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ORIGINAL ARTICLE

Biochemical and haematological differentiation of opiate addicts from healthy subjects. A case control study

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Summary
The biochemical and haematological parameters of nutritional interest were determined in the serum of opiate addicts in order to compare them with those obtained in healthy subjects. The blood of 106 opiate addicts in detoxification treatment (n=19) or in Methadone Maintenance Treatment Program (MMTP) (n=87) was studied. Opiate addicts presented lower levels in the number of red cells, cholesterol, albumin, retinol, α-tocopherol, folic acid, K and Se and higher levels in the number of leukocytes, GOT, GPT and Na, than the control group. The opiate addicts in MMTP had higher levels of glucose, triglycerides, Mg and P than the opiate addicts in the detoxification treatment and control groups. Significant correlations between the three vitamins (folic acid, retinal and α-tocopherol) were observed and the graphic representations suggest a biochemical differentiation between opiate addicts and healthy subjects. Factor analysis made it possible to select seven factors explaining 66.2% of the total variance, and representing the first and fourth factor, opiate addicts tended to separate from the control group.

Keywords: opiate addicts – correlation and factor analysis – detoxification – methadone

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INTRODUCTION

Evaluation of nutritional status is a complex question. Several types of parameters are often determined: food intake, clinical, anthropometric, biochemical and haematological parameters (Grant and DeHoog 1985). Haematological and biochemical classical parameters and vitamins and

mineral status are influenced by food intake. Moreover, the determination of these parameters provides important information relative to the nutritional status of the individual.

For most alcohol or drug abusers, money is often used to buy alcohol and drugs instead of food. Alcohol or drugs can cause poor appetite, loss of taste and apathy regarding diet and nutrition. Opiate addicts, and especially those with heavier consumption, develop anorexia which is determined by poor consumption of food and drink leading to malnutrition (Santolaria-Fernández et al. 1995). Morabia et al. (1989) have deduced that opiate addicts replace foods that are rich in fat and animal proteins with those that are rich in carbohydrates, especially sucrose and alcohol, which provide the so-called "empty" calories. In addition, frequent social and domestic problems as well as associated pathologies such as AIDS or several types of hepatitis may affect their nutritional status. Therefore, this section of the population is usually at nutritional risk and, corrective nutritional programs must be seriously considered as an integral part of the total services provided for the treatment of opiate addicts.

Methadone substitution treatment is regarded as an effective treatment for heroin addicts (Farrel et al. 1994) and is widely used in many countries. According to some authors (Gambera and Clarke 1976, Kabrtt et al. 1999), the food habits and life-style of the patients enrolled in Methadone Maintenance Treatment Programs (MMTP) largely improved with this treatment. Most opiate addicts in detoxification are said to consume only one meal (43%) or two meals (43%) per day, as opposed to the individuals included in MMTP, of the which a third indicated a consumption of three or more meals per day (Gambera and Clarke 1976). So, the nutrient intake of the opiate addicts in MMTP was nearer to an adequate intake than the corresponding intake in the heroin patients in detoxification (Gambera and Clarke 1976). However, many of these patients continue to use drugs and consume alcohol in excess. So, 61.5% of the population studied manifested a high consumption of alcoholic drinks, and 53.1% of them indicated a daily drug consumption (Verde Méndez et al. 2002).

In another paper (Díaz-Flores Estévez et al. 2003) we determined the levels of biochemical and haematologic classical parameters and some vitamins and minerals of nutritional importance in the blood samples of opiate addicts. Then, we applied the linear discriminant analysis to the matrix of data formed with all these parameters, and it was deduced that the opiate addicts were clearly differentiated from healthy subjects. Also, a tendency to differentiate the opiate addicts in MMTP from those in detoxification treatment was observed. In the present paper, we describe and compare the mean results obtained (ANOVA) between the three groups of individuals considered: opiate addicts in MMTP, opiate addicts in detoxification and the control group. In addition, a correlation study and factor analysis were applied to differentiate the opiate addicts included in MMTP or in detoxification treatment, and individuals belonging to the control group.

