Oral *Bacopa monnieri* is Antihypertensive in Rats Chronically Treated with L-NAME

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**Keywords:** hypertension, *Bacopa monnieri*, L-NAME, vasodilation, captopril

**Introduction.** We have previously reported that intravenous injection of *Bacopa monnieri* (Brahmi) reduced blood pressure in normotensive anaesthetised rats, suggesting that Brahmi could be an effective antihypertensive. Therefore, the present study sought to show that orally administered Brahmi could lower blood pressure in rats made chronically hypertensive by Nω-nitro-L-arginine methyl ester hydrochloride (L-NAME) which blocks endothelial nitric oxide synthase.

**Methods.** Male Wistar rats (200-250 g) were divided into 4 groups: 1) Normotensive control, 2) L-NAME (50 mg/kg), 3) L-NAME plus Brahmi (60 mg/kg/day), 4) L-NAME plus captopril (20 mg/kg/day). L-NAME was administered via the drinking water for 8 weeks. After 4 weeks, animal groups 3 and 4 also received Brahmi ethanolic extract or captopril in the drinking water for the remaining 4 weeks. Systolic blood pressure (SBP) and heart rate were measured weekly, whilst conscious, using an inflatable tail cuff. To elucidate the mechanism of action of Brahmi, we studied the vasodilator effects of Brahmi on phenylephrine (10 μM) pre-contracted isolated mesenteric artery by organ bath technique using a separate group of normotensive rats.

**Results.** L-NAME produced a sustained elevation of SBP from 94.7 ± 7.5 mmHg (week 0, n = 7) to 166.6 ± 3.5 mmHg (week 8, n = 6, P < 0.001). A further 4 weeks of Brahmi reduced blood pressure from 162.8 ± 4.9 mmHg (week 4) to 129.9 ± 6.8 mmHg (week 8, P < 0.01, n = 6-8) and captopril from 166.4 ± 7.2 mmHg (week 4) to 140.4 ± 5.8 mmHg (week 8, P < 0.01, n = 6-7) but had no effect on normotensives. There was no difference in heart rate among the 4 groups studied. In isolated mesenteric artery, Brahmi extract elicited endothelial independent vasorelaxation, suggesting that it acts directly on the vascular smooth muscle cells.

**Conclusion.** These data show that Brahmi is an effective antihypertensive animals, but unlikely to be nitric oxide-mediated. Thus Brahmi or its active ingredients may make a clinically efficacious antihypertensive treatment.


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Effect of Tetrahydrocurcumin on Aortic Stiffening in Mice Exposed to Cadmium

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Keywords: aortic elasticity, cadmium, hypertension, matrix metalloproteinase, oxidative stress, tetrahydrocurcumin

Introduction. Cadmium is a potential new risk factor for early atherosclerosis and cardiovascular diseases in humans and animals, however, the pathogenesis mechanisms are still a matter of debate. Our previous study demonstrated that Cd induces oxidative stress, vascular dysfunction and hypertension in mice. Tetrahydrocurcumin (THU), a major metabolite of curcumin found in turmeric, possesses a variety of biological activities, including vascular protective effect. The objective of this study was to investigate whether THU could protect against Cd-induced vascular dysfunction and aortic stiffening in mice.

Methods. Male ICR mice were randomly distributed into six groups (n = 14/group). Mice were received CdCl2 (100 mg/L) via drinking water alone or received THU supplementation at doses of 50 and 100 mg/kg/day for 8 consecutive weeks. After treatment, arterial blood pressure, aortic elasticity, endothelial nitric oxide synthase (eNOS) protein expression and oxidative stress were assessed. Moreover, the thoracic aortas were fixed and analyzed by using histomorphometry and immunohistochemistry techniques.

Results. Cd administration increased blood pressure and induced aortic stiffening and oxidative stress. Supplementation with THU reduced blood pressure and decreased aortic wall thickness and stiffness. THU also reduced collagen accumulation and elastin deposition in the aortic wall. Moreover, THU significantly alleviated MMP-9 and MMP-2 expressions in thoracic aorta. The improvement of these alterations was associated with increased aortic eNOS expression, suppressed oxidant formation and reduced Cd contents in blood and tissues. We also found that administration of THU did not change the normal levels of all parameter measurements in normal control mice.

Conclusion. Results of this study provide the first evidence that THU attenuates the detrimental effect of Cd by improving the hemodynamic status and aortic elasticity. THU might be used as a dietary supplement to protect against cardiovascular alterations in Cd-induced toxicity.

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Effect of Long-Term Exposure to Low Levels of Lead and Cadmium on Oxidative Stress and Vascular Function in Rats

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Keywords: lead, cadmium, oxidative stress, vascular dysfunction

Introduction. Lead (Pb) and cadmium (Cd), which are highly toxic metals, have been contaminated in human stuff and environment as a consequence of increasing industrialization. Long-term exposure to these metals causes adverse health effects. Toxic metal-induced oxidative stress has been proposed to be the risk factor for developing hypertension and diabetes. Therefore, this study aimed to determine the effects of Pb and Cd either alone or in combination on blood pressure, vascular responsiveness, oxidative stress, and blood glucose level.

Methods. Low dose of lead acetate (100 mg/l) and/or cadmium chloride (10 mg/l) were administered as drinking water to male Sprague-Dawley rats for 12 weeks (n = 6/group). Thereafter, oral glucose tolerance test (OGTT, 2 g glucose/BW) was performed in all animals. Arterial blood pressure and vascular reactivity to vasoactive agents were measured. Oxidative stress markers and antioxidant glutathione (GSH) were assessed.

Results. Increased arterial blood pressure and blunted vascular responses were found in rats exposed to Cd and/or Pb (P < 0.05). Lipid peroxidation as indicated by the levels of malondialdehyde in plasma and tissues (kidneys and heart) were also elevated, and this was concurrent with a rise of superoxide production in the thoracic aortas (P < 0.01). These deleterious effects are more pronounced in rats treated with Pb plus Cd than those treated with Cd or Pb alone. Impaired glucose tolerance was found in all Pb and/or Cd treated group. Moreover, increased fasting blood glucose level were also found in rats after exposed to Pb plus Cd for 12 weeks (P < 0.01).

Conclusion Long-term exposure to Pb and Cd induced high blood pressure and vascular dysfunction. These alterations might be associated with chronic complications of glucose metabolism disorders. The overall findings suggest that toxic metals may play a role in development of diabetes and cardiovascular disease.

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Curcumin Improves Hemodynamic Status and Attenuates Arterial Remodeling in L-NAME-Induced Hypertensive Rats

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Keywords: curcumin, L-NAME, hypertension, arterial remodeling, matrix metalloproteinase

Introduction. Hypertension is associated with structural and functional alterations in the vasculature that lead to hemodynamic disturbances and target organ damage. A recent study, performed by our group found that curcumin (CUR), a natural polyphenol compound isolated from Curcuma longa, possesses strong antioxidant and blood pressure lowering properties. However, the mechanism involved with the antihypertensive effect of CUR remains unclear. The aim of this study was to investigate whether CUR could improve hemodynamic alterations and reduce arterial remodeling in Nω-nitro-L-arginine methyl ester (L-NAME)-induced hypertensive rats.

Methods. Male Sprague-Dawley rats were divided to two main groups, normotensive and hypertensive groups. Rats with normal blood pressure were served as the normotensive group. Hypertension was induced by giving L-NAME (50 mg/kg) in drinking water for 5 weeks. CUR (50 or 100 mg/kg) or vehicle was intragastrically administered daily during the fourth and fifth weeks. The effects of CUR on hemodynamics, endothelial nitric oxide synthase (eNOS) protein expression and oxidative stress markers were assessed. Histopathological examination of large and small arteries was conducted. The levels of matrix metalloproteinase (MMPs) were also determined.

Results. Marked increases in blood pressure, peripheral vascular resistance, and oxidative stress were found in rats, treated with the L-NAME. Increases in wall thickness, cross-sectional area and wall/lumen ratio along with the elevations of MMP-2 and MMP-9 levels in aorta and mesenteric artery were found in L-NAME hypertensive rats. CUR significantly reduced hypertension and oxidative stress but increased aortic eNOS protein expression. CUR also attenuated pathological changes in aorta and mesenteric artery by reducing smooth muscle cells proliferation, collagen accumulation, and MMPs levels.

Conclusions. This study has shown that CUR exhibits antioxidative, antihypertensive and vascular protective effects through mechanisms that may involve generation of nitric oxide and improvement of arterial function and structural remodeling.

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The Alteration of Osteoblastic Insulin Receptor Signaling in Insulin Resistant Rats Induced by 12-week High-Fat Diet Consumption

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Keywords: insulin signaling, insulin resistance, obesity, osteoblast

Introduction. Type 2 diabetes mellitus has been shown to alter bone remodeling. We previously showed that 12-week high fat diet (HFD) fed rats had developed peripheral insulin resistance, a pre-diabetic stage. However, the alteration of osteoblastic insulin signaling in insulin resistant models induced by HFD consumption has not been investigated. Therefore, we hypothesized that 12-week HFD consumption causes the impairment of osteoblastic insulin signaling and leads to the abnormality of osteoblast proliferation and survival.

Objectives. To investigate the effect of 12-week HFD consumption induced insulin resistance on the alteration of osteoblastic insulin signaling, osteoblastic proliferation and osteoblastic survival.

Methods. Osteoblasts were isolated from calvariae of 12-week normal diet fed rats and 12-week HFD-fed rats (n = 15/group). Isolated osteoblasts were cultured in DMEM containing 10 % FBS for 7 days. Subsequently, cells were starved for 24 hours before stimulated with insulin (500 nM) for 5 minutes and resuspended in lysis buffer. The expression of osteoblastic insulin signaling molecules, including insulin receptor (IR), IR phosphorylation (IR-P), insulin receptor substrate (IRS), IRS phosphorylation (IRS-P), Akt/PKB and serine phosphorylation of Akt/PKB (Akt/PKB-ser), proliferative protein (cyclin D1) and apoptotic-related proteins (Bax and Bcl-2) were evaluated using western blot analysis.

Results. Rats fed with HFD for 12 weeks developed peripheral insulin resistance. Their osteoblasts illustrated an impairment of IR signaling: decreased in IR-P, IRS-P and Akt/PKB-ser. In addition, osteoblastic cells of HFD group reduced cell proliferation and increased cellular apoptosis as indicated by the reduction of cyclin D1 and increase of Bax to Bcl-2 ratio, compared to those of ND group.

Conclusions. The present study suggested that HFD consumption induced not only peripheral insulin resistance, but also the impairment of osteoblastic insulin signaling. Moreover, HFD consumption also led to the defect in osteoblast proliferation and osteoblast survival.

