科技部補助專題研究計畫成果報告
期末報告

維生素B-6與氧化壓力及抗氧化能力相關性之研究(第3年)

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報 告 附 件 ： 出席國際會議研究心得報告及發表論文

處 理 方 式 ：
1.公開資訊：本計畫可公開查詢
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中 華 民 國 104 年 10 月 16 日

中 文 摘 要 ： 維生素B-6可能直接清除自由基或藉由參與穀胱甘肽的合成而間接扮演抗氧化功能的角色。本研究第一年的目的是要探討以高濃度同半胱胺酸誘發小鼠氧化壓力後的維生素B-6與胱胺酸與穀胱甘肽濃度及其相關抗氧化酵素活性的關係。四週齡大的BALB/c小鼠經過7天的適應期後依其體重平均分配至控制組（n = 9）、同半胱胺酸組（n = 9）、維生素B-6缺乏組（n = 9）、維生素B-6補充組（n = 9），為期28天。之後除了控制組外，在其他各組的小鼠飲水中添加同半胱胺酸以便誘發小鼠的氧化壓力狀態，為期21天。結果顯示維生素B-6缺乏小鼠較其他組有顯著較高的血漿及肝臟同半胱胺酸濃度及肝臟的丙二醛濃度。在所有接受高同半胱酸飲水的各組中，維生素B-6缺乏的小鼠有最高的血漿穀胱甘肽濃度以及相對較低的肝臟穀胱甘肽濃度；但是，血漿及肝臟的穀胱甘肽過氧化酶活性在各組中則維持相對穩定的狀態。小鼠處在高氧化壓力狀態下，維生素B-6若缺乏時可能會造成穀胱甘肽從肝臟重新分布到血漿中利用，但是不會進一步的影響穀胱甘肽依賴的酵素活性。

電銲作業員在電銲作業的環境下經常暴露於粉塵中，而處在高氧化壓力的工作環境中，而可能增加對維生素B-6的利用，進而消耗體內儲存的維生素B-6。因此本研究第二年的目的是探討電銲作業員的維生素B-6和同半胱胺酸與氧化壓力及抗氧化能力的相關性。於台中市中科某公司依其工作性質募集電銲作業員(暴露組，57位)及同公司的辦公室白領員工(對照組，42位)。兩組的血漿維生素B-6及同半胱胺胺酸濃度皆無顯著差異。但暴露組相較對照組有顯著較高的氧化低密度脂蛋白濃度，較低的紅血球穀胱甘肽濃度及超氧岐化酶活性。血漿維生素B-6濃度不論在暴露組或對照組皆和氧化壓力指標及抗氧化能力無顯著相關性。但是調整相關干擾因子後，暴露組的血漿同半胱胺酸濃度則與總抗氧化能力(partial rs= -0.34, $p < 0.05$)及紅血球超氧岐化酶活性(partial rs = 0.29, $p < 0.05$)呈顯著相關性。只要暴露組的電銲作業員有足夠的維生素B-6營養狀況應不影響其氧化壓力狀態及抗氧化能力。但是，較高的同半胱胺酸濃度似乎是影響抗氧化能力的主要危險因子。

在我們實驗室之前的研究發現高血漿同半胱胺酸及血清葉酸濃度是增加大腸直腸癌發生的重要危險因子，且大腸直腸癌患者的高同半胱胺酸濃度是獨立於維生素B-6及葉酸外與罹患大腸直腸癌危險性有顯著相關性。在研究過程中發現大腸直腸息肉(大腸直腸癌前驅)患者相對無息肉受試者有較高的代謝症候群盛行率，因此本研究第三年目的是探討大腸直腸息肉患者的代謝症候群與血漿同半胱胺酸關係與分析這兩個因子對罹患大腸直腸息肉危險性的影響。以病例-對照研究模式於台中榮民總醫院大腸直腸外科募集135位有大腸直腸息肉受試者，其中59位有代謝症候群及110位無息肉受試者，其中36位有代謝症候群。結果顯示代謝症候群的指標中除了血清三酸甘油脂外，其餘指標(包括：腰圍、血壓、血糖及高密度脂蛋白)與血漿同半胱胺酸濃度都會分別增加罹患大腸直腸息肉的勝算比。但是若同時考慮代謝症候及其指標與同半胱胺酸對罹患大腸直腸息肉的影響時，同半胱胺酸對罹患大腸直腸息肉危險性的影響及消失，但是腰圍、收縮壓、舒張壓、高密度脂蛋白及有無代謝症候群仍會單獨顯著影響罹患大腸直腸息肉的勝算比。代謝症候群似乎是相較血漿同半胱胺酸在影響罹患大腸直腸息肉危險性有較獨立且重要的角色。

除了前述研究外，我們也注意到葉酸在癌化過程中有可能因為扮演雙重角色而改變與罹患大腸直腸癌危險性的關係。因此本研究第三年的另一目的是探討葉酸在健康受試者、大腸直腸腺瘤性息肉(可能演化成大腸直腸癌)患者以及大腸直腸癌患者的營養狀況以及與罹患大腸直腸腺瘤性息肉及大腸直腸癌的關係。以病例-對照研究模式於台中榮民總醫院大腸直腸外科募集156位大腸直腸癌患者，另外於健康管理中心募集70位經大腸鏡檢查及病理檢驗後確認為大腸直腸息肉的受試者，以及182位健康的受試者。將大腸直腸息肉及大腸直腸癌患者的葉酸濃度分層為四分位數後，若葉酸濃度位於第三個四分位數（OR, 3.46; 95% CI, 1.16 - 10.34; p = 0.03）與第四個四分位數(OR, 4.86; 95% CI, 1.42 - 16.58; p = 0.01)會顯著增加罹患大腸直腸癌的危險對比值，但是同樣的結果卻未在大腸直腸癌患者與健康受試者之間觀察到。高血清葉酸濃度可能在大腸直腸癌形成過程中扮演雙重的角色。

中文關鍵詞：維生素B-6、同半胱胺酸、氧化壓力、穀胱甘肽、抗氧化酵素活性、葉酸、大腸直腸癌

英文摘要：

英文關鍵詞：

科技部補助專題研究計畫成果報告

（□期中進度報告/■期末報告）

維生素 B-6 與氧化壓力及抗氧化能力相關性之研究

計畫類別：■個別型計畫　□整合型計畫
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計畫參與人員：陳芳霈、李宛儒、楊忞秀、藍郁淳、蕭郁樺、張慶隆

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■出席國際學術會議心得報告

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中　華　民　國　104　年 10　月 10　日

中文摘要

維生素 B-6 可能直接清除自由基或藉由參與穀胱甘肽的合成而間接扮演抗氧化功能的角色。本研究第一年的目的是要探討以高濃度同半胱胺酸誘發小鼠氧化壓力後的維生素 B-6 與胱胺酸與穀胱甘肽濃度及其相關抗氧化酵素活性的關係。四週齡大的 BALB/c 小鼠經過 7 天的適應期後依其體重平均分配至控制組（n = 9）、同半胱胺酸組（n = 9）、維生素 B-6 缺乏組（n = 9）、維生素 B-6 補充組（n = 9），為期 28 天。之後除了控制組外，在其他各組的小鼠飲水中添加同半胱胺酸以便誘發小鼠的氧化壓力狀態，為期 21 天。結果顯示維生素 B-6 缺乏小鼠較其他組有顯著較高的血漿及肝臟同半胱胺酸濃度及肝臟的丙二醛濃度。在所有接受高同半胱酸飲水的各組中，維生素 B-6 缺乏的小鼠有最高的血漿穀胱甘肽濃度以及相對較低的肝臟穀胱甘肽濃度；但是，血漿及肝臟的穀胱甘肽過氧化酶活性在各組中則維持相對穩定的狀態。小鼠處在高氧化壓力狀態下，維生素 B-6 若缺乏時可能會造成穀胱甘肽從肝臟重新分布到血漿中利用，但是不會進一步的影響穀胱甘肽依賴的酵素活性。

電銲作業員在電銲作業的環境下經常暴露於粉塵中，而處在高氧化壓力的工作環境中，而可能增加對維生素 B-6 的利用，進而消耗體內儲存的維生素 B-6。因此本研究第二年的目的是探討電銲作業員的維生素 B-6 和同半胱胺酸與氧化壓力及抗氧化能力的相關性。於台中市中科某公司依其工作性質募集電銲作業員(暴露組，57 位)及同公司的辦公室白領員工(對照組，42 位)。兩組的血漿維生素 B-6 及同半胱胺胺酸濃度皆無顯著差異。但暴露組相較對照組有顯著較高的氧化低密度脂蛋白濃度，較低的紅血球穀胱甘肽濃度及超氧岐化酶活性。血漿維生素 B-6 濃度不論在暴露組或對照組皆和氧化壓力指標及抗氧化能力無顯著相關性。但是調整相關干擾因子後，暴露組的血漿同半胱胺酸濃度則與總抗氧化能力(partial r_s= -0.34, $p < 0.05$)及紅血球超氧岐化酶活性(partial r_s= 0.29, $p < 0.05$)呈顯著相關性。只要暴露組的電銲作業員有足夠的維生素 B-6 營養狀況應不影響其氧化壓力狀態及抗氧化能力。但是，較高的同半胱胺酸濃度似乎是影響抗氧化能力的主要危險因子。

在我們實驗室之前的研究發現高血漿同半胱胺酸及血清葉酸濃度是增加大腸直腸癌發生的重要危險因子，且大腸直腸癌患者的高同半胱胺酸濃度是獨立於維生素B-6及葉酸外與罹患大腸直腸癌危險性有顯著相關性。在研究過程中發現大腸直腸息肉(大腸直腸癌前驅)患者相對無息肉受試者有較高的代謝症候群盛行率，因此本研究第三年目的是探討大腸直腸息肉患者的代謝症候群與血漿同半胱胺酸關係與分析這兩個因子對罹患大腸直腸息肉危險性的影響。以病例-對照研究模式於台中榮民總醫院大腸直腸外科募集135位有大腸直腸息肉受試者，其中59位有代謝症候群及110位無息肉受試者，其中36位有代謝症候群。結果顯示代謝症候群的指標中除了血清三酸甘油脂外，其餘指標(包括：腰圍、血壓、血糖及高密度脂蛋白)與血漿同半胱胺酸濃度都會分別增加罹患大腸直腸息肉的勝算比。但是若同時考慮代謝症候及其指標與同半胱胺酸對罹患大腸直腸息肉的影響時，同半胱胺酸對罹患大腸直腸息肉危險性的影響及消失，但是腰圍、收縮壓、舒張壓、高密度脂蛋白及有無代謝症候群仍會單獨顯著影響罹患大腸直腸息肉的勝算比。代謝症候群似乎是相較血漿同半胱胺酸在影響罹患大腸直腸息肉危險性有較獨立且重要的角色。

除了前述研究外，我們也注意到葉酸在癌化過程中有可能因為扮演雙重角色而改變與罹患大腸直腸癌危險性的關係。因此本研究第三年的另一目的是探討葉酸在健康受試者、大腸直腸腺瘤性息肉(可能演化成大腸直腸癌)患者以及大腸直腸癌患者的營養狀況以及與罹患大腸直腸腺瘤性息肉及大腸直腸癌的關係。以病例-對照研究模式於台中榮民總醫院大腸直腸外科募集156位大腸直腸癌患者，另外於健康管理中心募集70位經大腸鏡檢查及病理檢驗後確認為大腸直腸息肉的受試者，以及182位健康的

受試者。將大腸直腸息肉及大腸直腸癌患者的葉酸濃度分層為四分位數後，若葉酸濃度位於第三個四分位數 (OR, 3.46; 95% CI, 1.16 - 10.34; $p = 0.03$) 與第四個四分位數(OR, 4.86; 95% CI, 1.42 - 16.58; $p = 0.01$)會顯著增加罹患大腸直腸癌的危險對比值，但是同樣的結果卻未在大腸直腸癌患者與健康受試者之間觀察到。高血清葉酸濃度可能在大腸直腸癌形成過程中扮演雙重的角色。

關鍵詞：維生素 B-6、同半胱胺酸、氧化壓力、穀胱甘肽、抗氧化酵素活性、葉酸、大腸直腸癌

英文摘要

Vitamin B-6 may directly or indirectly play a role in oxidative stress and the antioxidant defense system. The purpose of the first year project was to examine the associations of vitamin B-6 status with cysteine, glutathione, and its related enzyme activities in mice with homocysteine-induced oxidative stress. Four-week-old male BALB/c mice were weighed and divided into one of four dietary treatment groups fed either a normal diet (as a control group and a homocysteine group), a vitamin B-6-deficient diet (as a B-6-deficient group), or a B-6-supplemented diet (a pyridoxine-HCl-free diet supplemented with 14 mg/kg of pyridoxine-HCl, as a B6 supplement group) for 28 days. Homocysteine thiolactone was then added to drinking water in three groups for 21 days to induce oxidative stress. At the end of the study, mice were sacrificed by decapitation and blood and liver samples were obtained. Mice with vitamin B-6-deficient diet had the highest homocysteine concentration in plasma and liver among groups. Significantly increased hepatic malondialdehyde levels were observed in the vitamin B-6-deficient group. Among homocysteine-treated groups, mice with vitamin B6-deficient diet had the highest plasma glutathione concentration and relatively lower hepatic glutathione concentration. The glutathione peroxidase activities remained relatively stable in plasma and liver whether vitamin B-6 was adequate, deficient, or supplemented. The vitamin B6-deficient status seems to mediate the oxidative stress in connection with the redistribution of glutathione from liver to plasma, but not further affect glutathione-related enzyme activities in mice with homocysteine-induced oxidative stress.

Welders are particularly susceptible to fume exposure during the welding process. In this high oxidative stress environment, the utilization and metabolic turnover of vitamin B-6 increase, and this lowers the body's pool of the vitamin. The purpose of the second year project was to examine the association of vitamin B-6 status and plasma homocysteine with oxidative stress and antioxidant capacities in welders. Workers were divided into either the welding exposure group (n = 57) or the nonexposure controls (n = 42) based on whether they were employed as welders. There were no significant differences in vitamin B-6 status and plasma homocysteine concentration between the welding exposure group and the nonexposure controls. The welding exposure group had significantly higher levels of oxidized low-density lipoprotein cholesterol and lower erythrocyte glutathione concentration and superoxide dismutase (SOD) activities when compared to nonexposure controls. Plasma pyridoxal 5'-phosphate concentration did not correlate with oxidative stress indicators or antioxidant capacities in either group. However, plasma homocysteine significantly correlated with total antioxidant capacity (TAC) (partial $r_s = -0.34$, $P < 0.05$) and erythrocyte SOD activities (partial $r_s = 0.29$, $P < 0.05$) after adjusting for potential confounders in the welding exposure group. In the welding exposure group, adequate vitamin B-6 status was not associated with oxidative stress or antioxidant capacities. However, elevated plasma homocysteine seemed to be a major contributing factor to antioxidant capacities in welders.

Our previous study results indicated that increased plasm homocysteine and serum folate concentrations were strongly associated with the risk of colorectal cancer (CRC), and plasma homocysteine was associated with the CRC risk independent of vitamin B-6 and folate status. In addition to the previous findings, we accidently observed that subjects with colorectal polyps (the precursor of CRC) had higher prevalence of metabolic syndrome when compared to subjects without metabolic syndrome. In the 3rd year of research, we

thus investigate the association between the metabolic syndrome and homocysteine and urther analyze the relationship between these two factors and the risk of colorectal polyps. This was a case-control study. A total of 135 subjects with colorectal polyps (59 subjects with metabolic syndrome) and 110 subjects (39 with metabolic syndrome) without colorectal polyps were recruited. The metabolic syndrome and its individual components, except for serum triglycerides, and homocysteine were associated with the risk of colorectal polyps. When the association of the metabolic syndrome and homocysteine with the risk of colorectal polyps was simultaneously considered, the association between homocysteine and the risk of colorectal polyps disappeared, but waist circumference, systolic and diastolic blood pressure, high-density lipoprotein cholesterol, and the metabolic syndrome itself were still significant risk factors for the development of colorectal polyps. Although the metabolic syndrome and plasma homocysteine were individually related to the risk of colorectal polyps, the metabolic syndrome was a major contributing factor in relation to the risk of colorectal polyps independent of plasma homocysteine.

In the 3rd year of research, we also found the possible dual role of serum folate in the development and progression of colorectal cancer has not been well established in human studies. Therefore, another purpose of the 3rd year project was to investigate the association between serum folate and the risk of CRC in subjects with CRC or colorectal adenomatous polyps (AP, a precursor of CRC), and healthy subjects. This was a case-control design. Two hundred and thirty-seven men and 171 women were recruited with 156 subjects in the CRC group, 70 subjects in the AP group and 182 healthy subjects in the control group. The risk of CRC was significantly increased in the third (OR, 3.46; 95% CI, 1.16e10.34) and fourth (OR, 4.86; 95% CI, 1.42e16.58) quartiles of serum folate concentration after adjusting for potential confounders among subjects with AP or CRC. Furthermore, serum folate concentration had no significant effect on the risk of CRC among subjects in the control and CRC groups. Higher serum folate concentration was significantly correlated with increased CRC risk in subjects with AP, while serum folate had no effect on CRC risk in healthy controls. Serum folate might possess potential dual modulatory effects on the risk of CRC.

Keywords: vitamin B-6, homocysteine, oxidative stress, glutathione, antioxidant enzyme activities, folate, colorectal cancer

第一年專題研究計畫結果已投稿並發表，茲將論文內容附註如下：

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ORIGINAL ARTICLE

Role of vitamin B6 status on antioxidant defenses, glutathione, and related enzyme activities in mice with homocysteine-induced oxidative stress

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Abstract

Background: Vitamin B6 may directly or indirectly play a role in oxidative stress and the antioxidant defense system.

Objective: The purpose of this study was to examine the associations of vitamin B6 status with cysteine, glutathione, and its related enzyme activities in mice with homocysteine-induced oxidative stress.

Design: Four-week-old male BALB/c mice were weighed and divided into one of four dietary treatment groups fed either a normal diet (as a control group and a homocysteine group), a vitamin B6-deficient diet (as a B6-deficient group), or a B6-supplemented diet (a pyridoxine-HCl-free diet supplemented with 14 mg/kg of pyridoxine-HCl, as a B6 supplement group) for 28 days. Homocysteine thiolactone was then added to drinking water in three groups for 21 days to induce oxidative stress. At the end of the study, mice were sacrificed by decapitation and blood and liver samples were obtained.

Results: Mice with vitamin B6-deficient diet had the highest homocysteine concentration in plasma and liver among groups. Significantly increased hepatic malondialdehyde levels were observed in the vitamin B6-deficient group. Among homocysteine-treated groups, mice with vitamin B6-deficient diet had the highest plasma glutathione concentration and relatively lower hepatic glutathione concentration. The glutathione peroxidase activities remained relatively stable in plasma and liver whether vitamin B6 was adequate, deficient, or supplemented.