SUBJECTS AND METHODS

Subject selection

From January to June 2000, the blood of opiate addicts undergoing treatment in the Youth Association Cooperation "San Miguel" of Santa Cruz de Tenerife was collected. One hundred and six opiate addicts, 86 males and 20 females, were studied, and they had the following characteristics: age 33.0±6.7 (19–53) years old; weight 70.6±15.5 (47–100) kg; height 172.1±9.1 (147–194) cm; and body mass index 23.4±3.8 (16.8–44.1) kg/m^2. These subjects were divided into two groups: 1) Current opiate addicts admitted for detoxification treatment (n=19, 15 males and 4 females). The objective of this treatment is to eliminate consumption and dependence. These individuals, especially those with heavier consumption patterns, develop anorexia which is determined by poor consumption of food and drink leading to malnutrition. The main criterion for inclusion in MMTP is state of health; and 2) Opiate addicts included in MMTP (opiate addicts) (n=87, 71 males and 16 females). The consumption of drugs in opiate addicts included in MMTP was exhaustively controlled. So, screening analysis of drugs in urine was carried out weekly on all the subjects. A positive result of heroin or other opiate was a suffucient reason for elimination of the subject from the MMTP.

The control group was formed of 186 apparently healthy individuals (72 males and 114 females), which were included in the Nutrition Survey of the Canary Islands (Henríquez Sánchez et al. 2000). They were selected within the age interval of the opiate addicts and presented the following characteristics: age 37.1±9.0 (19–53) years old; weight 70.0±13.7 (42–109) kg; height 165.8±10.1 (135–192) cm; body mass index 25.5±4.2 (16.8–44.1) kg/m^2 respectively.

Collection and storage of samples

The blood samples were collected by venepuncture in the morning (between 8.00 and 9.30 h) from the fasting subjects. Twenty ml of whole blood was extracted and divided in two parts. EDTA was added to one part as anticoagulant, and, this part was then used for the determination of

haematological parameters. The other fraction was left to spontaneous coagulate, and then, the blood samples were centrifuged at 300 x g for 10 min. Hemolyzed samples were excluded. The clean serum was used for the determination of the classical biochemical parameters, and an aliquot of serum was frozen (–40ºC) for storage and transportation to the laboratories for the determination of vitamins and minerals.