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Calorie Restriction Prevents the Development of Insulin Resistance and Impaired Insulin Signaling in Skeletal Muscle of Ovariectomized Rats

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Keywords: insulin resistance, calorie restriction, skeletal muscle, insulin signaling, MAPK, ovariectomy

Introduction. Insulin resistance of skeletal muscle glucose transport due to prolonged loss of ovarian function in ovariectomized (OVX) rats is accompanied by other features of the metabolic syndrome and may be confounded by increased calorie consumption.

Objectives. 1) To investigate whether the insulin resistance in OVX rats is directly attributed to ovarian hormone depletion or is secondary to increased caloric intake; 2) To examine the effects of calorie manipulation on the insulin-resistant condition in OVX rats; and 3) To examine whether the insulin resistance of glucose transport in skeletal muscle of OVX rats is associated with alterations in signaling molecules involved in the skeletal muscle glucose transport system.

Methods. Ten-week-old female Sprague-Dawley rats were OVX (n = 30) or sham-operated (SHAM, n = 10). OVX rats either had free access to food, pair feeding (PF) with SHAM or received a 35% reduction in calorie restriction (CR) for 12 weeks. Glucose tolerance, insulin-stimulated skeletal muscle glucose transport, serum lipid profile, and visceral fat content were determined. Moreover, the protein expression and functionality of the phosphatidylinositol 3-kinase (PI3-kinase) and mitogen-activated protein kinase (MAPK) pathways including insulin receptor (IR), insulin receptor substrate-1 (IRS-1), the p85 subunit of PI3-kinase, Akt, c-Jun NH2-terminal kinase (SAPK/JNK), and p38 MAPK were evaluated.

Results. Ovariectomy induced skeletal muscle insulin resistance, which was associated with decreases (32-70%) in tyrosine phosphorylation of IR and IRS-1, IRS-1/p85 subunit of PI3-kinase, and Akt Ser473 phosphorylation whereas insulin-stimulated phosphorylation of IRS-1 Ser307, SAPK/JNK Thr183/Tyr185, and p38 MAPK Thr180/Tyr182 was increased (24-62%). PF did not restore insulin-stimulated glucose transport. Contrary, impaired insulin sensitivity and defective insulin signaling were not observed in the skeletal muscle of OVX+CR rats.

Conclusion. Insulin resistance in OVX was caused by ovarian hormone deprivation, not over-feeding. CR effectively prevented the development of insulin resistance and impaired insulin signaling in the skeletal muscle of OVX rats.

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Role of Fibroblast Growth Factor-23 in the Regulation of Calcium Transport Across Intestinal Epithelium in Mice

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Keywords: Ussing chamber, 1,25(OH)2D3, calcium flux, duodenal calcium absorption.

Introduction. Recently, fibroblast growth factor-23 (FGF-23) has been identified as a hypophosphatemic hormone which is synthesized by bone cells. Its main function is to regulate phosphate (Pi) homeostasis by increasing urinary Pi excretion. However, whether FGF-23 regulates calcium (Ca) homeostasis is not known. Since FGF-23 receptors are present in the intestine and can antagonize 1,25(OH)2D3 action, FGF-23 should have direct action and may compromise 1,25(OH)2D3-dependent Ca absorption in the intestine. Therefore, the objective of this study is to investigate the effects and possible signaling pathways of FGF-23 on intestinal Ca transport in male ICR mice by using in vitro Ussing chamber experiment.

Methods. In vivo effect of FGF-23 on Ca transport. Mice were pretreated with 1 µg/kg 1,25(OH)2D3 or vehicle over 3 days and FGF-23. Duodenum was removed and mounted in an Ussing chamber for Ca transport measurement. Acute direct effect and signaling pathways of FGF-23 on Ca transport. Mice were pretreated with 1,25(OH)2D3. Duodenum was directly exposed to FGF-23 and/or various inhibitors of the signaling pathways in an Ussing chamber. Net flux of Ca and electrical parameters i.e., potential difference, short circuit current, and transepithelial resistance were measured throughout the 60-min experiment by 45Ca kinetics and voltage clamp techniques, respectively.

Results. Pretreatment with 1,25(OH)2D3 significantly enhanced total active duodenal Ca transport. Injection of FGF-23 and direct administration of FGF-23 into the Ussing chamber significantly decreased the 1,25(OH)2D3-enhanced duodenal Ca absorption. Inhibitors of MAPK/ERK, p38 MAPK and PKC, but not inhibitors of JNK, Src, JAK2, PI3K or Akt significantly abolished the effect of FGF-23 on 1,25(OH)2D3-induced intestinal Ca absorption.

Conclusion. This study showed for the first time that FGF-23 besides being a hypophosphatemic hormone, it could also be a hypocalcemic hormone through its inhibitory effect on Ca absorption. FGF-23’s action was mediated by MAPK/ERK, p38 MAPK, and PKC signaling pathways.

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DPP4-inhibitor Improves Neuronal Insulin Receptor Function and Brain Mitochondrial Function Caused by High-Fat Diet Consumption

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Keywords: fEPSP, vildagliptin, brain mitochondria, high-fat diet, insulin resistance.

\textbf{Introduction.} High-fat diet (HFD) consumption is one of several factors that can lead to peripheral insulin resistance as well as neuronal insulin resistance. Moreover, our previous study demonstrated that HFD caused the impairment of neuronal insulin signaling and brain mitochondria dysfunction. Dipeptidyl peptidase IV (DPP-4) inhibitor is a new anti-diabetic drugs used for glycemic control. DPP-4 inhibitor is known to inhibit the degradation of glucagon-like peptide-1 (GLP-1), an important peptide for glycemic control. At present, the effect of DPP-4 inhibitor on neuronal insulin resistance following HFD consumption has not yet been investigated. In this study, we tested the hypothesis that a DPP-4 inhibitor, vildagliptin, reverses the impaired function of neuronal insulin receptors and brain mitochondria caused by HFD consumption.

\textbf{Methods.} Rats were divided into 2 groups (n = 12/groups) to receive either HFD or normal diet (ND) for 12 weeks. After that, rats in each group were further divided into 2 subgroups (n = 6 /subgroups). Each subgroup was treated orally with either vildagliptin (3 mg/kg/day) or vehicle for 21 days. After the 15\textsuperscript{th} week, rats were sacrificed and each brain was removed to examine for insulin-induced long-term depression (LTD), neuronal insulin signaling, brain mitochondrial function and brain GLP-1 level.

\textbf{Results.} We found that vildagliptin significantly increased brain GLP-1 levels. It also improved insulin-induced LTD and increased neuronal Akt/PKB-ser phosphorylation in response to insulin. Vildagliptin also attenuated brain mitochondrial swelling, brain mitochondrial membrane potential changes and brain mitochondrial ROS production.

\textbf{Conclusion.} Our findings suggested that vildagliptin improves neuronal insulin resistance and attenuates brain mitochondria dysfunction caused by HFD consumption.

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Effects of Metformin on Learning Behaviors and Brain Mitochondrial Functions in 12-Week High-Fat Diet-Induced Insulin Resistant Rats

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Keywords: insulin resistance, high-fat diet consumption, metformin, memory decline, brain mitochondrial dysfunction

Introduction. Insulin resistance is related to the development of Type 2 Diabetes Mellitus (T2DM). Our previous study found that High-fat Diet (HFD) consumption caused not only peripheral insulin resistance, but also neuronal insulin resistance and brain mitochondrial dysfunction. Furthermore, HFD consumption has been shown to be associated with the cognitive impairment. Metformin is the first line drug therapy for T2DM. However, the effects of metformin on learning and memory behaviors and brain mitochondrial functions in HFD-induced insulin resistant rats have never been investigated. We tested hypothesis that metformin ameliorates the insulin resistance, prevents brain mitochondrial dysfunction, and improves cognitive function in HFD-induced insulin resistant rats.

Methods. Twenty-four male Wistar rats were divided into 2 groups, feeding with either normal diet or HFD for 12 weeks. Then, rats in each group were divided into 2 subgroups, and being treated with either vehicle or metformin (15 mg/kg twice daily) for 21 days. Morris Water Maze (MWM) test was used to test cognitive behaviors, and blood samples were collected for the plasma analysis. At the end of the study, the brain was removed to determine the brain mitochondrial function and malondialdehyde (MDA) levels.

Results. HFD-rats developed insulin resistance, increased oxidative stress, brain mitochondrial dysfunction, and impaired learning and memory behaviors. Metformin significantly improved peripheral insulin resistance by reducing plasma glucose level, plasma insulin level, and HOMA index. Moreover, metformin also attenuated both plasma and brain MDA levels. The cognitive function was also improved in the metformin-treated rats. Furthermore, metformin significantly improved brain mitochondrial dysfunction by reducing mitochondrial ROS production, decreasing mitochondrial membrane depolarization, and attenuating mitochondrial swelling.

Conclusion. Metformin improves peripheral insulin sensitivity, decreases oxidative stress, and restores brain mitochondrial function caused by HFD consumption. These beneficial effects of metformin could be responsible for improving the cognitive function in these insulin resistant rats.

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Trabecular Bone Loss and Impaired Bone Formation in Heterozygous β^{IVSII-654} Thalassemic Mice

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Keywords: thalassemia, bone mineral density, DXA, bone histomorphometry, Goldner’s trichrome, osteoblasts, osteoclasts

Introduction. The mutation of cysteine to tyrosine at nucleotide 654 of intron 2 (β^{IVSII-654}) which results in aberrant splicing of β-globin RNA and insufficient β-globin synthesis, is a major cause of β-thalassemia. Although bone loss has previously been reported in β-thalassemic mice, architectural bone change in this β^{IVSII-654} thalassemic mouse model has not been demonstrated. Therefore, this study aimed to investigate architectural bone change by using dual-energy X-ray absorptiometry (DXA) and histomorphometric analysis, respectively.

Methods. Twelve-week-old wild-type C57BL/6 male mice and heterozygous β^{IVSII-654} knockin thalassemic littermates (n = 4/group) were obtained from the National Laboratory Animal Center, Thailand. Microarchitectural bone changes were investigated by using dual-energy X-ray absorptiometry (DXA) and histomorphometric analysis.

Results. Dual-energy X-ray absorptiometry (DXA) revealed a significant decrease in bone mineral density (BMD) of trabecular bone such as L5,6 vertebrae and tibial metaphysis, but no change was found in the cortical bone such as femoral diaphysis, suggesting that osteoporosis associated with β^{IVSII-654} thalassemia was site specific and occurred predominantly at trabecular sites, where bone remodeling normally takes place. Histomorphometric analysis of the tibial metaphysis demonstrated that trabecular bone volume was significantly decreased with an expansion of bone marrow cavity. Bone loss was consistent with the observed decreases in osteoblast surface, osteoid surface, mineral apposition rate, mineralizing surface, and mineralized volume. On the other hand, trabecular bone resorption was markedly enhanced as indicated by increases in the osteoclast surface and eroded surface.

