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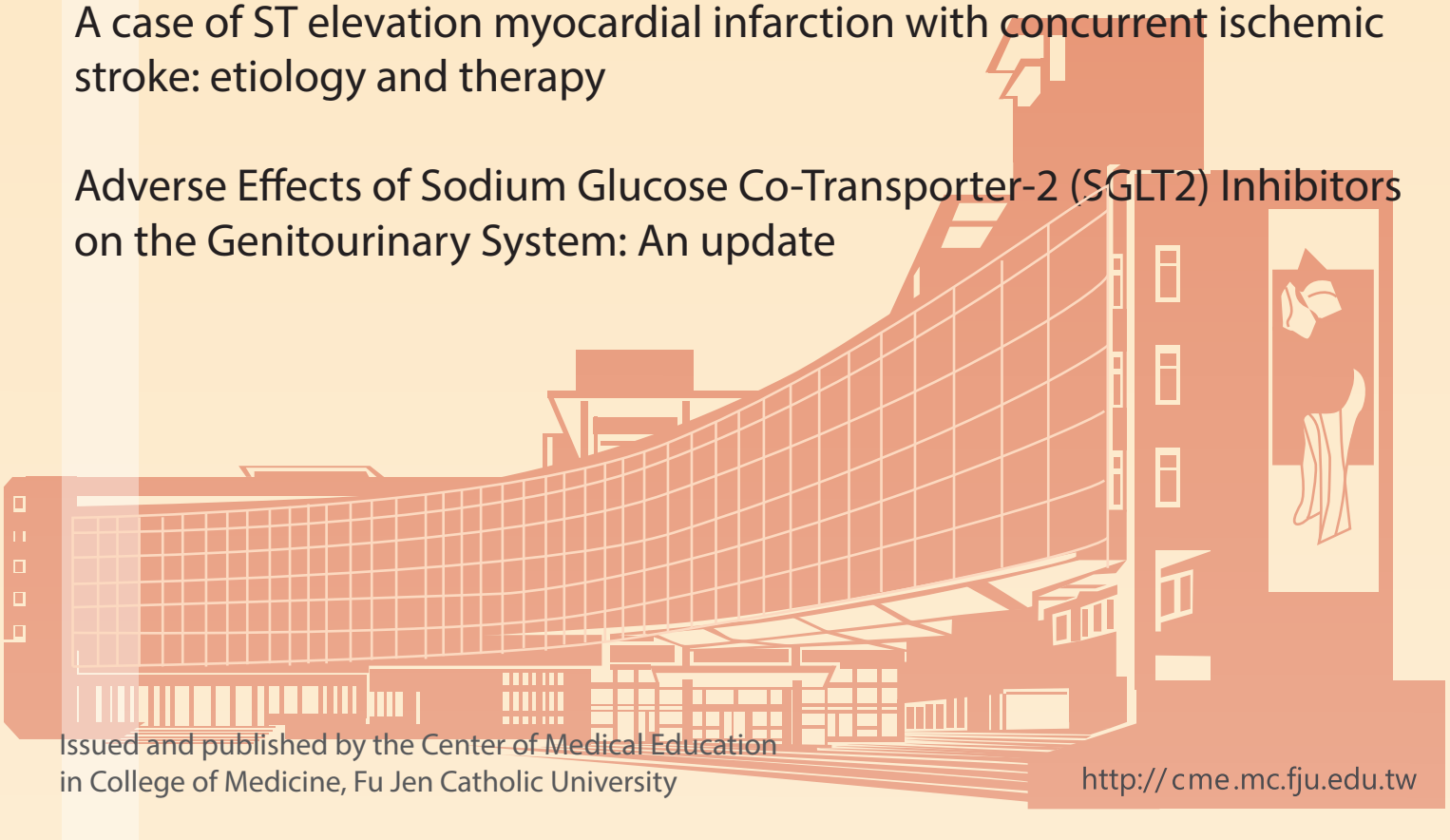

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Original Research Article

Lipopolysaccharide modified brown adipose tissue lipolysis and mitogenesis via up-regulation of cPLA2 and COX-2 expression

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ABSTRACT

Background and purpose: Obesity is a global health issue associated with various metabolic syndromes, influenced by factors including gut microbiota and adipose tissue dynamics. This study investigated how lipopolysaccharide (LPS) impair brown adipose tissue (BAT), focusing on its roles in lipolysis and mitogenesis, which are crucial for energy expenditure and obesity management. ***Methods:*** In this study, male C57BL/6 mice were fed a high-fat diet (HFD) and treated with LPS alone or LPS combined with various inhibitors to detect the specific proteins involved in BAT func-

tion. Immunohistochemical staining was used to investigate the changes in protein expression. ***Results:*** LPS-upregulated expression of cytosolic phospholipase A2 (cPLA2) and cyclooxygenase-2 (COX-2) in BAT was separately suppressed by AG490, BML-275, apocynin, and N-acetylcysteine, suggesting the participation of JAK, AMPK, and NADPH oxidase/ROS in mediating LPS effects. LPS treatment affected lipolysis and mitogenesis by downregulating the expression of adipose triglyceride lipase, PPAR-γ coactivator-1α, and uncoupling protein 2. Inhibition of cPLA2 or COX-2 reversed the effects of LPS, highlighting their pivotal roles in the LPS-induced reduction of lipolysis and mitogenesis. Thus, LPS treatment decreases expression of proteins related to metabolic functions in BAT, contributing to suppression of thermogenesis and promotion of obesity. ***Conclusion:*** The findings of this study indicate that LPS influences BAT functionality by modulating cPLA2 and COX-2 levels, which affects lipolysis and mitochondrial content. These findings highlight a potential association between bacterial endotoxins and obesity, suggesting that targeting these pathways may offer new therapeutic strategies for managing obesity.

Keywords: Lipopolysaccharide, cPLA2, COX-2, Adipocyte

INTRODUCTION

Obesity, which results from the accumulation and enlargement of adipose tissue, is a worldwide problem that increases spending on healthcare as it is associated to various pathological conditions, including type 2 diabetes mellitus, and dyslipidemia[1-4]. Two main types of adipose tissues (white and brown) have been detected. Unlike white adipose tissue (WAT), which primarily serves as a storage for lipid droplets, brown adipose tissue (BAT) stands out because of its high vascularization and abundance of mitochondria[5]. BAT is crucial for adaptive thermogenesis, a physiological mechanism that expends energy in reaction to environmental changes, including exposure to cold temperatures and changes in diet[6, 7]. An increase in the expression of PPAR-γ coactivator-1α (PGC-1α), a major regulatory protein of mitochondrial synthesis and function, drives the cell to mitochondrial synthesis[8]. PGC-1α promotes the transcription of nuclear respiratory factors (NRF)-1 and NRF-2, thereby stimulating mitochondrial DNA replication and the expression of mitochondrial genes, such as cytochrome c, uncoupling protein 1 (UCP1), and UCP2, through the regulation of mitochondrial transcription factor A (TFAM) expression[9]. UCP2 contributes to BAT thermogenesis by promoting use of nonesterified fatty acid, and loss of UCP2 impairs cold-induced nonshivering thermogenesis by promoting a shift from fatty acid toward glucose utilization in BAT[10]. On the other hand, adipose triglyceride lipase (ATGL) plays an important role in initiating lipolysis. Expression of ATGL promotes degradation of triglycerides within adipocytes into diacylglycerol, which is further metabolized into monoacylglycerol and subsequently glycerol and fatty acids by hormone-sensitive lipase and monoacylglycerol lipase. Apart from being released into the bloodstream as glycerol, the fatty acids are transported to the mitochondria for oxidation to produce heat in BAT[11]. Thus, BAT has an advantage over fat burning as it relies on ATGL-related lipolysis and PGC-1α-dependent mitogenesis.

There are two kinds of adipose tissue changes in obesity, hyperplasia and hypertrophy. Several previous studies report that the increase in adipocyte numbers in hyperplasic obesity are controlled by the action of various hormones and cytokines, such as macrophage colony-stimulating factor, transforming growth factor-β, basic fibroblast growth factor, and bone morphogenetic protein[12-21]. The lipopolysaccharide (LPS), also called endotoxin, is the outer membrane of Gram-negative bacteria and is thought to be a mediator linking gut microbiota, inflammation, and high-fat diet-related metabolic syndrome[22]. In our previous studies, we found that LPS contributed to hyperplasia of adipose tissue via promoting the proliferation and adipogenesis of preadipocytes[23, 24]. Cytosolic phospholipase A2 (cPLA2) and cyclooxygenase-2 (COX-2) promote the production of arachidonic acid and subsequently eicosanoids in the inflammatory responses. cPLA2 and COX-2 are involved in proliferating regulation of cells, such as keratinocytes[25], glioblastoma[26], mesenchymal stromal cells[27] and myoblasts[28]. Similarly, we found that the proliferation of adipogenesis of 3T3-L1 preadipocytes was mediated by LPS-stimulated cPLA2[24] and COX-2 expression[23]. However, the effects of cPLA2 and COX-2 on BAT are still unclear.

In this study, we found that LPS enhanced cPLA2 and COX-2 expression in high fat diet-fed obsess mice. Expression of cPLA2 or COX-2 was suppressed by treatment with an inhibitor of cPLA2 (AACOCF3), COX-2 (NS398), JAK (AG490), AMPK (BML-275), NADPH oxidase (apocynin), or scavenger of ROS (N-acetylcysteine), separately. LPS reduced the expression of proteins for lipolysis (ATCL) and mitogenesis (PGC-1α and UCP2), which was reversed by treatment with AACOCF3 and NS398. These results suggested that LPS decreases the lipolysis and mitogenesis of BAT via upregulation the expression of cPLA2 and COX-2. In summary, LPS contributes to hypertrophic obesity via decreasing the heat burning of BAT by upregulating cPLA2 and COX-2.