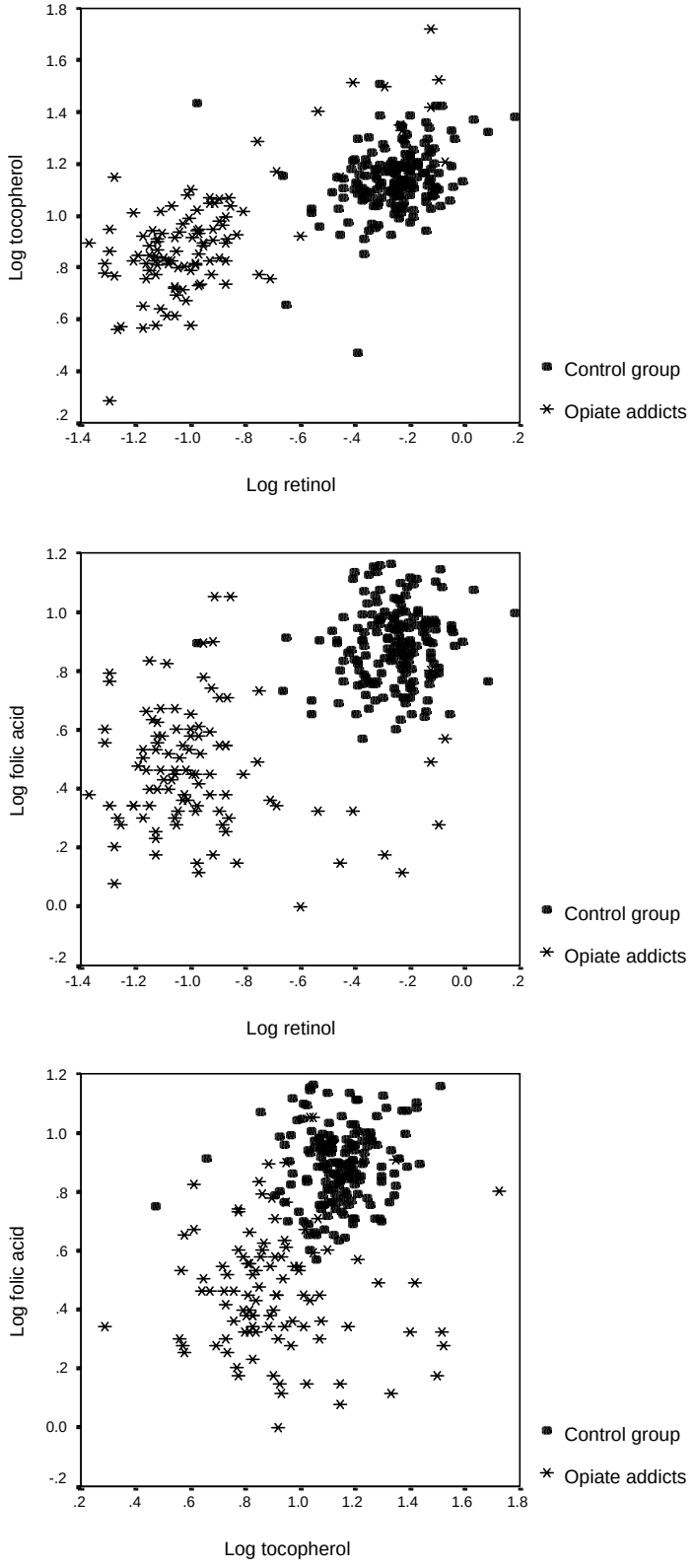

Fig. 1. **Plot of correlation between vitamins differentiating opiate addicts and control group**

Determination of biochemical and haematological parameters

The parameters analysed were divided into four groups, which are described below, together with the methods used for their determination.

Haematological parameters

The haemoglobin concentration, haematocrit, number of red cells, leukocytes and platelets were obtained using an interpretative and differential Haematologic Analyzer in five parts System 9000+ (Serono Baker Diagnostics, Inc).

Classical biochemical parameters

The parameters included within this group were the following: glucose, cholesterol, triglycerides, uric acid, GOT, GPT and albumin, and were determined with a autoanalizer BM/Hitachi 911 (enzymatic test in vitro). The methods for the determination of these parameters are commonly used in the Laboratory of the Youth Association Cooperation "San Miguel" of Santa Cruz de Tenerife and they were optimized and periodically submitted to internal and external quality control to maintain the quality of the analytical results.

Table 1. **Comparison of mean values in opiate addicts and control group**

Parameters	Control group	MMTP	Detoxification treatment
1) Haematological parameters			
Haemoglobin (g/dl)	15.1±1.2 (m) 12.9±1.1 (f)	15.0±1.3 13.3±1.3	15.2±1.0 13.5±1.5
Haematocrit (%)	45.7±3.2 (m) 39.8±3.0 (f)	44.6 ± 3.3^{c} 38.8±3.8	46.5 ± 3.0^{b} 41.3±4.9