Conclusion. This study indicated that bone loss observed in β^{IVSII-654} thalassemia was due to a decrease in osteoblastic bone formation and increase of bone osteoclastic bone resorption, especially at the trabecular site of tibial metaphysis.

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Prolactin Upregulated Calcium Transport-related Transcripts in Duodenal Epithelial Cells But Not in IEC-6 Crypt Cells

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Keywords: prolactin, calcium transport, real-time PCR

Introduction. Prolactin (PRL) has been known to enhance intestinal calcium absorption to compensate for negative calcium balance during placental calcium transfer and milk production. Although PRL receptors are expressed in both intestinal villous and crypt cells, these cells may differentially respond to PRL since calcium absorption mainly occurs in the villous tip.

Methods. We used quantitative real-time PCR to investigate mRNA expression of genes related to transcellular and paracellular calcium transport in (i) duodenal epithelial cells of anterior pituitary-grafted hyperprolactinemic rats, and (ii) 50 or 200 ng/mL PRL-exposed IEC-6 intestinal crypt cells. IEC-6 cells proliferation was demonstrated by MTT and BrdU techniques.

Results. It was found that both short and long isoforms of PRL receptors were strongly expressed in rat duodenal cells and IEC-6 cells. In duodenal epithelial cells, hyperprolactinemia upregulated several genes related to calcium transport, namely TRPV5, TRPV6, calbindin-D9k and claudin-3, whereas tight junction genes ZO-1 and occludin were downregulated. On the other hand, some genes such as calbindin-D9k, occludin and claudin-3, were downregulated in IEC-6 after 48-h exposure to 200 ng/mL PRL (comparable to plasma PRL levels during lactation), whereas claudin-2 was upregulated. However, neither 50 nor 200 mg/mL PRL affected IEC-6 cells proliferation.

Conclusion. It could be concluded that intestinal villous and crypt cells differentially responded to PRL. The upregulation of genes related to calcium transport was predominant in the villous epithelial cells, but not in crypt cells.

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Exercise Training Prevents the Cardiac Myofilament Hypersensitivity to Ca\textsuperscript{2+} in Angiotensin II-induced Hypertensive Rat

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Keywords: exercise training, ovariectomy, cardiac myofilament Ca\textsuperscript{2+} sensitivity, myosin heavy chain

Introduction. Angiotensin II (AII) is well-known to induce hypertension and hypertrophy of the heart. We have recently demonstrated an additive increase in cardiac myofilament Ca\textsuperscript{2+} sensitivity in ovariectomized (OVX) rat infused with AII. Based on our previous report showing the preventive effect of regular exercise on myofilament Ca\textsuperscript{2+} hypersensitivity in OVX rat heart, the question was raised whether exercise training also prevents the myofilament Ca\textsuperscript{2+} hypersensitivity in AII-induced hypertensive rat heart.

Method. Adult female rats were divided into two main groups, exercise and sedentary, in which each contained four subgroups, including sham and OVX rats with and without AII infusion. One week after sham-operation or ovariectomy, exercise rats were subjected to a nine-week moderate intensity program of treadmill running. In AII-infused groups, AII was introduced by mini-osmotic pump during last four weeks of 10-week study duration. Myofilament Ca\textsuperscript{2+} sensitivity was derived from mechanical measurements of skinned fiber preparation from left ventricular papillary muscle. Myosin heavy chain (MHC) isoforms and angiotensin receptor type 1 (AT1) were also determined.

Results. As expected in sedentary groups, hypersensitivity of cardiac myofilament to Ca\textsuperscript{2+} activation was clearly detected in both OVX and AII subgroups with an additive increase in AII-OVX rat. In exercise-trained groups, the cardiac myofilament Ca\textsuperscript{2+} hypersensitivity induced by either ovariectomy/AII alone or combination was all fully abolished. In contrast to OVX rat heart in which MHC was shift toward β-isoform, AII-infused rat demonstrated no shift in MHC isoforms. Surprisingly, exercise training reversed the MHC shift in the heart of OVX but not AII-OVX rats. In contrast to effects on MHC, exercise training completely normalized the upregulation of AT1 in the heart of both OVX and AII-OVX rats.

Conclusion. Results from the present study indicate a significant preventive impact of exercise training on cardiac contractile performance even under angiotensin II-induced hypertension.
Mechanistic Effects of Rosiglitazone on Its Facilitation of Ventricular Fibrillation in Ischemic/Reperfusion Rat Hearts

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Keywords: rosiglitazone; heart; ischemia/reperfusion; connexin 43

Introduction. Rosiglitazone has been used to improve insulin sensitivity in type II diabetes. However, evidence in both basic and clinical studies indicated that it could be harmful to the cardiovascular system. Our previous study demonstrated that rosiglitazone facilitated fatal arrhythmias in the heart exposed to ischemia-reperfusion (I/R) injury. However, the definite mechanisms responsible for these cardiac effects of rosiglitazone are still unclear. Since the phosphorylation of connexin 43 at serine 368 residue has been shown to attenuate the ventricular fibrillation (VF) in I/R models, we tested the hypothesis that rosiglitazone facilitates VF by decreasing cardiac connexin 43 phosphorylation during I/R.

Methods. Sixteen male Wistar rats were used. In each rat, either rosiglitazone (1 mg/kg) or normal saline was administered intravenously for 30 minutes. Then, the left anterior descending coronary artery (LAD) was ligated for 30 minutes, and released to promote reperfusion for 120 minutes. During the experiment, the time interval from LAD occlusion to the first spontaneous VF onset, and the occurrence of ventricular arrhythmias was characterized according to the Lambeth Convention. At the end of each experiment, the level of phosphorylated connexin 43 at serine 368 residues in myocardial tissues, and the infarct size were determined.

Results. Rosiglitazone decreased the time interval to the first occurrence of VF during I/R period. Furthermore, rosiglitazone increased both the arrhythmia scores and mortality rate in I/R rats. In rosiglitazone-treated rats, the level of connexin 43 phosphorylation was markedly decreased in the ischemic myocardium, compared to the vehicle-treated rats. The infarct size was also markedly decreased in rosiglitazone-treated rats.

Conclusions. Rosiglitazone facilitated fatal arrhythmia during I/R by decreased phosphorylated connexin 43 in the I/R heart. This proarrhythmic effect of rosiglitazone could be responsible for increased mortality that has been reported previously and in the present study.

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Dipeptidyl Peptidase-4 Inhibitor Attenuates Cardiac Ischemia-Reperfusion Injury and Cardiac Mitochondrial Dysfunction

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Keywords: DPP-4 inhibitor, ischemia-reperfusion injury, mitochondria, cardiac electrophysiology

Introduction. Dipeptidyl peptidase-4 (DPP-4) inhibitor is a new antidiabetic drug that has been used to treat type-2 diabetes mellitus. Despite its benefits on glycemic control, the effects of DPP-4 inhibitor on the heart during ischemia-reperfusion (I/R) periods are not known. We tested the hypothesis that DPP-4 inhibitor attenuates cardiac arrhythmias and reduces the infarct size during I/R, and that the cardioprotective effects of DPP-4 inhibitor is via its prevention of cardiac mitochondrial dysfunction caused by severe oxidative stress during I/R.

Methods. Fourteen pigs were randomized to receive either DPP-4 inhibitor (vildagliptin, Vil) 50 mg or normal saline (NSS) intravenously prior to a 90-min left anterior descending coronary artery occlusion, followed by a 120-min reperfusion period. The hemodynamic, cardiac electrophysiological parameters, and the infarct size were determined. Rat cardiac mitochondria from eight rats were used to study the protective effects of DPP-4 inhibitor on cardiac mitochondrial dysfunction caused by severe oxidative stress induced by H2O2 to mimic the I/R condition.

Results. When compared with NSS group, DPP-4 inhibitor mitigated effective refractory period (ERP) shortening, reduced the number of premature ventricular contractions, increased the ventricular fibrillation threshold (VFT) during the ischemic period, and also decreased the infarct size (see Figure). In addition, DPP-4 inhibitor significantly decreased the reactive oxygen species production and prevented cardiac mitochondrial depolarization caused by severe oxidative stress (see Figure).

Conclusions. During I/R, DPP-4 inhibitor preserves cardiac electrophysiology by attenuating the ERP shortening, and reducing the infarct size, resulting in decreasing myocardial vulnerability to cardiac arrhythmia. This cardioprotective effect could be due to its prevention of cardiac mitochondrial dysfunction caused by severe oxidative stress during I/R.

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Association of Microvascular Endothelial Dysfunction and Arterial Stiffness in Subjects with Metabolic Syndrome

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Keywords: arterial stiffness, endothelial dysfunction, forearm blood flow, pulse wave velocity, metabolic syndrome

Introduction. Individuals with metabolic syndrome (MetS) are thought to be prone to serious cardiovascular disease. Although impaired endothelial function and arterial stiffness have been demonstrated to be associated with MetS, however, the impact of MetS on both arterial variables has not yet been clarified in Thai population. The objective is to investigate microvascular endothelial function by measuring forearm blood flow (FBF) and arterial stiffness by measuring pulse wave velocity (PWV) in Thai subjects with MetS, and to explore the relationship among these changes.

Methods. 236 Thai adults with mean age of 59.5 ± 10 years (range 38 to 85 years and 57 % men) were enrolled in this study. Routine anthropometric and serologic data were collected. The two non-invasive techniques for measurements of FBF and PWV were used for assessments of endothelial function and arterial stiffness in all subjects, respectively.

Results. 145 subjects were classified as MetS according to the National Cholesterol Educational Program-Adult Treatment Panel III (NCEP-ATPIII). Brachial-ankle PWV (baPWV), aortic-ankle PWV (aaPWV) and aortic-femoral PWV (afPWV) were significantly increased in MetS patients ($P < 0.001$). The maximal change of FBF during reactive hyperemia in MetS was lower than non-MetS patients. There was a negative correlation between afPWV and maximum hyperemic FBF ($r = -0.267, P < 0.001$). This association remained significant ($r = -0.214, P = 0.003$) after adjusted for age and sex.

Conclusion. In subjects with MetS, a more impairment of response of FBF during reactive hyperemia showed a faster of afPWV. Data of this study suggest that monitoring of central PWV and microvascular FBF in patients with MetS may be helpful in identifying persons at risk of atherosclerosis-related disease.