MATERIALS AND METHODS

Materials

Antibodies targeting cPLA2 and COX-2 were sourced from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Antibodies for ATGL, PGC-1α, and UCP2 were obtained from Abclonal (Woburn, MA, USA). Compounds, such as AACOCF3, NS398, AG490, BML-275, apocynin, and N-acetylcysteine, were procured from Biomol (Plymouth Meeting, PA, USA). LPS, various enzymes, and additional chemicals were supplied by Sigma (St. Louis, MO, USA).

Animal manipulation

Male C57BL/6 mice, aged 3 weeks, were acquired from BioLASCO Taiwan Co., Ltd., Taiwan, ROC. These animals were kept individually in a room with controlled environmental conditions, under a 12-hour light/12-hour dark cycle. The mice had unrestricted access to food and water. Experimental activities were con-

ducted in adherence to the Guide for the Care and Use of Laboratory Animals, and they received approval from the Institutional Animal Care and Use Committee of Fu Jen Catholic University, Taiwan (IACUC No. A10525). The dietary regimen of this study included a standard chow diet comprising 13.5% calories from fat and a high-fat diet (HFD) with 60% of calories from fat, with a gradual transition from the chow diet to the HFD. Mice were allocated randomly into the following treatment groups: solely HFD; HFD combined with LPS administration at 100 μg/kg/week; and HFD combined with various inhibitors, such as AACOCF3 (AA), NS398 (NS), AG460 (AG), BML-275 (BML), apocynin (APO), and N-acetylcysteine (NAC) at 2 mg/kg/week, each administered separately prior to LPS treatment. The experiment spanned a period of 10 weeks. Post experimentation, adipose tissue from the interscapular region (BAT) was harvested for further analysis.

Immunohistochemical (IHC) staining

Paraffinized tissues were deparaffinized, rehydrated, and washed with TTBS as previously described[29]. Nonspecific binding was blocked by pre-incubation with a blocking solution for 1 h at room temperature. The sections were incubated with an anti-cPLA2 (1:200), anti-COX-2 (1:200), anti-ATGL (1:200), anti-PGC-1α (1:200) or anti-UCP2 antibody at 4°C for 16 h before incubation with anti-rabbit HRP (horseradish peroxidase) antibody at room temperature for 1 h. Bound antibodies were detected by incubation in 0.5 mg/mL with 3,3-diaminobenzidine (DAB)/0.01% hydrogen peroxide in 0.1 M Tris-HCl buffer as the chromogen (Vector Lab, Burlingame, CA, USA).

RESULTS

LPS upregulated cPLA2 expression in the BAT

Mice subjected to a HFD exhibited signs of endotoxemia, a condition characterized by the presence of bacterial endotoxins, specifically LPS, in the blood. This chronic metabolic endotoxemia is associated with obesity, insulin resistance, and diabetes[22]. Additionally, the enzyme cPLA2 plays a pivotal role in adipogenesis, the process by which fat cells (adipocytes) are formed[30]. We previously reported that stimulation of preadipocytes with LPS may increase proliferation and adipogenesis via JAK/STAT and AMPK-regulated cPLA2 expression[24]. To examine LPS-mediated cPLA2 expression in the BAT of HFD-fed obese mice, the protein expression levels of cPLA2 were studied. Compared to the group of mice fed with only HFD, administration of LPS increased cPLA2 expression at the protein levels (Figure 1). Similarly, treatment with a cPLA2 inhibitor (AACOCF3), but not a COX-2 inhibitor (NS398), reduced the effects of LPS on cPLA2 regulation in the BAT of obese mice (Figure 1). Moreover, administration of a JAK inhibitor (AG490) or an AMPK inhibitor (BML-275) also attenuated LPS-mediated cPLA2 expression in the BAT (Figure 1), indicating the role of JAK and AMPK in mediating LPS effects in vitro and in vivo. In summary, stimulation with LPS promoted cPLA2 expression in the BAT of HFD-fed mice via the JAK/STAT and AMPK pathways.

Effects of LPS on stimulating COX-2 expression in the BAT

Previously, we demonstrated that LPS enhanced the proliferation and adipogenesis of preadipocytes via a NADPH oxidase/ROS/p42/p44 MAPK-dependent mechanism that upregulated COX-2 expression[23]. To examine how LPS modulates COX-2 expression in the BAT of obese mice, IHC staining was used. Figure 2 shows the expression of COX-2 was upregulated at the protein levels in LPS-treated HFD mice compared to that in HFD-only mice. The expression of COX-2 protein was suppressed by treatment with the COX-2 inhibitor (NS398), but not with the cPLA2 inhibitor (AACOCF3) (Figure 2). Concurrently, administration of a NADPH oxidase inhibitor (apocynin) or a ROS scavenger (N-acetylcysteine) reduced LPS-stimulated COX-2 expression in the BAT (Figure 2). Overall, these data reveal that LPS-regulated COX-2 expression in the BAT of HFD-fed mice is governed by the NADPH oxidase/ROS pathway.

LPS modulated lipolysis of adipose tissue via upregulated cPLA2 and COX-2

Adipose triglyceride lipase (ATGL) and hormone-sensitive lipase play crucial roles in reducing the volume of adipose tissue by initiating the breakdown of triglycerides into diacylglycerol and subsequently into monoacylglycerol and fatty acids[11]. This metabolic cascade facilitates the decrease in triglyceride concentrations within fat cells, directly causing the reduction of adipose tissue mass. To investigate the effects of LPS-induced promotion of cPLA2 and COX-2 on the lipolysis process in BAT, HFD-fed mice were administered LPS 1 h after treatment with a cPLA2 inhibitor (AACOCF3) or a COX-2 inhibitor (NS398). The expression of ATGL was detected via IHC staining. We discovered that the

expression of ATGL was significantly lower in the LPS than in the HFD-fed-only groups (Figure 3). Reducing the expression of cPLA2 and COX-2 with the inhibitors AACOCF3 and NS398, respectively, suppressed the LPS-modulated effects on ATGL expression in the BAT (Figure 3). Overall, the results suggest that LPS reduces the process of lipolysis via cPLA2 and COX-2 mechanisms.

LPS modified mitogenesis of BAT via stimulation of cPLA2 and COX-2

Apart from being transported to the serum, the fatty acids from lipolysis may impair the function of mitochondria, which play important roles in generating heat by BAT. The synthesis and content of mitochondria are regulated by the protein PPAR-γ coactivator-1α (PGC-1α). PGC-1α stimulates mitochondrial DNA replication and the expression of mitochondrial genes, including uncoupling protein 2 (UCP2)[9]. To assess the impact of LPS-induced stimulation of cPLA2 and COX-2 on mitochondrial content in BAT, mice fed a HFD were treated with LPS 1 h after the administration of either a cPLA2 inhibitor (AACOCF3) or a COX-2 inhibitor (NS398). The expression of PGC-1α and UCP2 was investigated by IHC staining. We found that the increase in LPS reduced the expression of PGC-1α together with UCP2 in the BAT of obese mice (Figure 4A and B). Inhibition of cPLA2 or COX-2 reversed the LPS-decreased expression of PGC-1α and UCP2 in the BAT of obese mice (Figure 4A and B). In summary, LPS reduced the mitochondrial content of BAT via the expression of cPLA2 and COX-2.

DISCUSSION

The increase in obesity escalates healthcare expenses by increasing the risk of associated conditions, such as type 2 diabetes mellitus and dyslipidemia[1-4]. BAT is more efficient than WAT in metabolizing lipolysis-derived free fatty acids via mitochondrial activity. Although less prevalent in infants, adult BAT has been detected in various locations, including visceral BAT (e.g., perivascular BAT), perivisceral BAT, BAT surrounding solid organs, and subcutaneous BAT[31]. The latter was observed between the anterior neck muscles and in supraclavicular fossa, posterior to the brachial plexus, beneath the clavicles, in the axilla, on the anterior abdominal wall, and in the inguinal area[31]. In our previous studies, we discovered that LPS promoted the proliferation and adipogenesis of preadipocytes via JAK/STAT and AMPK-regulated cPLA2 expression[24] and NADPH oxidase/ROS/p42/p44 MAPK-dependent COX-2 expression [23]. In this study, we investigated the role of cPLA2 and COX-2 in the BAT of LPS-stimulated obese mice. We discovered that expression of cPLA2 was inhibited by treatment with AG490 and BML-275. This revealed the role of JAK and AMPK in regulating LPS-mediated cPLA2 expression in BAT. Similarly, treatment with apocynin and N-acetylcysteine reduced LPS-mediated COX-2 expression, which indicated that NADPH oxidase and ROS participated in regulating COX-2 expression in LPS-treated BAT. Moreover, we discovered that inhibition of cPLA2 and COX-2 reversed ATGL, PGC-1α, and UCP2 expression in LPS-treated BAT, which suggested that LPS decreased lipolysis and mitogenesis of BAT via cPLA2 and COX-2 expression. Overall, our results suggested that increasing LPS in circulation may diminish lipolysis and mitogenesis, thereby contributing to the worsening of obesity via upregulation of cPLA2 and COX-2 expression (Figure 5).