Conclusions: Mice with deficient vitamin B6 intakes had an aggravate effect under homocysteine-induced oxidative stress. The vitamin B6-deficient status seems to mediate the oxidative stress in connection with the redistribution of glutathione from liver to plasma, but not further affect glutathione-related enzyme activities in mice with homocysteine-induced oxidative stress.

Keywords: *vitamin B6; glutathione; antioxidant enzyme activities; homocysteine-induced oxidative stress; mice*

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Pyridoxal 5'-phosphate (PLP), the physiologically active coenzyme form of vitamin B6, is mainly involved in the metabolism of amino acids, nucleic acids, glycogen, porphyrin, and lipids. In addition, vitamin B6 may have a crucial role in antioxidant mechanism (1–6). Although the exact antioxidant mechanism of vitamin B6 has not been confirmed yet, vitamin B6 may directly react with the peroxy radicals and thereby scavenge radicals and inhibit lipid peroxidation (6–10). On the contrary, vitamin B6 may indirectly play an antioxidant role by serving as coenzyme in the glutathione antioxidant defense system. PLP serves as a coenzyme in the transsulfuration pathway of homocysteine to cysteine. Cysteine synthesized by this pathway is an important contributor to synthesis of reduced glutathione (GSH). The GSH-dependent antioxidant system, including glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione *S*-transferase (GST), plays a fundamental role in cellular defense against reactive free radicals and other oxidant species (6, 11). A decrease in antioxidant enzyme activity may disrupt the

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balance between pro- and anti-oxidants, leading to higher oxidative stress and cellular damage. It would then be reasonable to hypothesize that deficient vitamin B6 status might either directly cause higher oxidative stress or might affect cysteine and GSH synthesis and, as a consequence, the entire GSH-dependent antioxidant defense system. A previous study showed increased malondialdehyde (MDA) level and GR activity, and decreased GSH synthesis, GPx, and GSH activities in liver tissue of vitamin B6-deficient rats (4). However, unchanged GSH concentrations and GR activities in the liver, kidney, brain, lung, spleen, and plasma, and increased GPx activities in the liver were observed in vitamin B6-deficient rats when compared to control rats (12). Other studies of animals (13, 14) and healthy humans (15) indicated that dietary vitamin B6 restriction did not affect liver/plasma cysteine concentrations but increased liver/plasma GSH concentrations. There seems to be an inconsistency regarding the relationship between level of GSH and its related enzyme activities and vitamin B6 status in animals.

Although increased oxidative stress has been observed in vitamin B6-deficient animal models (1, 2, 4), the antioxidant roles of vitamin B6 have not been fully studied yet. It is unclear whether deficient vitamin B6 status would mediate the increased oxidative stress in connection with deficient cysteine and GSH synthesis and decreased GSH-related enzyme activities. The purpose of this study was to examine the associations of vitamin B6 status with cysteine, GSH, and its related enzyme activities in mice with homocysteine-induced oxidative stress.

Materials and methods

Animals and diets

Four-week-old male BALB/c mice were purchased from National Laboratory Animal Center (Taipei, Taiwan). Mice were housed in individual metal cages in an air-conditioned room at 23 ± 2°C, 55–60% relative humidity, and a 12 h light/dark cycle, and were given a laboratory rodent chow diet for 7 days to allow for acclimatization. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Chung Shan Medical University (IACUC Approval No. 1092), Taichung, Taiwan. After 7 days of acclimatization, mice were weighed and evenly divided into one of four dietary treatment groups fed a normal diet (AIN-93-M, ICN Biomedicals, Inc., USA) (as a control group, $n=9$; and a homocysteine group, $n=9$), a vitamin B6-deficient diet (AIN-93-M without pyridoxine (PN)-HCl, ICN Biomedicals, Inc., USA) (as a vitamin B6-deficient group, $n=9$), or a vitamin B6-supplemented diet (a PN-HCl free diet supplemented with either 14 mg/kg) (as a vitamin B6 supplement group, $n=9$) for 28 days. The composition of normal and experimental diets is shown in Table 1. Hyperhomocysteinemia has been considered to be a potential oxidative stress indicator (16, 17) and has been used to induce oxidative stress in rats (18). Homocysteine thiolactone was then added to drinking water (1.8 g/L) for 21 days to induce oxidative stress (16, 17) in three groups (except for the control group). At the end of the study, mice were sacrificed by decapitation and blood and liver tissue samples were obtained. The study protocol is shown in Fig. 1.

Biochemical measurements

During the experimental feeding, the animal weight and intakes were measured twice a week. Blood samples were withdrawn from inferior vena cava, transported on ice, and separated into plasma and red blood cells within 30 min by low speed centrifugation (3,000 rpm, 15 min, 4°C). Liver tissues were immediately homogenized in phosphate-buffered saline (PBS). The homogenized solution was then centrifuged (12,000 rpm, 4°C, 10 min). The supernatant were then carefully removed for analysis. All the samples were stored frozen (−20°C) until analysis.

Plasma and liver PLP concentrations were determined by high performance liquid chromatography (HPLC) as previously described (19). The inter- and intraassay variabilities of plasma and liver PLP were 5.1% ($n=8$) and 1.1% ($n=5$), respectively. Homocysteine and cysteine concentrations in plasma and liver were determined by HPLC using the method of Dudman (20). The interassay variabilities of homocysteine and cysteine were 6.2% ($n=8$) and 6.9% ($n=8$), respectively, and the intraassay variabilities of homocysteine and cysteine were 1.0 ($n=5$) and 0.9% ($n=5$), respectively. Vitamin B6 and homocysteine assays were carried out under yellow light to prevent photodestruction. Oxidative stress was estimated as the levels of plasma and liver MDA. Plasma and liver MDA was measured by thiobarbituric-acid-reactive substances (TBARs) according to a method previously described (21). The following reagents were used: PBS, 3% sodium dodecyl sulfate, 0.1 N hydrochloride, 10% phosphotungstic acid, 0.7% TBARs, and n-butanol. The excitation and emission wavelengths of fluorescence spectrophotometer (F-4500, Hitachi, Japan) were set at 515 and 555 nm, respectively. The GSH concentration in plasma and liver were measured using the method of Hissin and Hilf (22). Plasma or liver homogenates were diluted with PBS, mixed with 2% trichloroacetic acid, and then added to the reagent containing PBS-EDTA buffer, 5,5'-dithiobis-2-nitrobenzoic acid, nicotinamide adenine dinucleotide phosphate (NADPH), and GR. The enzyme-linked immunosorbent assay (ELISA) reader (PTL-3965, Jasco, Japan) was used to read the absorbance value at wavelength of 405 nm. The inter- and intraassay variabilities of plasma GSH were 3.5% ($n=3$) and 2.7% ($n=5$), respectively. The GSH-related enzyme (GPx and GR) activity levels in plasma and liver were measured using the method of Lawrence and Burk (23). For the

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Table 1. Composition of normal and experimental diets

Diet (ingredients)	%	Normal diet (g/kg dry matter)	Vitamin B6-deficient diet (g/kg dry matter)
Casein	14.00	140	140
Dextrinized cornstarch	15.50	155	155
Sucrose	10.00	100	100
Corn starch	46.60	466	466
Alphacel, non-nutritive bulk	5.00	50	50
Soybean oil	4.00	40	40
AIN-93M mineral mix[a]	3.50	35	35
L-Cystine	0.18	1.80	1.80
AIN-93-VX vitamin mix[b]	1.00	10	–
AIN-93-VX vitamin mix (without pyridoxine-HCl)	1.00	–	10
Choline bitartrate	0.25	2.50	2.50
tert-Butylhydroquinone	0.08×10^{-2}	0.08×10^{-1}	0.08×10^{-1}

All compounds used in the study were of analytical grade.
[a]Supplied (g/kg diet), $CaCO_3$, 357.00; KH_2PO_4, 250.00; $K_3C_6H_5O_7 \cdot H_2O$, 28.00; NaCl, 74.00; K_2SO_4, 46.60; MgO, 24.00; Ferric citrate, 6.06; $ZnCO_3$, 1.65; $MnCO_3$, 0.63; $CuCO_3$, 0.30; KIO_3, 0.01; Na_2O_3Se, 10.25×10^{-3}; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 7.95×10^{-3}; $Na_2O_3Si \cdot 9H_2O$, 1.45; $KCr(SO_4)_2 \cdot 12H_2O$, 2.75×10^{-1}; LiCl, 1.74×10^{-2}; $B(OH)_3$, 8.15×10^{-2}; NaF, 6.35×10^{-2}; $NiCO_3$, 3.18×10^{-2}; NH_4VO_3, 0.66×10^{-2}; powdered sugar, 209.81.
[b]Supplied (g/kg diet), nicotinic acid, 3; D-calcium pantothenate, 1.6; pyridoxine-HCl, 0.7; thiamine HCl, 0.60; riboflavin, 0.60; folic acid, 0.20; D-biotin, 0.02; vitamin B-12 (0.1% triturated in mannitol), 2.50; α-tocopherol powder (250 U/g), 30.00; vitamin A palmitate (250,000 U/g), 1.60; vitamin D-3 (400,000 U/g), 0.25; phylloquinone, 0.75×10^{-1}; powdered sucrose, 959.66.

analysis of GPx activity level, plasma or liver homogenates were diluted with PBS and NADPH, added to potassium phosphate buffer (PPB), GSH and GR, and then mixed with hydrogen peroxide. For the analysis of GR activity level, plasma or liver homogenates were mixed with PPB, NADPH, oxidized GSH, and hydrogen peroxide. The ELISA reader (PTL-3965, Jasco, Japan) was used to read the absorbance value at wavelength of 340 nm for GPx and GR activity levels. The interassay variabilities of plasma GPx and GR activities were 4.4% ($n = 3$) and 6.8% ($n = 3$), respectively, and the intraassay variabilities GPx and GR activities were 6.1% ($n = 5$) and 6.2% ($n = 5$), respectively. Protein content was determined using the method of Lowry et al. (24). Results were expressed in nmol/mg protein for GSH and nmol/min/mg protein for GPx and GR activity levels. All analyses were performed in duplicate.

Statistical analyses

Data were analyzed using the SAS statistical software (version 9.3; Statistical Analysis System Institute Inc., Cary, NC, USA). A Kolmogorov–Smirnov test was performed to determine the normal distribution. Biochemical values are compared for significant differences using

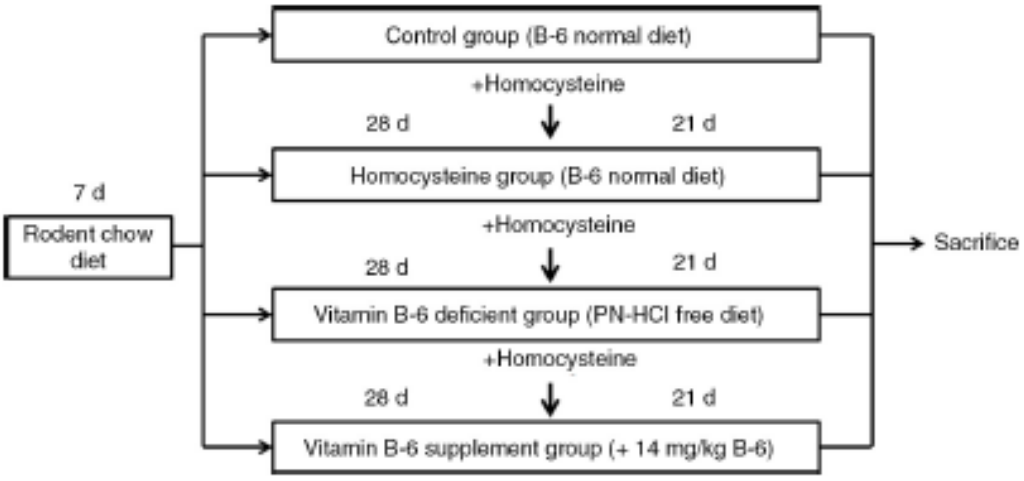

Fig. 1. The study design.

one way analysis of variance among groups. Because some data were skewed rather than normally distributed, differences among groups were then determined using the Kruskal–Wallis one way analysis of variance on ranks. Student–Newman–Keuls test was used for the post-hoc analysis. Correlations of PLP with cysteine, GSH, and antioxidant enzyme activities were determined using the Pearson correlation coefficient. Results were considered statistically significant at $p < 0.05$. Values presented in the text are means ± standard deviation (SD).

Results

Table 2 shows the PLP, homocysteine, and cysteine concentrations in plasma and liver. Mice fed the vitamin B6-deficient diet had significantly decreased plasma PLP concentrations among groups. On the contrary, plasma PLP significantly increased when the vitamin B6-deficient diet was supplemented with 14 mg/kg of vitamin B6. Although control and homocysteine groups had adequate vitamin B6 intake, plasma and liver PLP concentration significantly decreased in the homocysteine group with homocysteine added to the drinking water. Homocysteine-treated mice had significantly higher homocysteine concentration in plasma and liver when compared with mice not given homocysteine. Among the homocysteine-treated groups, mice with the vitamin B6-deficient diet had the highest homocysteine concentrations in plasma and liver when compared with those of mice with normal diet or vitamin B6-supplemented diet. Plasma cysteine concentrations were significantly reduced in the vitamin B6-deficient group when compared with other groups, but there were no significant changes in cysteine concentrations in liver.

Concentrations of the oxidative stress indicator, GSH, and its related enzyme activities are listed in Table 3. There were no significant changes in plasma MDA concentration among groups. Significantly increased hepatic MDA levels were only observed in the vitamin B6-deficient group. Among homocysteine-treated groups, mice with vitamin B6-deficient diet had the highest plasma GSH concentration and relatively lower hepatic GSH concentration. The GPx activities remained relatively stable in plasma and liver whether vitamin B6 was adequate, deficient, or supplemented.

Plasma and hepatic PLP significantly negatively correlated with plasma ($r = -0.74$, $p < 0.001$) and hepatic ($r = -0.46$, $p < 0.01$) homocysteine concentrations, respectively. Hepatic PLP significantly negatively correlated with hepatic MDA ($r = -0.35$, $p < 0.01$) levels. Plasma PLP positively correlated with plasma cysteine ($r = 0.41$, $p < 0.05$) concentration. Hepatic PLP positively correlated with hepatic cysteine ($r = 0.45$, $p < 0.01$) and hepatic GSH ($r = 0.48$, $p < 0.01$) concentration. However, plasma or hepatic PLP did not correlate with plasma or hepatic GPx and GR activities.

Discussion

A vitamin B6-deficient diet has been reported to increase plasma lipid peroxidation (TBARS) levels (1, 2, 4). Supplementation of vitamin B6 to a folic-acid-deficient diet with excess methionine prevented the elevation of oxidative stress markers (i.e. serum TBARS and advanced oxidation protein products levels) in homocysteinemic rats (18). The results of the present study were in accordance with previous findings (1, 2, 18). Homocysteine-induced oxidative stress could be moderated by an adequate vitamin B6 diet or a B6-supplemented diet but was aggravated by a vitamin B6-deficient diet.

Vitamin B6 has been shown to prevent the oxygen radical generation and lipid peroxidation caused by H_2O_2 in U937 monocytes (10) and endothelial cells (25). Keles et al., (6) evaluated lipid peroxidation and free radical scavenging activities in kidney tissue of vitamin B6-deficient rats; the results showed that levels of total and non-enzymatic superoxide scavenger activity and antioxidant potential in kidney tissue of vitamin B6-deficient rats were significantly lower than those of the control rats. A study of human indicated that lower plasma PLP was

Table 2. Vitamin B6, homocysteine, and cysteine in plasma and liver

	Control ($n = 9$)	Homocysteine ($n = 9$)	B6 deficient ($n = 9$)	B6 supplement ($n = 9$)
PLP (nmol/L)				
Plasma	243.98 ± 30.36^{b}	224.51 ± 15.83^{c}	26.43 ± 3.67^{d}	276.58 ± 16.22^{a}
Liver	6.66 ± 1.28^{a}	4.90 ± 0.90^{b}	3.73 ± 0.60^{b}	4.94 ± 1.33^{b}
Homocysteine (μmol/L)				
Plasma	3.53 ± 0.81^{c}	15.43 ± 1.93^{b}	55.41 ± 25.23^{a}	21.27 ± 4.74^{b}
Liver	1.32 ± 0.19^{d}	2.14 ± 0.21^{b}	3.08 ± 1.10^{a}	1.79 ± 0.15^{c}
Cysteine (μmol/L)				
Plasma	117.73 ± 21.34^{a}	120.42 ± 12.80^{a}	95.80 ± 10.37^{b}	112.46 ± 11.26^{a}
Liver	37.39 ± 11.36	32.01 ± 5.03	28.19 ± 3.81	27.54 ± 5.79

PLP, pyridoxal 5'-phosphate. Values with different superscript letters are significantly different among groups; $p < 0.05$.

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Table 3. Oxidative stress indicator, glutathione, and antioxidant enzyme activities in plasma and liver

	Control ($n=9$)	Homocysteine ($n=9$)	B6 deficient ($n=9$)	B6 supplement ($n=9$)
MDA (μmol/L)				
Plasma	3.53±0.64	3.43±0.43	3.50±1.13	3.40±0.46
Liver	1.40±0.47[b]	1.37±0.46[b]	3.33±1.45[a]	1.18±0.55[b]
GSH (nmol/mg protein)				
Plasma	0.56±0.20[a]	0.32±0.09[c]	0.43±0.18[b]	0.26±0.03[c]
Liver	15.39±3.15[a]	11.50±0.79[b]	9.07±1.26[c]	9.72±2.12[c]
GPx (nmol/min/mg protein)				
Plasma	21.21±2.02	20.89±3.84	21.51±1.36	22.44±2.81
Liver	332.94±43.31	299.11±47.93	315.31±18.69	348.57±55.29
GR (nmol/min/mg protein)				
Plasma	3.27±0.73[a]	2.15±0.48[b]	2.63±0.55[b]	2.33±0.77[b]
Liver	77.06±6.02[b]	76.78±5.17[b]	84.51±3.64[a]	77.90±2.50[b]

MDA, malondialdehyde; GSH, reduced glutathione; GPx, glutathione peroxidase; GR, glutathione reductase.
Values with different superscript letters are significantly different among groups; $p<0.05$.

associated with higher urinary 8-hydroxydeixyguanosine (an oxidative damage marker) concentration in older Puerto Rican adults (26), which also supports the association between lower vitamin B6 status and higher oxidative stress. In the present study, a significant relationship between vitamin B6-deficient status and higher lipid peroxidation was observed in mice livers. Although we could not demonstrate whether vitamin B6 had a free radical scavenging activity in this study, a possible direct antioxidant mechanism of vitamin B6 might be that vitamin B6 compounds have both the hydroxyl and amine group substitution on a pyridine ring which can react with the peroxy radicals and thereby scavenge radicals and inhibit lipid peroxidation (6–10).