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Mitochondrial Calcium Uniporter Blocker Effectively Prevents Brain Mitochondrial Dysfunction Caused by Iron Overload

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Keywords: iron dyshomeostasis, brain mitochondria, mitochondrial swelling, mitochondrial membrane potential, reactive oxygen species (ROS)

Introduction. Several studies have shown that iron overload can induce oxidative stress in brain mitochondria, and is associated with neurodegenerative diseases. However, brain mitochondrial iron uptake has been rarely investigated. We aimed to determine the role of mitochondrial calcium uniporter (MCU) in brain mitochondria as a major route for iron entry. We hypothesized that iron overload causes brain mitochondrial dysfunction including mitochondrial swelling, increased mitochondrial reactive oxygen species (ROS) production, and mitochondrial membrane potential depolarization, and that the MCU blocker prevents iron entry into mitochondria, thus attenuating brain mitochondrial dysfunction.

Methods. Isolated brain mitochondria from male Wistar rats were used. Fe^{2+} and Fe^{3+} forms (0-20 μg/ml) were applied onto brain mitochondria at various time courses (5-30 minutes). The amount of iron entry into brain mitochondria and mitochondrial function was determined. Furthermore, effects of pharmacological interventions using an MCU blocker (Ru-360), L-type calcium channel blocker (verapamil and nifedipine), and iron chelator (DFO) were studied.

Results. Both Fe^{2+} and Fe^{3+} entered brain mitochondria and caused mitochondrial swelling in a dose- and time-dependent manner, and subsequently caused mitochondrial depolarization and increased ROS production. Moreover, Fe^{2+} caused more severe mitochondrial dysfunction than Fe^{3+}. Although all pharmacological interventions attenuated mitochondrial dysfunction caused by iron overload, only an MCU blocker could completely prevent an increased ROS level and mitochondrial depolarization.

Conclusion. Iron overload induced oxidative stress, mitochondrial membrane depolarization and mitochondrial swelling in brain mitochondria. Furthermore, an MCU blocker effectively prevented brain mitochondrial dysfunction. These findings suggested that MCU could be the major portal for iron entry into brain mitochondria.

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Novel Biosignal Parameters Relate to the Brain-Heart Axis Concept in Ischemic Stroke Patients with Atrial Fibrillation: A Preliminary Study

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Keywords: sample entropy, atrial fibrillatory rate, heart rate variability, ischemic stroke, atrial fibrillation

Introduction. Atrial fibrillation (AF) is the most common cardiac arrhythmia associated with an increased risk of ischemic stroke. Meanwhile, an ischemic brain injury also affects autonomic control loops on the heart with particular relevance to arrhythmogenesis. Therefore, the insights in these brain-heart interactions (or axis) may play a major role in treatment and prevention of AF with ischemic stroke. It has been known that heart rate variability (HRV) determines cardiac autonomic function. Recently, atrial fibrillatory rate (AFR) and sample entropy (SampEn), surface ECG signal processing, represent the degrees of AF signal irregularity and have shown their potential prediction of AF recurrence after electrical cardioversion. In this study, we aimed to evaluate HRV, AFR, and SampEn in AF patients with acute ischemic stroke compared to those after stroke recovery (post-stroke) to test the hypothesis that recovery of cardiac autonomic control relates to improved degree of AF signal irregularity.

Methods. Patients (age 40-80 years) with AF and acute ischemic stroke (n = 12) including those with post-stroke (n = 6) were recruited. The ECG recordings (PowerLab, fs 1000 Hz, bandwidth 20-50 Hz) were performed within 24 hours after admission or at the OPD visit, thereafter HRV, AFR, and SampEn were analyzed.

Results. AFR and SampEn tend to decrease in post-stroke patients although there are no significant differences. HRV analysis shows an increase in LF/HF ratio (0.571 ± 0.459 vs. 0.477 ± 0.317) after stroke recovery, while both biosignals decrease in post-stroke patients compared to acute stroke group (AFR; 401 ± 90 fpm vs 408 ± 74 fpm, and SampEn; 0.129 ± 0.021 vs 0.132 ± 0.024).

Conclusion. This preliminary study indicates that the degrees of AF signal irregularity decrease consistently with HRV improvement in AF patients recovered from ischemic stroke, possibly due to the interactions in brain-heart axis.

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Anti-Obesity Effect of Rutin on Lipid Emulsion Induced Hypertriglyceridemic Rats

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Keywords: rutin, pancreatic lipase, triglyceride, anti-obesity, cholesterol

Introduction. Rutin, a flavonoid of the flavonol type, is an important dietary constituent of medicinal plants. Rutin could inhibit porcine pancreatic lipase activity in vitro. Reduced plasma triglyceride (TG) and total cholesterol (TC) levels by rutin were found in hypercholesterolaemic rats. However, there is no report on anti-obesity effect of rutin on lipid emulsion induced hypertriglyceridemic rats. Therefore, the effects of rutin on pancreatic lipase inhibitory activity in vitro and plasma TG and TC levels in male Wistar rats orally administered with lipid emulsion were investigated.

Methods. In in vitro study, porcine pancreatic lipase inhibitory activities of rutin (0.625–320 mg/ml) and orlistat (commercial anti-obesity drug; 40 mg/ml) were measured using tritiummetric method. In in vivo study, rats were orally administered with lipid emulsion alone (4 ml/kg of 20 % intralipid, n = 8), lipid emulsion with orlistat (45 mg/4 ml/kg, n = 8) or lipid emulsion with rutin (1000 mg/4 ml/kg, n = 8). Plasma TG and TC levels were measured at 0, 1, 2, 3 and 4 h after administration of lipid emulsion (alone, with orlistat or with rutin).

Results. At a concentration of 40 mg/ml, orlistat showed a strong inhibition on pancreatic lipase activity (75.47 %), while rutin showed 37.24 % of pancreatic lipase inhibitory activity. At concentration of 320 mg/ml, rutin inhibited 65.05 % of pancreatic lipase activity. Administration of lipid emulsion alone caused significantly increased in plasma TG and TC levels at 2 h and 3 h (P < 0.05 compared with baseline) and reached a peak at 3 h. Rutin administration significantly decreased incremental plasma TG and TC levels at 3 h (P < 0.05).

Discussion. These findings demonstrated for the first time regarding beneficial effect of rutin on suppression of plasma TG and TC in hypertriglyceridemic rats that may be caused by the inhibition of lipase activity. However, the underlying machanisms are needed to investigate.

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Effects of Curcumin Attenuate Hepatitis in Mice with Paracetamol Overdose

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Keywords: paracetamol, curcumin, hepatitis, oxidative stress, IL-12, IL-18

Introduction: N-acetyl-p-aminophenol (APAP) or paracetamol overdose causes increasing of toxic metabolites, which disrupting hepatocyte function, and liver injury occurs. This study aims to determine effects of curcumin attenuate hepatitis in mice with APAP overdose.

Methods: Male mice were divided into four groups. Group I (control); gavaged with distilled water. Group II (APAP); gavaged with a single dose of 400 mg/kg of APAP. Group III (APAP + CUR 200); gavaged with a single dose of 400 mg/kg of APAP and 200 mg/kg of curcumin. Group IV (APAP + CUR 600); gavaged with a single dose of 400 mg/kg of APAP and 600 mg/kg of curcumin.

Results: Serum transaminases were significantly increased in APAP when compared with control and significantly decreased in APAP + CUR 200 and APAP + CUR 600 when compared with APAP. Histological examination of APAP showed acute centrilobular hemorrhagic hepatic necrosis involving all zones and the improvement of liver pathology revealed in APAP + CUR 200 and APAP + CUR 600. Hepatic GSH was significantly decreased in APAP when compared with control and significantly increased in APAP + CUR 200 and APAP + CUR 600 when compared with APAP. Hepatic MDA, serum IL-12, and IL-18 were significantly increased in APAP when compared with control and significantly decreased in APAP + CUR 200 and APAP + CUR 600 when compared with APAP.

Conclusion: APAP toxicity to liver is related to depletion of hepatic GSH concomitant with the induction of oxidative stress, liver inflammation, and the damage of liver pathology. Our results show curcumin can prevent most of the damage caused by APAP overdose by induction of hepatic GSH, reduction of oxidative stress, attenuation of liver inflammation, and the improvement of liver pathology. In addition, curcumin at the dose of 600 mg/kg tends to be more potent than 200 mg/kg.
Cardioprotective of Vildagliptin in Long-Term High-Fat Diet Consumption-Induced Insulin Resistant Rats

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Keywords: vildagliptin, high-fat diet, insulin resistance, heart rate variability, cardiac function, mitochondria

Introduction. Long-term high-fat diet consumption has been shown to cause insulin resistance and was associated with depressed heart rate variability (HRV), cardiac contractile dysfunction, and cardiac mitochondrial dysfunction. Vildagliptin is a novel oral anti-diabetic drug which inhibits the action of dipeptidyl peptidase-4 (DPP-4) enzyme. Previous studies demonstrated that vildagliptin could improve insulin resistance in diabetic rats. We tested the hypothesis that DPP-4 inhibitor, vildagliptin, preserves HRV, attenuates cardiac contractile dysfunction, and prevents cardiac mitochondrial dysfunction in long-term high-fat diet consumption-induced insulin resistant rats.

Methods. Male Wistar rats weighing 180-200 g. were fed with either normal diet (ND; 19.77 %Energy from fat) or high-fat diet (HF; 59.28 %Energy from fat) for 12 weeks. Then, rats in each group were treated with either vehicle (0.9 % NSS, n = 6/group) or vildagliptin (3 mg/kg/day, n = 6/group) for 21 days. Plasma insulin, glucose, cholesterol, MDA, HRV, cardiac function, and cardiac mitochondrial function were determined in each rat.

Results. High-fat fed rats developed insulin resistance, characterized by increased body weight, visceral fat, plasma insulin, cholesterol, MDA levels, and HOMA index. High-fat fed rats also had increased LF/HF ratio, indicating depressed HRV. Vildagliptin attenuated insulin resistant condition and completely restored the HRV. Moreover, vildagliptin also improved cardiac function, including end-systolic and diastolic pressure, and stroke volume in high-fat fed rats. Vildagliptin attenuated cardiac mitochondrial dysfunction, indicated by decreased ROS production and restored cardiac mitochondrial membrane potential.

Conclusion. Rats chronically fed with high-fat diet developed insulin resistance, depressed HRV, left ventricular dysfunction and cardiac mitochondrial dysfunction. Vildagliptin improved insulin resistant condition. Furthermore, it prevented cardiac mitochondrial dysfunction caused by high-fat diet consumption, thus improving HRV and cardiac function.

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Amelioration of Cadmium-induced Kidney Mitochondrial Injury by Caffeic Acid Phenethyl Ester

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Keywords: cadmium, mitochondria, caffeic acid phenethyl ester, reactive oxygen species, nephrotoxicity.