Obesity, driven by excessive caloric intake and positive energy balance, involves more than just energy imbalances [32, 33]. It is characterized by increased inflammatory markers, leading to chronic low-grade inflammation, known as meta-inflammation, which disrupts normal metabolism[34]. Previous studies have reported that increased expression of cPLA2 and COX-2 promotes the proliferation and differentiation of preadipocytes, which may contribute to the development and expansion of adipose tissue in children[23, 24]. In this study, the involvement of cPLA2 and COX-2 in LPS-treated BAT was investigated. Increased cPLA2 protein expression in LPS-treated obese mice was regulated by JAK and AMPK, while COX-2 expression was modulated by NADPH oxidase and ROS. These findings are consistent with results from previous in vitro studies on preadipocytes. In fact, it is suggested that COX-2-mediated signaling may have dual roles on obesity and insulin resistance. Over-expression of the COX-2 gene in WAT has been shown to promote the formation of beige adipocytes within WAT, enhancing systemic energy expenditure and providing protection against high-fat diet-induced obesity. Conversely, COX-2 activation in epididymal adipose tissue is strongly associated with increased inflammation, insulin resistance, and fatty liver in rats subjected to a high-fat diet[35]. In addition, the potential modulation of cPLA2 or COX-2 in combating obesity is attracting increasing attention. cPLA2 is identified as a key molecular target of pyruvate, demonstrating that pyruvate mitigates

diet-induced obesity, white adipose tissue inflammation, and hepatic steatosis in a cPLA2-dependent manner, with studies in cPLA2 knockout mice confirming that the protective effects of pyruvate are largely diminished without cPLA2, highlighting the significance of the pyruvate/cPLA2 interaction in reducing inflammation and obesity[36].

Unlike WAT, BAT has two characteristics: abundance of mitochondria and numerous small multilocular lipid droplets[5, 37]. Fatty acids are liberated from small lipid droplets via lipolysis before they are transported into the mitochondria, serving as one of the energy sources[38]. This mechanism activates thermogenesis, thereby regulating body temperature and energy expenditure. Expression of ATGL facilitates the hydrolysis of triglycerides and the release of fatty acids. PGC-1α, acting as a transcription factor, plays crucial roles in mediating mitochondrial biogenesis, mitochondrial dynamics, and mitophagy to maintain a steady-state of mitochondria[39]. After being delivered to the mitochondria, fatty acids are used as an energy source to promote ATP production and heat generation by the action of UCP-2[40]. In this study, we discovered that administration of LPS reduced lipolysis and mitogenesis by downregulating the expression of ATGL, PGC-1α, and UCP-2 in BAT. Inhibition of cPLA2 or COX-2 attenuated LPS effects on BAT, indicating that LPS contributed to obesity via modulated lipolysis and mitochondrial contents of BAT through cPLA2 and COX-2. Previous studies that showed that systemic and adipocyte-specific ATGL knockout mice exhibited moderate obesity have reported that defects in triglyceride mobilization within adipocytes result in obesity. These studies have demonstrated an approximately twofold increase in WAT depots and five–sevenfold increase in BAT[[41]. Similarly, a defect in UCP-2 impairs cold-induced nonshivering thermogenesis, promoting a shift from non-esterified fatty acid utilization toward glucose utilization in BAT[10].

There is a correlation between gut microbial composition and metabolic syndromes, such as obesity[42]. Additionally, microbiota-related LPS influences both inflammation and metabolic outcomes[22]. Furthermore, a relationship between inflammation and obesity has been proposed. Treatment with dexamethasone decreased the expression of Early B-cell Factor 2 (EBF2), a marker of beige precursor cells, impairing the ability of WAT to differentiate into beige adipocytes via reducing the expressions of thermogenic response genes, Ucp-1, Dio2, and Pgc1α[43]. cPLA2 knock out mice on a normal diet have less adipose tissue and lower liver triglyceride levels than WT mice, associated with decreased serum PGE2[44]. In this study, we found that stimulation of BAT with LPS enhanced the expression of cPLA2 and COX-2, which were modulated separately by JAK, AMPK, or NADPH oxidase/ROS. These results corroborate the findings of[40] who reported that toll-like receptor (TLR4) knockout mice or pharmacological inhibition of TLR4 with Atorvastatin attenuate adipose tissue remodeling by reducing macrophage infiltration and adipocyte atrophy in Lewis lung carcinoma tumor-bearing mice. Ablation of TLR4 provides resistance to LPS-stimulated ER stress, resulting in a reduction of subcutaneous WAT browning and thermogenesis[45]. Thus, understanding the effects of LPS on BAT may increase opportunities for developing strategies against obesity.

CONCLUSIONS

In summary, based on literature and our findings, the effects of LPS on lipolysis and mitogenesis in BAT are shown in Figure 5. LPS-enhanced cPLA2 expression in BAT was reversed by AG490 and BML-275, suggesting that JAK and AMPK are involved in regulating LPS effects on BAT. Expression of COX-2 was suppressed by treatment with apocynin and N-acetylcysteine, which revealed the involvement of NADPH oxidase/ROS in LPS-stimulated COX-2 expression in BAT. cPLA2 and COX-2 participate in the LPS-mediated decrease in lipolysis and mitogenesis of BAT via downregulating ATGL, PGC-1α, and UCP-2 expression, leading to reduced thermogenesis and obesity. The mechanisms of LPS-stimulated cPLA2 and COX-2 expression provide a link between bacteria and obesity, suggesting novel strategies for treatment.

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FIGURE AND FIGURE LEGENDS

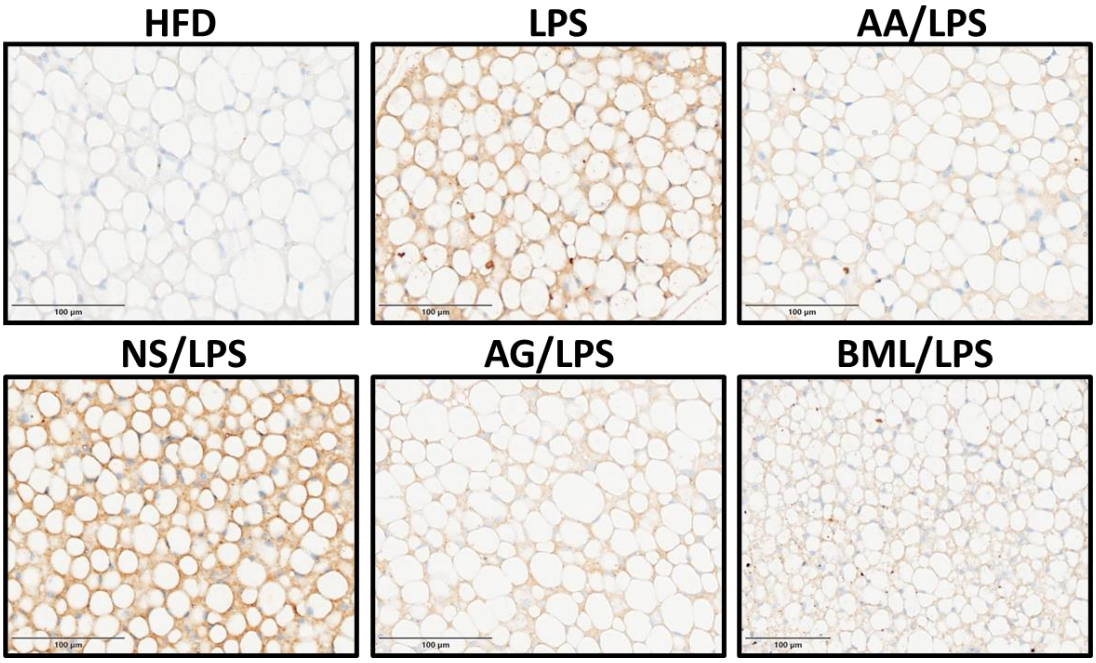

Figure 1. Expression of cPLA2 gene in the brown adipose tissue of High fat diet-fed mice. Mice were pretreated with or without of AACOCF3 (AA), NS398 (NS), AG460 (AG) or BML-275 (BML) (2 mg/kg/week) for 1 hour and then incubated with LPS (100 g/kg/week). Mice were fed with high fat diet (HFD) for 10 weeks and interscapular adipose tissues were isolated after sacrificed. Protein expression of cPLA2 were analyzed by immunohistochemical (IHC) staining.

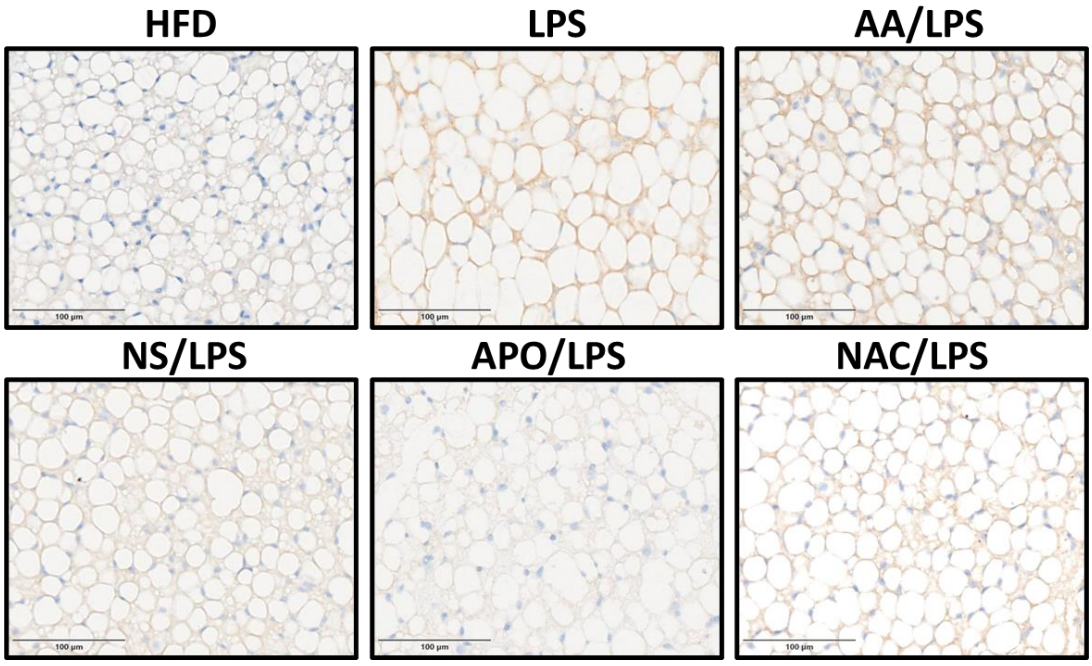

Figure 2. COX-2 expression in the brown adipose tissue of High fat diet-fed mice. Mice were pretreated with or without of AA, NS, apocynin (APO) or N-acetyl cysteine (NAC) (2 mg/kg/week) for 1 hour and then incubated with LPS (100 g/kg/week). All the mice were fed with HFD for 10 weeks and interscapular adipose tissues were isolated after sacrificed. Protein expression of COX-2 were analyzed by IHC staining.

