行政院國家科學委員會補助

大專學生參與專題研究計畫研究成果報告

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＊ 計 畫 名 稱 ： 冠狀動脈心臟病人輔酵素Q10及維生素B-6之營養狀況與氧化壓力及抗氧化酵素相關性之探討 ＊

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學生計畫編號： NSC 100-2815-C-040-020-B

研 究 期 間 ： 100 年 07 月 01 日至 101 年 02 月 28 日止，計 8 個月

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處理方式： 本計畫涉及專利或其他智慧財產權，2 年後可公開查詢

執 行 單 位： 中山醫學大學營養學系（所）

中華民國 101 年 03 月 01 日

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冠狀動脈心臟病人輔酵素Q10及維生素B-6之營養狀況與氧化壓力及抗氧化酵素相關性之探討

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Abstract
Objective: The purpose of this study was to investigate the relationship between the coenzyme Q10 and vitamin B-6 statuses and the risk of CAD.
Methods: Patients who were identified by cardiac catheterization as having at least 50% stenosis of one major coronary artery were assigned to the case group (n = 45). The control group (n = 89) comprised healthy individuals with normal blood biochemical values. The plasma concentrations of coenzyme Q10 and vitamin B-6 (pyridoxal 5'-phosphate) and the lipid profiles were measured.
Results: Subjects with CAD had significantly lower plasma levels of coenzyme Q10 and vitamin B-6 compared to the control group. The plasma coenzyme Q10 concentration were positively correlated with CAT and GPx activities and negatively correlated with the levels of SOD. However, there was no correlation between plasma vitamin B-6 and antioxidant enzymes. In addition, the plasma coenzyme Q10 concentration was positively correlated with the vitamin B-6 status.
Conclusion: There was a significant correlation between the plasma levels of coenzyme Q10 and vitamin B-6. Further study is needed to demonstrate the beneficial of administer vitamin B-6 or coenzyme Q10 to CAD patients, especially those with low coenzyme Q10 levels.
Keywords: Coenzyme Q10; Vitamin B-6; Coronary artery disease; Case-control study

Introduction

Coenzyme Q10 (also known as ubiquinone) is a lipid-soluble benzoquinone with 10 isoprenyl units on the side chain and is a key component of the mitochondrial respiratory chain for adenosine triphosphate (ATP) synthesis.[1, 2] Tissues with high energy requirements, such as the heart, kidney, liver, and skeletal muscle cells, need a higher amount of coenzyme Q10 to synthesize ATP. Coenzyme Q10 can be synthesized in tissues from farnesyl diphosphate and tyrosine. Human synthesis of coenzyme Q10 begins with the conversion of tyrosine into 4-hydroxybenzoic acid. 4-Hydroxybenzoic acid is the key precursor of the benzoquinone core of coenzyme Q10 and vitamin B-6 (pyridoxal 5'-phosphate) is required for the production of 4-hydroxyphenylpyruvic acid from tyrosine.[3]

Cardiovascular disease (CVD) is the leading cause of death worldwide. A lower plasma coenzyme Q10 concentration[4-7] or a lower vitamin B-6 status[8-10] may contribute to the higher susceptibility of some individuals to coronary artery disease (CAD). However, the relationships between coenzyme Q10 and vitamin B-6 statuses and the prevention of CAD are controversial. Some studies have reported that there was no correlation of the levels of the compounds with the risk of coronary atherosclerosis and no significantly beneficial effect of coenzyme Q10 to patients with CAD.[11, 12] Recently, two larger clinical trials reported there was no correlation between the vitamin B-6 level and CAD.[13, 14] The purpose of the present study was to investigate the correlation between the coenzyme Q10 and vitamin B-6 concentrations and to examine the association between the coenzyme Q10 and vitamin B-6 statuses, and antioxidant.