Introduction. Cadmium is an environmental and industrial pollutant that impacts greatly on human health. The kidney is the critical target organ for cadmium toxicity. Evidence has accumulated implicating the reactive oxygen species (ROS) generation with subsequent oxidative stress in mediating cadmium nephrotoxicity. Since, mitochondria are major source of ROS production and defective mitochondria are related to various disease conditions, this study was carried out to determine whether caffeic acid phenethyl ester (CAPE), a potent phenolic antioxidant, could protect kidney mitochondria against the toxic effect of cadmium.

Method. Three sets of experiments (n = 8 each) were performed using mitochondria isolated from rat kidney. Each set consisted of untreated group, CAPE (10 µM)-treated group, cadmium (30 µM)-treated group, and CAPE plus cadmium-treated group. The first set investigated the effects of CAPE and cadmium on mitochondrial function by determination of mitochondrial swelling, mitochondrial ROS production, and mitochondrial membrane potential change. The second set examined the alterations of mitochondrial structure using electron microscopy. The last set explored the impacts of cadmium and CAPE on mitochondrial oxidative status by assessments of nitric oxide, malondialdehyde, antioxidant thiols and superoxide dismutase activity.

Result. Cadmium significantly (all \( P < 0.001 \)) caused mitochondrial damage as indicated by mitochondria swelling, increased mitochondrial ROS production, dissipation of membrane potential, and impaired mitochondrial ultrastructure. Mitochondrial injury was accompanied by a marked rise in nitric oxide and malondialdehyde levels, as well as a substantial fall in antioxidant thiols and superoxide dismutase activity (all \( P < 0.001 \)). Pretreatment with CAPE significantly (all \( P < 0.001 \)) attenuated all the changes caused by cadmium.

Conclusion. This study demonstrates that CAPE can directly act on the mitochondria to block cadmium-induced oxidative stress mediated renal injury by inhibiting ROS generation, decreasing lipid peroxidation and/or maintenance of the mitochondrial antioxidant capacity, and suggests that it may be useful to unravel the renal toxic effect of this heavy metal.

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Gallic Acid Reduced Blood Pressure and Oxidative Stress in Insulin Resistant Rats Induced by High Fructose Diet

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Keywords: blood pressure, insulin resistance, oxidative stress, gallic acid

Introduction. High fructose diet (HFD) induced insulin resistance (IR) in rat has been associated with high blood pressure (BP) and increased oxidative stress. Gallic acid is a powerful antioxidant agent. Several studies have shown the therapeutic effect of the phenolic compounds on BP which was related with its antioxidant activity. Therefore, this study was to investigate the effect of gallic acid on BP and oxidative stress status in IR rats induced by a HFD.

Methods. Male Sprague-Dawley rats (120-140 g) were fed with a HFD for 14 weeks to induce IR status. Gallic acid (20 mg/kg) or vehicle was given further for 4 weeks with HFD feeding. Indirect systolic blood pressure (SP) was determined once a week during 4 weeks of treatments. At the end of experimental day, fasting blood glucose (FBG) and serum insulin, BP, and vascular tissue superoxide production were evaluated.

Results. Rats fed with HFD significantly showed the increase in FBG (112.50 ± 1.69 mg/dl vs control FBG; 81.67 ± 1.29 mg/dl) and serum insulin (1.63 ± 0.25 ng/ml vs control group 0.25 ± 0.06 ng/ml, P < 0.05). This indicated IR status in rat received HFD. High SP was found in IR rats (152.54 ± 1.82 mmHg vs control group 117.29 ± 1.82 mmHg, P < 0.05). Superoxide production in carotid arteries was significantly increased in IR rats (108.92 ± 3.57 counts/min/mg dry weigh, P < 0.05). However, gallic acid significantly reduced FBG (92.88 ± 5.30 mg/dl), serum insulin (0.35 ± 0.06 ng/ml), SP (123.30 ± 1.76 mmHg), and the superoxide production (64.098 ± 3.91 counts /min/mg dry weight, P < 0.05) in IR rats.

Conclusion. This finding supports that gallic acid exhibited antihyperglycemic and improved IR status in rat induced by HFD. The antihypertensive effect of gallic acid in IR rat might be related to decrease an oxidative stress marker.

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Physiological and Protective Roles of Androgen in Cardiac Contractile Activities in Normal and Diabetic-Induced Cardiomyopathic Rats

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Keywords: testosterone, diabetes, cardiac myofilament Ca\textsuperscript{2+} activation, myosin heavy chain, myocardial stiffness

Introduction. A higher incidence of heart disease in hypogonadal than normal men indicates a crucial role of androgen in the cardiac performance. Two hypotheses were then tested: 1) androgen plays a regulatory effect on the cardiac contractile function, and 2) androgen exerts a protection against the pathological insult to the heart.

Methods. To investigate the physiological effect of androgen, adult male rats were divided into sham and orchidectomized (ORX) rats with and without testosterone supplementation. To evaluate the cardioprotective role of androgen, the three groups of rat were diabetes induced using streptozotocin. Measurements of myofilament Ca\textsuperscript{2+} activation and myocardial stiffness for systolic and diastolic properties, respectively, were performed using skinned-fiber preparation from left ventricular papillary muscle. Myosin heavy chain (MHC) and titin isoforms, and collagen deposition were also determined.

Results. Ten-week testicular hormone deprivation induced a significant decrease in maximum active force contraction without affecting myofilament Ca\textsuperscript{2+} sensitivity. The declined force contraction was accompanied by a shift in MHC isoforms from a fast activity \(\alpha\)-MHC toward a slow \(\beta\)-MHC. These changes were disappeared in testosterone-supplemented ORX rats. On the other hand, there was no effect of androgen on the myocardial stiffness, titin isoforms, and collagen deposition in the heart. Diabetes induced a significant suppression in the cardiac myofilament Ca\textsuperscript{2+} sensitivity in every experimental group with the same magnitude. In contrast, a profound shift of \(\alpha\)- toward \(\beta\)-MHC in the heart of diabetic-ORX rat (12 ± 5 \% of \(\alpha\)-MHC/total MHC) was partly reversed by testosterone supplementation (40 ± 5 \%). Testosterone also partly prevented increases in the compliant N2BA titin isoform and in collagen deposition in diabetic-ORX rat heart.

Conclusion. Results from the present study demonstrated a physiological impact of male sex hormones on the systolic function of the heart and the hormones also possess partially protective role in diabetic rat heart.
Genistein Acts Like Estrogen on Inhibiting Intimal Hyperplasia from Vascular Injury in Ovariectomized Rats

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Keywords: genistein, intimal hyperplasia, vascular injury, ovariectomy, endogenous NOS inhibitor

Introduction. Hormone replacement therapy can reduce cardiovascular disease incidence in menopausal women. However, hormone replacement is still controversial. In animal model, the ovariectomy leads to estrogen deficiency and nitric oxide (NO) reduction from endothelial cells. Ballooning injury at carotid arteries created intimal hyperplasia and the event was inhibited by estrogen administration. Genestein – an estrogen substitute was able to prevent neointimal changes and therefore conducted this experimental research to prove it.

Methods. Female Wistar rats weighing 240-270 g (n = 32) were randomly divided into 4 groups:- i) the ovariectomized rats treated with dimethyl sulfoxide (DMSO) 100 µl/day subcutaneously (sc) (n = 8; OVX+DMSO), ii) the ovariectomized rats treated with Genistein 0.25 mg/kg/day,sc (n = 8; OVX+Genistein), iii) the ovariectomized rats treated with 17β-estradiol 0.2 µg/kg/day,sc (n = 8; OVX+E2) and iv) the sham group treated with DMSO 100 µl/day,sc (n = 8; Sham+DMSO). Three groups of ovariectomized rats underwent balloon injury of left carotid artery. The left carotid arteries were harvested two weeks after the surgery event for histological and immunohistochemical studies.

Results. Intimal hyperplasia was seen in the controlled arm (1.3 ± 0.2 in thickness). The estrogen and Genestein-administration groups showed (0.58 ± 0.0 and 0.6 ± 0.0 in thickness). Immunohistochemistry study disclosed that the number of von Willebrand factor and endothelial nitric oxide synthase (eNOS)-positive cells were not significantly different among groups. The immunostaining for inducible nitric oxide synthase (iNOS) and asymmetric dimethylarginine (ADMA), the eNOS inhibitor, showed more positive cells in the OVX+DMSO group compared with the Sham+DMSO group (77.5 ± 7.9 %, 82.5 ± 7.9 % respectively; P < 0.05). Meanwhile, the number of iNOS-and ADMA-positive cells were significantly decreased in the OVX+Genistein group and the OVX+E2 group compared with the OVX+DMSO group (12.5 ± 5.2 %, 10 ± 3.7 % and 25 ± 5 %, 18.7 ± 6.3 % respectively; P < 0.05).

Conclusion. The findings suggest that Genistein could inhibit intimal hyperplasia after balloon injury of carotid artery in ovariectomized rats via reducing iNOS and endogenous NOS inhibitor similar to estrogenic action.

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**Endothelium-Dependent Vasodilating Effect of Rice Bran Peptides**

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**Keywords:** endothelium-dependent, rice bran peptides, vasodilating effect

**Introduction.** Rice bran is the major by-product generated during milling. We observed that rice bran peptides (RBP) at dose of 500 mg/kg significantly reduced arterial blood pressure of two-kidney, one-clip (the 2K-1C) hypertensive rats. This finding suggests the antihypertensive property of RBP. In the present study, our goal was to investigate mechanisms involving blood pressure reduction of RBP in the 2K-1C rat aorta.

**Methods.** The relaxant effect of RBP was assessed in the presence or absence of the endothelium of the aortic rings from 2K-1C rats. To determine whether relaxant effects of RBP involved with nitric oxide (NO), cyclic guanosine monophosphate (cGMP), ATP-sensitive K⁺-channel (KATP) or prostacyclin (PGI₂), the aortic rings were pre-incubated with NG-nitro-L-arginine methyl ester (L-NAME, 10 µM), methylene blue (1 µM), glibenclamide (3 µM) or indomethacin (10 µM), respectively. The aortic rings were preconstricted with phenylephrine before testing the relaxation of RBP.

**Results.** RBP produced a concentration-dependent vasodilatation (EC₅₀ 0.46 ± 0.07 mg/ml), which was absent in endothelium-denuded vessels. The endothelium-dependent vasodilatation induced by RBP was abolished by L-NAME, a NO synthase inhibitor and methylene blue, a guanylate cyclase inhibitor, but not by indomethacin, a cyclooxygenase inhibitor, or glibenclamide, an ATP-sensitive K⁺-channel blocker.