In addition to the potential direct free radical scavenging capability of vitamin B6, vitamin B6 status might affect cysteine and GSH synthesis and be indirectly involved in the antioxidant defense system. We observed a significant reduced plasma cysteine concentration in mice with a vitamin B6-deficient diet; however, the same observation did not exist in liver. This finding may indicate that plasma cysteine concentration would be rapidly affected by vitamin B6-deficient intake, while hepatic cysteine concentration could be counterbalanced by entering from GSH pool or being kept out of the taurine pool (15, 27–30). However, animal and human studies have reported that cystathionine synthesis is more susceptible to dietary vitamin B6 restriction than cysteine concentration in the transsulfuration pathway (15, 31). Since we did not measure cystathionine concentrations in mice plasma and liver, the relationships among cystathionine, cysteine, and vitamin B6 status could not be discussed in the present study. In contrast with the changes of cysteine in plasma and liver, significantly increased plasma GSH and decreased hepatic GSH concentrations were observed in mice with a vitamin B6-deficient diet. However, previous studies of animals (13, 14) and healthy humans (15) indicated that a dietary vitamin B6 restriction increased hepatic glutathione concentrations. A possible explanation for the increased plasma GSH synthesis might be that vitamin B6 deficiency induces greater oxidative stress (1, 2, 4, 6), which may trigger the elevation of GSH transport from the liver to plasma (32, 33). It is worth noting that the ratio of plasma GSH (nmol/mg protein) to hepatic GSH (nmol/mg protein) was 4.74% in the vitamin B6-deficient group, compared to 2.78% in the control and 2.67% in the vitamin B6-supplemented group. The results of the present study seemed to support the hypothesis that the redistribution of GSH from liver to plasma could have occurred in the vitamin B6-deficient state.

Marginal vitamin B6 contents not only increased lipid peroxidation but also considerably stimulated the activity of GSH-related enzymes (2, 4, 6). In contrast to the previous findings (2, 4, 6), our results showed that GPx and GR activities were not correlated with the changes of vitamin B6 status. A previous study also indicated that the GR activities were not altered by vitamin B6 deficiency in rat tissues (12). In the antioxidant defense system, superoxide dismutase (SOD) is the first line of defense against oxygen free radicals, and it catalyzes the dismutation of the superoxide anion into hydrogen peroxide. Hepatic cytosol SOD activities of B6-deficient rats have been observed to be lower when compared to those of control rats regardless of exercise (5). Although we did not measure SOD activities, we assume that SOD would respond more quickly than GSH-related enzymes to the vitamin B6-deficient or supplemented status under homocysteine-induced oxidative stress. Further study is warranted to investigate the responses of SOD, GPx, and

GR activities to the homocysteine-induced oxidative stress under vitamin B6-deficient and supplemented status.

The data herein indicate that mice with vitamin B6-deficient intakes had a aggravate effect while mice with adequate or supplemented vitamin B6 intake had a protective effect under homocysteine-induced oxidative stress. The vitamin B6-deficient status seems to mediate the oxidative stress in connection with the redistribution of GSH from liver to plasma, but could not further affect GSH-related enzyme activities in mice with homocysteine-induced oxidative stress.

Conflict of interest and funding

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Research Article

Plasma Homocysteine Is Associated with Increased Oxidative Stress and Antioxidant Enzyme Activity in Welders

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The purpose of this study was to examine the association of vitamin B_6 status and plasma homocysteine with oxidative stress and antioxidant capacities in welders. Workers were divided into either the welding exposure group ($n = 57$) or the nonexposure controls ($n = 42$) based on whether they were employed as welders. There were no significant differences in vitamin B_6 status and plasma homocysteine concentration between the welding exposure group and the nonexposure controls. The welding exposure group had significantly higher levels of oxidized low-density lipoprotein cholesterol and lower erythrocyte glutathione concentration and superoxide dismutase (SOD) activities when compared to nonexposure controls. Plasma pyridoxal 5′-phosphate concentration did not correlate with oxidative stress indicators or antioxidant capacities in either group. However, plasma homocysteine significantly correlated with total antioxidant capacity (TAC) (partial $r_s = -0.34$, $P < 0.05$) and erythrocyte SOD activities (partial $r_s = 0.29$, $P < 0.05$) after adjusting for potential confounders in the welding exposure group. In the welding exposure group, adequate vitamin B_6 status was not associated with oxidative stress or antioxidant capacities. However, elevated plasma homocysteine seemed to be a major contributing factor to antioxidant capacities (TAC and erythrocyte SOD activities) in welders.

1. Introduction

Welders constitute a large work force in Taiwan and worldwide. Welding is a process of joining metals by melting and fusing, and this process generates fumes. The fumes contain many metals and toxic gases [1], which produce free radicals and cause lipid peroxidation [2–4] and further cause a variety of adverse health effects. Welders exposed to these metals and toxic gases during the welding process have been shown to be associated with increased oxidative stress and alterations in antioxidants or antioxidant capacities when compared to controls [5–9]. Fortunately, the human body has antioxidant enzyme and nutrient systems that protect it against free radical attacks. The main antioxidant enzymes responsible for controlling oxygen free radicals are superoxide dismutase (SOD), catalase, and glutathione-dependent enzymes [i.e., glutathione peroxidase (GPx), glutathione reductase, and glutathione *S*-transferase (GST)] [10]. In addition to antioxidant enzymes, major antioxidant nutrients include vitamin A, C, and E. Recently, the potent antioxidant ability of vitamin B_6 has been recognized [11–18].

Pyridoxal 5′-phosphate (PLP), the physiologically active coenzyme form of vitamin B_6, may play a crucial role in antioxidant mechanism. Although the exact antioxidant

mechanism has not been confirmed yet, PLP may directly scavenge radicals and inhibit lipid peroxidation [11, 16, 19–21] or may indirectly play an antioxidant role through serving as coenzymes in the glutathione antioxidant defense system. Plasma PLP serves as a coenzyme in the transsulfuration pathway of homocysteine to cysteine. Cysteine synthesized by this pathway is an important contributor to glutathione synthesis. It would then be reasonable to hypothesize that greater oxidative stress might be associated with lower vitamin B_6 status, a higher homocysteine concentration, and an impaired glutathione-dependent antioxidant defense system [22–24].

Welders are particularly susceptible to fume exposure during the welding process. In this high oxidative stress environment, the utilization and metabolic turnover of vitamin B_6 increase, and this lowers the body's pool of the vitamin. Although the effects of welding fume exposures on oxidative stress and antioxidant capacities have been studies in welders, no data on the associations of vitamin B_6 status and homocysteine with oxidative stress and antioxidant capacities have been reported. The purpose of this study was to examine the associations of vitamin B_6 status (plasma and erythrocyte PLP) and plasma homocysteine with oxidative stress and antioxidant capacities in welders.

2. Materials and Methods

2.1. Participants. This cross-sectional study enrolled workers from the industry (Changhua, Taiwan), which offers a complete line of innovative fitness products in central Taiwan. This study was approved by the Institutional Review Board of Chung Shan Medical University hospital (Taichung, Taiwan), and each participant signed the informed consent form.

Welders were recruited into the welding exposure group if they were older than 20 years and had been employed full time for at least 3 months. Welders did not work in a specific area but were involved in various welding-related processes, including formulating, mixing, loading, and welding application and were exposed to variable levels of fumes. The control participants were white-collar office workers employed in the same company who were not exposed to welding fumes, and they were assigned into the nonexposure control group. Exclusion criteria were pregnancy or lactation, illness, history of gastrointestinal disorder, cardiovascular disease, liver and renal diseases, diabetes, cancer, alcoholism, or other metabolic disease.

All subjects' age, smoking status and drinking habits, welding exposure time, and duration of employment were recorded. Body weight and height were measured; the body mass index (BMI; kg/m^2) was then calculated. Fasting venous blood specimens were collected in vacutainer tubes (Becton Dickinson, Rutherford, NJ, USA) containing EDTA as an anticoagulant or without anticoagulant and were centrifuged (2500 rpm, 15 min) to separate serum/plasma and red blood cells then analyzed immediately or stored frozen (−80°C) until analysis. Spot urine samples were collected from each participant.

2.2. Biochemical Analyses. Hematological entities (i.e., albumin, hemoglobin, creatinine, triglycerides, total cholesterol, low-density lipoprotein cholesterol (LDL), and high-density lipoprotein cholesterol) were measured by using an automated biochemical analyzer. Plasma and erythrocyte PLP were determined by high performance liquid chromatography (HPLC) according to a method described by Talwar et al. [25]. The inter- and intra-assay variabilities were 4.38% ($n = 11$) and 1.23% ($n = 5$) for plasma PLP and 7.16% ($n = 12$) and 3.33% ($n = 5$) for erythrocyte PLP, respectively. A plasma PLP level ≥ 20 nmol/L has been suggested as an indicator of adequate vitamin B_6 status [26, 27]. Plasma homocysteine was measured by using HPLC with a modified method as described previously [28]. Hyperhomocysteinemia was defined as a plasma homocysteine concentration ≥ 15 μmol/L [29]. The inter- and intra-assay variabilities of plasma homocysteine were 4.03% ($n = 8$) and 1.72% ($n = 5$), respectively. Plasma lipid peroxidation was measured as the levels of malondialdehyde (MDA) according to a modified method as described by Lapenna et al. [30]. The MDA level was measured at an excitation wavelength of 515 nm and an emission wavelength of 555 nm using a fluorescence spectrophotometer. Oxidized LDL (ox-LDL) was measured with ox-LDL ELISA kit (Mercodia AB, Uppsala, Sweden). Among the methodologies used to evaluate total antioxidant capacity (TAC), the most widely used colorimetric method for serum and plasma samples are 2′-2′-azinobis-3-ethylbenzothiazoline-6-sulfonate-based methods. Therefore, TAC was measured according to a method described by Erel [31], who developed a novel colorimetric and automated direct assay. Reduced glutathione concentration in erythrocyte was measured by using glutathione assay kit (Cayman Chemical Company, Michigan, USA). GPx catalyzes the reduction of hydroperoxides, including hydrogen peroxide, by reducing glutathione and functions to protect the cell from oxidative damage [32]. Erythrocyte GPx levels were measured by using GPx assay kit (Cayman Chemical Company, Ann Arbor, MI, USA). GST is a ubiquitous multifunctional enzyme, which plays a key role in cellular detoxification [32]. Erythrocyte GST was determined by using GST assay kit (Cayman Chemical Company, Ann Arbor, MI, USA). SOD catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide, after which the peroxide can be destroyed by catalase and GPx. SOD is an important antioxidant defense in nearly all cells exposed to oxygen. Erythrocyte SOD was determined by using SOD assay kit (Cayman Chemical Company, Ann Arbor, MI, USA). Urinary creatinine and 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-OHdG) levels were measured. Urinary 8-OHdG concentration is a biomarker of oxidative DNA damage and repair and was determined using a competitive enzyme-linked immunoassay (Genox Corporation, Baltimore, MD, USA).

2.3. Statistical Analysis. Data were analyzed using the SAS statistical software (version 9.2, Statistical Analysis System Institute, Inc., Cary, NC, USA). A sample size of 47 subjects would allow detecting at least as well significantly correlated ($r = 0.4$) between plasma PLP or homocysteine and antioxidant enzyme activities with 80% statistical power and a two-sided α level of less than 0.05. A Kolmogorov-Smirnov test was performed to test the normal distribution. Differences

in participants' demographic characteristics and biochemical values were compared for significance using student t-test or Mann-Whitney Rank Sum test between groups. Chi-square test was used for the analysis of categorical variables. Spearman correlation coefficient (r_s) was used to analyze the association of vitamin B_6 status and plasma homocysteine with oxidative stress indicators and antioxidant capacities in the welding exposure group and nonexposure controls. Spearman partial correlation coefficient (partial r_s) was further used to analyze the association of vitamin B_6 status and plasma homocysteine with oxidative stress indicators and antioxidant capacities in the welding exposure group and nonexposure controls after adjusting for age, gender, serum albumin and creatinine, smoking and drinking status, and duration of employment. Results were considered statistically significant at $P < 0.05$. Values presented in the text are means standard deviation (SD).

3. Results

Characteristics of participants are shown in Table 1. Ninety-nine subjects completed this study. Subjects' ages ranged from 20 to 61 years, with a mean age of 33.4 years. Fifty-seven welders were classified into the welding exposure group, while there were 42 white-collar office workers in the nonexposure control group. There were no significant differences in age, weight, height, BMI, serum triglycerides, total cholesterol, and LDL concentrations between the two groups. Welders had significantly higher levels of serum hemoglobin and creatinine and higher percentage of smoking and drinking habits when compared with the controls.

Table 2 shows subjects' vitamin B_6 status, homocysteine, oxidative stress, and antioxidant enzyme activities during the study. Welders and nonexposure controls had similar vitamin B_6 status (i.e., plasma and erythrocyte PLP), and none of welders or controls had deficient vitamin B_6 status (plasma PLP < 20 nmol/L). No significant differences in plasma homocysteine, MDA and TAC levels, erythrocyte GPx and GST activities and urinary 8-OHdG concentration were observed between the two groups. However, welders had significantly higher ox-LDL levels and lower erythrocyte glutathione concentrations and erythrocyte SOD activities than did nonexposure controls.

Plasma PLP strongly correlated with erythrocyte PLP in exposure welders ($r_s = 0.68$, $P < 0.001$) and nonexposure controls ($r_s = 0.57$, $P < 0.001$). The correlations between the mean of vitamin B_6 status, plasma homocysteine, oxidative stress, and antioxidant capacities in welders and nonexposure controls are shown in Table 3. Plasma and erythrocyte PLP concentrations did not correlate with oxidative stress indicators (i.e., MDA, ox-LDL, and 8-OHdG) and antioxidant capacities (TAC, SOD, GPx, and GST activities) in welders. However, plasma homocysteine significantly positively correlated with the MDA level and erythrocyte SOD activity in welders. We further adjusted potential confounders including age, gender, serum albumin and creatinine, smoking and drinking status, and duration of employment which might affect oxidative stress and antioxidant capacities; plasma homocysteine still significantly positively correlated with erythrocyte SOD activity and negatively correlated with TAC in welders (Table 4).

4. Discussion

It has been reported that welders exposed to welding fumes have higher oxidative stress and lower antioxidant capacities than that of controls [5–9]. Our welders had significantly higher ox-LDL concentrations and lower erythrocyte SOD activities when compared to controls. The finding indicates that welders are under greater oxidative stress and lower antioxidant capacity during welding process. In addition to oxidative stress indicators in plasma, we also measured urinary 8-OHdG concentration. Nuernberg et al. [9] observed that welders had a significantly higher rise in urinary 8-OHdG excretion when comparing preshift with postshift change. Urinary 8-OHdG concentration might normalize back to baseline by 24 hours from the start of the exposure [9]. Since we only collected urine sample from each subject at preshift time, this might explain why we did not observe the difference in urinary 8-OHdG concentration between welders and controls.

In the past decades, vitamin B_6 status and oxidative stress responses were mostly studied in animal models; very little data in humans have been reported. Recently, the association between higher oxidative stress and lower vitamin B_6 status has been observed in older individuals [18], which might suggest the potent antioxidant ability of vitamin B_6 in humans. Unfortunately, we did not observe the significant association of vitamin B_6 status with oxidative stress indicators and antioxidant capacities in our subjects. Since none of our welders had inadequate vitamin B_6 status (plasma PLP concentration < 20 nmol/L), other potential risk factors might affect welders' oxidative stress and antioxidant capacities. Although the exact role which the vitamin B_6 compounds play as antioxidants is not clear yet, as long as welders maintain an adequate plasma PLP concentration, their vitamin B_6 status is unlikely to affect their oxidative stress or antioxidant capacities.

In the transsulfuration pathway of homocysteine metabolism, it requires plasma PLP as a coenzyme. Plasma homocysteine concentration, therefore, might be associated with vitamin B_6 status. In addition to vitamin B_6 status, plasma homocysteine concentration was measured in our subjects. Higher oxidative stress due to higher homocysteine concentration through homocysteine oxidation has been observed [33–35]. Elevated plasma homocysteine concentration may induce excessive production of reactive oxygen species and impair the glutathione-related antioxidant defense system thus leading to greater oxidative stress and lower antioxidant enzymatic activities [22, 36–38]. Our welders with higher homocysteine concentration had increased MDA level and erythrocyte SOD activities when potential confounders were not adjusted. Since smoking may be an important cause of oxidative stress and antioxidant capacities and this is known to be potentiated by exposure to fumes/toxic gases in the work place, smoking was forced into all models as

Table 1: Demographic and clinical characteristics of participants.

Characteristics	Welding exposure ($n = 57$)	Nonexposure controls ($n = 42$)
Age (y)	33.29 ± 10.40	33.67 ± 7.46
Gender (male/female)	46/11[a]	9/33[b]
Height (cm)	165.15 ± 8.19	162.96 ± 7.49
Weight (kg)	61.94 ± 16.13	57.78 ± 12.31
Body mass index (kg/m^2)	22.64 ± 5.69	21.55 ± 3.23
Duration of employment (yr)	1.99 ± 2.03[a]	3.64 ± 3.83[b]
Welding exposure time (hr/d)	7.54 ± 3.14	—
Serum albumin (g/dL)	4.61 ± 0.24[a]	4.51 ± 0.23[b]
Serum hemoglobin (g/dL)	15.20 ± 1.22[a]	13.88 ± 1.51[b]
Serum creatinine (mg/dL)	0.92 ± 0.16[a]	0.81 ± 0.16[b]
Lipid profiles		
Triglycerides (mg/dL)	86.58 ± 55.91	72.07 ± 36.17
Total cholesterol (mg/dL)	167.60 ± 31.12	176.17 ± 28.02
High-density lipoprotein (mg/dL)	60.18 ± 12.97[a]	67.69 ± 14.54[b]
Low-density lipoprotein (mg/dL)	101.70 ± 29.14	102.95 ± 23.94
Smoking (n, %)	23 (40.35%)[a]	1 (2.38%)[b]
Drinking (n, %)	6 (10.53%)[a]	1 (2.38%)[b]

Values are means ± SD.
Values with different superscript letter are significantly different between two groups; $P < 0.05$.

Table 2: Vitamin B_6 status, oxidative stress indicators, glutathione, and antioxidant capacity.