Number of red cell ($x10^6/mm^3$)	5.10 ± 0.39 (m)^{b} 4.51 ± 0.32 (f)^{b}	4.88 ± 0.38^{a} 4.16 ± 0.48^{a}	4.91±0.48 4.47±0.43
Leukocytes ($x10^3/mm^3$)	$6.60\pm1.93^{b,c}$	7.52 ± 2.04^{a}	8.29 ± 2.43^{a}
Platelets ($x10^3/mm^3$)	244±59	221±78	250 ± 64^{a}
2) Biochemical parameters			
Cholesterol (mg/dl)	209 ± 45^{c}	196 ± 42^{c}	163 ± 43^{a}
Triglycerides (mg/dl)	116 ± 65^{b}	$152\pm103^{a,c}$	113 ± 48^{b}
GOT (U/l)	$24.7\pm8.5^{b,c}$	50.9 ± 57.2^{a}	54.5 ± 51.0^{a}
GPT (U/l)	$20.6\pm13.4^{b,c}$	69.9 ± 88.9^{a}	76.5 ± 75.3^{a}
Uric acid (mg/dl)	5.44±1.33 (m) 4.17±1.00 (f)	5.37±1.25 4.09±1.04	4.91±1.19 4.38±1.51
Glucose (mg/dl)	83.5±21.0	91.8±16.5	85.6±13.5
Albumin (g/dl)	$4.65\pm0.60^{b,c}$	$4.30\pm0.31^{a,c}$	$4.06\pm1.30^{a,b}$
3) Vitamins			
Retinol (mg/l)	$0.58\pm0.17^{b,c}$	0.15 ± 0.16^{a}	0.13 ± 0.18^{a}
α-tocopherol (mg/l)	$14.4\pm4.2^{b,c}$	9.7 ± 7.9^{a}	8.7 ± 3.1^{a}
Folic acid (µg/l)	$8.13\pm2.37^{b,c}$	3.23 ± 1.76^{a}	4.02 ± 2.39^{a}
B_{12} (ng/l)	523±295	582±196	542±170
4) Minerals			
Na (mmol/l)	$146\pm10^{b,c}$	156 ± 12^{a}	157 ± 17^{a}
K (mmol/l)	$5.36\pm0.67^{b,c}$	$4.87\pm0.51^{a,c}$	$4.51\pm0.56^{a,b}$
Ca (mg/l)	95.1±8.3	96.1±5.2	94.8±4.4
Mg (mg/l)	25.3 ± 4.3^{b}	$28.7\pm3.3^{a,c}$	25.9 ± 3.9^{b}
P (mg/l)	35.6 ± 5.9^{b}	38.9 ± 9.8^{a}	35.9±7.9
Fe (µg/dl)	111±38 (m) 85.2±39.1 (f)	103±42 74.2±46.2	93.3±47.2 87.0±54.1
Cu (mg/l)	1.00 ± 0.15 (m)^{b,c} 1.20±0.31 (f)	1.09 ± 0.20^{a} 1.12±0.19	1.09 ± 0.24^{a} 1.21±0.43
Zn (mg/l)	1.17±0.56	1.09±0.93	0.93±0.64
Se (µg/l)	$76.1\pm25.5^{b,c}$	59.1 ± 19.3^{a}	59.9 ± 18.6^{a}