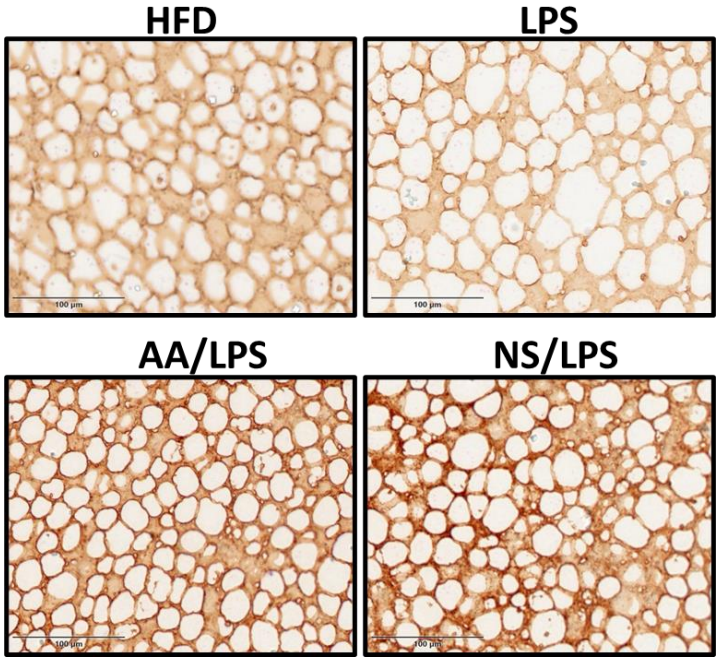

Figure 3. Expression of lipolysis-related ATGL gene in the brown adipose tissue of High fat diet-fed mice. Mice were pretreated with or without of AA or NS (2 mg/kg/week) for 1 hour and then incubated with LPS (100 g/kg/week). Mice were fed with HFD for 10 weeks and interscapular adipose tissues were isolated after sacrificed. The proein expression of ATGL were analyzed by IHC staining.

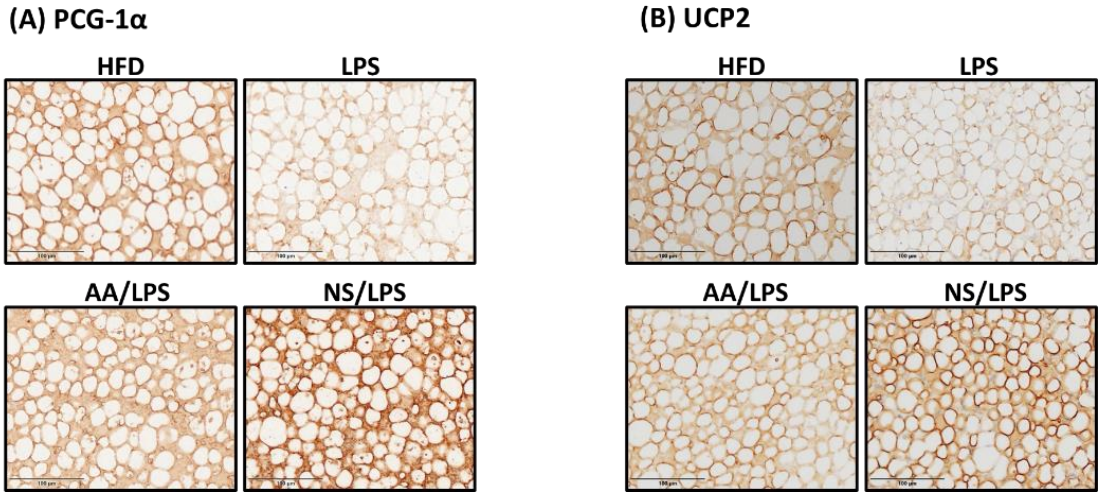

Figure 4. Expression of mitogenesis-related gene, PCG-1 α and UCP2 in the brown adipose tissue of High fat diet-fed mice. Mice were pretreated with or without of AA or NS (2 mg/kg/week) for 1 hour and then incubated with LPS (100 g/kg/week). Mice were fed with HFD for 10 weeks and interscapular adipose tissues were isolated after sacrificed. The protein expression of (A) PCG-1α and (B) UCP2 were evaluated by IHC staining.

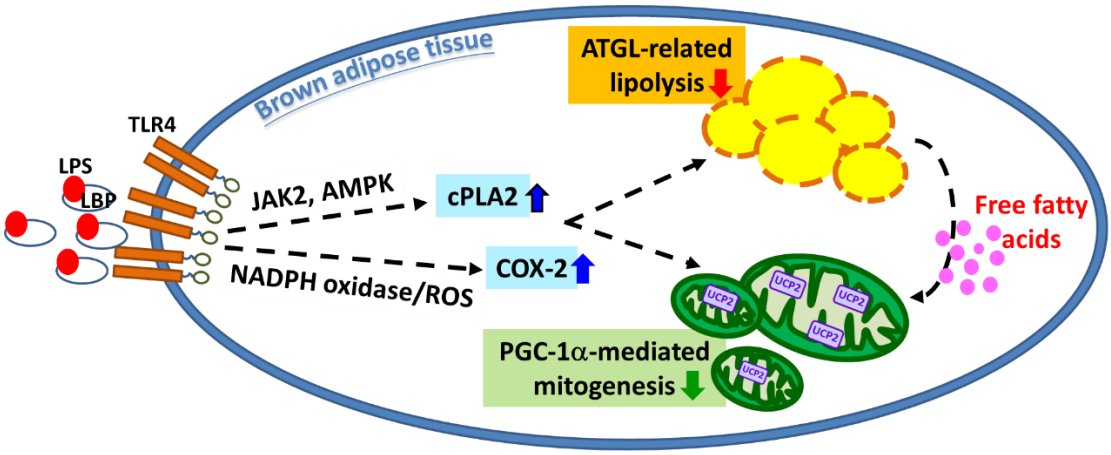

Figure 5. LPS modulated lipolysis and mitogenesis of BAT via upregulation of cPLA2 and COX-2. LPS stimulated expression of cPLA2 and COX-2 via JAK, AMPK pathway or NADPH oxidase-regulated ROS, separately. Upregulated cPLA2 and COX-2 participated in ATGL-dependent lipolysis and PCG-1α-regulated mitogenesis in LPS-treated BAT.

Lipopolysaccharide modified brown adipose tissue lipolysis and mitogenesis via up-regulation of cPLA2 and COX-2 ex-pression

內毒素透過上調 cPLA2 與 COX-2 表現改變棕色脂肪組織粒線體生成作用

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中文摘要

背景和目的：肥胖是一個全球性的健康問題與各種代謝症候群有關，會受到腸道微生物群改變和脂肪組織組成等因素的影響。本研究目的是探討內毒素(lipopolysaccharide，LPS)如何影響棕色脂肪組織(BAT)在脂解作用和粒線體增生作用中的作用，對於能量消耗和肥胖管理至關重要。**方法：**實驗中使用雄性 C57BL/6 小鼠進行高脂飲食(HFD) 餵食，並腹部施打 LPS 或結合各種抑制劑處理，之後取 BAT 並評估特定的蛋白質表現。使用的技術包括免疫組織化學染色、即時定量 PCR 和統計分析。**結果：**LPS 的處理增加 BAT 中 cPLA2 和 COX-2 的表現量，並分別被 AG490、BML-275、apocynin 或 N-acetylcysteine 所抑制，說明 JAK/STAT、AMPK 和 NADPH oxidase/ROS 參與調控 LPS 的作用。LPS 處理通過減少 adipose triglyceride lipase (ATGL)、PAR-γ coactivator-1α (PGC-1α)和 uncoupling protein 2 (UCP2)的表現量，影響脂肪分解和粒線體生成作用。抑制 cPLA2 或 COX-2 可以逆轉 LPS 對於脂肪分解與粒線體生成的影響，顯示 cPLA2 與 COX-2 在 LPS 調控 BAT 功能中的影響。因此，LPS 處理可以降低 BAT 中與代謝功能相關的基因表現導致熱生成效率降低和增加肥胖形成。**結論：**LPS 通過調節 cPLA2 和 COX-2 的表現量影響脂肪分解與粒線體含量進而改變 BAT 的功能。這些發現顯示細菌內毒素和肥胖之間的潛在聯結，針對這些途徑可能提供肥胖處理的新治療策略。

關鍵字：脂多醣、細胞質磷脂酶 A2、環氧化酶-2、脂肪細胞

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Original Research Article

Application of Content Language and Integrated Learning in a Medical Terminology Course

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ABSTRACT

Background and purpose：Taking the medical terminology course is usually problematic for students with worse language learning confidence. Content and language-integrated learning (CLIL) is a student-centered and practical teaching approach. Few studies have examined the effect of CLIL on students' confidence while learning medical terminology. This study explored the influence of CLIL on the student's confidence in the medical terminology course. ***Methods：***This study was quasi-experimental with a convenience sample that used a single-group, pre-post research design. This research was conducted at a university in northern Taiwan. The demographics, learning confidence, academic performance, and student satisfaction were evaluated using the structured questionnaire. Levels of learning confidence were analyzed by using the generalized estimating equation. ***Results：***A total of 36 undergraduate students were recruited. Academic performance on the midterm was positively correlated with learning confidence on the final test ($p = .033$). With learning confidence divided into four subscales, the scores of skills enhancements, goal achievement, and speaking confidence showed an increase between the pretest and midterm ($p < .001$, $< .001$, and $< .001$, respectively) and between the pretest and final test ($p = .033$, $.005$, and $< .001$, respectively). ***Conclusions：***This result identified the benefit of teaching medical terminology via CLIL based on improving students' learning confidence. CLIL is a practical design for enhancing students' confidence in the medical terminology course.