**Conclusion.** RBP induces endothelium-dependent vasodilating effect that does not involve PGI₂ release or opening of the KATP. Part of the vasodilating effect of RBP might be modulated by NO-cGMP activation. We conclude that RBP possess antihypertensive property which might be due to an endothelium-dependent vasodilatory action.

This study was supported by grants from National Research Council of Thailand and Faculty of Medicine, Khon Kaen University. P. Tuangpolkrung was supported by Development and Promotion of Science and Technology Talents Project, The Institute for the Promotion of Teaching Science and Technology.
Effects of a Novel Beta-Glucan on Tumor Angiogenesis in CaSki Cells-Implanted Nude Mice

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Keywords: β-glucan, cervical cancer, vascular endothelial growth factor (VEGF), CD31, capillary density

Introduction. β-glucans (BGs) are naturally occurring glucose polymers found in yeasts, mushrooms and plants. BGs have gained interest due to their bioactive properties, including immuno-modulating and anti-tumor activities. Tumor angiogenesis is required for tumor growth, especially in the cervical cancer. We thus determined the effects of our novel BG on angiogenesis in cervical cancer (CaSki cells)-implanted nude mice.

Methods. BALB/c nude mice were divided into 2 groups: control (CON) and CaSki groups. The CaSki group was subcutaneously injected with CaSki cells within dorsal skin. Two months after injection, mice were daily oral fed with BG (16 mg/kg) or distilled water. One-month post-treatment, the tumor microvasculature was determined by capillary density (CD) and expression of CD31 protein. VEGF protein was also detected by immunohistochemistry.

Results. In control groups, the CD of both CON+vehicle and CON+BG were similar (30.23 and 28.96 %). The CD in CaSki+vehicle group was significantly increased (55.76 %) compared to the CON+vehicle group. Interestingly, treatment with our novel BG in the CaSki group significantly attenuated the increase of CD (40.13 %, P < 0.001). However, the CD in this group was higher than both of the control groups (P < 0.005). In the same fashion, the CD31 expression, which was higher in the CaSki-group than the control, markedly attenuated when treated with BG. VEGF protein expressions, which were found stronger in the CaSki group, also attenuated by the BG treatment.

Conclusion. Our novel BG expressed the anti-angiogenesis in CaSki-implanted mice. Expression of VEGF, tumor angiogenesis biomarker, decreased in the CaSki mice treated with BG. It is likely that this anti-angiogenic activity of this novel BG is mediated by the reduction of VEGF protein expression. This study has shed light on the anti-angiogenic activity of the novel BG. It may be considered as a new therapeutic agent against cancers in the future.

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Cashew apple juice supplementation enhanced immune function at rest and after a heavy exercise in healthy sedentary men

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Keywords: cashew, lymphocyte, white blood cell, cortisol, exercise

Introduction. Moderate exercise can promote immune function whereas heavy exercise can cause immunosuppression. Cashew apple juice comprises vitamin C, glucose, and anacardic acids which may exert its effect on enhancement of immune function. However, there is a lack of evidence to show that cashew apple juice can enhance immune response to exercise. Therefore, this study investigated the effect of cashew apple juice supplementation on immune function at rest and in response to a heavy exercise. We hypothesized that supplementation with the cashew apple juice for 4 weeks may enhance immune function both at rest and in response to a heavy exercise.

Methods. This study was randomized crossover design. Ten healthy sedentary men aged 19-28 years old randomly ingested either 40% cashew apple juice or placebo 3.5 ml/kg BM/day everyday for 4 weeks with 4-week interval to eliminate carry over effect. They performed a cycling exercise at 85% of peak oxygen consumption for 20 min before and after of each ingestion period. Venous blood samples were obtained at rest and immediately after the exercise to determine total white blood cells (WBC), neutrophil, lymphocyte and monocyte counts and cortisol concentration.

Results. After the supplementation with cashew apple juice for 4 weeks, there were significant increases in WBC (9.36 vs 8.53 x 10^3 cells/μl; P < 0.05) and lymphocyte count (4.53 vs 3.37 x 10^3 cells/μl; P < 0.05) both at rest and after the exercise without significant differences in neutrophil and monocyte counts and cortisol concentration compared to placebo.

Conclusion. The findings suggest that a 4-week supplementation with the cashew apple juice enhanced immune function in response to a heavy exercise in healthy sedentary men through the augmentation of WBC and lymphocyte. This cannot be explained by the decreased corticosteroid-induced suppression of the immune response.

This study was supported by Graduate School and Exercise and Sport Sciences Development and Research Group, Khon Kaen University (2010).
Deep Sedation for Colonoscopy in Overweight Patients: A Comparison Between Propofol Alone Versus Propofol and Ketamine

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Keywords: deep sedation, propofol, ketamine, complication, colonoscopy, obesity

Introduction. The aim of this study was to evaluate and compare the clinical efficacy of propofol alone and propofol and ketamine for deep sedation (DS) for colonoscopy in overweight (BMI > 25) patients in a teaching hospital in Thailand.

Methods. We undertook a retrospective review of the sedation service records of overweight patients who underwent colonoscopic procedures from November 2007 to May 2009. All patients were premedicated with intravenous midazolam before the procedure. The primary outcome variable was the successful completion of the endoscopy. The secondary outcome variables were sedation and procedure-related complications during and immediately after the procedure, and mortality rate.

Results. There were 104 overweight patients who underwent colonoscopic procedure by using deep sedation technique during the study period. After matching age, gender, ASA physical status and indications of procedure, 38 patients were sedated by using propofol alone (group P) and 42 patients were sedated with propofol and ketamine (group PK). All sedation was given by residents or anesthetic nurses directly supervised by staff anesthesiologist in the endoscopy room. There were no significant differences in patients’ characteristics, sedation time, indication, anesthetic personnel, mortality rate, success rate and sedative agents used between the two groups. Sedation-related complication including hypotension in group P was significantly higher than in group PK. However, all complications were easily treated, with no adverse sequelae.

Conclusion. Deep sedation in both regimens for colonoscopy in overweight patients provided effective and safe. No serious adverse events were observed. However, the combination of propofol and ketamine used for DS had significantly lower complication than the propofol alone.
Complication Rate During and Immediately After Propofol-Based Deep Sedation for Colonoscopy in Marked Obesity Patients

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Keywords: deep sedation, propofol, complication, colonoscopy, marked obesity

Introduction. The aim of this study was to evaluate and compare the complication rate of propofol-based deep sedation (PBDS) for colonoscopy in marked obesity (BMI > 30) and non-obesity (BMI < 25) patients in a teaching hospital in Thailand.

Methods. We undertook a retrospective review of the sedation service records of elderly patients who underwent colonoscopic procedures from December 2008 to April 2010. All patients were classified into two groups according to the body mass index (BMI). In group A, the patients had BMI < 25. In group B, the patients had BMI > 30. The primary outcome variable was the overall complication rate. The secondary outcome variables were sedation and procedure-related complications during and immediately after the procedure, and mortality rate.

Results. After matching age, gender, ASA physical status and indications of procedure, there were 100 colonoscopic procedures in group A and 33 colonoscopic procedures in group B. All sedation was given by residents or anesthetic nurses directly supervised by staff anesthesiologist in the endoscopy room. There were no significant differences in patients’ characteristics, sedation time, indication of procedure, overall complication rate, anesthetic personnel and mortality rate between the two groups. However, upper airway obstruction in group B was relatively higher than in group A. All complications were easily treated, with no adverse sequelae.

Conclusion. PBDS for colonoscopy procedure in marked obesity patients by trained anesthetic personnel with appropriate monitoring was relatively safe and effective. The complication rate of this technique in marked obesity (BMI > 30) patients was not different or worse than in non-obesity (BMI < 25) patients. Serious adverse events were rare in our population.
P-18

Anticancer Activity of MUC-30 in Breast Cancer Cells

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Keywords: MUC-30, apoptosis, human breast cancer cells (MCF-7), PARP-1

Introduction. Currently, a number of semi-synthetic of natural plant compounds have been developed to improve its effectiveness for treatment of cancers. In the present study, we investigated the anti-proliferative activity and apoptosis induction of MUC-30 in human breast cancer cells (MCF-7). MUC-30, a semi-synthetic analog of lignan, isolated from Phyllanthus taxodiifolius Beille (Krai Hang Nak).

Methods. MCF-7 cells were treated with various concentrations of MUC-30 (0.01-10 µM) for 24-72 h and cell viability was evaluated by MTT assay. The anti-proliferative effect of the compound to MCF-7 cells via apoptosis pathway was determined by using DAPI assay, flow cytometry analysis, and the protein expression of PARP-1.

Results. MUC-30 potently inhibited the proliferation of MCF-7 cells in a concentration- and time-related manners. Its IC50 (concentration that inhibits 50% of cell growth) was approximately 0.23 ± 0.07 µM at 72 h. The compound induced morphological changes of nucleus, caused an accumulation of apoptotic cell in subG0 phase, and induced the expression of cleaved PARP-1.

Conclusion. MUC-30 is a promising potent anti-proliferative agent for MCF-7 cells by inducing apoptosis. The result of this study is important for further development of this compound as a new therapeutic agent for cancer treatment.


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Piperine is Anti-Hyperlipidemic and Improves Endothelial-Dependent Vasorelaxation in Rats on High Cholesterol Diet

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Keywords: piperine, cholesterol, lipid, vasodilatation

**Introduction.** Piperine is a major ingredient of black pepper and long pepper, which are widely used as a spice and in Ayurvedic medicine. As an anti-hyperlipidemic, some reports show clear blood lipid reductions whilst others failed to show any effect. Therefore, we aimed to resolve this discrepancy and to show whether piperine could improve vascular endothelial function in cholesterol fed rats.

**Methods.** Male Sprague-Dawley rats (180-250 g) were made hypercholesterolemic by daily intragastric gavage of emulsified cholesterol for 8 weeks and piperine was given 8 h after cholesterol as appropriate to prevent digestive/absorptive interactions. Animals were divided into 4 groups: (i) sham (control), (ii) cholesterol (HC), (iii) the cholesterol plus 40 mg/kg piperine (Pip40) and (iv) cholesterol plus 80 mg/kg piperine (Pip80). Serum total cholesterol (TC), triglycerides (TG) and high density lipoprotein (HDL) were measured at week 0 and week 8. At week 8, rats were killed and endothelium-dependent vasorelaxation induced by acetylcholine in isolated aortae.

**Results.** Throughout the 8 week trial, treatment with piperine (40, 80 mg/kg) reduced body weight gain and food intake per day compared with control. The HC group exhibited elevation of both TC and TG. Piperine at 80 mg/kg but not low dose (40 mg/kg) partially reduced TC, while both doses effectively normalised the elevated TG. HDL was decreased in all animals including controls. Hypercholesterolemic and hypertriglyceridemic rats showed significant reduction of acetylcholine-induced vasorelaxation of isolated aortae and this was prevented by treatment with piperine.