m=males; f=females

a=significant as compared with the control group; b=significant as compared with individual in MMTP; c=significant as compared with individuals in detoxification treatment

Table 2. **Matrix correlation (coefficient correlation of Pearson) of the studied parameters for all the individuals**

	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1. Log haemoglobin	**0.874**	**0.698**	**0.237**	**-0.240**	-0.050	**0.147**	**0.211**	**0.391**	**0.387**	**0.142**	0.063	**-0.182**	-0.074	**-0.174**	**0.215**	**0.204**	0.039	**0.162**	**0.198**	-0.023	**0.371**	**-0.218**	-0.003	0.058
2. Log haematocrit		**0.821**	**0.162**	**-0.171**	-0.032	0.102	**0.225**	**0.390**	**0.362**	**0.128**	0.105	**-0.132**	-0.067	-0.097	**0.174**	**0.138**	0.031	0.115	**0.211**	-0.064	**0.385**	**-0.208**	0.041	0.088
3. Log red cell			0.112	**-0.137**	0.000	0.074	0.115	**0.247**	**0.347**	0.086	**0.183**	-0.017	-0.009	0.031	**0.121**	0.045	0.069	**0.123**	**0.139**	-0.062	**0.187**	**-0.174**	0.067	0.100
4. Log leukocytes				**0.170**	0.063	**0.221**	-0.013	0.063	**0.122**	0.041	-0.103	**-0.165**	0.009	**-0.177**	**0.131**	0.100	0.053	-0.021	**0.213**	0.071	-0.034	**0.128**	-0.069	-0.030
5. Log platelets					0.071	-0.045	**-0.188**	**-0.255**	-0.042	**-0.161**	0.041	**0.172**	**0.220**	**0.155**	-0.063	**-0.149**	**0.119**	-0.035	-0.091	0.057	-0.079	0.066	0.105	-0.030
6. Log cholesterol						**0.370**	-0.056	-0.093	0.097	**0.191**	**0.398**	**0.280**	**0.389**	**0.118**	-0.016	-0.074	**0.143**	**0.306**	0.051	0.067	**0.116**	**0.147**	0.027	**0.205**
7. Log triglycerides							0.088	**0.175**	**0.365**	**0.157**	0.100	-0.118	0.012	**-0.234**	-0.052	**0.168**	-0.047	**0.197**	0.090	**0.167**	0.056	0.001	0.047	-0.008
8. Log GOT								**0.768**	**0.250**	**0.165**	0.016	**-0.314**	**-0.315**	**-0.196**	**0.267**	**0.189**	**-0.189**	**0.175**	**0.148**	0.044	**0.282**	-0.090	-0.060	**-0.120**
9. Log GPT									**0.339**	**0.215**	-0.093	**-0.456**	**-0.317**	**-0.301**	**0.273**	**0.281**	**-0.171**	0.107	**0.178**	0.014	**0.181**	**-0.172**	**-0.126**	**-0.156**
10. Log uric acid										**0.184**	**0.239**	-0.117	0.023	-0.104	0.092	**0.146**	-0.072	**0.291**	0.098	**0.148**	**0.193**	**-0.159**	0.090	0.099
11. Log glucose											**0.241**	**-0.215**	**-0.149**	**-0.179**	0.107	0.078	-0.041	**0.356**	**0.177**	0.056	0.083	0.008	**-0.167**	-0.059
12. Log albumin												**0.222**	**0.123**	**0.235**	-0.028	-0.078	0.054	**0.735**	0.006	**0.156**	**0.275**	-0.050	0.069	**0.260**
13. Log retinol													**0.722**	**0.671**	**-0.168**	**-0.431**	**0.380**	-0.065	**-0.248**	-0.112	0.049	0.026	**0.303**	**0.241**
14. Log tocopherol														**0.446**	**-0.124**	**-0.242**	**0.280**	-0.040	-0.108	-0.092	0.015	0.081	**0.234**	**0.128**
15. Log folic acid															-0.033	**-0.425**	**0.311**	-0.043	**-0.307**	**-0.137**	0.064	-0.051	**0.304**	**0.255**
16. Log vit B_{12}																**0.134**	0.023	0.065	0.027	0.035	0.090	-0.064	0.042	-0.025
17. Log Na																	0.090	0.099	**0.416**	**0.134**	-0.025	0.077	**-0.146**	0.002
18. Log K																		-0.066	0.010	-0.067	0.055	**0.160**	**0.132**	**0.279**
19. Log Ca																			0.105	**0.267**	**0.246**	-0.048	-0.020	0.025
20. Log Mg																				**0.161**	0.034	0.072	-0.094	0.040
21. Log P																					-0.074	-0.033	-0.028	-0.042
22. Log Fe																						**-0.176**	0.063	**0.116**
23. Log Cu																							0.009	0.107
24. Log Zn																								**0.158**
25. Log Se																								

* Bold letter indicates the significant correlations

Table 3. **Factor matrix obtained after a varimax rotation**

	Factor 1	***Factor 2***	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7
Cumulative variance (%)	18.5	30.7	39.9	47.9	54.7	60.6	66.2
Retinol	**0.771**	-0.197	-0.008	-0.306	0.160	-0.071	0.062
Folic acid	**0.717**	-0.049	0.108	-0.358	-0.165	-0.030	0.040
K	0.636	-0.028	0.015	0.160	-0.044	0.322	-0.016
Tocopherol	0.596	-0.100	-0.159	-0.164	0.491	0.066	0.040
Se	0.504	-0.161	0.271	0.273	-0.168	-0.289	0.253
GOT	-0.156	**0.916**	-0.014	0.048	-0.060	-0.149	-0.065
GPT	-0.184	**0.911**	-0.021	0.095	0.014	-0.135	-0.078
Vitamin B_{12}	0.147	0.522	0.121	0.159	-0.123	0.374	0.287
Ca	-0.060	0.095	**0.875**	0.020	0.150	-0.028	-0.059
Albumin	0.240	-0.105	**0.856**	-0.068	0.047	-0.112	-0.058
P	-0.346	0.030	0.454	0.128	0.032	0.277	0.112
Mg	-0.037	0.012	0.064	**0.731**	0.055	0.011	-0.163
Na	-0.235	0.195	-0.025	**0.727**	0.020	0.029	0.044
Triglycerides	-0.242	0.001	0.089	0.133	**0.821**	0.019	0.103
Cholesterol	0.335	-0.028	0.316	0.047	0.655	0.020	-0.123
Platelets	0.076	-0.218	0.022	-0.146	-0.075	**0.765**	-0.072
Leukocytes	-0.034	0.014	-0.125	0.364	0.206	0.537	0.001
Zn	0.095	0.045	0.072	0.003	0.127	-0.015	**0.819**
Glucose	0.035	0.124	0.242	0.240	0.129	0.038	-0.511