Keywords: CLIL, learning confidence, medical terminology

INTRODUCTION

Confidence is a feeling of trust and belief in oneself or one's ability. Therefore, learning confidence is an individual's confidence in learning behaviors[1]. Most teachers' experiences show that those with poorer English ability usually have less confidence in learning English[2]. Moreover, medical terminology is a problematic professional subject for students at various levels. Therefore, applying content and language-integrated learning (CLIL) in a medical terminology course might increase students' learning confidence.

Students who are digital natives use smartphones and tablet computers, and teaching models should be adjusted accordingly. Teaching a medical terminology course is difficult due to students' varying degrees of background knowledge. Therefore, we applied CLIL in a medical terminology course. Our aim to improve students' learning confidence was grounded in well-designed content and related activities conducted before the commencement of the class.

Background

CLIL is an educational approach where subjects are taught in a secondary language to allow students to learn both the content and the language simultaneously[3]. The content aspect involves analyzing the contents of teaching materials and setting teaching goals related to the content. Moreover, it is necessary to understand students' learning situations to design suitable teaching materials. The language aspect involves teachers helping students use vocabulary correctly and improving pronunciation. It allows students to acquire professional knowledge, enhance their vocabulary, and improve their oral expression. Learning refers to enhancing students' class participation and interaction with teachers and classmates through the course design. It allows students to internalize and further apply the knowledge they have gained through assignments and related activities[4]. In short, CLIL improves students' language abilities and gives them the tools to acquire professional knowledge[2].

The CLIL teaching model is different from traditional language learning. The purposes of CLIL are to practice student-driven teaching and to make students recognize the significance of the learning[5-6]. Students may fear using incorrect grammar but can improve their ability through cooperation, communication, and competition with peers and teachers. When students realize their ability improves, their learning confidence will also increase[7]. CLIL is an interdisciplinary approach that combines professional subjects with language learning, and it helps students facilitate effective and professional communication by using precise language.

CLIL could improve students' learning confidence, academic performance, and satisfaction. Therefore, the purposes of this study were 1) to explore the learning results in a medical terminology course based on students' academic performance, satisfaction, and learning confidence and 2) to examine the factors correlated with students' learning confidence when using a CLIL.

MATERIALS AND METHODS

Design and ethical issues

This one-group, pre-posttest study using convenience sampling was approved in 2016 by the institutional review board (IRB, no. C105006) of the target university in Taiwan. Regardless of whether students participated in this study, the Institutional Review Board protected their rights to education. The participants could end participation in this study at will.

Before the course, one of our authors had learned CLIL at the University of Queensland in 2016. Further, two of our authors had been participating in the College of Medicine for three years. After discussion, the teachers of this course decided to use videos as the preview materials. CLIL was used to develop students' English language ability and professional medical terminology skills. CLIL includes three pillars: Suitable content, practical language expression, and multiple learning skills. The components and strategies associated with CLIL are shown in Figure 1. The figure shows that CLIL incorporates various teaching strategies to improve content understanding, language acquisition, and learning skills through diverse activities like video watching, discussions, and group work.

Study participants and sampling procedure

This study adopted the convenience sampling method. According to the G Power for F-test analysis, the total sample size was 28 based on setting the effect size as 0.25 with the alpha level at 0.05 and the power at 0.8. The data collection area was the classroom in the target university in northern Taiwan. The study participants matched the following criteria: 1) were above age 20, 2) had to take the medical terminology course, and 3) were willing to participate in this study. Participants were excluded if they returned an incomplete questionnaire. Data collection was conducted at the beginning of the

course, after the midterm test, and after the final test. Although participants, the first and third authors, were not blinded, the second author, who keyed in and analyzed data, was blinded.

A total of 36 students participated in the study from September 2016 to January 2017. The students were recruited and received an explanation of the study process in the first class. The students could drop out of the study anytime for any reason.

CLIL intervention

The 16-week course is typically used in CLIL designs to achieve the course objectives. After finishing this course, learners should be able to 1) define medical word-building systems, 2) spell medical words correctly, 3) pronounce medical words correctly, and 4) know how to use medical terminology. The research framework is shown in Figure 1. Based on the principles of CLIL, we designed concise and illustration-combined teaching materials. In terms of language expression, the teachers defined medical terminology and taught students how medical terminology evolved through the variation in etymons and prefixes.

The electronic teaching materials demonstrating correct pronunciation were recorded by the teaching assistant for the nursing department, who grew up in the United States and is proficient in English. This teaching assistant had participated in the overseas service of Fu Jen Catholic University several times and had six months of overseas exchange experience in the nursing department.

From the philosophical perspective of CLIL, students exhibit better performance if appropriate content, language, and learning skills are supported. Therefore, in this study, the teachers used some of the strategies presented in Table 1 to implement the three principles of CLIL. First, we provided appropriate content to students by setting learning objectives, delivering accessible texts, and understanding students' English language abilities. Second, we offered a vocabulary list at least one week before class to reduce the language barrier by supplying glossaries and highlighting keywords. Finally, we satisfied students' diverse learning needs and created a cooperative environment. For visual-auditory learning, we asked students to watch a video before class and used Kahoot and Zuvio to inspire their learning motivation. We utilized gestures and body movements for kinesthetic learning to help students memorize critical terminology. We designed role modeling and group activities to build a peer-learning environment.

We provided students with electronic materials that were easy to understand. We presented the basic medical terminology concepts for each unit, and students could preview the content they had to know before class. Despite the different learning conditions of each student, all the students could achieve the same pre-class level through self-learning before class.

Instrument

A structured questionnaire collected information on demographics, learning confidence, and satisfaction. Data were collected at three time points: at the beginning of the course, after the midterm test, and after the final exam. Furthermore, qualitative data were collected when participants completed the questionnaire for the last time. Open-ended questions included: 1) What impressed you when you took this course? 2) What are the differences between before and after taking this course when you took other nursing professional courses or did clinical practicum? 3) What were the barriers you faced when studying this course? 4) What were the facilitators for you to study this course?

Each individual required approximately 10 minutes to complete the questionnaire except for open-ended questions. Additionally, all participants' midterm and final exam scores were collected.

Demographics

Data were collected on the participants' department, gender, and years in the degree program.

Learning confidence

Data were collected on the participants' confidence in learning medical terminology by modifying "The Scale of Technology University Students' English Learning Self-efficacy," which consists of 21 items in four categories: enhancement, goal achievement, autonomous learning, and speaking confidence[8]. The original scale is not a dedicated measure of self-confidence in learning medical terminology. Six items were deleted because they were not related to medical terminology, only related to fundamental English abilities, such as speaking and cultural awareness. Therefore, the scale was validated by experts after removing six items as mentioned above. We revised this scale to suit our participants' situation, reducing it from 21 items to 15 items related to skills enhancement (items 1-5), goal achievement (items 6-9, 12), autonomous learning (items 10-11), and

speaking confidence (items 13-15). The response scores ranged from 1 to 5. Higher scores indicated greater confidence in learning. The Cronbach's α coefficient of the scale in this study was 0.89.

Satisfaction

There were five items for evaluating students' satisfaction with the course: 1) Did the course raise your learning interest? 2) Were you satisfied with the teachers' teaching style, which means the teacher's verbal and nonverbal emotional expression in the classroom? 3) Were you satisfied with the design of the teaching materials? 4) Were you satisfied with the arrangement of the activities in the course? And 5) did you prefer this teaching method? Responses ranged from 1 (very unsatisfied) to 5 (very satisfied). The Cronbach's α coefficient in this study was 0.94. We also provided an open question to collect participants' opinions.

Data analysis

Using SPSS 20.0 (software SPSS, Inc., Chicago, IL, USA) for Windows, descriptive statistics were calculated to describe the participants' demographic profile (age, gender, and department), self-confidence, academic performance, and satisfaction with CLIL. The association between learning confidence (as measured on the pretest, midterm, and final test) with demographics (as reported on the pretest), academic performance (as measured on the midterm and final test), and satisfaction with CLIL (as reported on the final test) was examined by nonparametric analysis (the Mann–Whitney U test or Spearman's rho). After adjusting for significant variables, a generalized estimating equation (GEE) analyzed the change distribution of learning confidence.

On the other hand, qualitative data were analyzed by following content analysis strategies [9]. This study used qualitative findings as supplements to support the arguments resulting from quantitative findings examined by statistics. Hence, qualitative data were coded and inductively clustered based on each open-ended question rather than done thematic analysis across different questions.

RESULTS

The distribution of the demographic variables, learning confidence, academic performance, and satisfaction among the students taking the course is shown in Table 2. 86.1% of the students were from the nursing department, 88.9% were female, and 80.6% were at the 2-year level. Their learning confidence was 51.33 (±7.18), or 68.44% (standard from 51.33/75 *100%), on the pretest; 56.92 (±6.99), or 75.89% (standard from 56.92/75 *100%), on the midterm; and 56.52 (±9.47), or 75.36% (standard from 56.52/75 *100%), on the final test. Their academic performance was 83.61 (±16.82) on the midterm and 88.78 (±6.37) on the final test. The sum of their satisfaction scores for this course was 21.02 (±2.88), or 84.08% (standard from 21.02/25 *100%), on the final test. Students expressed the most satisfaction with the "teaching method." Its standard score was 87.8% (4.39/5*100%). Students were least satisfied with the "teaching materials," with a standard score of 82.2% (4.11/5*100%).