Indicators	Welding exposure ($n = 57$)	Nonexposure controls ($n = 42$)
Vitamin B_6 status		
Plasma PLP (nmol/L)	75.48 ± 72.23	71.16 ± 91.77
<20 nmol/L (%)	0	0
Erythrocyte PLP (pmol/g Hb)	178.36 ± 191.65	238.29 ± 462.87
Plasma homocysteine (μmol/L)	12.13 ± 4.14	12.78 ± 4.75
≥15 μmol/L (n, %)	11 (19.30%)	9 (21.43%)
Oxidative stress indicators		
Malondialdehyde (μM)	0.61 ± 0.13	0.62 ± 0.11
Oxidized low-density lipoprotein (mU/L)	30329.74 ± 7507.95[a]	27521.15 ± 5891.29[b]
8-oxo-7,8-dihydro-2′-deoxyguanosine (ng/mg creatinine)	2.93 ± 1.21	3.03 ± 1.47
Antioxidant capacities		
Total antioxidant capacity (μmol/L)	4278.05 ± 322.82	4280.41 ± 246.07
Erythrocyte glutathione (μmol/g Hb)	0.65 ± 0.29[a]	0.73 ± 0.38[b]
Erythrocyte SOD (U/g Hb)	8461.28 ± 3202.72[a]	13391.24 ± 4729.43[b]
Erythrocyte GPx (nmol/min/g Hb)	61858.44 ± 15727.25	59021.55 ± 16498.69
Erythrocyte GST (nmol/min/g Hb)	10306.87 ± 6537.18	10943.85 ± 4201.24

Values are means ± SD. PLP: pyridoxal 5′-phosphate; Hb: hemoglobin; SOD: superoxide dismutase; GPx: glutathione peroxidase; GST: glutathione S-transferase.
Values with different superscript letter are significantly different between two groups; $P < 0.05$.

a likely confounder. Our welders with higher homocysteine concentration were more likely to have lower TAC and higher erythrocyte SOD activity after potential confounders were adjusted. However, elevated homocysteine concentration has been found to be associated with decreased erythrocyte SOD activities in patients with cardiovascular heart diseases [39]. In a similar vein, Wilcken et al. [40] observed a strikingly positive relationship between excellular SOD and homocysteine in patients with homocystinuria. In agreement with the results of previous studies, elevated homocysteine

Table 3: Spearman correlation of vitamin B_6 status and homocysteine with each indicator of oxidative stress and antioxidant capacity.

	Plasma PLP (nmol/L)		Erythrocyte PLP (pmol/g Hb)		Plasma homocysteine (μmol/L)	
	r_s					
	Welding exposure	Nonexposure controls	Welding exposure	Nonexposure controls	Welding exposure	Nonexposure controls
Plasma homocysteine (μmol/L)	−0.26*	−0.27	−0.51†	−0.32*	—	—
Oxidative stress indicators						
Malondialdehyde (μM)	0.14	0.15	−0.09	−0.06	0.32*	0.14
Oxidized low-density lipoprotein (mU/L)	0.04	0.13	−0.06	−0.06	0.14	−0.19
8-oxo-7,8-dihydro-2′-deoxyguanosine (ng/mg creatinine)	−0.05	0.06	−0.04	0.08	−0.14	0.09
Antioxidant capacities						
Total antioxidant capacity (μM)	0.24	−0.02	0.08	−0.15	−0.18	0.23
Erythrocyte glutathione (μmol/g Hb)	−0.04	0.04	0.11	−0.13	−0.09	0.36*
Superoxide dismutase (U/g Hb)	0.04	0.11	0.03	0.40**	0.34*	0.02
Glutathione peroxidase (nmol/min/g Hb)	−0.15	0.11	0.04	0.34*	−0.08	−0.23
Glutathione S-transferase (nmol/min/g Hb)	−0.11	−0.16	0.00	−0.15	−0.23	0.07

PLP: pyridoxal 5′-phosphate; Hb: hemoglobin. *$P < 0.05$. **$P < 0.01$. †$P < 0.001$.

Table 4: Spearman partial correlation of vitamin B_6 status and homocysteine with each indicator of oxidative stress and antioxidant capacity.

	Plasma PLP (nmol/L)		Erythrocyte PLP (pmol/g Hb)		Plasma homocysteine (μmol/L)	
	Partial r_s					
	Welding exposure	Nonexposure controls	Welding exposure	Nonexposure controls	Welding exposure	Nonexposure controls
Plasma homocysteine (μmol/L)	−0.44†	−0.38*	−0.58†	−0.18	—	—
Oxidative stress indicators						
Malondialdehyde (μM)	0.03	0.13	−0.16	0.13	0.15	−0.04
Oxidized low-density lipoprotein (mU/L)	0.04	0.25	−0.06	0.06	−0.04	−0.38*
8-oxo-7,8-dihydro-2′-deoxyguanosine (ng/mg creatinine)	−0.04	0.03	−0.08	0.22	−0.13	0.14
Antioxidant capacities						
Total antioxidant capacity (μM)	0.20	0.09	0.12	0.04	−0.34*	0.08
Erythrocyte glutathione (μmol/g Hb)	−0.09	0.14	0.04	0.15	0.04	0.19
Superoxide dismutase (U/g Hb)	−0.13	0.10	−0.05	0.41*	0.29*	0.28
Glutathione peroxidase (nmol/min/g Hb)	−0.07	0.07	0.01	0.15	0.08	−0.05
Glutathione S-transferase (nmol/min/g Hb)	−0.14	−0.18	−0.01	−0.18	−0.11	0.06

PLP: pyridoxal 5′-phosphate; Hb: hemoglobin. Adjusting for age, gender, albumin, creatinine, smoking, drinking, and duration of employment. *$P < 0.05$. **$P < 0.01$. †$P < 0.001$.

concentration may cause the release of heparan sulfate-bound extracellular SOD into the blood [41] and thus constitute a protective mechanism with the effect of combating oxidative stress [40]. This would explain why our welders simultaneously had higher homocysteine concentration and increased SOD activity. Since we have observed that our welders with higher homocysteine concentration had lower TAC status, we could not rule out the possibility that welders with higher homocysteine concentration might have lower SOD activity if their welding exposure lasts for a longer period of time. It should be pointed out that this study had a cross-sectional design, so we could only observe the relationship between homocysteine and SOD activity at one point in time. Therefore, it was not possible to discriminate the short-term and long-term effects of elevated homocysteine concentration on antioxidant enzymatic activities in welders.

There were some limitations in this study. Although we calculated the sample size to meet the statistical power criteria, a larger sample size might be needed to increase the significance of the associations between vitamin B_6 and oxidative stress indicators and antioxidant capacities. The other limitation was that this was a cross-sectional design study, so the long-term associations of vitamin B_6 and homocysteine with oxidative stress and antioxidant capacities in welders could not be assessed.

5. Conclusion

To the best of our knowledge, the present study is the first to show the associations of vitamin B_6 status and homocysteine with oxidative stress indicators and antioxidant capacities in welders. The data herein indicate that, among welders, adequate vitamin B_6 status was not associated with oxidative stress or antioxidant capacities. In addition to vitamin B_6 status, elevated plasma homocysteine seemed to be a major contributing factor in relation to decreased TAC and increased erythrocyte SOD activity in welders. Further research into the long-term association of vitamin B_6 and homocysteine concentration with oxidative stress and antioxidant enzymatic activities during welding exposure is warranted.

Conflict of Interests

All authors have no conflict of interests.

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The Metabolic Syndrome Is Associated with an Increased Risk of Colorectal Polyps Independent of Plasma Homocysteine

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Key Words
Metabolic syndrome · Homocysteine · Colorectal polyps

Abstract
Background/Aims: The links between the metabolic syndrome and homocysteine in relation to the risk of colorectal polyps are not understood. The purpose of this study was to investigate the association between the metabolic syndrome and homocysteine and further analyze the relationship between these two factors and the risk of colorectal polyps. ***Methods:*** This was a case-control study. A total of 135 participants with colorectal polyps (cases) and 110 participants without polyps (controls) were recruited. ***Results:*** There were 59 participants with the metabolic syndrome in the case group and 36 participants with the metabolic syndrome in the control group. The metabolic syndrome and its individual components, except for serum triglycerides, and homocysteine were associated with the risk of colorectal polyps. When the association of the metabolic syndrome and homocysteine with the risk of colorectal polyps was simultaneously considered, the association between homocysteine and the risk of colorectal polyps disappeared, but waist circumference, systolic and diastolic blood pressure, high-density lipoprotein cholesterol, and the metabolic syndrome itself were still significant risk factors for the development of colorectal polyps. ***Conclusion:*** Although the metabolic syndrome and plasma homocysteine were individually related to the risk of colorectal polyps, the metabolic syndrome was a major contributing factor in relation to the risk of colorectal polyps independent of plasma homocysteine.

Introduction

Colorectal polyps are classified as hyperplastic or adenomatous based on the histologic type and are considered precursors of most colorectal cancers, especially adenomatous polyps [1–3]. A high-fat diet, low vegetable consumption and some lifestyle habits may contribute to the development of colorectal polyps [4–7]. The metabolic syndrome, a cluster of central obesity, hypertension, dyslipidemia, and impaired glucose tolerance, has more recently been associated with the recurrence of colorectal adenomas [8] and has been considered an independent risk factor for colorectal cancer [9–12] and polyps [13–15]. Not only the metabolic syndrome itself but also its individual components, such as overweight status and dyslipidemia, were associated with the risk of colorectal

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polyps [16–18]. Although the exact pathogenetic involvement of the metabolic syndrome in the development of colorectal polyps is unknown, the metabolic syndrome may play a role in this development.

Homocysteine is a well-known risk factor for cardiovascular diseases. In the last decade, much attention has also been paid to the relationship between high plasma homocysteine levels and the risk of colorectal polyps [19–22]. Besides the many known risk factors (i.e. age, enzyme deficiencies and mutations, vitamin deficiencies, disease, and drugs) for developing hyperhomocysteinemia, the metabolic syndrome has been recently reported to be associated with increased plasma homocysteine concentrations [23–25]. In contrast with the results reported in some previous studies indicating that the metabolic syndrome or its components could increase homocysteine concentrations [23–25], a lack of a correlation between metabolic syndrome components and increased homocysteine concentrations was observed in other studies [26–28]. Since there is no consistent evidence to demonstrate the association between plasma homocysteine and the metabolic syndrome, further study is thus warranted.

Although the metabolic syndrome and homocysteine have both individually been shown to be potential risk factors for the development of colorectal polyps, the links between the metabolic syndrome and the plasma homocysteine level in relation to the risk of colorectal polyps are not well understood. Thus, the purpose of this study was to investigate the association between the metabolic syndrome and homocysteine and further analyze the relationship between these two factors and the risk of colorectal polyps.

Methods

Study Design and Subjects

This study was a case-control design. The sample size of 124 subjects was based on detecting significantly correlated ($r = 0.25$) between homocysteine and the metabolic syndrome itself with 80% statistical power and a two-sided α level <0.05.

Participants were recruited from the Gastroenterology Clinic at Chung Shan Medical University Hospital and the Health Management Center of Taichung Veterans General Hospital. Each participant was referred for colonoscopy. Eligible case participants were those identified by colonoscopy as having at least one or more histologically confirmed colorectal adenomatous or hyperplastic polyps. Healthy controls were those who were identified by colonoscopy as having no colorectal polyps. Participants were excluded if they were pregnant, lactating, had a history of colorectal cancer, attenuated adenomatous polyposis coli or inflammatory bowel disease, or were taking any medication which could influence homocysteine, folate and vitamin B_6 and B_{12} status, such as H_2 blockers, proton pump inhibitors, metformin, phenytoin, or methotrexate. Informed consent was obtained from each participant. The study was jointly approved by the Institutional Review Board of Chung Shan Medical University Hospital and Taichung Veterans General Hospital.

Data Collection and Measurements

Participants' age, gender, height, weight, waist circumference (WC), smoking status, alcohol consumption, and current medication uses were recorded. BMI was calculated from height and weight measurements. Blood pressure [systolic and diastolic blood pressure (SBP and DBP)] was measured after a resting period of at least 5 min.

The metabolic syndrome was defined according to the criteria of the Health Promotion Administration, Ministry of Health and Welfare, Taiwan (2007). Participants were assigned to the metabolic syndrome subgroup if they fulfilled 3 or more of the following criteria: (1) central obesity with WC ≥90 cm in men or ≥80 cm in women; (2) elevated blood pressure ≥130/85 mm Hg or use of antihypertensive agents; (3) fasting plasma glucose ≥100 mg/dl or use of diabetic medications; (4) triglycerides ≥150 mg/dl or treatment for lowering triglycerides, and (5) high-density lipoprotein cholesterol (HDL) <40 mg/dl in men or <50 mg/dl in women.

Fasting venous blood samples were drawn and collected in vacutainer tubes (Becton Dickinson, Rutherford, N.J., USA) containing an appropriate anticoagulant or no anticoagulant as required for hematological (i.e. serum creatinine, glucose, total cholesterol, HDL, and triglycerides) and biochemical (i.e. plasma homocysteine) measurements. Hematological entities were measured using an automated biochemical analyzer. Plasma homocysteine was quantified by high-performance liquid chromatography using fluorescence detection according to the method of Araki and Sako [29]. The interassay variability for plasma homocysteine was 7.62% ($n = 15$).

Statistical Analyses

Data were analyzed using the SAS statistical software package (version 9.2, Statistical Analysis System Institute Inc., Cary, N.C., USA). A Kolmogorov-Smirnov test was performed to test the normal distribution. Demographic characteristics and biochemical data were compared for significance using the Student's t test or the Mann-Whitney rank sum test between and within groups. The χ^2 test was used for the analysis of categorical variables. The Spearman correlation coefficient was used to analyze the association between the components of the metabolic syndrome and plasma homocysteine. A multiple logistic regression analysis was used to evaluate the linear association between each component of the metabolic syndrome, plasma homocysteine and the risk of colorectal polyps while not adjusting or adjusting for potential confounders. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. Statistical results were considered to be significant at $p < 0.05$. Values presented in the text are means and standard deviations.

Results

Table 1 shows the demographic characteristics of all participants. 245 participants were recruited in this study; 110 participants with either adenomatous ($n = 70$) or hyperplastic polyps ($n = 40$; case group) and 135 healthy

Table 1. Characteristics of the participants in the case and control groups

Characteristics	Controls (n =135)		Cases (n = 110)	
	non-MS (n = 99)	MS (n = 36)	non-MS (n = 51)	MS (n = 59)
Age, years	45.5±9.8[a]	52.1±10.5	49.9±9.6[a, b]	55.0±8.1
Female/male, n	60/39	14/22	20/31	13/46
Height, cm	164.0±8.7	164.5±9.9	164.9±7.0	165.8±8.0
Weight, kg	60.7±11.0[a]	73.3±12.5	64.8±9.7[a, b]	72.5±10.9
BMI	22.5±3.1[a]	27.0±3.6	23.8±3.1[a, b]	26.3±2.7
Creatinine, mg/dl	0.8±0.2[a]	0.9±0.2	0.8±0.2[a, b]	1.0±0.2
Drinking, yes/no[1]	23/73	10/26	10/31	15/38
Smoking, yes/no[1]	11/85	8/28	11/30	14/36
Colorectal polyps, n				
Adenomatous	–	–	30 (58.8%)	40 (67.8%)
Hyperplastic	–	–	21 (41.2%)	19 (32.2%)

Values are means ± standard deviation. [a] $p < 0.05$: significant difference between the non-metabolic syndrome and metabolic syndrome subgroups within the case and control groups; [b] $p < 0.05$: significant difference between the case and control groups within the non-metabolic syndrome and metabolic syndrome groups. MS = Metabolic syndrome.

[1] There were missing data on smoking and drinking habits.

Table 2. Homocysteine concentration and metabolic syndrome components in the case and control groups

Characteristics	Controls (n = 135)		Cases (n = 110)	
	non-MS (n = 99)	MS (n = 36)	non-MS (n = 51)	MS (n = 59)
Homocysteine, μmol/l	11.2±4.3	11.9±2.9	12.8±6.6[b]	14.0±6.1
Metabolic syndrome components				
WC, cm	75.4±9.3[a]	89.6±9.7	81.4±9.5[a, b]	92.0±9.5
Blood pressure, mm Hg				
Systolic	111.0±14.2[a]	129.9±14.8	119.6±11.9[a, b]	144.0±22.4
Diastolic	71.6±9.9[a]	83.0±13.3	76.6±10.6[a, b]	92.8±14.3
Glucose, mg/dl	85.5±12.2[a]	107.8±39.0	89.5±10.1[a, b]	115.8±32.2
HDL, mg/dl	63.3±16.1	47.0±10.7[a]	54.7±11.6[b]	43.8±13.9[a]
Triglyceride, mg/dl	97.3±45.2[a]	222.4±181.0	119.3±70.7[a]	206.9±225.6

Values are means ± standard deviation. [a] $p < 0.05$: significant difference between the non-metabolic syndrome and metabolic syndrome subgroups within the case and control groups; [b] $p < 0.05$: significant difference between the case and control groups within the non-metabolic syndrome and metabolic syndrome groups. MS = Metabolic syndrome.

participants without colorectal polyps (control group) were identified. Of the 110 cases and 135 controls, 59 participants (53.6%) in the case group and 36 participants (26.7%) in the control group were identified as having the metabolic syndrome. Participants with the metabolic syndrome were older and heavier and had significantly higher BMI and serum creatinine values than those without the metabolic syndrome in the two groups.

Table 2 lists the values of plasma homocysteine and the components of the metabolic syndrome. There were no significant differences in the plasma homocysteine concentration between the non-metabolic syndrome and metabolic syndrome subgroups within the case and control groups. Case participants without the metabolic syndrome had significantly higher plasma homocysteine concentrations than control participants without the

Table 3. Spearman correlation (r_s) of homocysteine with each component of the metabolic syndrome in three groups

Characteristics	Homocysteine, μmol/l		
	controls (n = 135)	cases (n = 110)	pooled (n = 245)
Waist circumference (cm)	0.29***	0.25**	0.34***
SBP (mm Hg)	0.29***	0.21*	0.31***
DBP (mm Hg)	0.24**	0.21*	0.29***
Glucose (mg/dl)	0.11	0.06	0.14*
HDL (mg/dl)	–0.34***	–0.31**	–0.37***
Triglyceride (mg/dl)	0.21*	0.04	0.17**
Metabolic syndrome (yes/no)	0.17*	0.15	0.20**

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table 4. The ORs for the risk of colorectal polyps

Characteristics	OR	95% CI	p
Unadjusted logistic regression			
Homocysteine (μmol/l)	1.10	1.03–1.17	0.004
WC (cm)	1.07	1.04–1.09	<0.001
SBP (mm Hg)	1.05	1.03–1.07	<0.001
DBP (mm Hg)	1.06	1.04–1.09	<0.001
Blood glucose (mg/dl)	1.02	1.01–1.03	0.001
HDL (mg/dl)	0.95	0.94–0.97	<0.001
Triglyceride (mg/dl)	1.00	1.00–1.00	0.087
Metabolic syndrome (yes/no)	3.18	1.86–5.43	<0.001
Age-, gender-, BMI- and serum creatinine-adjusted logistic regression			
Homocysteine (μmol/l) +	1.08	0.98–1.19	0.115
WC (cm)	1.08	1.02–1.14	0.011
Homocysteine (μmol/l) +	1.08	0.98–1.18	0.142
SBP (mm Hg)	1.04	1.02–1.06	<0.001
Homocysteine (μmol/l) +	1.09	0.99–1.20	0.087
DBP (mm Hg)	1.05	1.02–1.07	<0.001
Homocysteine (μmol/l) +	1.08	0.99–1.19	0.095
Blood glucose (mg/dl)	1.01	1.00–1.02	0.204
Homocysteine (μmol/l) +	1.07	0.98–1.18	0.148
HDL (mg/dl)	0.96	0.94–0.99	0.003
Homocysteine (μmol/l) +	1.08	0.99–1.19	0.092
Triglyceride (mg/dl)	1.00	1.00–1.01	0.330
Homocysteine (μmol/l) +	1.08	0.99–1.19	0.094
Metabolic syndrome (yes/no)	2.10	1.01–4.37	0.048

metabolic syndrome. As for the components of the metabolic syndrome, it was not surprising that participants with the metabolic syndrome had significantly higher WC, blood pressure, serum glucose, HDL, and triglyceride levels than participants without the metabolic syndrome within the case and control groups. Case participants without the metabolic syndrome had significantly higher WC, blood pressure and serum glucose but lower HDL levels when compared to control participants without the metabolic syndrome.