Vitamins

The preparation of samples for the determination of retinol and α-tocopherol by high performance liquid chromatography (HPLC) was made according to the procedures described by Steghens et al. (1997). The determination was carried out by HPLC with a diode-array detector using a method proposed by us (Rodríguez-Delgado et al. 2002), which is based on the method described by Steghens et al. (1997). Recoveries of the extraction procedure were calculated analyzing ten replicate assays on spiked serum samples on different days obtaining values above 93% for both vitamins. Acceptable precisions were also obtained for both retinol (2.1%) and α-tocopherol (2.3%). The serum levels of vitamin B_{12} and folic acid were measured using a commercially available recombinant DNA technology (U.S. Patent Nº 4708929. Roche® diagnostics) to produce an unique homogeneous enzyme assay system.

Minerals

Phosphorous was determined using a colorimetric method with ammonium molybdate. The rests of the minerals were determined after mineralization of serum samples. Eight hundred to 900 µl of serum measured exactly was introduced into a vessel-tube for mineralization with 4 ml of HNO_3:$HClO_4$ (3.5:0.5 v/v). The temperature of this mixture was slowly increased to 170°C until fumes of $HClO_4$ appeared. The mixture was heated using a heating block according to the following sequence (temperature/time): 100°C/15 min, 125°C/15 min, 150°C/60 min, 160°C/60 min, 170°C/15 min. After cooling to room temperature, this solution was quantitatively transferred and adjusted in a flask of 10 ml with milli-Q water. All samples and standards were analysed in duplicate. Na and K were measured after dilution in milli-Q water (1:10) by flame emission spectrometry and Ca and Mg were measured after dilution in $LaCl_2$ (10 g/l). The Ca, Mg, Fe, Cu and Zn were determined using the air-acetylene flame atomic absorption spectrometry technique and the Se was determined using the hydride generation atomic absorption spectrometry technique (Atomic absorption spectrometer Varian Spectra AA-10 Plus equipped with D_2 lamp background correction system). Accuracy was evaluated by analysing a commercially available sample of lyophilized human serum (Seronorm™ Trace Elements Serum; SERO AS N-1375 Billingstad, Norway). Precision was established by analyzing ten replicate assays of a pooled serum sample. Recoveries near 100% and good precision were obtained for all the metals and therefore it can be deduced that the methods applied are adequate for these samples.

Statistical analysis

All statistical analysis has been performed by means of the SPSS version 12.0 software for Windows. The Kolmogorow-Smirnov's test was applied to verify if the variable had a normal distribution, with a significance level $2\alpha=0.05$. The mean values obtained in the different groups were compared by a One-Way ANOVA and *t*-test, assuming that there were significant differences between mean values at the significance level $2\alpha=0.05$. Simple linear and logarithmic correlation analysis was used to indicate a measure of the correlation and the strength of the relationship between two variables. Factor analysis, using principal components for extraction of factors, was used to summarise the information in a reduced number of factors and graphically differentiate the individuals according to the group under consideration,

