of WC. In the analysis conducted only in women, it was verified that those with B_{12} deficiency exhibited values of BMI, body adiposity index, WC, total body fat (% and kg) and trunk body fat (% and kg) significantly higher than women with B_{12} sufficiency (Table 3) even after adjustment for age, estimated glomerular filtration rate, time from transplantation, type of graft donor and physical activity (Table 4).

The frequency of B_{12} deficiency was not significantly higher in KTR using MMF (16 %) *v.* AZA (9 %) ($P = 0·25$) in the analysis considering all the participants included in the present study. However, in the analysis restricted to individuals who had adequate intake of B_{12} there was a significantly higher frequency

Table 4. Risk for vitamin B_{12} deficiency according to parameters of body adiposity in kidney transplant recipients (Odds ratios and 95 % confidence intervals)

	OR	95 % CI	*P*	OR*	95 % CI	*P*
Anthropometry						
BMI (kg/m^2)	1·04	0·97, 1·12	0·28	1·04	0·94, 1·15	0·46
Men	0·97	0·86, 1·09	0·57	0·94	0·81, 1·09	0·39
Women	1·13	1·01, 1·25	0·03	1·22	1·01, 1·48	0·04
Body adiposity index (%)	1·04	0·98, 1·11	0·21	1·02	0·94, 1·11	0·58
Men	1·02	0·90, 1·16	0·71	1·01	0·86, 1·17	0·94
Women	1·18	1·05, 1·33	0·007	1·20	1·01, 1·43	0·04
Waist circumference (cm)	1·02	0·99, 1·05	0·09	1·01	0·97, 1·05	0·71
Men	0·99	0·95, 1·03	0·49	0·96	0·91, 1·01	0·15
Women	1·06	1·02, 1·11	0·005	1·08	1·01, 1·16	0·04
Neck circumference (cm)	1·07	0·95, 1·20	0·26	1·04	0·90, 1·19	0·63
Men	0·96	0·81, 1·14	0·64	0·93	0·75, 1·16	0·53
Women	1·31	1·00, 1·72	0·05	1·12	0·80, 1·56	0·52
Dual-energy X-ray absorptiometry						
Total body fat (%)	1·03	0·98, 1·08	0·27	1·02	0·97, 1·09	0·42
Men	1·00	0·92, 1·08	0·96	1·00	0·91, 1·10	0·99
Women	1·18	1·03, 1·36	0·02	1·20	1·01, 1·43	0·04
Total body fat (kg)	1·03	0·98, 1·07	0·23	1·03	0·98, 1·09	0·28
Men	0·97	0·90, 1·05	0·45	0·97	0·89, 1·06	0·49
Women	1·08	1·01, 1·14	0·02	1·13	1·02, 1·26	0·02
Trunk body fat (%)	1·03	0·98, 1·07	0·22	1·02	0·97, 1·07	0·39
Men	1·00	0·94, 1·06	1·00	1·00	0·93, 1·07	0·91
Women	1·13	1·02, 1·25	0·03	1·13	1·02, 1·26	0·03
Trunk body fat (kg)	1·04	0·97, 1·12	0·24	1·04	0·95, 1·13	0·43
Men	0·97	0·87, 1·08	0·54	0·96	0·84, 1·09	0·49
Women	1·13	1·02, 1·26	0·02	1·17	1·01, 1·36	0·04
Visceral fat (Kg)	1·17	0·67, 2·03	0·56	0·98	0·50, 1·94	0·96
Men	0·91	0·45, 1·85	0·79	0·71	0·28, 1·77	0·46
Women	2·11	0·76, 5·88	0·15	2·08	0·53, 8·17	0·29

* Adjusted for age, estimated glomerular filtration rate, time from transplantation, type of graft donor and total Baecke score.

of B_{12} deficiency in patients using MMF (17 %) *v.* AZA (2 %) ($P = 0·01$).

Discussion

To our knowledge, three studies have described the occurrence of B_{12} deficiency in KTR[45–47]. Födinger *et al.*[45] reported B_{12} deficiency in 8·9 % of 733 Austrians KTR using <160 pg/ml as the cut-off to determine the vitamin deficiency. Karakus *et al.*[46] evaluating ninety KTR, who developed anaemia, found B_{12} deficiency in 40 % of the individuals with macrocytic and in 8·3 % of those with normocytic anaemia. Scott *et al.*[47] registered less than 1 % of both B_{12} and folic acid deficiency in 584 North American and Canadian KTR using the cut-off <200 pg/ml. In the present study, including KTR with a minimum of 6 months of transplantation, B_{12} deficiency prevalence (cut-off < 200 pg/ml) was 14 %.