To determine whether the metabolic syndrome and its individual components were associated with plasma homocysteine, the correlation coefficients were calculated (table 3). Plasma homocysteine concentration correlated significantly with each component of the metabolic syndrome and the metabolic syndrome itself in the pooled (case plus control) group. Plasma homocysteine correlated significantly with WC, SBP, DBP, and HDL levels in cases and controls. To determine the association of the metabolic syndrome, its individual components and plasma homocysteine in relation to the risk of colorectal polyps, a logistic regression model was performed (table 4). Homocysteine, WC, SBP, DBP, glucose, HDL, and the metabolic syndrome itself, but not serum triglycerides, were associated with the risk of colorectal polyps. When the association of the metabolic syndrome, its individual components and plasma homocysteine with the risk of colorectal polyps was simultaneously considered, the association between homocysteine and the risk of colorectal polyps disappeared. However, WC (OR 1.08, 95% CI 1.02–1.14), SBP (OR 1.04, 95% CI 1.02–1.06), DBP (OR 1.05, 95% CI 1.02–1.07), HDL (OR 0.96, 95% CI 0.94–0.99), and the metabolic syndrome itself (OR 2.10, 95% CI 1.01–4.37) were still significant risk factors for the development of colorectal polyps after adjusting for plasma homocysteine and other potential confounders.

Discussion

The relationship between the metabolic syndrome and homocysteine is intriguing but controversial. Although the present study did not attempt to investigate the mechanisms between homocysteine and the metabolic syndrome, hyperhomocysteinemia has been proposed to cause insulin resistance [30–32], hypertension [33–36] and dyslipidemia [37–39], which are the underlying pathophysiological features of the metabolic syndrome. Björck et al. [30] reported that homocysteine was dependent on serum insulin, providing a possible link between the metabolic syndrome and increased plasma homocysteine concentration. An elevated plasma homocysteine concentration may lead to insulin resistance through the

inhibition of insulin receptor kinase activity [31, 32]. Furthermore, an increased plasma homocysteine concentration induces oxidative injury of vascular endothelial cells, arteriolar constriction and renal dysfunction, diminishes vasodilation by nitric oxide and increases sodium reabsorption and arteriolar stiffness, which can cause hypertension [33–36]. A relationship between hyperhomocysteinemia and dyslipidemia has been established [37–40]. Increased homocysteine levels might stimulate the oxidation of low-density lipoprotein [39] and decrease activities of hepatic thiolase and serum lecithin cholesterol acyltransferase, two important enzymes involved in the HDL metabolism [40]. In agreement with some previous studies [23–25], the results for our control and pooled, but not case, participants supported the implication that the metabolic syndrome and its components are associated with increased plasma homocysteine concentrations. However, other studies did not support this implication [26–28].

A systematic review and meta-analysis indicated there was a significant association between the presence of the metabolic syndrome and the risk of development of colorectal neoplasms [13]. Several mechanisms have been proposed regarding the effect of the metabolic syndrome on the risk of colorectal adenomas, including obesity [41], oxidative stress [42], inflammation [43], and insulin resistance [44, 45]. In support of results reported in previous studies [13–18], the present study showed that the metabolic syndrome itself and its individual components (i.e. higher WC, SBP, DBP, serum glucose and triglyceride levels, and lower HDL concentration) significantly increased the risk of colorectal polyps. Hyperhomocysteinemia, a well-known risk factor for cardiovascular diseases, can increase the production of oxygen free radicals through homocysteine oxidation and can diminish the DNA methylation in critical tissues through a simultaneous increase in intracellular S-adenosylhomocysteine and is thus a risk factor for cancer [46, 47]. Although some previous studies did not show that high plasma homocysteine was significantly associated with the development of colorectal polyps [48, 49], the present study, in agreement with other studies [19–22], demonstrated that an increased plasma homocysteine concentration was associated with the development of colorectal polyps.

Since the metabolic syndrome, its components and increased plasma homocysteine had significant individual effects on the risk of colorectal polyps, we wanted to investigate whether the metabolic syndrome and increased plasma homocysteine concentration have a synergistic effect on the risk of colorectal polyps. Although the association of the metabolic syndrome or increased plasma homocysteine concentration with the risk of colorectal polyps was individually observed, the association of plasma homocysteine with this risk disappeared after the combined effect of the metabolic syndrome and increased plasma homocysteine was analyzed. On the other hand, the effect of the metabolic syndrome and its components on the risk of colorectal polyps still remained after adjusting for plasma homocysteine. The reasons why the metabolic syndrome and its components play a more prominent role than homocysteine in the development of colorectal polyps are not clear. Although the cutoff values required for the plasma homocysteine concentration to show a dose response between homocysteine and adenoma recurrence were 11.58 μmol/l [49] and 12.2 μmol/l [50], respectively, our participants' plasma homocysteine levels might not have been high enough to confirm an association with the risk of developing colorectal polyps when the metabolic syndrome was simultaneously considered. The other possibility might be that the metabolic syndrome is a cause of hyperhomocysteinemia and has a more important role than plasma homocysteine in mediating the process of colorectal polyp development even though we could not demonstrate the cause-effect or effect-cause of the metabolic syndrome and hyperhomocysteinemia because this was only a case-control study. Further study is warranted to investigate the link between the metabolic syndrome and homocysteine and the risk of colorectal polyp development.

There were some limitations to this study. Although we calculated the sample size to meet the statistical power criteria, a larger sample size might be needed to increase the significance of the association of the metabolic syndrome and plasma homocysteine with the risk of colorectal polyps. Another possible limitation might be that participants with adenomatous polyps or hyperplastic polyps were combined into the case group, which might cause misclassification and misjudgment. However, when we conducted a subgroup analysis stratified by types of polyps, participants with adenomatous polyps and hyperplastic polyps had comparable demographic and health characteristics as well as comparable levels of metabolic syndrome components and homocysteine (data not shown), and thus the two groups were combined to form a single colorectal polyps group to increase the statistical power. Therefore, misclassification and misjudgment would not be of concern.

The role of the metabolic syndrome and homocysteine together or independent of each other in the risk of colorectal polyps has not been fully investigated. To the best of our knowledge, the present study is the first at-

tempt to determine whether the metabolic syndrome and homocysteine exert an independent or synergistic effect in relation to the risk of colorectal polyps. The data herein indicate that the metabolic syndrome and plasma homocysteine were not only associated with each other but were also individually related to the risk of colorectal polyps. However, the metabolic syndrome was predominant and was associated with the risk of colorectal polyps independent of plasma homocysteine.

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Disclosure Statement

The authors declare that they have no competing interests.

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Original article

High serum folate might have a potential dual effect on risk of colorectal cancer

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SUMMARY

Background & aims: The possible dual role of serum folate in the development and progression of colorectal cancer (CRC) has not been well established in human studies. This study investigated the association between serum folate and the risk of CRC in subjects with CRC or colorectal adenomatous polyps (AP, a precursor of CRC), and healthy subjects.

Methods: This study has a case–control design. Two hundred and thirty-seven men and 171 women were recruited with 156 subjects in the CRC group, 70 subjects in the AP group and 182 healthy subjects in the control group.

Results: The risk of CRC was significantly increased in the third (OR, 3.46; 95% CI, 1.16–10.34) and fourth (OR, 4.86; 95% CI, 1.42–16.58) quartiles of serum folate concentration after adjusting for potential confounders among subjects with AP or CRC. Furthermore, serum folate concentration had no significant effect on the risk of CRC among subjects in the control and CRC groups.

Conclusions: Higher serum folate concentration was significantly correlated with increased CRC risk in subjects with AP, while serum folate had no effect on CRC risk in healthy controls. Serum folate might possess potential dual modulatory effects on the risk of CRC.

1. Introduction

Colorectal cancer (CRC) is the 3rd and 2nd most commonly diagnosed cancer worldwide in men and women, respectively [1]. Colorectal polyps are classified as hyperplastic or adenomatous based on the histologic type, and adenomatous polyps (AP) are considered precursors of most colorectal cancers [2,3]. Among the various nutritional factors which are related to the CRC risk, the feature of folate in the development and progression of CRC warrants attention. To investigate the association between folate status and the risk of CRC, dietary folate intake rather than serum folate concentration has been extensively studied [4]. However, there are few data on the effects of serum folate levels on the risk of CRC. Low serum folate level has been shown to be a significant risk factor for the risk of CRC [5,6], while other studies indicated that low serum folate level had a protective effect [7,8] or no effect on the risk of CRC [9,10]. Further studies are therefore warranted to verify the relationship between serum folate concentration and CRC risk.

Folate is an essential cofactor for the de novo biosynthesis of purines and thymidylate; therefore, folate deficiency may cause a defect in DNA synthesis in tissue with rapidly replicating cells. This may explain why low serum folate concentration increased the risk of CRC [6,8]. However, folate might possess dual modulatory effects on CRC development and progression; folate deficiency may inhibit while folate supplementation may promote the progression of established colorectal neoplasms [11–15]. Studies indicated that tumor growth might be inhibited by ineffective DNA synthesis resulting from folate deficiency [13,16,17]. Although the dual modulatory effect of folate on colorectal carcinogenesis has been established in animal studies [18–20], the possible dual feature of serum folate in CRC development and progression has not been well described in human studies. Thus, the study presented here

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investigated the association of serum folate with the risk of CRC in subjects with CRC or AP (a precursor of CRC), and healthy subjects.

2. Subjects and methods

2.1. Subjects

Consecutive patients were recruited from the division of colorectal surgery of Taichung Veterans General Hospital, Taiwan if they were confirmed to have either colon or rectal cancer (International Classification of Diseases 9, codes 153 and 154, respectively) (CRC group). Patients were excluded if they were pregnant, lactating, received chemotherapy, had history of cardiovascular disease, liver or renal diseases, diabetes, alcoholism, attenuated adenomatous polyposis coli or inflammatory bowel disease, or were taking any medication which could influence homocysteine and folate status. Two study oncologists reviewed patients' medical records for diagnostic confirmation and staging. Healthy subjects were recruited from the health management center of Taichung Veterans General Hospital, Taiwan. Each subject was referred for colonoscopy. If subjects were identified by colonoscopy as having at least one or more histologically confirmed colorectal AP, they were assigned to the AP group; otherwise, they were allocated to the control group. Subjects in the AP and control groups were excluded when they had history of gastrointestinal disorder, cardiovascular diseases, liver or renal diseases, diabetes, cancer, alcoholism, or other metabolic diseases. Each subject signed the informed consent. The Institutional Review Board of Taichung Veterans General Hospital approved this study.

2.2. Data collection and biochemical measurements

All subjects' age, gender, height, weight, smoking and drinking habits and use of medications were recorded. Subjects' height and weight were used to calculate their body mass index (BMI, kg/m^2). Systolic and diastolic blood pressure (SBP and DBP) was measured after a resting period of at least 5 min.

Each subject's fasting venous blood samples were drawn in vacutainer tubes containing an appropriate anticoagulant or no anticoagulant. Serum or plasma was separated within 30 min after blood was collected and then frozen (−80 °C) until analysis. Serum creatinine was assessed using an automated biochemical analyzer. Samples of plasma homocysteine and PLP were prepared under yellow light to avoid photodestruction and measured by high performance liquid chromatography as previously described [21,22]. Serum folate was analyzed using standard competitive immunochemiluminometric methods on a Chiron Diagnostics ACS:180 Automated Chemiluminescence System (Chiron Diagnostics Corporation, USA).

The genomic DNA purification kit (Blood total DNA auto kit, TANBead, Taiwan) was used to extract DNA from frozen peripheral blood lymphocytes. The 5, 10-methylenetetrahydrofolate reductase (MTHFR) 677C→T gene polymorphism was amplified by polymerase chain reaction. The amplified DNA fragment (198 bp) was then digested by the *Hin*FI restriction enzyme (New England BioLabs, Ipswich, MA) and subsequent electrophoresis in a 3% agarose gel [23].

2.3. Statistical analyses

The SAS statistical software package (version 9.3; Statistical Analysis System Institute Inc., Cary, NC, USA) was used for all data analyses. Demographic characteristics and biochemical data were compared for significance using one-way analysis of variance or Kruskal–Wallis test. Chi-square or Fisher's exact tests were used for the analysis of categorical variables. Adjusted odds ratios (OR) with 95% confidence intervals (CI) for CRC risk were calculated from unconditional logistic regression models using serum folate and the quartiles of serum folate concentration, based on the distribution of the AP and CRC subjects or the control and CRC subjects. Statistical significance was defined as a two-sided $p < 0.05$.

3. Results

Table 1 shows subjects' demographic and health characteristics. Two hundred and thirty-seven men and 171 women were in this study with 156 subjects in the CRC group, 70 subjects in the AP group and 182 subjects in the control group. Subjects' ages ranged from 30 to 79 years with a mean age of 53.4 ± 11.0 y. No significant differences were observed in gender, BMI, plasma PLP concentrations, and distribution of the three variants of the MTHFR 677C→T genotypes among the 3 groups. Subjects in the CRC group had significantly older age, higher SBP and plasma homocysteine levels compared with subjects in the other two groups. A significantly higher serum folate level was observed in CRC subjects when compared to AP and control subjects. The distribution of genotypes among the 3 groups of subjects was conformed with the Hardy–Weinberg equilibrium.

We calculated the risks of CRC according to the distribution of subjects in the AP and CRC groups (Table 2) and subjects in the control and CRC groups (Table 3). Increased plasma homocysteine and serum folate had significant positive effects on the risk of CRC among subjects in the AP and CRC groups with or without adjusting for confounders (Table 2). We then further calculated the OR using the quartiles of serum folate based on the distribution of AP and CRC subjects. The CRC risk was significantly increased in the third (serum folate concentration, 13.55–23.61 ng/mL; OR, 3.46; 95% CI, 1.16–10.34) and fourth (serum folate concentration > 23.61 ng/mL; OR, 4.86; 95% CI, 1.42–16.58) quartiles of serum folate concentration following adjustment for age, gender, BMI, SBP, serum creatinine concentration, smoking and drinking habits, MTHFR 677C→T mutation, and plasma homocysteine and PLP concentrations (Table 2). Interestingly, serum folate concentration had no effect on the risk of CRC among subjects in the control and CRC groups (Table 3). However, plasma homocysteine was still a significant risk factor for CRC after adjusting for potential confounders among subjects in the control and CRC groups (Table 3).

Table 1
Demographic and health characteristics of subjects.[a]

Parameters	CRC ($n = 156$)	AP ($n = 70$)	Control ($n = 182$)
Age (y)	59.12 ± 10.92a	52.73 ± 9.5 1c	48.75 ± 9.24b
Male/Female	94/62	52/18	91/91
BMI (kg/m^2)	24.13 ± 3.25	25.07 ± 3.23	24.09 ± 3.74
Blood pressure			
SBP (mmHg)	140.37 ± 17.62a	135.25 ± 24.46b	118.12 ± 16.98c
DBP (mmHg)	82.71 ± 11.29a	85.54 ± 15.6 1a	75.78 ± 12.18b
Serum creatinine (mg/dL)	0.84 ± 0.31b	0.92 ± 0.22a	0.81 ± 0.20b
Plasma homocysteine (μmol/L)	15.27 ± 5.61a	13.28 ± 4.94b	11.63 ± 4.97c
Serum folate (ng/mL)	19.78 ± 12.72a	13.29 ± 9.14b	15.27 ± 8.31b
Plasma PLP (nmol/L)	91.51 ± 85.93	103.43 ± 95.98	84.47 ± 77.39
MTHFR 677C→T (n) CC/CT/TT	84/64/8	44/26/0	91/73/18
Smoking habit (n, %)	25, 16.03%	24, 34.29%	34, 18.68%
Drinking habit (n, %)	13, 8.33%	23, 32.86%	48, 26.37%

CRC, colorectal cancer; AP, adenomatous polyps. *MTHFR*, methylenetetrahydrofolate reductase; BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure.
Values in a row with different superscript letters are significantly different, $p < 0.05$.
[a] Values are means ± standard deviation.

Table 2
Adjusted odds ratios of colorectal cancer according to the distribution of subjects in the adenomatous polyps and colorectal cancer groups.[a]

	No factors adjusted for			Additional factors adjusted for[b]			Additional factors adjusted for[c]		
	OR	95% CI	p	OR	95% CI	p	OR	95% CI	p
Plasma homocysteine (μmol/L)	1.09	1.02–1.16	0.01	1.13	1.03–1.24	0.01	1.16	1.05–1.27	<0.01
MTHFR 677C→T genotypes									
CC	1	–	–	1	–	–	1	–	–
T-allele[c]	1.45	0.81–2.59	0.21	1.52	0.76–3.04	0.23	1.58	0.76–3.29	0.23
Plasma PLP (nmol/L)	1.00	1.00–1.00	0.36	1.00	0.99–1.00	0.22	1.00	0.99–1.00	0.16
Serum folate (ng/mL)	1.06	1.03–1.09	<0.01	1.04	1.00–1.08	0.03	1.07	1.02–1.11	<0.01
Serum folate (ng/mL)									
≤8.86	1			1			1		
8.87–13.54	1.12	0.53–2.36	0.76	0.91	0.36–2.32	0.85	1.21	0.45–3.31	0.71
13.55–23.61	2.64	1.18–5.95	0.02	2.14	0.79–5.76	0.13	3.46	1.16–10.34	0.03
>23.61	4.08	1.69–9.88	<0.01	2.09	0.73–5.98	0.17	4.86	1.42–16.58	0.01

CI, 95% confidence interval; OR, odds ratio; MTHFR, methylenetetrahydrofolate reductase; PLP, pyridoxal 5′-phosphate.
[a] n = 226 (n = 156 in the colorectal cancer group plus n = 70 in the adenomatous polyps group). Subjects in the adenomatous polyps group were considered to be the reference group.
[b] Adjusted for age, gender, body mass index, systolic blood pressure, serum creatinine concentration, smoking and drinking habits.
[c] Additional adjustment for genotypes [CC = 0; T-allele carrier (677CT plus 677 TT genotypes) = 1], homocysteine, pyridoxal 5′-phosphate or folate.