Students were impressed by interactive or visual-auditory learning in this course. Students also thought these teaching methods facilitated them to learn medical terminology. Students A and B wrote, *"I became less tense and more interested in learning medical terminology through interactive learning in this course."* Student C wrote, *" It was easy to understand and remember vocabulary for me when teachers used visual-auditory materials, such as watching a video or singing and dancing self-made melody."* Student D also wrote, *"That video inspired me, and I would like to watch more other videos to learn how to use medical terminology appropriately."*

Students' reports demonstrated that taking this course was helpful for them in studying nursing knowledge matter in the classroom or a clinical setting. These reports were consistent with the quantitative findings as above. Student E wrote, *"After I learned medical terminology, I understood the contents of other nursing professional courses more and was more confident about discussing with healthcare providers when I did clinical practicum." Student F wrote, "I read medical chat more quickly because I could conjecture the meaning of unfamiliar vocabulary according to prefix or suffix." "I know more about what the nursing staff reported in English because I learned the correct medical terminology pronunciation."*

As determined via nonparametric analysis, the relationship of learning confidence with participants' demographics, academic performance, and satisfaction is shown in Table 3. Their academic performance on the midterm positively correlates with their learning confidence on the final test ($p = .033$). Their total satisfaction score positively correlates with their learning confidence on the final test ($p = .006$).

As calculated with the GEE, the change distribution of learning confidence among the

students is shown in Table 4. After adjusting academic performance on the midterm and course satisfaction items no. 1, 3, 4, and 5 on the final test for covariates, the total learning confidence score showed a significantly increasing trend between the pretest and midterm or pretest ($p < .001$) and final test ($p = .002$).

After learning confidence was divided into four subscales, the score for skills enhancement was 18.03 (±3.06) on the pretest, 19.94 (±2.60) on the midterm, and 19.42 (±3.53) on the final test. The goal achievement score was 17.11 (±2.72) on the pretest, 18.78 (±2.42) on the midterm, and 18.67 (±3.33) on the final test. The autonomous learning score was 7.72 (±1.19) on the pretest, 8.28 (±1.14) on the midterm, and 8.03 (±1.56) on the final test.

The speaking confidence score was 8.47 (±1.95) on the pretest, 9.92 (±2.23) on the midterm, and 10.14 (±2.10) on the final test. Only autonomous learning showed no significant increase between the pretest and final test. Other subscales showed a significant increase between the pretest and midterm and between the pretest and final test.

Students reported several barriers to learning medical terminology. These barriers may reveal why autonomous learning did not significantly increase at the final test. Student G wrote, *"I have to study required courses harder than elective courses after the midterm test. As we know, the final examination was a critical test to determine a course to be passed or fail. Therefore, I decide to take less time to study elective courses, such as this course (medical terminology)."* Student H wrote, *"I learned more difficult things after midterms because the vocabulary was longer and more complicated."* Student I wrote, *"I gradually felt frustrated when I was required to remember more and more vocabulary."*

DISCUSSION

According to the students' statements, they perceived less stress and would like to study harder after enjoying CLIL. The approach resulted in good academic performance. Good learning outcomes can increase learning confidence significantly. These results were consistent with those of prior research. Confidence is boosted when students' capability increases and anxiety decreases; motivation and ability are consequently stimulated[10].

Analysis of the subscales of learning confidence revealed that skills enhancement, goal achievement, and speaking confidence were significantly higher on the mid-test. These findings are consistent with the results of other studies demonstrating that the learning confidence of students who engaged in interactive learning with peers and teachers through CLIL was improved[2,7]. By using CLIL methods, such as kinesthetic activities and video watching, the learning confidence of students was enhanced in this study. Watching videos between classes motivated students to learn and increased students' involvement in the class, comprehension of the material, and confidence in understanding it[11].

Moreover, autonomous learning significantly improved between the pretest and midterm. However, this improvement did not continue between the midterm and final tests. Based on the statements from the students who completed the semester, most students focused on studying for their required courses instead of their elective courses, such as medical terminology. The finding was consistent with a previous result revealing the positive correlation between work engagement and autonomous motivation[12]. On the other hand, students thought vocabulary was more extended and more difficult in the final test than in the mid-term test. When applying the CLIL methods, teachers must respect students' voices, allow them to learn at their own pace, and provide guidance and support to struggling [5,13-14]. Therefore, long-term autonomous learning may not be maintained. Thus, the appropriate course duration to motivate students' autonomous learning should be further studied when CLIL processing.

Generally, students showed 84.08% (standard score) satisfaction with this course. Of the five satisfaction items, the "teaching method" was the most satisfactory (standard score 87.8%). The CLIL approach was thus acceptable to the students in this course. However, the least satisfactory item was "teaching materials" (standard score 82.2%). Some students said they disliked and resented the excessive paper learning materials. They expressed a desire for more video lessons in the curriculum.

Moreover, students' satisfaction scores positively correlated with their learning confidence on the final test. Based on the analysis of the satisfaction results, there was no significant correlation between the teachers' teaching style and students' learning confidence. In this study, teachers' teaching style was defined as the teacher's verbal and nonverbal patterns about personal traits. The finding was consistent with the preceding results: students evaluated their teachers' performance rather than specific social characteristics[15]. Therefore, teachers who

follow the principles of CLIL can improve students' learning confidence even though teachers have different types of personal traits.

Limitations

In this study, we collected small samples from a classroom setting, which may have led to bias. Additionally, this one-group study cannot be compared with traditional teaching methods. It is recommended that prospective researchers increase the number of samples and construct a two-group experimental design to provide high-quality evidence. Additionally, we only helped students memorize medical terminology using kinesthetic learning and did not evaluate how to apply the terms in a clinical context appropriately. However, CLIL emphasizes assisting learners in acquiring content and language in a situational context[5]. Therefore, future studies should objectively evaluate students' use of medical terminology by practicing nurse-patient conversations in virtual clinical settings or their clinical practicum.

CONCLUSIONS

Generally, CLIL is a practical design for improving students' confidence in learning in a medical course. Students' satisfaction was positively associated with their learning confidence. We suggest that researchers or educators can, based on the principles of the CLIL, test different course durations in the future. Based on our findings, shortening the duration of CLIL in half-semester might increase students' autonomous learning confidence. Improving students' autonomous learning skills is an important issue that must be addressed in future research. Based on CLIL, continuing to modify and enhance teaching methods at the right time may effectively increase students' learning confidence.

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TABLES

Table1. Examples of each strategy

Teaching strategies in this course	Examples of each strategy
Content	
1. Understanding students	1. students' various levels of preparedness were considered.
2. Setting objectives	2. structures and uses of medical terminology were introduced, and the goals were micro-adjusted according to student performance
3. Delivering accessible text	3. photos and subheadings added to the text
Language	
1. Providing a glossary	1. practicing correct pronunciation through conversation
2. Highlighting keywords	2. practicing spelling of common words through micro-tests
Learning skills	
1. Visual-auditory learning	1. online video lessons, Kahoot, and Zuvio
2. Kinesthetic learning	2. utilizing gestures, body movement
3. Group activities	3. collaborating in feedback and dialogue with classmates
4. Role modeling	4. Video lessons were prepared by the teaching assistant, who was an expert in English

Table2. The distribution of demographic characteristics, learning confidence, academic performance, and satisfaction among the students taking the course (N = 36)

Variables			**Pre**		**Mid**		**Post**	
	n	%	M	SD	M	SD	M	SD
Demographics								
Department			0.9	0.4				
Nurse	31	86.1						
RT	5	13.9						
Gender			1.9	0.3				
Female	32	88.9						
Male	4	11.1						
Year in degree program			2.2	0.5				
2	29	80.6						
3	7	19.5						
Learning confidence			**51.33**	**7.18**	**56.92**	**6.99**	**56.52**	**9.47**
Academic performance					**83.61%**	**16.82**	**88.78%**	**6.37**
Satisfaction							**21.02**	**2.88**
S1							4.14	0.68
very unsatisfied	0	0						
unsatisfied	0	0						
fair	6	16.7						
satisfied	19	52.8						
very satisfied	11	30.6						
S2							4.19	0.62
very unsatisfied	0	0						
unsatisfied	0	0						
fair	4	11.1						
satisfied	21	58.3						
very satisfied	11	30.6						
S3							4.11	0.71
very unsatisfied	0	0						
unsatisfied	0	0						
fair	7	19.4						
satisfied	18	50.0						
very satisfied	11	30.6						
S4							4.19	0.67
very unsatisfied	0	0						
unsatisfied	0	0						
fair	5	13.9						
satisfied	19	52.8						
very satisfied	12	33.3						
S5							4.39	0.64
very unsatisfied	0	0						
unsatisfied	0	0						
fair	3	8.3						
satisfied	16	44.4						
very satisfied	17	47.2						

Note. S1 = Does the course raise your learning interest? S2 = Are you satisfied with the teaching style? S3 = Are you satisfied with the design of the teaching materials? S4 = Are you satisfied with the arrangement of the activities in the course? S5 = Do you prefer this teaching method?