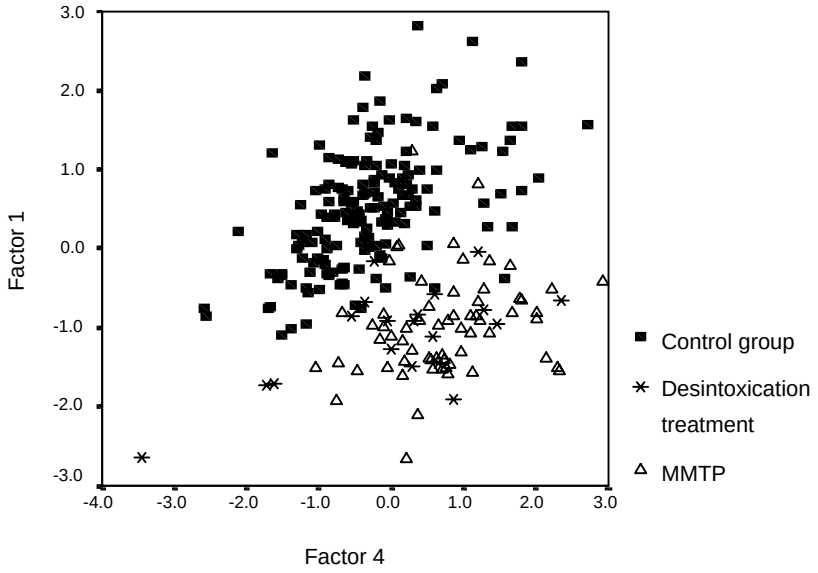

Fig. 1. **Plot of correlation between vitamins differentiating opiate addicts and control group**

RESULTS

To organise this section in a more comprehensive manner, it has been divided into three parts: Variance analysis, correlation study and factor analysis.

Variance analysis

Table 1 shows the mean values and standard deviation of the haematological and classical biochemical parameters and micronutrients levels, vitamins and minerals, for the two groups of opiate addicts considered, according to treatment (in

MMTP and in detoxification treatment) and for the control group. The results of ANOVA analysis are also included. Some differences among the mean values reached statistical significance, in particular, when the control group was compared with both groups of opiate addicts. In relation to the haematological parameters, it was observed that males ($p<0.05$) and females ($p<0.05$) belonging to the control group had a higher number of red cells than the corresponding opiate addicts. In contrast, no significant differences were found between the mean values obtained for haemoglobin and, in the females, for haematocrit. The number of leukocytes in the blood samples of the control group was significantly lower than those mean values in the groups of opiate addicts, which could be due to the frequent infectious processes observed in the opiate addicts. Considering the biochemical parameters analyzed, the transaminases (GOT and GPT) presented significantly higher enzymatic activities in both groups of opiate addicts. The mean levels of albumin made it possible to statistically differentiate the three groups of individuals considered. So, the opiate addicts in detoxification treatment had lower levels than the opiate addicts in MMTP, and these had lower levels than the individuals in the control group. The opiate addicts in detoxification treatment and the individuals of the control group presented similar values of glucose and triglycerides, however, the opiate addicts in detoxification had the lowest level of cholesterol and the opiate addicts included in MMTP had significantly higher levels of glucose and triglycerides than the individuals in the control group.

Large differences were observed in the mean values found for retinol, α-tocopherol and folic acid, however, no significant differences were observed for vitamin B_{12}. So, the control group presented higher levels of these three vitamins than both groups of opiate addicts considered, with no significant differences between them.

Sodium and K mean concentrations in the serum of the individuals in the control group were significantly lower and significantly higher respectively than those concentrations obtained for both groups of opiate addicts considered. Mean Mg and P concentrations for the opiate addicts in MMTP were significantly higher than the corresponding mean values for opiate addicts in detoxification treatment and for the control group, with no significant differences between these groups. No significant differences were observed in the mean concentrations of Fe and Ca for both sexes considering the three groups of individuals. However, the male individuals belonging to the control group tended to present a higher mean serum concentration for iron. Copper mean concentration in males in control group was significantly lower than the corresponding mean concentrations in both groups of opiate addicts considered, however, no significant differences were found for females. Selenium mean concentrations for both groups of opiate addicts were significantly lower than the mean concentration in the control group.

Correlation study

Table 2 shows the double logarithmic correlation matrix for all the parameters studied considering all the individuals, the opiate addicts and the individuals in the control group. A high number of significant correlations were observed. There are many significant correlations among the haematological parameters, which is due to the fact that these parameters are interrelated. Besides which these haematological parameters correlated with classical biochemical parameters emphasizing the GPT and GOT with uric acid with haemoglobin, haematocrit and red cells. Few significant correlations were observed between haematological parameters and vitamins and minerals, except with Fe. Cholesterol presented relatively high correlation coefficients with triglycerides albumin, and α-tocopherol. The transaminases were logically correlated between each other and GPT showed a high and negative correlation with retinol and α-tocopherol. Besides which, the albumin was positively and highly correlated with Ca. The three vitamins, retinol, α-tocopherol and folic acid were interrelated (Figure 1). These correlations made it possible to differentiate the control group from the opiate addicts, because these parameters presented very important differences in both groups. Few significant correlations were observed with the minerals, however, it is interesting saying that sodium strongly correlated with Mg and negatively with retinol.