4. Discussion

An important finding in this study was that serum folate had a different effect on subjects' risk of CRC that was dependent on whether they were with or without AP. Higher serum folate concentration (≥13.55 ng/mL) might promote the transition from AP to CRC, while serum folate levels had no effect on the CRC risk in healthy subjects without colorectal AP. This finding is inconsistent with the previous study which indicated that subjects in the highest quartile of serum folate had half the risk of CRC than that of subjects in the lowest quartile [6]. A nested case control study within the Physicians' Health study also indicated that subjects with plasma folate concentration less than 3 ng/mL (as folate deficiency) had a marginally significant increase in CRC risk when compared to subjects with adequate folate level [5]. However, subject's plasma folate concentration in the highest quintile was positively associated with CRC risk than did those subjects in the lowest quintile of plasma folate concentration when subjects were followed up for the median of 4.2 years (multivariate OR, 3.87; 95% CI, 1.52–9.87) [7]. Similarly, a lower CRC risk was observed in subjects having lower plasma folate concentrations in 3 large prospective studies [8]. The mechanisms underlying the correlation of serum folate levels and CRC risk in subjects with different physiological statuses require further elucidation.

The relationship between folate and CRC risk seemed to be critically affected by the dose and timing of folate administration [11–13,15,19]. In an Aspirin-Folate Polyp Prevention study [24], the results indicated that subjects with a recent history of adenomas could not be benefited by taking folic acid supplementation (1 mg/d) to reduce their adenoma recurrence. Previous animal studies have suggested that moderate dietary folate deficiency may promote the development and progression of CRC before the occurrence of neoplastic foci in the intestine, while folic acid supplementation may suppress this process [25,26]. It is therefore reasonable to speculate that high serum folate concentration may also promote the transition from AP to CRC in human studies. As such, high folate intake or high dose of folic acid supplementation in subjects with AP may be inadvisable.

Folic acid fortification of foods is compulsory in more than 50 countries including the United States and Canada, but many Asian countries including Taiwan have yet to implement this policy. However, the high dietary folate intake has been promoted by public media in Taiwan to prevent neutral tube defect, decrease plasma homocysteine level, and further to decrease the risk of cardiovascular disease. Although we did not assess subjects' folate intake, some of our subjects might have taken folic acid supplements regularly. This may possibly explain why serum folate concentrations were considerably higher in our subjects compared with data reported in previous studies [5–7]. However, the synthetic form of folic acid supplementation could not be completely metabolized in plasma and has been suggested to decrease the cytotoxicity of natural killer cells when folic acid supplementation is in excess of 400 μg [27]. The consequence of a high amount of daily folic acid supplementation is a concern, especially for subjects who are potentially in the pre-stage of colorectal carcinogenesis.

The present investigation was not a cohort study and therefore the cause–effect relationship between serum folate and CRC risk could not be established. However, one of the strengths of this study was that subjects with AP were compared with a control group comprising healthy subjects. Another strength of this study was that it was possible to control for a number of covariates. The association between serum folate concentrations and CRC risk has been considered to be less compatible than that between dietary folate intake and CRC risk [14,16], because plasma homocysteine is regard as a more sensitive indicator of cellular folate depletion than serum folate levels [28] and plasma homocysteine might be a more sensitive marker of CRC risk [29–31]. In the present study, plasma homocysteine was also significantly associated with CRC risk. In addition to plasma homocysteine, genetic polymorphisms may affect folate metabolism. The folate-dependent enzyme MTHFR catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. The MTHFR 677C→T mutation [32,33] has been shown to correlate well with risk of CRC, although a significant association was not found in the present study. In addition, there was no association between plasma PLP and CRC risk in the present study; however, plasma PLP is involved in the transsulfuration of homocysteine metabolism and was associated with CRC risk [34–36]. Thus, in the analysis of the association between serum folate and CRC risk, we adjusted plasma homocysteine and PLP concentrations, MTHFR 677C→T mutation and other various confounders (such as age, gender, BMI, SBP, serum creatinine, smoking and drinking habits).

One of the limitations of this study was that erythrocyte folate concentration was not measured. Erythrocyte folate concentration seemed to be more sensitive to tissue folate stores and could be used for a long-term folate status indicator [37]. However, plasma homocysteine was correlated more strongly with serum folate than with erythrocyte folate, and serum folate concentration seems to

Table 3
Adjusted odds ratios of colorectal cancer according to the distribution of subjects in the control and colorectal cancer groups.[a]

	No factors adjusted for			Additional factors adjusted for[b]			Additional factors adjusted for[c]		
	OR	95% CI	p	OR	95% CI	p	OR	95% CI	p
Plasma homocysteine (μmol/L)	1.18	1.11–1.25	<0.01	1.12	1.04–1.21	<0.01	1.14	1.05–1.23	<0.01
MTHFR 677C→T genotypes									
CC	1	–	–	1	–	–	1	–	–
T-allele[c]	0.84	0.55–1.29	0.43	1.04	0.58–1.86	0.91	1.05	0.57–1.94	0.87
Plasma PLP (nmol/L)	1.00	1.00–1.00	0.43	1.00	1.00–1.00	1.00	1.00	1.00–1.00	0.72
Serum folate (ng/mL)	1.04	34.34–1.06	<0.01	1.01	0.98–1.05	0.37	1.03	1.00–1.07	0.09
Serum folate (ng/mL)									
≤9.65	1			1			1		
9.66–13.66	0.62	0.33–1.15	0.13	0.49	0.20–1.23	0.13	0.64	0.24–1.67	0.36
13.67–21.88	0.93	0.51–1.70	0.81	0.62	0.26–1.52	0.30	0.88	0.34–2.28	0.78
>21.88	2.23	1.20–4.13	0.01	1.17	0.44–3.12	0.76	1.74	0.61–4.95	0.30

CI, 95% confidence interval; OR, odds ratio; MTHFR, methylenetetrahydrofolate reductase; PLP, pyridoxal 5′-phosphate.
[a] $n = 338$ ($n = 156$ in the colorectal cancer group plus $n = 182$ in the control group). Subjects in the control group were considered to be the reference group.
[b] Adjusted for age, gender, body mass index, systolic blood pressure, serum creatinine concentration, smoking and drinking habits.
[c] Additional adjustment for genotypes [CC = 0; T-allele carrier (677CT plus 677 TT genotypes) = 1], homocysteine, pyridoxal 5′-phosphate or folate.

adequately explain the association with CRC risk in an epidemiological study [38]. Another limitation was that dietary folate intake and folic acid supplement use were not assessed and thus it was not possible to estimate optimal folate intake for subjects with AP.

5. Conclusion

Higher serum folate concentration (≥13.55 ng/mL) appeared to be associated with increased CRC risk in subjects with AP while serum folate had no effect on CRC risk in healthy controls. We speculate that serum folate might play a dual role with respect to risk of CRC.

Statement of authorship

FFC assisted with the study design, was in charge the screening and recruitment of subjects and interpreted the results. SCH performed statistical analyses, interpreted the results and critically revised the manuscript. HMW screened and recruited subjects. FPC analyzed biochemical measurements and performed data coding. YCH developed intellectual content and the study design, interpreted the results and wrote the manuscript drafting. All authors read and approved the final manuscript.

Conflict of interest

All authors have no conflict of interests.

Acknowledgments

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科技部補助專題研究計畫出席國際學術會議心得報告

日期：104 年 4 月 15 日

計畫編號	NSC 101－2320－B－040－016 － MY3		
計畫名稱	維生素 B-6 與氧化壓力及抗氧化能力相關性之研究 (第三年)		
出國人員 姓名	黃怡嘉	服務機構 及職稱	中山醫學大學營養系 / 教授
會議時間	2015 年 3 月 28 日 至 2015 年 4 月 1 日	會議地點	美國波士頓
會議名稱	Experimental Biology 2015		
發表題目	Low serum folate is associated with increased risk of chronic kidney disease independently of oxidative stress and antioxidant capacities (poster)		

一、參加會議經過

2015 年 Experimental Biology 會議於 3 月 28－4 月 1 日在美國波士頓召開。Experimental Biology 是一年一度由美國的 6 個學會（解剖、生理、生化、病理、藥理及營養學會）共同召開的科學性國際會議。此次因獲得科技部專題研究計畫（NSC 101-2320-B-040-016-MY3）補助出席國際學術會議，計畫主持人榮幸的能參與此次的國際研討會（圖一），與營養及醫學等相關領域學者齊聚一堂，分享彼此研究心得及聆聽大會邀請的國際著名講者精湛的演說。

因為計畫主持人的專長及研究是在營養相關的領域，且本身也是美國營養學會（American Society for Nutrition）的會員（會員編號#30314），因此主要是參與美國營養學會所舉辦的 symposium 以及 mini-symposium。每天議程以 symposium 揭開序幕，邀請在其研究領域中的佼佼者做其專題演講，內容包括：Creating the Future of Evidence-based Nutrition Recommendations Using Lipid Research Case Studies, Nutrition and Developmental Origins of Health and Disease, Dietary Fiber, Gut Microbiota and Kidney Function, Sweeteners and Health: What Does the Science Tell Us, An Interdisciplinary Examination of Potential Effects of Maternal Obesity on Lactation Physiology and the Human Milk Microbiome 等。下午的議程主要是從 symposium 的主題所延伸出來的多個相關的副題（表一）；另外，有全天候的 poster 展示。大會對其內容的安排非常多元、緊湊且充實，讓參與者有如置身學術研究的殿堂，透過聆聽演講與其他研究者的心得交流，讓計畫主持人獲益良多。除了美國營養學會安排的主題外，今年主辦單位還特地提供一個 session (The International Forum - Taiwan) 給我們台灣營養學會介紹台灣的營養現況及營養研究的發展及成果。在這個 session 中有

很多關注台灣營養研究的外國學者們來參與，藉由這個交流的機會促成台灣及外國營養研究學者們將來可研究合作的機會。

此次除了參加每日的演講外，也以壁報的形式（poster presentation）發表研究成果，發表的作者及標題為：Yi-Chia Huang, Cheng-Hsu Chen, Yu-Hua Hsiao, Wen-Ching Yang. Low Serum Folate is Associated with Increased Risk of Chronic Kidney Disease Independently of Oxidative Stress and Antioxidant Capacities. FASEB J 2015. 在展示的過程中有許多位的國外學者提出他們對我們的研究結果的問題及見解，並展開熱烈的討論，事後並且互留聯絡方式，期待將來也許有國際合作的機會。

二、與會心得

Experimental Biology，為每年在美國不同城市所舉行的國際聯合會議，此會議共有 6 個學會一同參與，除了自己本身所參與的學會外，也可以同時參與其他學會所舉辦的 symposium 或 mini-symposium，是一個非常大型且多元的國際研討會。此次非常榮幸能獲得科技部專題計畫的出席國際會議的差旅費用補助，讓計畫主持人可以至美國波士頓參與此次的學術盛會，有幸能與食品、營養與生化等相關領域學者齊聚一堂，共同聆聽台上講者精湛的演講並參與討論，不僅增加與國外學術研究學者的交流，也開拓對自己的研究深度及廣度，真是不虛此行。

三、發表論文全文或摘要

Low Serum Folate is Associated with Increased Risk of Chronic Kidney Disease Independently of Oxidative Stress and Antioxidant Capacities

Yi-Chia Huang[1], Cheng-Hsu Chen[2], Yu-Hua Hsiao[1], Wen-Ching Yang[3]. [1]School of Nutrition, Chung Shan Medical University, [2]Division of Nephrology, Taichung Veterans General Hospital; Department of Internal Medicine, Chiayi Branch, Taichung Veterans General Hospital, [3]Department of Food and Nutrition, Taichung Veterans General Hospital, Taichung, Taiwan.

Increased oxidative stress and decreased antioxidant defense function may cause later cancer development in chronic kidney disease (CKD) patients. Folate involves in DNA synthesis and its deficiency may cause cancer development. However, the associations of folate with oxidative stress and antioxidant capacities in CKD patients are unclear. The purpose of this study was to determine the association of folate with oxidative stress indicators and antioxidant capacities, and to further analyze their relationships with respect to risk for CKD. Ninety-three CKD patients and 135 healthy subjects were recruited. Patients with CKD had significantly higher levels of malondialdehyde and total antioxidant capacities, but had significantly lower activities of glutathione peroxidase, glutathione *S*-transferase and superoxide dismutase when compared to healthy subjects. There were no any significant associations of folate with oxidative stress indicators and antioxidant capacities in CKD patients and healthy subjects. Subjects with lower serum folate concentration exhibited significantly increased risk of CKD with (OR, 0.87; 95% CI, 0.76 – 0.99) or without (OR, 0.93; 95% CI, 0.90 – 0.97) adjustment for potential confounders. Decreased folate was strongly associated with the risk of CKD independently of oxidative stress indicators and antioxidant capacities.

四、攜回資料名稱及內容

表一：美國營養學會的大會議程

American Society for Nutrition at Experimental Biology 2015 – Boston, MA

All sessions listed are in the Boston Convention & Exhibition Center unless otherwise noted.

	SATURDAY, March 28, 2015				SUNDAY, March 29, 2015			
	8:00 – 10:00 AM	10:30 AM – 12:30 PM	12:45 – 2:45 PM	3:00 – 5:00 PM	8:00 – 10:00 AM	10:30 AM – 12:30 PM	12:45 – 2:45 PM	3:00 – 5:00 PM
Ballroom East	**Creating the Future of Evidence-based Nutrition Recommendations Using Lipid Research Case Studies (ILSI)** *K.H. Rubin and J.T. Dwyer*			**5:00 – 7:00 PM Emerging Leaders in Nutrition Science Poster Competition**	**Translational and Transformational Concepts in Amino Acid Sensing** *S. Hutson and T.G. Anthony*	**Presidential Symposium: Nutrition and Developmental Origins of Health and Disease** *S.K. Meydani*		**Human Variability & Physiological Extremes: Blessing or Curse?** *S.H. Adams and D.B. Allison*
157 ABC		**Maternal/Fetal Interactions: Looking at Various Animal Models** *G.M. Hill and T.A. Davis*		**Nutritional Approaches for Osteosarcopenic Obesity: Interrelationships between Bone, Muscle and Fat** *B.H. Arjmandi and C. Castaneda-Sceppa*	**Determinants of Disease Risk in the Postprandial Period** *B.O. Schneeman and T.M. Rains*			**Do We Need Preconception Nutrition Interventions to Improve Birth Outcomes Beyond the Prevention of Neural Tube Defects?** *U. Ramakrishnan and J.C. King*
Education Track Room 151 AB	**8:00 AM – 9:30AM Clinical Emerging Leaders Award Competition**		**11:30 – 1:00 PM ASN Young Minority Investigator Oral Competition**	**1:30 – 4:30 PM Graduate Student Research Award Competition**	**The eNutrition Academy: Supporting a New Generation of Nutritional Scientists Around the World** *S.M. Donovan and C.A. Geissler*		**NOF/ASN Statement: Peak Bone Mass Development and Lifestyle Factors**	***International Forum-Korea***
152				Global Nutrition: Understanding and Predicting Program Impact	EMM: Energy Balance, Macronutrients and Weight Management			EMM: Protein and Amino Acid Metabolism
153A		**9:30 AM – 11:30 PM The Postdoctoral Research Award Competition**			DBC: Mechanism of Action and Molecular Targets of Dietary Bioactive Components I			DBC: Mechanisms of Action and Molecular Targets of Dietary Bioactive Components II
153B					Nutr. Epi: Epidemiological Research Addressing Diet and Health Outcomes			Nutr. Epi: Innovation and Validation of Dietary Assessment Tools and Their Applications
153C	Global Nutrition: Nutrition and Cognitive and Neurological Outcomes	Global Nutrition: Recent Insights into Growth and Growth Monitoring			Comm Pub Hlth Nutr: Community and Public Health Nutrition Interventions			Comm Pub Hlth Nutr: Food Environment
154					Lactation: Biology of Lactation Including Bioactive Components and Other Milk Constituents and Their Effect on the Infant			Lactation: Lactation and Maternal and Infant Health
156A		Carotenoids and Retenoids in Human Health			Vit Min: Micronutrient Bioavailability and Antioxidant Function			Vit Min: B Vitamins and One-Carbon Metabolism
156B					Animal: Animal Models of Fetal Nutrition, Programming and Neonatal Development			Nutr Edu: Childhood Obesity Prevention
156C					Nutr Trans: Nutrition Science for Public Policy, Practice and the Consumer			Animal: Comparative Animal Nutrition and Physiology

This overview includes sessions programmed by ASN's Scientific Program Committee.
View ASN's *Society Highlights* and *Guest Society Highlights* in the onsite program for Council, RIS and other activities.

American Society for Nutrition at Experimental Biology 2015 – Boston, MA

All sessions listed are in the Boston Convention & Exhibition Center unless otherwise noted.