Materials and Methods

Subjects

The current study was designed as a case-control study. CAD patients were recruited from the cardiology clinic of Taichung Veterans General Hospital in Taiwan. Patients who were identified by cardiac catheterization as having at least 50% stenosis of one major coronary artery or who were receiving percutaneous transluminal coronary angioplasty (PTCA) were assigned to the case group (n = 45). Case subjects with diabetes, liver, or renal diseases or undergoing statin therapy were excluded. None of our subjects had experienced an acute myocardial infarction within the previous 6 months. Control subjects (n = 89) were recruited from the physical examination unit of Taichung Veterans Hospital and exhibited normal blood biochemical values, including fasting blood glucose < 6.11 mmol/L, blood urea nitrogen (BUN) < 7.9 mmol/L, creatinine < 123.8 μmol/L, alkaline phosphates < 190 U/L, glutamic oxaloacetic transaminase (GOT) < 35 U/L, and glutamic pyruvate transaminase (GPT) < 45 U/L. Control subjects did not have any illnesses or a history of gastrointestinal disorders, cardiovascular disease (normal electrocardiogram), hypertension, hyperlipidemia, liver and renal diseases, diabetes, cancer, alcoholism, or other metabolic diseases. Subjects currently taking vitamin supplements were excluded. Informed consent was obtained from each subject. This study was approved by the Institutional Review Board of Taichung Veterans General Hospital in Taiwan. The age, blood pressure, smoking, and exercise habits of all subjects were recorded. Blood pressure was measured in each patient after resting for at least 5 min. Body weight, height, waist, and hip circumferences were measured. The body mass index (BMI; kg/m^2) and the waist to hip ratio were then calculated.

Laboratory analyses

Fasting venous blood samples (15 mL) were obtained to determine the hematological and vitamin statuses. Blood specimens were collected in Vacutainer tubes (Becton Dickinson, Rutherford, NJ, USA) with or without EDTA as an anticoagulant as needed. Serum and plasma were prepared and then frozen (-80℃) for storage until analysis. Hematological parameters [i.e., BUN, serum creatinine, total cholesterol (TC), triacylglyceride (TG), low density lipoprotein-cholesterol (LDL-C), and high density lipoprotein-cholesterol (HDL-C)] were measured using an automated biochemical analyzer. Automated measurements of the high sensitivity C-reactive protein (hs-CRP) concentration were performed using particle-enhanced immunonephelometry with an Immage analyzer.

Plasma coenzyme Q10 concentration was measured using high-performance liquid chromatography (HPLC) according to the methods of Chu et al.[15] and Littarru et al.[16] The mean intra- and inter-assay coefficients of variability for the fasting plasma coenzyme Q10 concentration were 1.8% and 4.4%, respectively. The mean analytical recovery of plasma coenzyme Q10 was 99.8%. The vitamin B-6 status was measured the plasma pyridoxal 5'-phosphate according to the method of Bates et al.[17] using HPLC system under yellow light to prevent photo-destruction. The mean intra- and inter-assays coefficients of variability for pyridoxal 5'-phosphate were 1.8% and 4.2%, respectively. The mean analytical recovery of pyridoxal 5'-phosphate was 99.4%. All analyses were performed in duplicate.

Plasma MDA was determined using the thiobarbituric acid reactive substances (TBARs) method, as described by Botsoglou (1997)[18]and Chung *et al*. (2009) [19]. The mean intra- and inter-assay coefficients of plasma MDA variability were 1.9% and 3.9%, respectively. Red blood cells (RBCs) were diluted with 25x sodium phosphate buffer for SOD and GPx measurements and 250x sodium phosphate buffer for CAT measurement. The methods for measuring CAT, SOD and GPx in RBCs have previously been described [19] and measurements were performed spectrophotometrically at 240 nm, 325 nm and 340 nm, respectively. Protein contents of RBCs were determined based on the Biuret reaction of the BCA kit (Thermo, Rockford, IL, USA). The mean intra- and inter-assay coefficients of protein variability were 0.2% and 3.3%, respectively, in RBCs. The antioxidant enzymes activity levels were expressed as unit/mg of protein. All analyses were performed in duplicate and the variations of repeated determinations were within 10% of the same sample. The analyses of plasma MDA and antioxidant enzymes activities were completed within 7 days.