Table3. The relationship of learning confidence with the demographic characteristics, academic performance, and satisfaction of the students (N = 36)

Learning confidence		***Pre***				***Mid***				***Post***			
	n	*M*	*SD*	*U*/ρ	*p*	*M*	*SD*	*U*/ρ	*p*	*M*	*SD*	*U*/ρ	*p*
Demographics													
Department				35.5	.054			66.0	.598			37.0	.063
Nurse	31	50.42	7.05			56.74	7.15			55.29	9.76		
RT	5	57.00	5.57			58.00	6.52			62.20	4.44		
Gender				58.5	.781			49.0	.449			63.5	.980
Female	32	51.34	7.24			57.31	6.92			56.03	9.96		
Male	4	51.25	7.72			53.75	7.81			58.00	4.08		
Years in the degree program				89.5	.631			77.5	.336			100.5	.968
2	29	51.59	7.45			56.21	7.16			56.00	10.32		
3	7	50.29	6.29			59.86	5.76			57.29	4.99		
Academic performance													
Mid								.328	.050			.36	.033
Post												.11	.518
Satisfaction												.48	.006
S1				86.0	.865			86.5	.882			.43	.010
S2				58.5	.781			63.5	.980			.32	.057
S3												.38	.024
S4												.41	.012
S5												.36	.029

Note. The p-value was determined by the nonparametric analysis (Mann–Whitney U test or Spearman's rho). S1 = Does the course raise your learning interest? S2 = Are you satisfied with the teaching style? S3 = Are you satisfied with the design of the teaching materials? S4 = Are you satisfied with the arrangement of the activities in the course? S5 = Do you prefer this teaching method?

Table4. The change distribution of learning confidence among the students (N = 36)

Learning confidence	*Pre*		*Mid*		*Post*		*p-value*	
	M	*SD*	*M*	*SD*	*M*	*SD*	Pre vs. Mid	Pre vs. Post
Skills enhancement	**18.03**	**3.06**	**19.94**	**2.60**	**19.42**	**3.53**	**<.001**	**.033**
1. My understanding of the prefixes and suffixes of medical terminology is better than before.	3.94	0.75	4.22	0.59	4.00	0.76	.016	.705
2. My listening ability for medical terminology is better than before.	3.39	0.69	3.78	0.64	3.81	0.79	.001	.009
3. My speaking ability for medical terminology is better than before.	3.42	0.87	3.61	0.73	3.56	0.74	.167	.431
4. My reading ability for medical terminology is better than before.	3.75	0.69	4.28	0.51	4.06	0.86	<.001	.073
5. I can correctly write more medical words than before.	3.53	0.88	4.06	0.83	4.00	0.86	<.001	.003
Goal achievement	**17.11**	**2.72**	**18.78**	**2.42**	**18.67**	**3.33**	**<.001**	**.005**
6. I am satisfied with the achievement of my medical terminology learning.	3.69	0.75	3.97	0.70	3.97	0.81	.016	.055
7. I think my test scores are good.	3.19	0.71	3.69	0.67	3.58	0.69	.001	.007
8. I will set the learning goals for medical terminology myself.	3.44	0.81	3.56	0.65	3.67	0.79	.366	.132
9. I have achieved my learning goals for medical terminology.	3.17	0.76	3.53	0.56	3.53	0.74	.002	.011
12. Overall, I am satisfied with my performance in the class.	3.61	0.73	4.03	0.61	3.92	0.84	.002	.030
Autonomous learning	**7.72**	**1.19**	**8.28**	**1.14**	**8.03**	**1.56**	**.006**	**.293**
10. I know how to use learning resources to improve my medical terminology knowledge.	3.44	0.77	3.92	0.73	3.78	0.87	.001	.064
11. I think learning medical terminology actively is essential.	4.28	0.78	4.36	0.64	4.25	0.87	.488	.869
Speaking confidence	**8.47**	**1.95**	**9.92**	**2.23**	**10.14**	**2.10**	**<.001**	**<.001**
13. I can do handovers and communicate with medical staff using medical terminology during the internship.	2.69	0.67	3.28	0.85	3.31	0.67	<.001	<.001
14. I won't be nervous when using medical terminology with medical staff during the internship.	2.50	0.85	3.06	0.75	3.14	0.83	<.001	<.001
15. I will test my understanding of medical terminology during physician's visits or by listening to seniors' handovers during the internship.	3.28	1.00	3.58	1.00	3.69	0.86	.088	.019
Total score	**51.33**	**7.18**	**56.92**	**6.99**	**56.25**	**9.47**	**<.001**	**.002**

Note: Learning confidence is grouped into four subscales: skills enhancement (items 1-5), goal achievement (items 6-9 & 12), autonomous learning (items 10-11), and speaking confidence (items 13-15). After adjusting for covariates, the GEE determines the p-value, including academic performance on the midterm and satisfaction items no. 1, 3, 4, and 5 on the posttest.

FIGURE AND FIGURE LEGEND

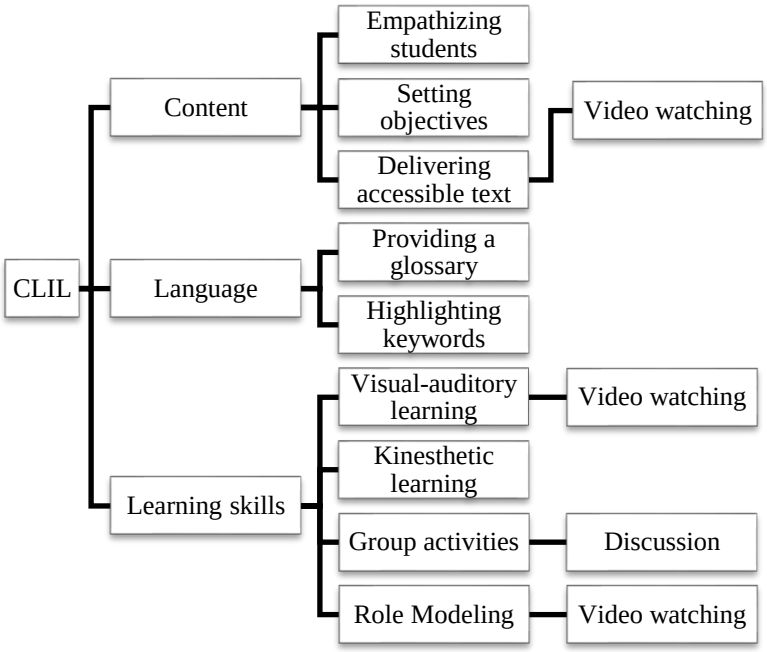

Figure 1. Conceptual framework of the medical terminology course design

Note.

CLIL consists of three components: content, language, and learning skills. In this study, the contents included empathizing with students, setting objectives, and delivering accessible text through Video watching. Second, the language means providing a glossary and highlighting keywords. Third, the learning skills contained visual-auditory learning, kinesthetic learning, group activities through discussion, and role modeling through video watching.

Application of Content Language and Integrated Learning in a Medical Terminology Course

應用內容語言與整合學習於醫學術語課程

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中文摘要

背景與目的：對於語言學習信心較差的學生來說，修習醫學術語課程通常是一個問題。內容與語言整合學習（CLIL）是一種以學生為中心且具實踐性的教學方法，但少有研究檢驗 CLIL 對於醫學術語修課學生的學習信心之成效。本研究旨在探討於醫學術語課程中使用 CLIL 對於提升學生學習信心的成效。**方法：**本研究採用準實驗性設計，使用單一組別、前後測研究設計，以方便取樣方法，選取該台灣北部某一所大學合乎條件的研究對象。以結構式問卷評估學生的人口學資料、學習信心、學業表現和滿意度。學習信心的提升則使用廣義估計方程式進行分析。**結果：**本研究共招募 36 名大學生，期中考的學業表現與期末考試的學習信心呈正相關（p＝.033）。相較前測而言，期中考的技能增強、目標達成和口語信心的得分有增加且達統計差異（p＜.001,＜.001 和 ＜.001）；此外，上述三項於期末考的得分也較前測增加且達統計差異（p＝.033，.005 和 ＜.001）。**結論：**本研究確認應用 CLIL 於醫學術語課程的好處，即提高學生的學習信心。CLIL 是一個實用的設計可提升學生修習醫學術語課程的學習信心。

關鍵字：內容及語言整合學習、學習信心、醫學術語

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Case Report

Improved Exercise Capacity with Closed Loop Stimulation Programming in a Patient with Chronotropic Incompetence: A Case Report

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ABSTRACT

This case report describes a 67-year-old male who underwent pacemaker implantation for bradycardia and was diagnosed with chronotropic incompetence after exercise stress test revealed a maximal heart rate less than 80% of predicted heart rate. The study evaluated the patient's exercise capacity using cardiopulmonary exercise (CPX) testing before and after the pacemaker adjustment, from DDD (dual chamber sensing; dual chamber pacing; dual chamber inhibition and stimulation of pacing with movement detection) to CLS (closed loop stimulation) mode. The results of the study showed that the patient's maximal aerobic capacity improved significantly from 11.86 to 16.15 ml/min/kg or 3.4 to 4.6 MET after changing the pacemaker mode, indicating an improvement in aerobic capacity. The patient reported a better quality of life after the adjustment. The study highlights the influence of cardiac pacing modes on the heart rate response to physical exercise, and how CLS pacemakers are designed to be more sensitive towards exercise detection, which can increase heart rate accordingly. Few studies have used CPX testing to evaluate exercise capacity in patients with chronotropic incompetence, and this case report provides evidence that changing pacemaker settings to CLS mode can improve aerobic capacity and thus improve the patient's quality of life.

Keywords: cardiac pacemaker, pacemaker programming modes, closed loop stimulation, chronotropic incompetence, cardiopulmonary exercise test, exercise capacity

INTRODUCTION

Cardiac pacing modes influence atrioventricular synchronicity and the heart rate response to physical exercise.[1] Closed loop stimulation (CLS) uses a rate-adaptive sensor to determine the appropriate heart rate based on continuous intracardiac impedance measurements, even during mental stress or physical exercises.[2] Changing dual-chamber pacemakers from a fixed rate to a rate-variable has been documented to increase oxygen uptake (VO2) at anaerobic threshold (AT) and peak VO2.[3] Here, we report a case of improved cardiopulmonary exercise (CPX) test outcomes achieved after altering the patient's pacemaker settings from DDD (dual chamber sensing; dual chamber pacing; dual chamber inhibition and stimulation of pacing with movement detection) to CLS.