	MONDAY, March 30, 2015				TUESDAY, March 31, 2015			
	8:00 – 10:00 AM	10:30 AM – 12:30 PM	1:45 – 2:45 PM	3:00 – 5:00 PM	8:00 – 10:00 AM	10:30 AM – 12:30 PM	12:45 – 2:45 PM	3:00 – 5:00 PM
Ballroom East	**Dietary Fiber, Gut Microbiota and Kidney Function** *C.J. Ketchum and C. Pelkman*	**Low-Calorie Sweeteners and Health: What Does the Science Tell Us?** *D. Greenberg and J.D. Fernstrom*	**G.A. Leveille Lecture** **1:45-2:45 PM**	**Gut Microbes and the Brain: What is the Effect on Human Behavior?** *E. Mayer and W. A. Walker*	**Diet and Immunometabolism** *S. R.Shaikh and L. Coleman*	**An Interdisciplinary Examination of Potential Effects of Maternal Obesity on Lactation Physiology and the Human Milk Microbiome** *C. Lovelady and L. Nommsen-Rivers*	***W.O. Atwater Lecture*** ***12:45-1:45 PM***	**Pediatric Neurocognitive Development: Emerging Insights and Applications in Nutrition** *M. Alonso-Alonso and E. C. Radlowski*
157 ABC	**Integrated Nutrition and Early Child Development Interventions: Preventing Health and Economic Disparities** *K. Hurley and S. Fernandez-Rao*	**Research Reporting in the 21 Century: How it is Different and Why You Should Care** *N. Fukagawa*		**Approaches to Account for the Effects of Inflammation on Nutrient Biomarkers: Nutrition Determinants of Anemia (BRINDA) Project** *R. Flores and D. Raiten*	**Is "When" We Eat as Important as "What" We Eat? - Chronobiological Aspects of Food Intake** *L. Sanders and C.E. O'Neil*	**Food and Nutrition Board Update: New Directions in Food, Nutrition and Population Health** *A. Yaktine and S. P. Murphy*		**Improving Cardio-Skeletal Health by Exploring the Heart - Bone Connection** *C. M. Weaver*
151 AB	**Being "Social": How Scientists Can Find their Way in a 24-Hour Digital World** *M.A. Johnson and V. Vieira-Potter*	***International Forum- ICAN/South America***			***International Forum- China***	**Establishing Yourself as an Expert** *E. Clappio and M. N. Henderson*	***International Forum- Taiwan***	**History of Nutrition: The Long Road Leading to the Dietary Reference Intakes** *A. Yaktine and F.A. Nielsen*
152	EMM: Lipid and Fatty Acid Metabolism and Transport	EMM: Obesity and the Metabolic Syndrome		EMM: Protein Intake and Health Implications	EMM: Carbohydrate Metabolism	EMM: Metabolic Phenotyping, Metabolomics and Biomarkers		EMM: Dietary Fatty Acids and Health
153A	DBC: Bioavailability, Metabolism, and Biomarkers of Dietary Bioactive Components	DBC: Dietary Bioactive Components and Markers of Chronic Disease: Human Intervention Studies		DBC: Effects of Dietary Bioactive Components in Animal Models of Chronic Disease Risk	DBC: Dietary Bioactive Compounds of Medicinal, Functional, and Fermented Foods	DBC: Antioxidant and Anti-Inflammatory Effects of Dietary Bioactive Components		DBC: Effects of Dietary Bioactive Components in Animal Models of Obesity and Cardiometabolic Risk
153B	Nutr. Epi Research with Dietary Supplements and Bioactive Components	Nutr. Epi: Nutrition and Chronic Disease Epidemiology		Nutr. Epi: Advancing Nutritional Epidemiology with Public Use and Commercial Data Sets	Nutr. Epi: Epidemiologic Methods in Examining Health Outcomes in Diverse Populations	Global Nutrition: Understanding Pathways to Intervention Impact		Global Nutrition: Recent Advances in Biomarker Development and Use
153C	Comm Pub Hlth Nutr: Health Disparities and Promoting Health in Diverse Populations	Comm Pub Hlth Nutr: Food Security and Its Connections to Nutrition and Health		Comm Pub Hlth Nutr: Policy and Systems Approaches in Community and Public Health Nutrition	Comm Pub Hlth Nutr: Food Environment II	Nutrition Across the Lifespan (Co-sponsor: Aging, Obesity and Nutrition Translation)		Nutrition Across the Lifespan: Early Childhood Nutrition
154	Nutr Immunology: Nutrition, Immunity and Infection	MNC: Nutrition and the Microbiome		MNC: Interventions for the Treatment and Prevention of Nutrition-Related Diseases	Aging: Risk Factor Modification for Healthy Aging	MNC: Nutrition and Inflammation		Nutrient-Gene Interactions: Obesity and Inflammation
156A	Vit Min: Water and Fat Soluble Vitamins and Chronic Disease	Vit Min: Micronutrient Interventions		Nutrient-Gene Interactions: Genomics, Proteomics, and Metabolomics	Nutrition Policies, Programs, and Public-Private Partnerships	Diet and Cancer: Fat versus Fiber in Colon Cancer: Opposite in the End?		Diet and Cancer: Uncovering the Role of Diet in Cancer Prevention: Population Based Studies
156B	Obesity: Chronic Diseases	Nutr Edu: Nutrition Education and Behavior Change		Obesity: Childhood Obesity Management	Obesity: Gut Microbiome and Obesity	Nutr Edu: Developing Healthy Eating and Physical Activity Behaviors Across the Lifespan		
156C		Animal: Animal Models of Nutrition and Intestinal Disease						

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American Society for Nutrition at Experimental Biology 2015 – Boston, MA

All sessions listed are in the Boston Convention & Exhibition Center unless otherwise noted.

	WEDNESDAY, April 1, 2015	
	8:00 – 10:00 AM	10:30 AM – 12:30 PM
Ballroom East	**Resistant Starch, Microbiota and Gut Health** *D.F. Birt*	**Moderate Alcohol Use, Nutrition and Chronic Diseases: What We Know and Where to Go Next** *N. Parekhand L. M. Troy*
157 ABC	**What's New in Natural Products Analysis? Cutting-edge Methods and Available Resources for Nutrition Research** *J. M. Harnly and B.C. Sorkin*	**"One Nutrition": Clinical Nutrition Across Species** *L.M. Freeman and S.N. Meydani*

ASN Satellite Programs: *Satellite programs are planned and conducted by external groups in conjunction with the American Society for Nutrition's Scientific Sessions and Annual Meeting.*

Friday		**Whole-Milk Dairy Foods in Nutrition and Health: An Evaluation of the Current State of the Science** Organized and sponsored by the Dairy Research Institute 9:30 am – 12:00 pm Renaissance Boston Waterfront Hotel, Pacific Grand Ballroom ABCD
	Science and Policy: Adopting a Fruitful Vegetable Encounter for Our Children Organized by the Alliance for Potato Research and Education 1:00 – 5:00 pm Room: Renaissance Boston Waterfront Hotel, Pacific Grand Ballroom ABCD	**Neural - Physiologic Mechanisms Regulating Sodium Appetite** Sponsored and organized by the Grocery Manufacturers Association 1:00 – 5:00 pm Room: Renaissance Boston Waterfront Hotel, Pacific Grand Ballroom EFG
Saturday	**Sweeteners and Health: Current Understandings, Recent Research Findings and Directions for Future Research** Organized and sponsored by the Rippe Lifestyle Institute 6:30 – 8:00 am Room: 153 AB	**Body Water Regulation: Vasopressin as New Predictor of Disease Risk?** Organized and sponsored by Danone Nutricia Research 6:30 – 8:00 am Room: Boston Convention Center & Exhibit Center, 156 BC
	Current Perspective on Protein Quality and Practical Implications for Policy Sponsored by DuPont Nutrition and Health 12:45 – 2:45 pm Room: 153 AB	**Yogurt in Nutrition: The Role of Yogurt in Weight Management** Organized and sponsored by the Danone Institute International and ASN 12:45 – 2:45 pm Room: Boston Convention Center & Exhibit Center, 156 BC
Sunday	**Oats. More Than Just a Whole Grain** Organized and sponsored by PepsiCo Global R&D Nutrition 6:30 – 8:00 am Room: Renaissance Boston Waterfront Hotel, Pacific Grand Ballroom E	**Running on Empty: Is There a Metabolic or Cognitive Benefit to the Morning Meal?** Organized and sponsored by the Kellogg Company 6:30 – 8:00 am Room: Renaissance Boston Waterfront Hotel, Pacific Grand Ballroom ABCD
Monday	**A Healthy Approach to Dietary Fat Consumption: Understanding the Science and Taking Action to Clear Up Consumer Confusion** Organized and sponsored by the Haas Avocado Board 6:30 – 8:00 am Room: Renaissance Boston Waterfront Hotel, Pacific Grand Ballroom D	**Breakfast Bioactives** Organized and sponsored by PepsiCo 6:30 – 8:00 am Room: Boston Convention Center & Exhibit Center, Pacific Ballroom E
Tuesday	**Pairing Nuts and Dried Fruit for Cardiometabolic Health** Organized and sponsored by the Sun-Maid Growers of California and American Pistachio Growers 6:30 – 8:00 am Room: TBD	**Smart Snacking: When Science Meets Nutrition** Organized and sponsored by PepsiCo 6:30 – 8:00 am Room: Renaissance Boston Waterfront Hotel, Atlantic Ballroom 123
Wednesday	**Moving Towards a More Effective National Food and Nutrition Policy: Balancing the Role of Research, Nutrition Science and Public Health** Organized and sponsored by the Corn Refiners Association 6:30 – 8:00 am Room: Boston Convention Center & Exhibit Center, 151 AB	
	Phenotypic Flexibility Organized and sponsored by Nutritech, a European Commission-funded Project 9:00 am – 12:30 pm Room: Boston Convention Center & Exhibit Center, 151 AB	

American Society for Nutrition at Experimental Biology 2015 – Boston, MA

All sessions listed are in the Boston Convention & Exhibition Center unless otherwise noted.

Sunday Posters:

- Global Nutrition: Anthropometry
- Lactation: Relationships of Maternal Diet and Health to Lactation Performance and Infant Health
- Lactation: Predictors of Lactation Initiation and Duration; and Interventions to Improve Lactation Success
- Lactation: Bioactive Components and Other Milk Constituents and Their Effect on the Infant
- Lactation: Biology of Lactogenesis, Lactation and Milk Composition
- Comm Pub Hlth Nutr: Community and Public Health Nutrition Interventions
- Comm Pub Hlth Nutr: Food Security and Its Connections to Nutrition and Health
- Nutr. Epi.: Research with Dietary Supplements and Bioactive Components
- Nutr. Epi.: Advancing Nutritional Epidemiology with Public Use and Commercial Data Sets
- Aging: Risk Factor Modification for Cardiometabolic Health and Chronic Disease
- Aging: Nutritional Needs and Assessment in Older Adults/Populations
- Nutrition Across the Lifespan: Nutrition and Reproductive Health -
- MNC: Personalized Nutrition
- MNC: Prenatal Nutrient Programming in Humans
- Nutr Immunology: Nutritional Immunology
- EMM: Energy Balance, Macronutrients and Weight Management
- EMM: Obesity and the Metabolic Syndrome (Co-sponsor: Obesity RIS)
- EMM: Carbohydrate Metabolism
- EMM: Diet and/or Exercise Regulation of Food Intake (Co-sponsor: Obesity RIS)
- EMM: Dietary Fatty Acids and Health
- EMM: Protein Intake and Health Implications
- Obesity: Diet, Devices, Medications and Surgery
- Obesity: Gut Microbiome and Obesity
- Obesity: Chronic Diseases
- CARIG: Carotenoids and Health/ Carotenoids & Retinoids Molecular Mechanisms of Action
- CARIG: Bioavailability and Metabolism of Carotenoids and Vitamin A
- CARIG: Biofortification of Staple Crops with Micronutrients
- DBC: Bioavailability, Metabolism and Biomarkers of Dietary Bioactive Components
- DBC: Mechanisms of Action and Molecular Targets of Dietary Bioactive Components
- DBC: Effects of Dietary Bioactive Components on Experimental Models of Chronic Disease Risk

Monday Posters:

- Global Nutrition: Micronutrients
- Nutrition and Sustainability
- Nutr Edu: Childhood Obesity Prevention
- Nutr Edu: Developing Healthy Eating and Physical Activity Behaviors Across the Lifespan
- Sports Nutr: Nutrition, Dietary Supplements and Human Performance
- Dairy and Yogurt: Health and Nutrition Effects
- Nutr. Epi.: Epidemiologic Methods in Examining Health Outcomes in Diverse Populations (Co-sponsor: Minority and Diversity Affairs Committee)
- Nutr. Epi.: Epidemiological Research Addressing Diet and Health Outcomes
- Aging: Nutrition and Sarcopenia
- Aging: Nutrition and Osteoporosis
- Nutr Trans: Nutrition Science for Public Policy, Practice and the Consumer
- Nutr Trans: Food Science and Technology in Nutrition
- Nutrition Policies and Programs: Implementation, Evaluation, and Monitoring
- EMM: Protein and Amino Acid Metabolism
- EMM: Lipid and Fatty Acid Metabolism and Transport
- EMM: Energy and Macronutrient Metabolism and the Gut
- EMM: Metabolic Phenotyping, Metabolomics and Biomarkers
- Obesity: Macronutrients and Obesity
- Obesity: Body Composition
- Nutrient-Gene Interactions: Genomics, Proteomics, and Metabolomics
- Nutrient-Gene Interactions: Epigenetics
- Nutrient-Gene Interactions: Obesity and Inflammation
- Nutrient-Gene Interactions: Biomarkers
- Diet and Cancer: Molecular Targets
- Diet and Cancer: Animal Studies
- Exp Animal Nutr: Animal Models of Fetal Nutrition, Programming and Neonatal Development
- Exp Animal Nutr: Animal Models of Nutrition and Disease
- Exp Animal Nutr: Animal Models for Nutrition across Physiological States
- Nutr Immunology: Nutrition and Infection
- Vit Min: Water and Fat Soluble Vitamins and Chronic Disease
- Vit Min: Selenium
- Vit Min: Micronutrient Bioavailability and Antioxidant Function
- Vit Min: Zinc

Tuesday Posters:

- Global Nutrition: Diets and foods
- Global Nutrition: Cognitive
- Nutrition Across the Lifespan: Nutrition, Neurobiology, Mood and Behavior (Co-sponsor: Aging, Obesity and Nutrition Translation)
- Nutrition Across the Lifespan: Early Childhood Nutrition
- Comm Pub Hlth Nutr: Health Disparities and Promoting Health in Diverse Populations
- Comm Pub Hlth Nutr: Food Environment
- Comm Pub Hlth Nutr: Policy and Systems Approaches in Community and Public Health Nutrition
- Nutr. Epi.: Innovation and Validation of Dietary Assessment Tools and Their Applications
- Nutr. Epi.: Nutrition and Chronic Disease Epidemiology
- Edu: Nutrition Education in Medical and Other Professional Schools
- Edu: Nutrition Education for Practicing Clinicians
- Edu: Innovations in Undergraduate, Graduate and Medical Nutrition Education
- Nutr Edu: Evidence-Based Nutrition Education: Development, Testing, and Evaluation
- Nutr Edu: Nutrition Education and Behavior Change
- MNC: Interventions for the Treatment and Prevention of Nutrition-Related Diseases
- MNC: Nutrition and Inflammation
- MNC: Nutrition and the Microbiome
- Nutrient-Gene Interactions: Chronic Disease
- Nutrient-Gene Interactions: Exercise
- Nutrient-Gene Interactions: Bioactives
- Diet and Cancer: Clinical and Human Studies
- Vit Min: B Vitamins and One-Carbon Metabolism
- Vit Min: Micronutrient Interventions
- Vit Min: Trace Element Transport and Homeostasis in Health and Disease
- DBC: Antioxidant and Anti-inflammatory Effects of Dietary Bioactive Components
- DBC: Cardiovascular Effects of Dietary Bioactive Components
- DBC: Dietary Bioactive Components of Medicinal, Functional and Whole Foods (including Probiotics and Fermented Foods)

Posters will be displayed 8:30 AM – 5:00 PM Sunday – Tuesday. Authors MUST be present by their boards during their designated time between 12:45 PM – 2:45 PM.
Late Breaking posters will be displayed Wednesday, April 1, 2015 starting at 8:30 AM. Authors MUST be present by their boards 10:00 AM – 11:00 AM.
Late Breaking Posters will include: Variability in Responses to Diet and Food, Nutrition on Healthy Growth, Development and Reproduction, Disease Prevention, Progression and Treatment, Nutrition-Related Behaviors, Food Supply and Environment, Vitamins, Minerals, and Bioactives, Energy and Nutrient Metabolism, and Community, Public Health and Global Nutrition

表二：

C410 I **758.10** Decreases in Circulating Uncarboxylated Osteocalcin Are Not Associated with HOMA-IR Changes in Humans **A.J. Centi, K. Shea, C.M. Gundberg, E. Saltzman and S.L. Booth.** USDA at Tufts Univ. and Yale Sch. of Med.

C411 II **758.11** Effect of Phylloquinone Supplementations on Inflammatory Markers in High-Fat Diet-Induced Obese Mice **C. Sohn and M. Kim.** Wonkwang Univ., South Korea.

C412 I **758.12** Serum 25-Hydroxyvitamin-D and Prostate Cancer U-Shaped Risk Curves: Does Latitude Play A Role? A Meta-Analysis of Case-Control Studies **T.S. Moreira-Lucas, T.M.S. Wolever and R. Vieth.** Univ. of Toronto.

C413 II **758.13** Biofortification of Eggs and Pork with Vitamin D as a Means of Increasing Dietary Supply **K.D. Cashman, S. Duffy, A. Hayes, K. Seamans, J. Kerry, A. Kelly, J. Jakobsen and J. O'Doherty.** University Col. Cork; University Col. Dublin and Tech Univ. of Denmark.

C414 I **758.14** Low Serum Folate Is Associated with Increased Risk of Chronic Kidney Disease Independently of Oxidative Stress and Antioxidant Capacities **Y-C. Huang, C-H. Chen, Y-H. Hsiao and W-C. Yang.** Chung Shan Med. Univ.; Chiayi Branch, Taichung Veterans Gen. Hosp. and Taichung Veterans Gen. Hosp., Taiwan.

C415 II **758.15** Whole Egg Consumption Completely Prevents Vitamin D Deficiency in Type 2 Diabetic Rats **S.K. Jones and K.L. Schalinske.** Iowa State Univ.

759. VITAMINS AND MINERALS: SELENIUM

Poster

(*Sponsored by:* Vitamins and Minerals RIS)

MON. 7:30 AM—BOSTON CONVENTION & EXHIBITION CENTER, EXHIBIT HALLS A & B

Presentation time: 12:45 PM–1:45 PM (I); 1:45 PM–2:45 PM (II)

C416 I **759.1** Potential Role of the 15kDA Selenoprotein in Colorectal Inflammation **P.A. Tsuji, B.A. Carlson, J.A. Canter, C. Onyewu, C.V. Saylor, R. Tobe, H.E. Seifried, Y. Yu, L. Cao, V.N. Gladyshev, C.D. Davis and D.L. Hatfield.** Towson Univ.; NCI, NIH; Brigham and Women's Hosp., Harvard Med. Sch. and ODS, NIH.

C417 II **759.2** Conditional Effect of Selenium on the Mammalian Hind Gut Microbiota **D. Taussig and G.F. Combs, Jr.** USDA, Grand Forks.

C418 I **759.3** Knockout of Gpx1 Exerted Differential Impacts on Responses of Lipid Metabolism-Related Genes to High Dietary Se Intake between Liver and Adipose Tissue **X. Lei, Z. Zhao and J. Kim.** Cornell Univ.

C419 II **759.4** Selenium Status Biomarkers and Selenium Requirements of Chickens (*G. gallus*) **J. Li and R.A. Sunde.** Univ. of Wisconsin-Madison and Northeast Agr. Univ., Harbin, China.

C420 I **759.5** Dietary Products Consumption in Relation to Serum 25-Hydroxyvitamin D and Selenium Level in Saudi Children and Adults **M. Alokail, N. Al-Daghri, O. Al-Attas, S. Yakout, N. Aljohani and H. Alfawaz.** King Saud Univ. and King Saud bin Abdulaziz Univ. for Hlth. Sci.

C421 II **759.6** Disease Associated Variations in Glutathione Peroxidase-1 Affect Its Subcellular Localization and Function **D.N. Ekoue, S. Bera, F. Weinberg, K.A. Fricano, M. Mao, M.G. Bonini and A.M. Diamond.** Col. of Med., Univ. of Illinois at Chicago.