Statistical analyses

Data were analyzed using SigmaStat statistical software (version 2.03; Jandel Scientific, San Rafael, CA, USA). The normal distribution of variables was evaluated using the Kolmogorov-Smirnov test. Differences in subjects' demographic data and the hematological measurement data between the case and control groups were analyzed using the Student's t-test or the Mann-Whitney rank sum test. For categorical response variables, differences between two groups were assessed using the Chi-square test or the Fisher's exact test. To examine the relationship between the plasma coenzyme Q10, vitamin B-6 concentrations, lipid peroxidation, and antioxidant enzymes simple linear regression analyses were used. Data were expressed as means ± standard deviations (SD), and the results were considered statistically significant at $p < 0.05$.

Results

Table 1 shows the demographic data and health characteristics of the subjects. Subjects in the case group had significantly higher values for age, SBP, waist to hip ratio, hematological parameters (i.e., BUN, creatinine, LDL-C, TC/HDL-C, hs-CRP), the number of males, and smoking habits and a lower value for HDL-C than the control group.

The concentrations of plasma coenzyme Q10, vitamin B-6, oxidative stress, and antioxidant enzymes activities in case and control groups are shown in Figure 1 and 2. Subjects in the case group had significantly lower concentrations of plasma coenzyme Q10 (263.5 ± 125.9 vs. 490.8 ± 192.8 nM, $p < 0.01$), pyridoxal 5-phosphate (41.7 ± 18.9 vs. 55.3 ± 31.3 nM, $p = 0.03$), and antioxidant enzyme activities (CAT and GPx), but higher MDA and SOD levels.

The correlations between plasma coenzyme Q10 and vitamin B-6, lipid peroxidation and antioxidant enzyme activities are shown in Table 2. The plasma coenzyme Q10 concentration were positively correlated with CAT and GPx activities and negatively correlated with the levels of SOD. However, there was no correlation between plasma vitamin B-6 and antioxidant enzymes. The correlation between the concentration of coenzyme Q10 and vitamin B-6 is shown in Figure 3. The plasma pyridoxal 5-phosphate status was significantly positively correlated with coenzyme Q10 concentration (Figure 3, $\beta = 1.06$, $p = 0.02$).

Discussion

The present study demonstrated that subjects with CAD had significantly lower levels of coenzyme Q10 and vitamin B-6 (Figure 1) and that there is a significantly positive association between the vitamin B-6 and coenzyme Q10 concentrations (Figure 3). Coenzyme Q10 is a lipid-soluble antioxidant that is transported by lipids in the blood.[20] Patients suffering from CAD might experience losses of coenzyme Q10 under the higher oxidative stress condition.[21-23] The administration of coenzyme Q10 can reduce oxidative stress and increase the activities of antioxidant enzymes in CAD patients.[24] A clinical study conducted by Willis et al.[25] suggested that the administration of vitamin B-6 to patients receiving supplemental coenzyme Q10 may induce greater endogenous synthesis of coenzyme Q10 to supplement the exogenous coenzyme Q10.Although our CAD subjects were stable and had no experience of acute myocardial infarction within the previous 6 months, their plasma coenzyme Q10 concentration was significantly lower than that of control subjects (Figure 1) and the reference values (0.46 μmol/L)[26]. The plasma coenzyme Q10 concentration has been shown to be reduced under statin therapy[27], and we therefore excluded patients who were being treated with statin from this study. Patient suffering from CAD might suffer loss of coenzyme Q10 under higher oxidative stress. Subjects in the case group showed a significant higher lipid peroxide (MDA) level than control (Figure 2, $p < 0.01$), which is an indicator of free radical-induced damage during myocardial ischemia.[28,29] However, we indentified a negative correlation between the plasma coenzyme Q10 and MDA levels, but it was not significant (Table 2).

In addition to oxidative stress, we assessed the activities of the major antioxidant enzymes directly involved in the neutralization of ROS. The activities of CAT and GPx were significantly lower in the case group compared to those of the control group (Figure 2). As shown in Table 2, there was a significantly positive relationship between the levels of plasma coenzyme Q10 and CAT or GPx. On the other hand, the activities of SOD were significantly higher in the case group and negative correlated with the concentration of plasma coenzyme Q10. The role of antioxidant enzymes defense against the ROS is controversial. In CAD patients, SOD activity may increase to protect against lipid peroxidation and against ROS.[30, 31] Coenzyme Q10 may assist SOD in the uptake of superoxide radical to form oxygen and hydrogen peroxide.