**Conclusion.** This study showed that piperine reduced body weight gain, lowered TC and fully normalised TG and endothelial-mediated vasorelaxation of aorta. Thus piperine could provide beneficial effects in weight control, anti-hyperlipidemia and counteracted the poor vascular endothelial function in hyperlipidemia.

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Blood Pressure Elevation Associated with Alteration in Vascular Reactivity in the Chronic Mild Stress Animal Model of Depression

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Keywords : chronic mild stress, depression, blood pressure, vascular reactivity, endothelial function

Introduction. The pathogenesis of depression is associated with hyperfunction of the hypothalamic-pituitary-adrenocortical axis and sympathetic hyperactivity. These neurohormonal changes may influence vascular tone regulation.

Objective. The aim of this study is to assess the effects of chronic mild stress, an experimental model of depression, on blood pressure and vascular reactivity.

Methods. Conscious blood pressure were obtained from male Sprague Dawley rats by a tail-cuff method. Then rats were exposed to either 4 weeks of mild and unpredictable of stressors (CMS group) or standard housing condition (control group). At the end of the test period, the tail-cuff blood pressure were examined and all rats were subjected to forced swimming test to evaluate immobility time as an index of depression. Thoracic aorta was isolated for the assessment of vascular responses to phenylephrine (α-adrenergic receptor agonist; 10^{-10}-10^{-6} M), isoproterenol (β-adrenergic receptor agonist; 10^{-8}-10^{-4} M), and acetylcholine (muscarinic receptor agonist; 10^{-9}-10^{-5} M).

Results. At the end of the treatment period, CMS group showed significantly increased immobility time in the FST ($P < 0.05$ vs control), reflecting a state of lowered mood in these rats. The CMS also showed less body weight gain throughout the test period, implying the existence of poor appetite. The percentage of increased systolic pressure in CMS was significantly higher than that of control ($P < 0.05$). Assessment of functional vascular reactivity revealed that the response to vasoconstrictor phenylephrine of CMS was significantly increased, whereas the response to vasodilator isoproterenol was significantly attenuated ($P < 0.05$ vs control). The aortic rings of CMS also exhibited a significantly lowered response to acetylcholine compared to control ($P < 0.05$), suggesting a decrease in endothelial function.

Conclusion. The CMS rat model of depression showed vascular changes in response to adrenergic agonist and impairment of endothelium-dependent relaxation, which may contribute to the increase in blood pressure.

This work was supported by Faculty of Allied Health Sciences, Burapha University.
Sensitization of KB Cells to Doxorubicin by MAG 9-19 Extracted from *Micromonospora* in Marine Sediments

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*Keywords: KB cells, MAG 9-19, Micromonospora, apoptosis, caspase-3*

**Introduction.** The search for new anticancer drugs to induce apoptosis is urgently required. The marine actinomycetes genus *Micromonospora* has long been recognized as an important source of its secondary metabolites, including therapeutic antibiotics for antibacterial, anticancer, antioxidant, and anti-inflammatory. The coastal marine sediments are largely unexplored source for *Micromonospora*, however, little is known about its ability to produce anticancer activity and molecular mechanisms. The aim of the study was to investigate the apoptosis-modulating activities of MAG 9-19 from *Micromonospora* extraction in human carcinoma of nasopharynx (KB cells).

**Methods.** *Micromonospora* strains were extracted (MAG 9-19) and treated with KB cells. The viable cell number is based on MTT colorimetric assay. Apoptosis was assessed by nuclear staining with DAPI, agarose gel electrophoresis for DNA fragmentation assay and quantified by flow cytometry analysis of cells stained with propidium iodide. Caspase-3 activity was measured by a colorimetric assay.

**Results.** MAG 9-19 extraction (>1,000 μg/ml) was not cytotoxic in KB cells. Nevertheless, the combined MAG 9-19 (300 μg/ml) and doxorubicin (DOX) (1 μg/ml) treatment increased significantly the number of nuclei fragmentation with chromatin condensation, DNA fragmentation and hypodiploid cells (sub-G1 phase) compared with MAG 9-19, DOX alone. Apoptosis enhancement of combination treatment was accompanied by increasing in the relative activity of caspase-3 by (2.7 ± 0.25) fold which was significantly attenuated in a caspase-3 inhibitor. The morphological evidence indicated a diminished size, rounded and easily detached when compared with polygonal adherent cells in normal shape.

**Conclusions.** The induction of apoptosis by combined MAG 9-19 and DOX treatment involves the activation of effector caspases-3. MAG 9-19 significantly sensitized KB cells to death induced by DNA damage and kill cancer cells by mainly apoptosis, therefore, it is suggested that this compound is a promising anticancer agent for nasopharynx cells.

*Supported by Office of the Higher Education Commission (2011)*
**Introduction.** Neurovascular oxidative stress in acute stroke has been proposed a crucial roles in ischemic brain injury. Major causes of acute ischemic stroke are from large artery atherosclerosis and from small vessel occlusion caused by lipohyaline degeneration or micro-atheroma in deep perforating arteries within lacunar area. We hypothesize that cerebral endothelial dysfunction through NO release and sLOX-1 cleaved by protease of LOX-1 may be distinguished markers of acute stroke.

**Methods.** Changes of plasma NO and sLOX-1 were studied within the first 3, 6, 12, 18, 24 hours after the onset in 65 acute ischemic stroke patients and 18 control subjects. We investigated the influence of hemodynamics through stenotic flow velocity by carotid duplex to identify two groups of carotid blood flow patterns; non-significant stenosis flow (NSSF) and significant stenosis flow (SSF). Patients were divided into two groups, large vessel atherosclerosis (NSSF : n = 48, SSF : n = 6) and lacunar infarction (NSSF : n = 11). Blood samples were collected in EDTA-containing tubes and promptly centrifuged for 15 minutes at 3000 g. Plasma was frozen at -70°C until analysis. Plasma NO concentration was measured by electrochemistry and sLOX-1 was assayed by ELISA.

**Results.** In patients with atherosclerosis, plasma NO level significantly reduced in NSSF (55.01 ± 1.00 nmol/l) compared with control (77.12 ± 1.64 nmol/l). Remarkably reduced NO was showed in SSF (47.53 ± 1.72 nmol/l). sLOX-1 increased significantly in SSF (2.07 ± 0.03 ng/ml) compared with control (0.78 ± 0.03 ng/ml) whereas NSSF was 1.10 ± 0.02 ng/ml. In lacunar stroke, sLOX-1 (0.89 ± 0.03 ng/ml) was significantly lower than atherosclerotic group while NO (57.47 ± 0.58 nmol/l) was slightly lower.

**Conclusion.** These findings confirm the potential role of neurovascular oxidative stress in large artery atherosclerotic and lacunar stroke. sLOX-1 was extremely influenced by large arterial stenosis.

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The Journal Writing and the Follow-Up Classes Enhance Physiology Learning of the Weak Students

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Keywords: health science students, physiological education, instructional tools

Introduction. Physiology is an essential subject for health science students. To have a clear understanding of physiology, students need a deep understanding of its underlying concepts rather than to memorize disjointed pieces of information. This requires various higher order cognitive skills which are less important in other subjects. Non-medical students find learning these physiological concepts a struggle as reflected in their low scores and complaints about the difficulty of the subject. We therefore aimed to investigate whether the journal writing and follow-up classes would enhance the physiology learning for these weaker students.

Methods. Two cohorts of students (Section (S)1: n = 141, S2: n = 110) had matched educational backgrounds and the same low average midterm scores (14 ± 4, 14 ± 3 % out of 35 %, respectively) in the basic physiology course. The S1 students studied the subject without any intervention whereas the S2 students had been advised to write a journal after each lecture throughout the course and those students having had scores below the midterm mean (14 %) were encouraged to attend four 3-hours follow-up classes before the final examination. The average of the final (Fn) scores - the midterm (Mt) scores and the number of students in each grade of these two sections were compared.

Results. Using the T-score for the summative assessment, the S2 students attained more A-C grades compared to the S1 cohort and fewer of the lower grades D− F (see graph). The average of the (Fn-Mt) scores of the S2 was higher than the S1 students. The below-Mt-means students of the S2 achieved the highest grade at C+ but of the S1 at C.

Conclusion. This classroom research shows that the journal writing and the follow up classes improve the learning capacity, especially the weak students, in the basic physiology course.

0 5 10 15 20 25 30
Percentage of students (%)

No intervention

A B+ B C+ C D+ D F
**Inhibitory Effects of the Rambutan Rind Extract \(Nephelium lappaceum\) L. and Tannin on Lipase Activity \textit{In Vitro}\**

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**Keywords:** rambutan, \(Nephelium lappaceum\) L., tannin, lipase activity

**Introduction.** Obesity has become an important public health problem that can cause a number of obesity-related diseases, such as heart disease, stroke, diabetes, gallbladder disease, and certain types of cancer. The use of commercial anti-obesity drugs, such as orlistat and sibutramine, can cause unpleasant side effects such as mouth dryness, sleepless, agitation, restlessness, hallucination, constipation and steatorrhea. To overcome these problems, the use of plant extracts is probably a better way to replace these anti-obesity drugs. Rambutan (\(Nephelium lappaceum\) L.) is a medicinal plant containing phenolic compounds (anthocyanins, flavonoids, tannins, ellagic acid, corilagin and geraniin) that can prevent obesity. This study investigated the effects of ethanol rambutan rind extract (RRE) and tannin on lipase activity \textit{in vitro}.

**Methods.** Total phenolic content and DPPH determination for antioxidant activity of the RRE were determined. The inhibitory effects of various concentrations the RRE (5-80 mg/ml), tannin (1.25-20 mg/ml) and orlistat (40 mg/ml; positive control) on porcine pancreatic lipase activity were determined by measuring the rate of the release of oleic acid from triolein using titrimetric method.

**Results.** RRE had total phenolic content of 18.69 ± 0.2 mg gallic acid/g dry extract and antioxidant activity values (IC\textsubscript{50}) of 0.288 ± 0.04 mg/ml extract. Orlistat (40 mg/ml) showed strong inhibition of lipase enzyme activity (62.49 %). The RRE and tannin inhibited lipase enzyme activity in a dose-dependent manner. The percentages of lipase enzyme activity inhibition of RRE (20, 40 and 80 mg/ml) and tannin (20 mg/ml) were 9.65 %, 33.46 %, 72.68 % and 52.27 %, respectively.