C422 I **759.7** Modulation of Intestinal Microbiota by Dietary Selenium and 15kDa Selenoprotein Expression in Inflammatory Colon Cancer **J.A. Canter, S. Sheckells, C.V. Saylor, A. Patterson, B.A. Carlson, V.N. Gladyshev, Y. Yu, L. Cao, M. May, C.D. Davis, D.L. Hatfield and P.A. Tsuji.** Towson Univ; NCI, NIH; Brigham and Women's Hosp., Harvard Med. Sch.; NCI. NIH; Univ. of New England and ODS, NIH.

C423 II **759.8** Effect of Dietary Bioactive Compounds on Thioredoxin Reductase 1 and 15kDa Selenoprotein Expression in Colon Cancer Cells **L.E. Rosso, S.E. Galinn, B.A. Carlson, R. Tobe, S. Naranjo-Suarez and P.A. Tsuji.** Towson Univ.; NCI, NIH and Johns Hopkins Univ.

C424 I **759.9** The 15kDa Selenoprotein Expression May Be Regulated through the AhR Pathway **S.E. Galinn, L.E. Rosso, B.A. Carlson, R. Tobe, S. Naranjo-Suarez and P.A. Tsuji.** Towson Univ; NCI, NIH and Johns Hopkins Univ.

C425 II **759.10** Effect of Long-Term Dietary Selenium Deprivation and Aging on Gut Microbiota in Short Telomere Mice **H-Y. Lu, R. Wu and W-H. Cheng.** Mississippi State Univ. and Univ. of Maryland College Park.

C426 I **759.11** Selenium Surveillance: A New Option for China to Examine Selenium Contents in Major Sources of the Chinese Diet **L-p. Liu, X-w. Li, N-n. Zhang and S. Du.** Beijing Ctr. for Dis. Prevent. and Control; China Natl. Ctr. for Food Safety Risk Assessment, Beijing and Univ. of North Carolina at Chapel Hill.

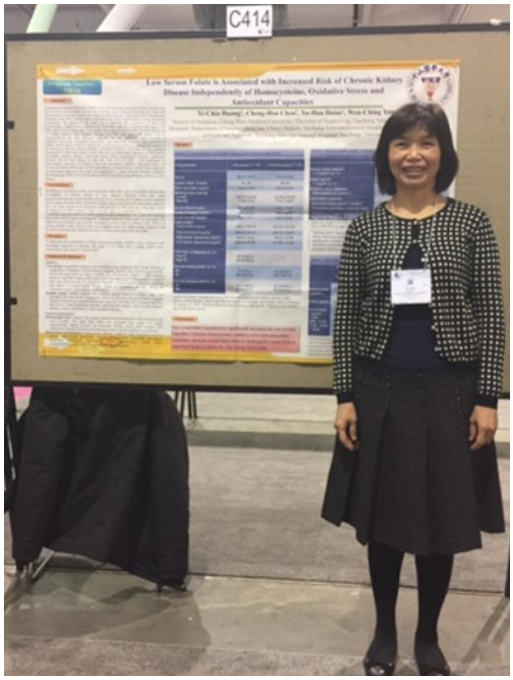
C427 II **759.12** Roles of Nutritional Selenium in Mouse Aging and Age-Related Degenerations **R.T. Wu, L. Cao and W-H. Cheng.** Univ. of Maryland College Park and Mississippi State Univ.

C428 I **759.13** Effect of Long-Term Dietary Selenium Deprivation and Aging on Selenoprotein Transcriptome in Short Telomere Mice **L. Cao, R.T.Y. Wu and W-H. Cheng.** Mississippi State Univ. and Univ. of Maryland College Park.

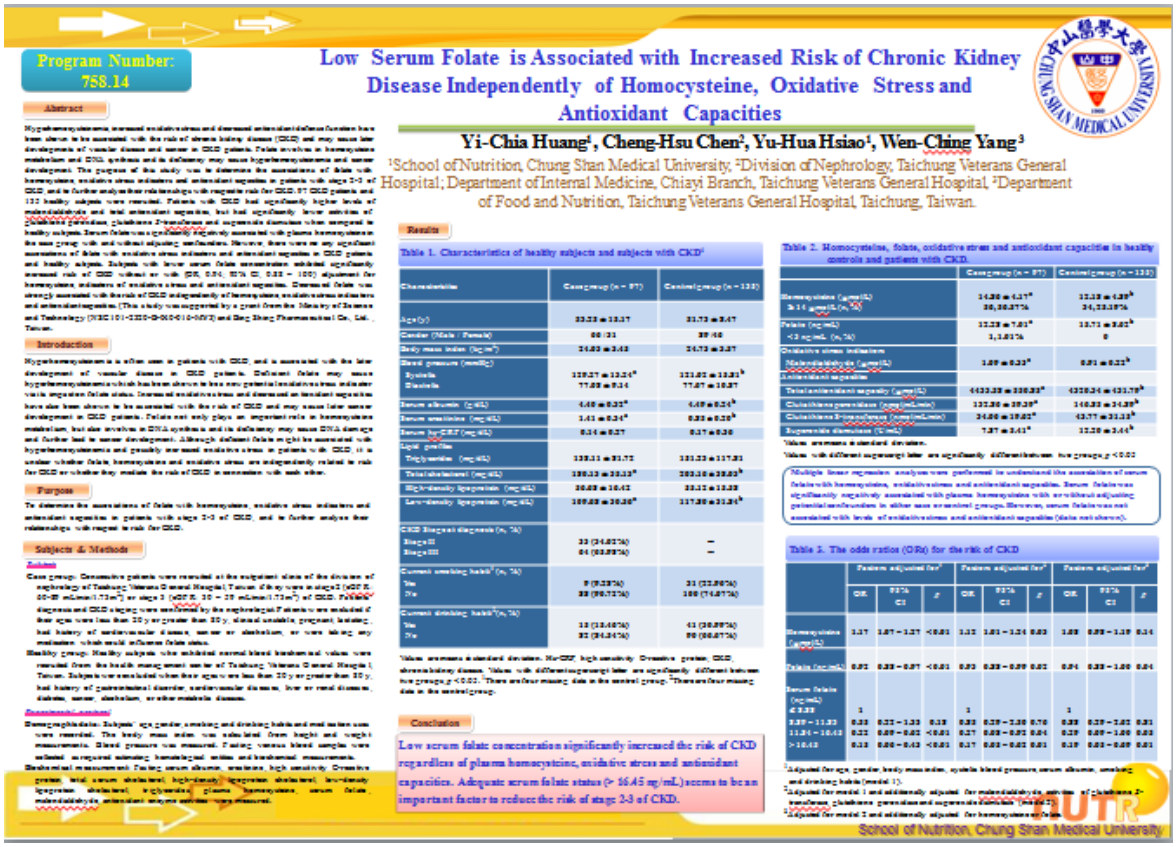
C429 II **759.14** Selenium and Soy Modulate Different Processes in Tumor Progression in Transgenic Adenocarcinoma of Mouse Prostate Mice **L.A. Spencer, B.H. Bahme, J.K. Worden, P.M. Urie and M.J. Christensen.** Brigham Young Univ. and Utah Valley Reg. Med. Ctr., Provo.

C430 I **759.15** Selenium Status in Cognitively Impaired Korean Elderly Using INAA-Method **O. Lee, S.Y. Moon, S-U. Lee, J.H. Moon and Y.S. Chung.** Yongin Univ.; Sch. of Med., Ajou Univ.; Jungsun Clin., South Korea and Korea Atomic Energy Res. Inst., Daejeon.

圖一：研討會會場



圖二：壁報發表



圖三：壁報內容

五、其他

1. 研討會邀請函

March 28 – April 1, 2015
Boston, MA

Annual Meeting of:

American Association of Anatomists (AAA)

The American Physiological Society (APS)

American Society for Biochemistry and Molecular Biology (ASBMB)

American Society for Investigative Pathology (ASIP)

American Society for Nutrition (ASN)

American Society for Pharmacology and Experimental Therapeutics (ASPET)

Guest Societies

Future Meetings

San Diego, CA
April 2 – 6, 2016

San Francisco, CA
April 22 – 26, 2017

San Diego, CA
April 21 – 25, 2018

Orlando, FL
April 6 – 10, 2019

San Diego, CA
April 4 – 8, 2020

February 05, 2015

Yi-Chia Huang
CHUNG SHAN MEDICAL UNIVERSITY
No. 110, Sec. 1, Jianguo N. Rd
Taichung
TAIWAN

Dear Yi-Chia Huang:

We would like to extend to you an invitation to attend and participate in the Experimental Biology 2015 Annual Meeting scheduled March 28 – April 1, in Boston, MA. Much thought and effort has gone into the planning and organization of this meeting to make it one of the premier scientific meetings for researchers. The scientific program will cover current topics in many areas including anatomy, biochemistry, physiology, pathology, nutrition and pharmacology. For detailed program information, please visit our website at www.experimentalbiology.org.

As part of U.S. security procedures, applications for visas are being sent to the State Department where they are reviewed. The website for the State Department is http://travel.state.gov. We advise scientists traveling to the United States to apply for a visa as early as possible (**at least three months before visa is needed**). Because of the number of visas being processed and the need to be thorough with the reviews, this can take as long as 8 - 10 weeks. Please check with your local U.S. consulate or embassy to find out the earliest that you may apply.

All visitors traveling to the U.S. from visa waiver countries (i.e., Europe, Japan, Australia, etc.) must meet all requirements. For more information on the Electronic System for Travel Authorization (ESTA), as well as link to a list of visa waiver countries, please visit: http://travel.state.gov/visa/temp/without/without_1990.html.

You should begin the visa process as early as possible. If your visa is denied, you will not be issued a refund of your paid registration fee if the cancellation is received after Friday, March 2, 2015.

If you followed the abstract submission guidelines please do not wait until you receive your program confirmation before applying for your visa.

Although the meeting organizers do not have funds available to assist with your travel, housing, and registration, we hope you are able to attend. We look forward to your participation. If you have any questions or require further assistance, please contact management@experimentalbiology.org.

Sincerely,

Yvette E. Clark, CMP
Meeting Manager
FASEB Scientific Meetings and Conferences

2. 論文被接受發表之大會證明文件

EXPERIMENTAL BIOLOGY 2015
ABSTRACT CONFIRMATION OF POSTER PRESENTATION - ASN
Boston Convention and Exhibition Center – 415 Summer Street, Boston, Massachusetts 02210

The following will confirm the day, date, time, and location of your poster presentation. Please advise co-authors of the time and place of the presentation as they will not receive a separate notification. A copy of this email has been sent to your sponsor. ***Please review the important note to presenters below.***

POSTER PRESENTATION INFORMATION: (read carefully)
Abstract Number: 2313
Abstract Title: Low Serum Folate is Associated with Increased Risk of Chronic Kidney Disease Independently of Oxidative Stress and Antioxidant Capacities
First Author: Yi-Chia Huang
Poster Session Title:Vitamins and Minerals: Water and Fat Soluble Vitamins and Chronic Disease
Day of presentation:March 30, 2015
Program Number:758.14
Poster Board Number:C414
Authors must be present at their posters from*12:45 pm - 1:45 pm
Location: Boston Convention and Exhibition Center, Exhibit Hall

The early registration deadline is Monday, February 9.

IMPORTANT NOTE TO POSTER PRESENTERS
All poster presentations are scheduled in Exhibit Hall AB at the Boston Convention and Exhibition Center. Presenters must hang their posters on the appropriate poster board no later than 8:30AM on the day of presentation and posters must remain on display all day from 8:30AM – 5:00PM (except on Wednesday). Poster viewing hours are: 7:30 AM – 6:00 PM Sunday and Monday; 7:30 AM – 4:00 PM Tuesday and Wednesday. Posters must be removed at the end of the day. Presentation times for each author are listed above as well as at the beginning of the session in the daily program. Presenters are expected to stand at their poster boards during the assigned session presentation time. Your poster board number is the alpha/numerical listing next to your abstract number. Please do not leave your belongings, poster containers or any materials under the poster boards or in the poster area. EB is not responsible for articles left in the poster area. Remember to bring your own pushpins. Details for preparing your poster are available at Poster Abstract Presentation - Experimental Biology

Call4Abstracts is offering a poster printing and onsite delivery service for you to pick up your poster at the convention center. Additional information, ordering and payment instructions will be sent to poster presenters directly from Call4Abstracts.

Following the EB 2015 meeting, all registered attendees will be able to access PDFs of the posters online through the e-poster link on the EB 2015 website. All presenters are requested to upload a PDF file of their poster prior to the meeting. For your convenience, you will also have the opportunity to upload your PDF file during the meeting. Please visit the CTT Publishing desk in the North Lobby to upload your file prior to 11:00AM on Wednesday, April 1. Only registered attendees will be able to access the e-poster site.

FINANCIAL INFORMATION:
Housing, transportation, registration and any additional expenses are your responsibility unless you have been otherwise notified. If you have not already registered or made your hotel arrangements, please do so immediately!! Forms for registration, hotel accommodations and travel discount information are located online at General Attendee Information

VISA INFORMATION FOR INTERNATIONAL REGISTRANTS:
Experimental Biology encourages you to start your visa application process as soon as possible. Because of new U.S. State Department regulations, U.S. embassies and consulates may require a face-to-face interview for most non-immigrant visa applications. You should apply

3. 研討會註冊證明

EB 2015
Experimental Biology
BOSTON

1. Name & Address | 2. Your Profile | 3. Package Selection | 4. Review Information | 5. Payment Info | **6. Thank You!**

REGISTRATION SUCCESSFUL

Name: **Yi-Chia Huang, PhD.**

Company/Institution: Chung Shan Medical University

Badge: 204119

PLEASE PRINT OUT THIS SCREEN FOR YOUR RECORDS. IT IS YOUR CONFIRMATION AND RECEIPT. YOU MAY USE THE QR CODE ON THIS RECEIPT TO ACCESS YOUR BADGE.

Registration Type: Member Scientist

Name: **Yi-Chia Huang, PhD.**

Department: School of Nutrition

Company/Institution: Chung Shan Medical University

Mailing Address: No. 110, Sec. 1, Jianguo N. Rd
Taichung 402
TAIWAN

Phone (Daytime): 886933512102

Email: ych@csmu.edu.tw

Individuals/Items Purchased

Badge	Last Name	First Name	Registration Type	Item Total
204119	Huang	Yi-Chia	Member Scientist	$390.00
	Member Scientist (1 x $390.00) Program/Meeting Bag Pick-up Card (1 x $0.00)			

Item Total: $390.00

Payment Records

Date	Payment Type	Reference #	Amount Paid
1/25/2015 10:31:07 PM	VI	XXXXXXXXXXXX8904	$390.00

Total Amount Paid: $390.00

科技部補助計畫衍生研發成果推廣資料表

日期:2015/10/05

科技部補助計畫	計畫名稱: 維生素B-6與氧化壓力及抗氧化能力相關性之研究
	計畫主持人: 黃怡嘉
	計畫編號: 101-2320-B-040-016-MY3　　學門領域: 保健營養

無研發成果推廣資料

101年度專題研究計畫研究成果彙整表

計畫主持人：黃怡嘉	**計畫編號：**101-2320-B-040-016-MY3
計畫名稱：維生素B-6與氧化壓力及抗氧化能力相關性之研究	

成果項目			量化			單位	備註（質化說明：如數個計畫共同成果、成果列為該期刊之封面故事...等）
			實際已達成數（被接受或已發表）	預期總達成數（含實際已達成數）	本計畫實際貢獻百分比		
國內	論文著作	期刊論文	0	0	0%	篇	
		研究報告/技術報告	0	0	0%		
		研討會論文	6	0	10%		
		專書	0	0	0%	章/本	
	專利	申請中件數	0	0	0%	件	
		已獲得件數	0	0	0%		
	技術移轉	件數	0	0	0%	件	
		權利金	0	0	0%	千元	
	參與計畫人力（本國籍）	碩士生	5	0	10%	人次	
		博士生	1	0	10%		
		博士後研究員	0	0	0%		
		專任助理	0	0	0%		
國外	論文著作	期刊論文	4	0	60%	篇	
		研究報告/技術報告	0	0	0%		
		研討會論文	3	0	10%		
		專書	0	0	0%	章/本	
	專利	申請中件數	0	0	0%	件	
		已獲得件數	0	0	0%		
	技術移轉	件數	0	0	0%	件	
		權利金	0	0	0%	千元	
	參與計畫人力（外國籍）	碩士生	0	0	0%	人次	
		博士生	0	0	0%		
		博士後研究員	0	0	0%		
		專任助理	0	0	0%		

其他成果（無法以量化表達之成果如辦理學術活動、獲得獎項、重要國際合作、研究成果國際影響力及其他協助產業技術發展之具體效益事項等，請以文字敘述填列。）	計畫主持人(黃怡嘉教授)2014年獲得台灣營養學會學術研究傑出獎。

	成果項目	量化	名稱或內容性質簡述
科教處計畫加填項目	測驗工具(含質性與量性)	0	
	課程/模組	0	
	電腦及網路系統或工具	0	
	教材	0	
	舉辦之活動/競賽	0	
	研討會/工作坊	0	
	電子報、網站	0	
	計畫成果推廣之參與（閱聽）人數	0	

科技部補助專題研究計畫成果報告自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估
   ■達成目標
   □未達成目標（請說明，以100字為限）
   　□實驗失敗
   　□因故實驗中斷
   　□其他原因
   說明：

2. 研究成果在學術期刊發表或申請專利等情形：
   論文：■已發表 □未發表之文稿 □撰寫中 □無
   專利：□已獲得 □申請中 ■無
   技轉：□已技轉 □洽談中 ■無
   其他：（以100字為限）
   此3年的多年期計畫已發表4篇SCI期刊論文、1篇國際研討會口頭報告、2篇國際研討會壁報、5篇國內研討會口頭報告以及1篇國內研討會壁報。

3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）（以500字為限）
   本研究結果顯示小鼠處在高氧化壓力狀態下，維生素B-6若缺乏時可能會造成穀胱甘肽從肝臟重新分布到血漿中利用，但是不會進一步的影響穀胱甘肽依賴的酵素活性。電銲作業員若有足夠的維生素B-6營養狀況應不影響其氧化壓力狀態及抗氧化能力。但是，較高的同半胱胺酸濃度似乎是影響抗氧化能力的主要危險因子。相較於同半胱胺酸，代謝症候群似乎是相較血漿同半胱胺酸在影響罹患大腸直腸息肉危險性有較獨立且重要的角色。除了高同半胱胺酸與大腸直腸癌的顯著正相關外，高血清葉酸濃度可能在大腸直腸癌形成過程中扮演雙重的角色。此三年的研究成果應可提供動物及人體在血漿同半胱胺酸、葉酸及維生素B-6與氧化壓力及其相關的抗氧化能力關係的完整概念。