Patients with CAD exhibited lower coenzyme Q10 and vitamin B-6 concentrations. Our results demonstrated a significant correlation between the levels of plasma coenzyme Q10 and vitamin B-6 and CAD. Further study is needed to demonstrate the beneficial of administer vitamin B-6 or coenzyme Q10 to CAD patients, especially those with low coenzyme Q10 levels.

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Table 1 Characteristics of subjects

	Case (n = 45)	Control (n = 89)	*p* values
Male / Female (n)	41 / 4	46 / 43	< 0.01
Age (y)	74.6 ± 8.9	49.8 ± 8.3	< 0.01
SBP (mmHg)	133.3 ± 14.0	119.6 ± 17.0	< 0.01
DBP (mmHg)	74.3 ± 10.5	74.7 ± 6.6	0.25
BMI (kg/m^2)	25.6 ± 3.2	24.5 ± 3.5	0.08
Waist to hip ratio	0.9 ± 0.1	0.8 ± 0.1	< 0.01
BUN (mmol/L)	14.9 ± 4.4	10.3 ± 2.7	< 0.01
Creatinine (μmol/L)	114.9 ± 26.5	88.4 ± 26.5	< 0.01
TC (mmol/L)	4.9 ± 0.9	5.1 ± 0.9	0.18
TG (mmol/L)	1.4 ± 0.8	1.5 ± 0.9	0.49
LDL-C (mmol/L)	3.2 ± 0.8	2.9 ± 0.8	0.02
HDL-C (mmol/L)	1.0 ± 0.3	1.4 ± 0.4	< 0.01
TC / HDL-C	5.3 ± 1.7	3.9 ± 1.1	< 0.01
hs-CRP (mg/L)	2.0 ± 2.0	1.5 ± 2.0	< 0.01
Current smoker[2], n (%)	10 (22.2%)	12 (13.5%)	< 0.01

[1]Mean ± SD. [2]Current smoker: individuals currently smoking one or more cigarettes per day. BMI, body mass index; BUN, blood urea nitrogen; DBP, diastolic blood pressure; HDL-C, high-density lipoprotein-cholesterol; hs-CRP, high sensitivity C-reactive protein; LDL-C, low density lipoprotein-cholesterol; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride.

Table 2 Correlations between plasma coenzyme Q10 and vitamin B-6, lipid peroxidation and antioxidant enzyme activities

	Plasma coenzyme Q10 (μmol/L)	Vitamin B-6 (nmol/L)
	r [1] (*p* value)	
MDA (μmol/L)	-0.03 (0.77)	-0.07 (0.43)
CAT (U/mg protein)	0.25 (< 0.01)	0.11 (0.24)
SOD (U/mg protein)	-0.33 (< 0.01)	-0.02 (0.86)
GPx (U/mg protein)	0.39 (< 0.01)	0.15 (0.10)

[1]Correlation coefficient (n = 134). CAT, catalase activity; GPx, glutathione peroxidase;; LDL-C, low density lipoprotein-cholesterol; MDA, malondialdehyde; SOD, superoxide dismutase; TC, total cholesterol; TG, triglyceride.