**Conclusion.** The present findings suggested that the RRE and tannin displayed beneficial effects in the treatment of obesity, possibly by lipase inhibition. The phenolic compounds may play a key role in lipase inhibition. However, further investigations are needed to elucidate the effectiveness of the RRE \textit{in vivo}.

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Diarylheptanoid, a Phytoestrogen from *Curcuma comosa* Roxb, Increases Femoral Arterial Blood Flow in Ovariectomized Rats via Nitric Oxide Signalling

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Keywords: *Curcuma comosa* Roxb, femoral arterial blood flow, nitric oxide signaling, rats

**Introduction.** Postmenopausal estrogen therapy is associated with lower risk of cardiovascular diseases attributed, in part, to the favorable hemodynamic action of estrogen. The compound (3R)-1-7-diphenyl-(4E,6E)-4,6-heptadien-3-ol (D12) present in *Curcuma comosa* Roxb (*C comosa*) enhances relaxation of rat aortic ring via nitric oxide (NO). We have shown that the extract of *C comosa* increased femoral blood flow (FBF) likely via NO signaling. This study aimed to investigate an acute effect and mode of action of D12 on the vascular hemodynamic responses in ovariectomized rats.

**Methods.** Sprague-Dawley female rats (8 weeks old) obtained from the National Animal Centre, Mahidol University were bilaterally ovariectomized and allocated to 3 groups: 1) control; 2) D12; 3) estradiol (E2) group. Rats were intra-arterially injected with vehicle (7% ethanol in saline) in control group, D12 at doses of 100, 200, 400 and 800 µg/kg in D12 group, or E2 at doses of 1, 2, 4, and 8 µg/kg in E2 group. Measurements of arterial blood pressure (mABP) via carotid catheter, heart rate, and FBF using a laser Doppler probe were made at every 15 min intervals. To elucidate the mechanism by which D12 enhance FBF, rats were pretreated with L-NAME (10 mg/kg), ODQ (quanylyl cyclase inhibitor, 900 µg/kg) or ICI 182,780 (estrogen receptor antagonist, 900 µg/kg) by infusion via left femoral artery for 30 min prior to D12 (800 µg/kg) or E2 (4 µg/kg).

**Results.** E2 and D12 dose-dependently increased FBF whereas mABP were not changed, suggesting decreases in femoral vascular resistance (FVR). Pretreatment with various blockers (L-NAME, ODQ, ICI 128,780) prevented the effects of both D12 and E2 on FBF and FVR.

**Conclusion.** This study indicates that D12 increases FBF in ovariectomized rats, in part, via estrogen receptor and nitric oxide signaling.

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The Suggested Medical Physiology Learning Contents from the Six Affiliated Hospitals, Faculty of Medicine, Naresuan University

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Keywords: medical students, physiological education, preclinical-clinical relations

Introduction. Medical physiology is essential knowledge for practicing physicians but few physicians have direct input to Physiology curricula. Most physiology teaching staff are from science research backgrounds and thus judging the appropriate content, amount and depth of physiological knowledge applicable to the clinic is a challenge. Therefore, we questioned our current clinical teaching staff about what they considered appropriate core medical physiological knowledge.

Methods. The questionnaire was based on our current medical physiology course but also drew from the Medical Physiology Core Learning Objective Project (edited 2010) of The Physiological Society (UK) and The American Physiological Society. The questions related to each organ system were about: (1) their general opinion about physiological knowledge of the students they teach, (2) differentiating between what “must know”, “should know” and “good to know” and (3) most appropriate teaching scenario. About four hundred questionnaires were distributed to the clinical teaching staff in twelve clinical departments throughout our six affiliated hospitals including Buddhachinaraj Phitsanulok Hospital, King Taksin the Great Hospital, Phichit Hospital, Phrae Hospital, Uttaradit Hospital and Naresuan University Hospital.

Results. The results were analyzed from 162 questionnaires (returned rate 40 %), 12-28 papers/system with the highest (20 %) from department of internal medicine. Most staff were moderately satisfied with the student physiological knowledge but otherwise suggested increased clinical relevance and application. The learning contents in each body system were returned as “must know” and “should know” except the muscular system was given as “should know.” 100 % of “must know” was hypoxia and the menstrual cycle. The first three ranks of suggested scenarios in each system were reported.

Conclusion. Most medical physiology learning contents closely aligned with later clinical needs but regular updates will track changes in medical practice.

We thank all faculty and hospital staff who contributed to this survey.
Twelve Weeks of Brisk Marching Decreases Oxidative Stress and Improves Antioxidant Status in Sedentary Thai Woman

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Keywords: brisk marching, oxidative stress, antioxidant, sedentary, exercise

Introduction. Sedentariness refers to behaviors with low energy expenditure. It is one of the major risk factors of cardiovascular disease (CVD) and the metabolic syndrome. It is well established that exercise training improves cardiovascular fitness and promotes the antioxidant defense capacity against the oxidative stress. However, the exercise training by brisk marching exercise has not been evaluated. Therefore, the aim of this study was to evaluate the changes in antioxidant glutathione (GSH) and oxidative stress status in sedentary women after performing 12 weeks of moderate intensity exercise by brisk marching.

Methods. Twenty sedentary Thai women (aged 23-55 years, BMI 18.5 to 24.9 kg/m²) was performed exercise training by brisk marching. All volunteers were randomly divided into 2 groups: Exercise group (n = 10) engaging to brisk marching at 75 to 80% of HRmax, 30min/session, 3-5 sessions/week for 12 consecutive weeks; Control group (n = 10) with no brisk marching. Blood samples were collected for measurement of oxidative stress and antioxidant markers at before (baseline) and 12 weeks after exercise session.

Results. There were no significant differences in all parameters measured at the baseline period. After 12 weeks, a significant decrease in plasma malondialdehyde, and protein carbonyl levels was found in the exercise training group (P < 0.05). The brisk marching also enhanced the antioxidant defense as shown by the increase in GSH levels and the GSH redox ratio.

Conclusions. Brisk marching exercise training should be recommended for daily exercise in sedentary individuals in order to reduce CVD risk.

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P-28

Weight Loss Effects of the Fruit Hull of Mangosteen (Garcinia mangostana L.) Extract in Aged Male Wistar Rats

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Keywords: obesity, body weight, mangosteen, Garcinia mangostana

Introduction. The fruit hull of mangosteen (Garcinia mangostana L.) is the rich source of polyphenols (xanthones, tannins, flavonoids, anthocyanins) that have anti-oxidant properties. Traditionally, mangosteen has been used for treating diarrhea, skin infections, and diabetes. Little is known about the anti-obesity effect of mangosteen. Therefore, the effect of mangosteen extract (GME) on body weight changes of adult and aged male Wistar rats was investigated.

Methods. Total anthocyanin content and antioxidant activity (DPPH assay) of GME were determined. Eight weeks old rats (n = 32) and 10 months old rats (n = 24) were daily orally administered with vehicle or GME (500 and 1000 mg/kg) for 30 days. Body weight of all rats was recorded weekly. At day 30, rats were fasted overnight and plasma samples collected by cardiac puncture were used for monitoring liver function by measuring levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT).

Results. The total anthocyanin content in GME was 168.2 μg/g dry sample extract and IC50 value for anti-oxidation were 3.55 mg/ml sample extract. At day 8, 15, 22 and 29, significant increases in body weight were found in all adult groups while significant decreases in body weight were found only in 1000 mg/kg GME aged rats, compared with day 1 (P < 0.05). Body weight of adult and aged rats did not differ between treatments over 30 days. There was no significant difference in AST and ALT plasma levels among groups in adult and aged rats.

Conclusion. The present study provided the first evidence of weight loss effects of the GME in aged rats. High anthocyanin content and strong anti-oxidation activity of GME has potential to be obesity-preventive candidate in aging. Further studies are required to determine the most effective dose of GME for losing weight in aging rats and to confirm the benefits of GME in other obesity models.

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Neurotonic Effects of Mango and Papaya on Psychomotor and Cognitive Function in Aged Rats.

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Keywords: Thai fruits, physical ability, cognition, aging

Introduction. Ripe mango (“Nam dok mai” variety) and ripe papaya (“Holland” variety) are local Thai fruits which contain high antioxidant agents such as polyphenols, vitamin C and beta-carotene. So far, there is no health report concerning the long-term consumption of these fruits on body weight, psychomotor performance, and cognitive function in elderly people. We, therefore, investigated this issue by conducting a series of experiments in aging male and female rats.

Methods. Aging rats (16-18 month old) of both sexes were randomly divided into 3 groups: a control group, mango-treated group, and papaya-treated group. Prior to and at the end of treatment session, each rat was subjected to psychomotor (beam walking, grip strength, and rotarod treadmill tests) and cognitive function (Novel Object Recognition and Morris Water Maze tests) evaluations. During treatment session, the treated group was orally administered with a single bout of blended ripe mango or papaya at a volume of 1 ml/100 g b.w. everyday for 90 consecutive days.

Results. After feeding ripe mango or ripe papaya for 90 days, both male and female rats showed a slight decrease in their body weights when compared to the old control group which showed no change. In addition, there is no improvement of the psychomotor performance in either mango- or papaya-treated group. Nevertheless, these treated groups showed better recognition and spatial memories when compared to the control group.

Conclusion. Long-term consumption of ripe mango or papaya can restore a memory deficit in aging rats. These preliminary data indicate a nootropic effect of ripe mango or papaya which may be beneficial for elderly people who are suffering from a dementia.

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Neurotonic Effects of Young Coconut Juice on Psychomotor and Cognitive Function in Aging Rats.

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Keywords: coconut water, physical ability, cognition, aging

Introduction. Young coconut juice (YCJ) has long been a popular natural drink as it enriches of health beneficial substances such as carbohydrate, fat, protein, vitamins, minerals and antioxidants. A recent study by Radenahmad et al. (2010) has into 2 groups: a control (water-treated) group and YCJ-treated group. Prior to and at the end of treatment session, each rat was subjected to psychomotor (beam walking, grip strength, and rotarod treadmill tests) and cognitive function (Novel Object Recognition and Morris Water Maze tests) evaluations. During treatment session, the treated group was orally administered with a single bout (1 ml/100 g b.w.) of YCJ or distill water in everyday for 90 consecutive days.

Results. After feeding YCJ for 90 days, both male and female rats showed a slight increase in their body weights when compared to the control group. However, there is no improvement of the psychomotor performance in CYJ-treated group. In addition, both sexes that received YCJ showed better recognition memory, but only YCJ-treated female rats showed an improvement of spatial memory.

Conclusion. Long-term consumption of YCJ can help to improve the cognitive function especially in female aging rats. These preliminary data indicate a nootropic effect of coconut water which may beneficial for menopausal women who are suffering from Alzheimer’s disease.

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