Factor analysis

Factor analysis was applied to the matrix of data studied to obtain a more simplified view of the relationship among the parameters considered. This procedure permits us to achieve a reduction of dimensionality, a data exploration investigating how many factors (a linear combination of original variables) are necessary to explain the greater part of the variance with minimum loss of information. In this work, we have performed three studies. In the first study all the individuals (males and females) and only the parameters not influenced by sex were included. The second and third studies were carried out with males and females in an independent manner and all the parameters studied were included.

a) *All the individuals.* Using only the variables that do not change with sex - leukocytes, platelets, cholesterol, triglycerides, GOT, GPT, glucose, albumin, retinal, α-tocopherol, vitamin B_{12}, folic

acid, Na, K, Ca, Mg, P, Zn and Se - seven factors were chosen (66.2% of the total variance) because their eigenvalues were higher than 1, and therefore, they explain more variance than the original variables. A Varimax rotation was carried out to minimize the number of variables influencing each factor, and then to facilitate the interpretation of the results (Table 3). The first factor that explains the higher percentage of variance (18.5%) is associated with retinol and folic acid. The second factor is strongly related with the transaminases (GOT and GPT), and the third and fourth factors are associated with Ca and albumin and with Mg and Na, respectively. The score plot for all the individuals of the representation of the first and fourth factors is set out in Figure 2. It can be seen that the opiate addicts tended to separate from the control. However, no separation was observed between the opiate addicts included in MMTP and in detoxification treatment.

b) *Males and females.* The factor analysis was also carried out on the males and females in an independent manner. For the male opiate addicts eight factors were selected which explained 67.4% of the total variance. The first factor, that explained 15.6% of the variance was related to K, and the second factor (12.1% of the total variance) was associated with haemoglobin and haematocrit. The third and fourth factors were mainly related to GOT and GPT and to Mg respectively. When the female group was considered with all the variables studied, nine first factors explaining 69.3% of the total variance were extracted. The first factor that explains the higher percentage of variance (13.2%) is associated with Ca and to a lesser degree with albumin. The second factor, explaining 11.6% of the total variance, is strongly related with haematocrit and to a lesser degree with haemoglobin. The third factor was related with the transaminases (GOT and GPT), and the fourth factor with α-tocopherol.

DISCUSSION

Many differences in the biochemical and haematological parameters were observed between the groups of individuals studied. These could be due to physiological or biochemical alterations in opiate addicts, to different food habits or, in the case of the opiate addicts in MMTP, to the effects of the use of methadone. Data relative to haematologic parameters could reflect a tendency to megaloblastic anaemia of opiate addicts and, it is consistent with the low levels of folic acid observed. The elevated leukocytes count in the blood samples of opiate addicts is probably due to the frequent infectious processes observed in opiate addicts.

With respect to the biochemical parameters, the higher levels of trasaminases in heroin addicts is a logical consequence of the typical hepatic alterations of these individuals. The significant differences in the albumin levels could be related to differences in the nutritional status of these groups of individuals. So the opiate addicts in MMTP have a better food intake than the opiate addicts in detoxification.

Christakis et al. (1973) reported low and deficient levels of folic acid and retinol of considerable significance in patients included in MMTP. These results agree with the results obtained by us, and they could be related to a lower consumption of vegetables and legumes in the addicted population studied (Verde Méndez et al. 2002). The low consumption of fish in the opiate addicts (Verde Méndez et al. 2002), and as a consequence, the relatively low Se intake, could explain the lower Se concentrations in their .

In the correlation study, one can emphasize the correlation between albumin, a nutritional indicator of visceral protein, and Ca. This suggests that the main source of protein in the studied individuals could be the milk products as the contribution of these products to the Ca intake is obviously high. Data previously published (Verde Méndez et al. 2002) confirm a high consumption of milk products in the opiate addicts and the Canary population, representing this consumption a high contribution to the protein intake, and of course, to the Ca intake.

The application of factor analysis indicates that the biochemical and haematological profiles of the opiate addicts, particularly vitamins, such as retinol and folic acid, and minerals, such as Mg and Na, allow us to differentiate the opiate addicts from healthy people. However, no separation was found between both groups of opiate addicts.

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