Some authors have evaluated the dietary intake in KTR[48–50], but only one study evaluated the intake of B_{12}[48], that was considered adequate in 94 % of the patients. In the present study, we found that a lower percentage of the participants (64 %) presented adequate B_{12} intake. Furthermore, the intake of protein presented a tendency to be lower in the B_{12}-deficient group (Table 2) and was significantly lower in patients with inadequate *v.* adequate intake of B_{12} (median 71·5 (IQR 52·1–91·8) *v.* 94·4 (IQR 81·6–115·0) g/d, respectively, $P < 0·0001$) (data not shown). These findings suggest that B_{12} dietary intake needs to be carefully monitored in KTR, especially in those with lower protein intake.

We checked the within-subject consistency of dietary intake evaluated through the three 24 h recalls (data not shown). The dietary intake was similar for the same participant when we considered the two weekday recalls. However, as expected, the dietary intake evaluated through these two recalls differed from that assessed through the weekend day recall. The recalls obtained by telephone call compared with those obtained by face-to-face interviews did not present systematic difference. We believe that the use of trained interviewers contributed to the lack of difference. As described in the literature[51,52], the dietary intake estimated through 24 h recalls collected by phone is comparable to the dietary intake that is collected face-to-face.

Although some studies have described the association between increased body adiposity and B_{12} deficiency in the general population[14–19,21], to our knowledge, the present study is the first to register this association in KTR. We observed that B_{12} deficiency was associated with increased central body adiposity (evaluated by WC) in the analysis including the whole cohort of KTR and with increased total and central body adiposity in the analysis including only women (considering both the anthropometric measures and DXA).

The mechanism by which individuals with excessive adiposity may exhibit decreased levels of B_{12} has not been completely elucidated. Guéant & Alpers[5] and Garcia *et al.*[53] using an

animal model demonstrated that the deficiency of methyl radical and other cofactors, such as B_{12}, impairs the oxidation of the fatty acids, increases the stress on the endoplasmic reticulum and decreases the expression of sirtuin 1, a protein which plays a key role in molecular mechanisms of obesity. Li *et al.*[20] proposed that the impaired conversion of methylmalonic acid (MMA) to succinyl-CoA, a B_{12}-dependent process, may be associated with MMA accumulation, which could lead to an increase in lipogenesis, in addition to insulin resistance. In a study that included both *in vivo* and *in vitro* observations, Adaikalakoteswari *et al.*[18] suggested that the B_{12} plays a role in epigenetic regulation by altering circulating microRNA during adipocyte differentiation that results in adipogenesis and adverse metabolic phenotype.

Since in the present study, a percentage of the individuals with adequate B_{12} intake presented B_{12} deficiency, we tested if immunosuppressive regimen including MMF was associated with the deficiency of B_{12} in these participants. We observed that in KTR with adequate B_{12} intake, the frequency of B_{12} deficiency was higher in patients using MMF than in those using AZA. This finding suggests that KTR using MMF may be at increased risk of B_{12} deficiency even if they present adequate B_{12} intake. However, in our opinion, additional studies are necessary to confirm this finding.

The evaluation of B_{12} status might include not only serum B_{12} but also markers of cellular B_{12}, such as homocysteine and MMA[54]. However, in individuals with renal dysfunction, these markers may be falsely increased, while the evaluation of serum B_{12} is not altered[54–57]. Considering that in the present study 68 % of the participants presented an estimated glomerular filtration rate <60 ml/min, we used solely serum B_{12} to evaluate B_{12} status.

The strengths of the present study include the evaluation of body adiposity with a 'gold standard' method (DXA). This is the first study to evaluate the prevalence of B_{12} deficiency in KTR and to observe an association between B_{12} deficiency with body adiposity and the use of MMF. As limitation, it is worth mentioning that the present study was conducted in only one centre and has a cross-sectional design not allowing to infer cause-and-effect relationships.

Conclusion

In conclusion, the prevalence of B_{12} deficiency in KTR was estimated as 14 % and was associated with reduced dietary intake of B_{12} as well as with higher adiposity, especially in women, and with the use of MMF in individuals with adequate B_{12} intake.

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