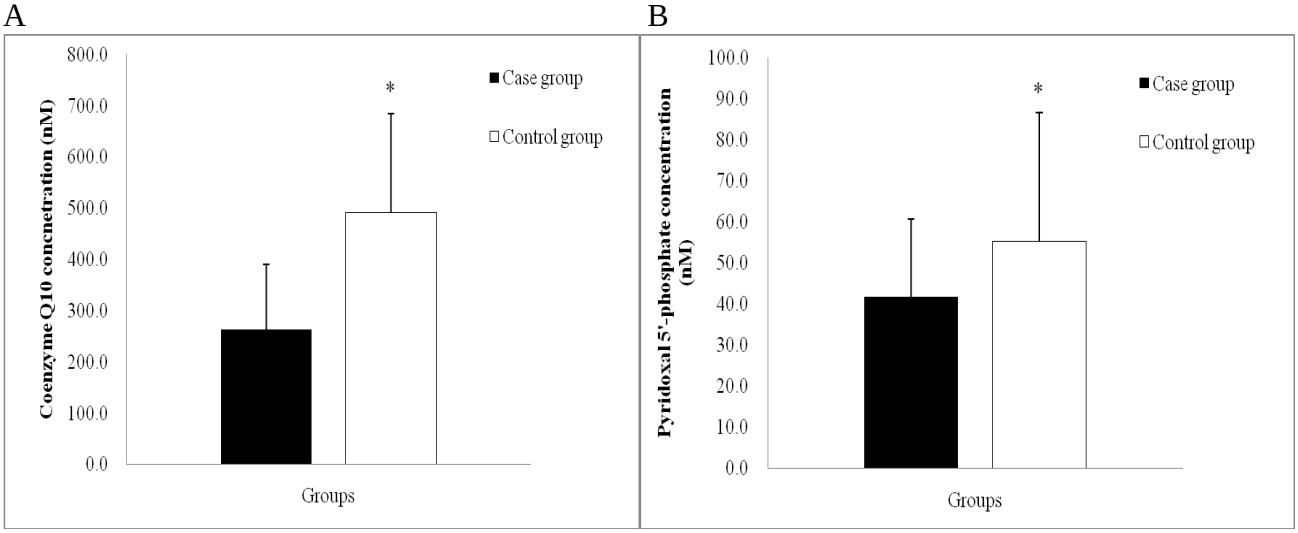

Figure 1 Concentrations of coenzyme Q10 (A) and vitamin B-6 (B)
*Values were significantly different between case and control groups

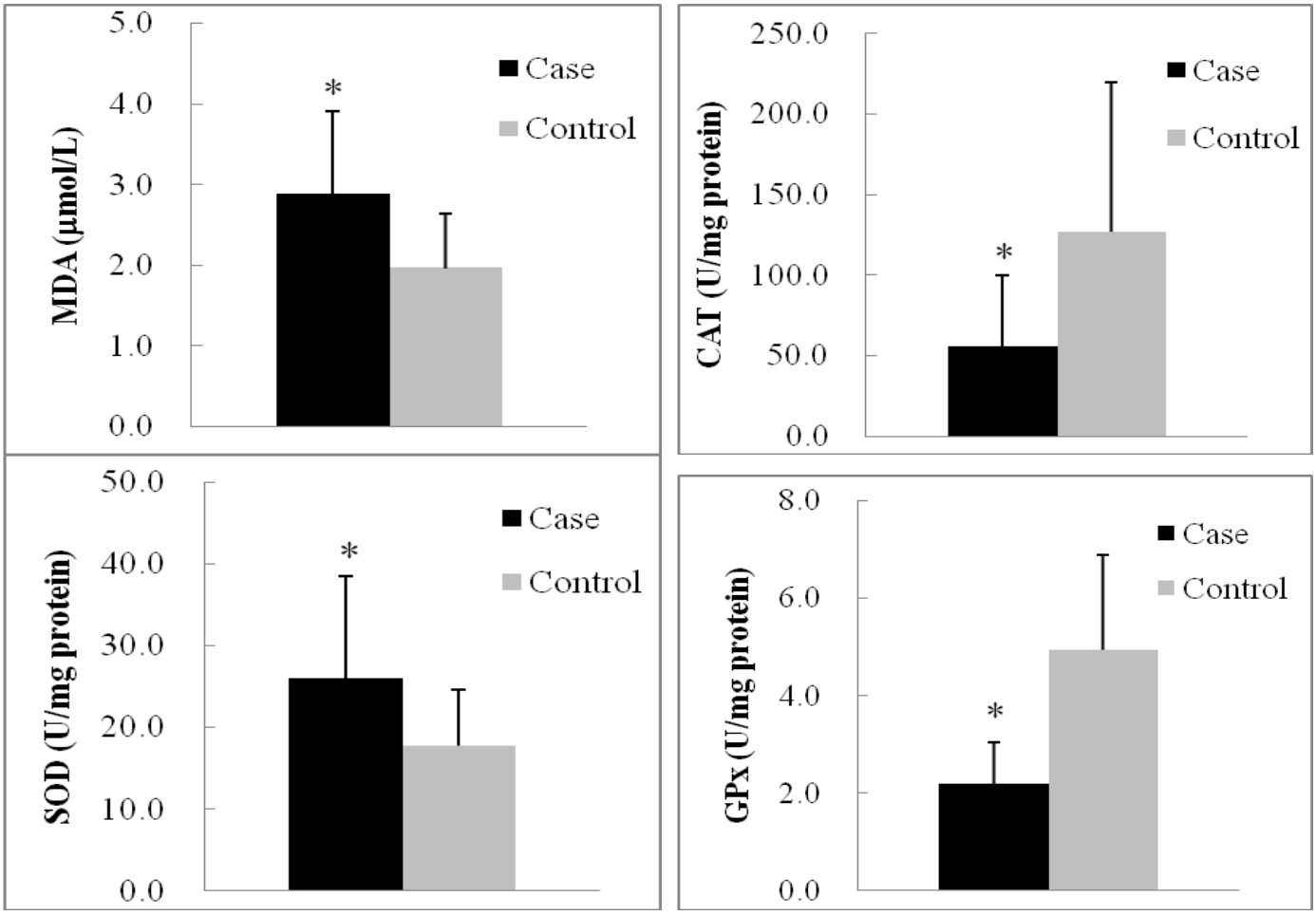

Figure 2 Concentration of lipid peroxidation and antioxidant enzymes activities.
Abbreviation: CAT, catalase; GPx, glutathionine peroxidase; MDA, malondialdehyde; SOD, superoxide dismutase. *Values were significantly different between case and control groups; $p < 0.01$.

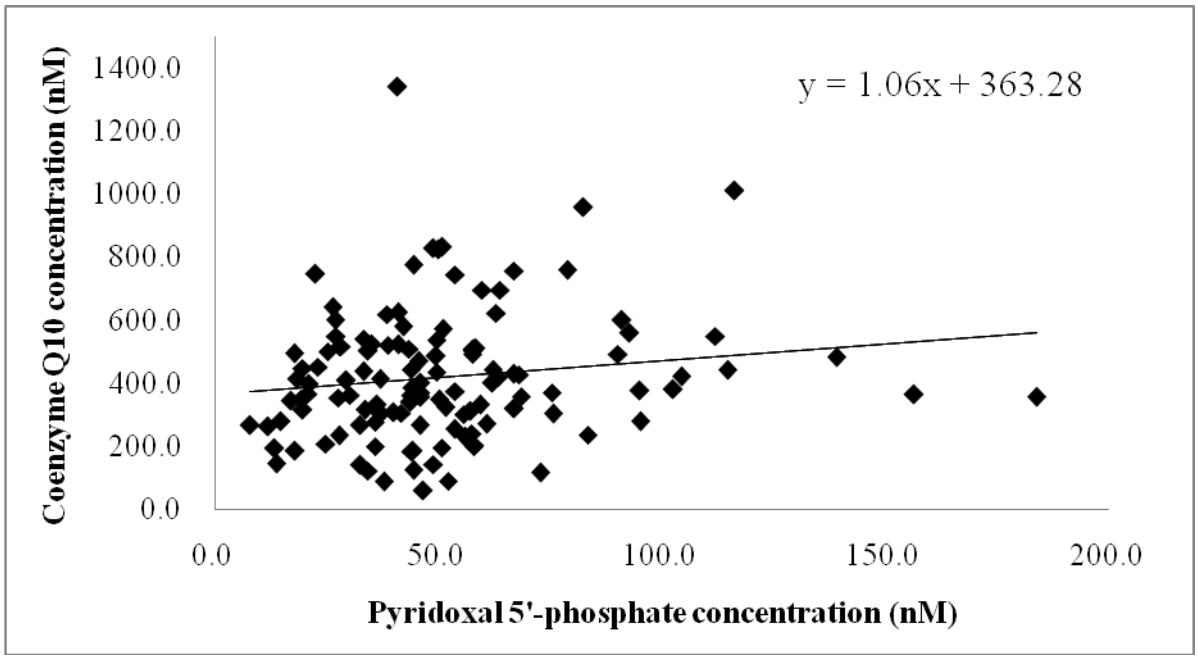

Figure 3 Correlation between the coenzyme Q10 and vitamin B-6 concentrations
Correlation between the coenzyme Q10 and pyridoxal 5'-phosphate concentrations ($p = 0.02$).