CASE REPORT

A 67-year-old male with a past history of gout, hyperlipidemia, and diabetes mellitus was admitted to the urology ward with hematuria from prostate cancer. He had suffered from general weakness and fatigue for months, and preoperative evaluation disclosed bradycardia (40 bpm) with sinus bradycardia shown on ECG. Exercise stress test was suggested after cardiologist consultation for bradycardia, which revealed the patient's maximal heart rate to be less than 80% of predicted heart rate and inconclusive for ischemia; therefore, chronotropic incompetence was diagnosed, and pacemaker implantation was prescribed.

Different pacemaker mode settings (DDD vs. CLS) were attempted and evaluated using CPX testing before and after the adjustment. CPX testing to maximal exertion was carried out on an electronically braked cycle ergometer using a 10W/min ramp protocol, and oxygen uptake (VO2) and carbon dioxide (VCO2) output were measured using a breath-by-breath analyzer. The following parameters were recorded during CPX tests: ECG, workload, oxygen delivery at AT, and peak oxygen delivery (Table 1).

The pacemaker was first programmed in DDD mode with a lower heart rate threshold of 60 bpm and an upper heart rate threshold of 120 bpm, resulting in a maximal aerobic capacity of 11.86 ml/min/kg or 3.4 MET. However, the maximal aerobic capacity increased to 16.15 ml/min/kg or 4.6 MET after shifting to CLS mode, indicating a significant improvement.

After an uneventful hospitalization course, the patient was discharged with regular outpatient clinic follow-up and reported a better quality of life with more confidence participating in activities of daily living as well as leisure activities.

This study was approved by Institutional Ethics Review Board (permission number: 20190508D).

DISCUSSION

Pacemakers employ various mechanisms to detect increased metabolic demand to alter the pacing rate accordingly, which are called rate-responsive pacing.[4] The most common detection is through a piezoelectric sensor, also known as an accelerometer.[5] This device detects movement and vibration and translates the signal into an increase in pacing rate. However, this tends to give an inadequate response when metabolic demand increases in the absence of movement, such as riding a stationary bike or during surgery. In contrast, the CLS pacemaker is designed to be more sensitive towards exercise detection and can increase heart rate accordingly, which emulates the healthy sinus node by adapting heart rates and thus cardiac output based on actual metabolic demand.[6]

CPX testing is a well-established diagnostic and prognostic method in heart failure, but few studies have used CPX testing to evaluate exercise capacity in patients with chronotropic incompetence.[7] Our patient was evaluated with CPX testing and showed a significant improvement (36%) in aerobic capacity after adjusting to CLS pacing, resulting in an improved aerobic capacity with a better quality of life.

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TABLE

Table1. CPX test results before and after pacemaker adjustment

	DDD mode	CLS mode
Exercise Time	8 min 3s	9 min 53s
Workload (W)	64	79
MET	3.4	4.6
VO2 AT (ml kg−1 min−1)	8.5	9.76
VO2 peak (ml kg−1 min−1)	11.86	16.15
RER	1.08	1.1
Max HR (bpm)	108	124
peak VO2/HR (ml/beat)	8	10

(MET= metabolic equivalent of task; RER= respiratory exchange ratio; HR= heart rate)

Pacemaker Mode

Incidental finding of a patient with gastrointestinal follicular lymphoma: a case report

改變閉環刺激心臟節律器模式來提高心律無法隨生理需要而加快之患者的運動能力：病例報告

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中文摘要

本篇病例報告探討一位 67 歲男性，因心律無法隨生理需要而加快，而裝設心臟節律器以治療竇性心搏過緩。透過極限心肺運動測試，研究病人在不同的心臟節律器設定下，對比閉環刺激(Closed loop stimulation,縮寫為 CLS)和雙腔式感應起博(Dual chamber pacemaker, 縮寫為 DDD)模式所呈現的最大攝氧量、無氧閾值、活動代謝當量的變化。結果顯示，使用 CLS 模式下病人的最大攝氧量由 11.86 進步至 16.15 每分鐘每公斤每毫升，達百分之 36 的變化。本研究結果顯示，通過改變心臟節律器模式為閉環刺激可提高心律無法隨生理需要而加快之患者的運動能力。

關鍵字：心臟節律器、節律器模式、閉環刺激、心律無法隨生理需要而加快、極限心肺運動試驗、運動能力

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Case Report

A case of ST elevation myocardial infarction with concurrent ischemic stroke: etiology and therapy

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ABSTRACT

Post-myocardial infarction (MI) ischemic stroke is rare but increases mortality than those not complicated with stroke. Here, we present a 64-year-old man suffered from ST segment elevation myocardial infarction and complicated with cardiogenic shock. Primary percutaneous coronary intervention was done for left main to left anterior descending. Inotropic agent, intra-arterial balloon pump (IABP) and mechanical ventilator support were used for unstable hemodynamics. Dual anti-platelet and anticoagulation were used as guideline recommendation. However, ischemic stroke happened with left side hemiplegia and aphasia after removing IABP. Intra-arterial thrombectomy was done for right middle cerebral infarct. National Institutes of Health Stroke Scale improved from 14 to 2 after thrombectomy. The pathogenesis of post-MI stroke includes extensive anterior wall akinesia leading to blood stasis, inflammatory changes by subendocardial injury, new onset atrial fibrillation (AF), and hypercoagulable status. Preexisting AF with acute coronary syndrome has clear recommendation with antithrombotic therapy with anti-platelet and non-vitamin K antagonist oral anticoagulant (NOAC) use. However, stroke prevention after acute MI is limited for those without AF. Guidelines suggest considering anticoagulation for 3 to 6 months once LV thrombus observed, but there was no randomized controlled trial to confirm which anticoagulant is better.

Keywords: STEMI, myocardial infarction, stroke

INTRODUCTION

Post-myocardial infarction (MI) ischemic stroke is rare but increases mortality than those not complicated with stroke. The prevailing etiologies include left ventricle (LV) thrombi due to anterior wall infarction and new-onset atrial fibrillation (AF)[1]. Mortality rate significantly increases due to stroke after acute MI[2].

CASE REPORT

We present a 64-year-old man arrived our emergency room for acute onset chest pain with blood pressure 84/58 mmHg. Electrocardiography (ECG) revealed sinus tachycardia with premature atrial contraction (PAC), poor R wave progression and ST elevation at V2 to V4 with reciprocal change at inferior leads (Figure 1A). Under the diagnosis of ST elevation myocardial infarction (STEMI) complicated with cardiogenic shock, primary percutaneous coronary intervention (PCI) was arranged. Coronary angiography showed left main (LM) to left anterior descending (LAD) artery and left circumflex (LCX) artery total occlusion (Figure 1B). After primary PCI, a bare metal stent was deployed at LM to LAD with revascularization (Figure 1C). Post-PCI ECG revealed sinus rhythm with PAC, QS pattern with T wave inversion at precordial leads (Figure 1D). Inotropic agent, intra-arterial balloon pump (IABP) and mechanical ventilator support were used for unstable hemodynamics. Echocardiography revealed LV ejection fraction 35% with apical cap-septal and mid anteroseptal akinesia. Dual anti-platelet (DAPT) and anticoagulation were used as guideline recommendation. IABP and ventilator were removed after condition improving. However, ischemic stroke happened with left side hemiplegia and aphasia after removing IABP. Brain computed tomography revealed right middle cerebral artery (MCA) infarct (M2) (Figure 1E) and only mild stenosis at left carotid siphon. Intra-arterial (IA) thrombectomy was done successfully for the lesion (Figure 1F and 1G). National Institutes of Health Stroke Scale (NIHSS) improved from 14 to 2 after IA thrombectomy. Muscle power of left side limbs recovered as right sides and bedside rehabilitation program initiated after 4 days. There was no AF episode monitored at intensive care unit. Hypercoagulable status was evaluated and there was no abnormal finding from lab data.

This study was approved by Institutional Review Board (permission number: CGH-P112003).

DISCUSSION

The incidence of MI-related stoke is rare. It had been reported around 1.07% from a case-control study in Sweden[3] and 0.88% from a single center in the USA[4]. The post-MI stroke significantly increases mortality and the symptoms of heart failure and neurologic signs deteriorate each other. We detailed reviewed the mechanisms of post-MI stoke. The most possible cause is LV thrombi due to extensive anterior wall akinesia leading to blood stasis and inflammatory changes by subendocardial injury during acute MI[5]. One study performing serial echocardiographic studies showed that about 80% of thrombi developed within the first 4 days after acute MI[6]. Other potential mechanism includes new onset AF or atrial dysfunction that may result in thromboembolism in the absence of AF[7]. Non-cardiac mechanism includes thromboembolism from the aortic arch or carotid arteries due to systemic inflammation change by MI[8]. The stroke risk after acute MI was highest during the first month, but remained heightened during the following 2 months[9]. The 1-year mortality was double for acute MI complicated by ischemic stroke than MI without stroke[2]. Preexisting AF with acute coronary syndrome has clear recommendation with one week triple antithrombotic therapy with DAPT and non-vitamin K antagonist oral anticoagulant (NOAC) and then de-escalation to single anti-platelet and NOAC for 12 months[10]. However, stroke prevention after acute MI is limited for those without AF. There was no randomized trial available to guide antithrombotic therapy to prevent LV thrombus formation during acute MI. Guidelines suggest considering anticoagulation for 3 to 6 months once LV thrombi observed[11]. Major adverse cardiac events and mortality were less common in patients with over 3 months anticoagulants[12].

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FIGURE AND FIGURE LEGEND

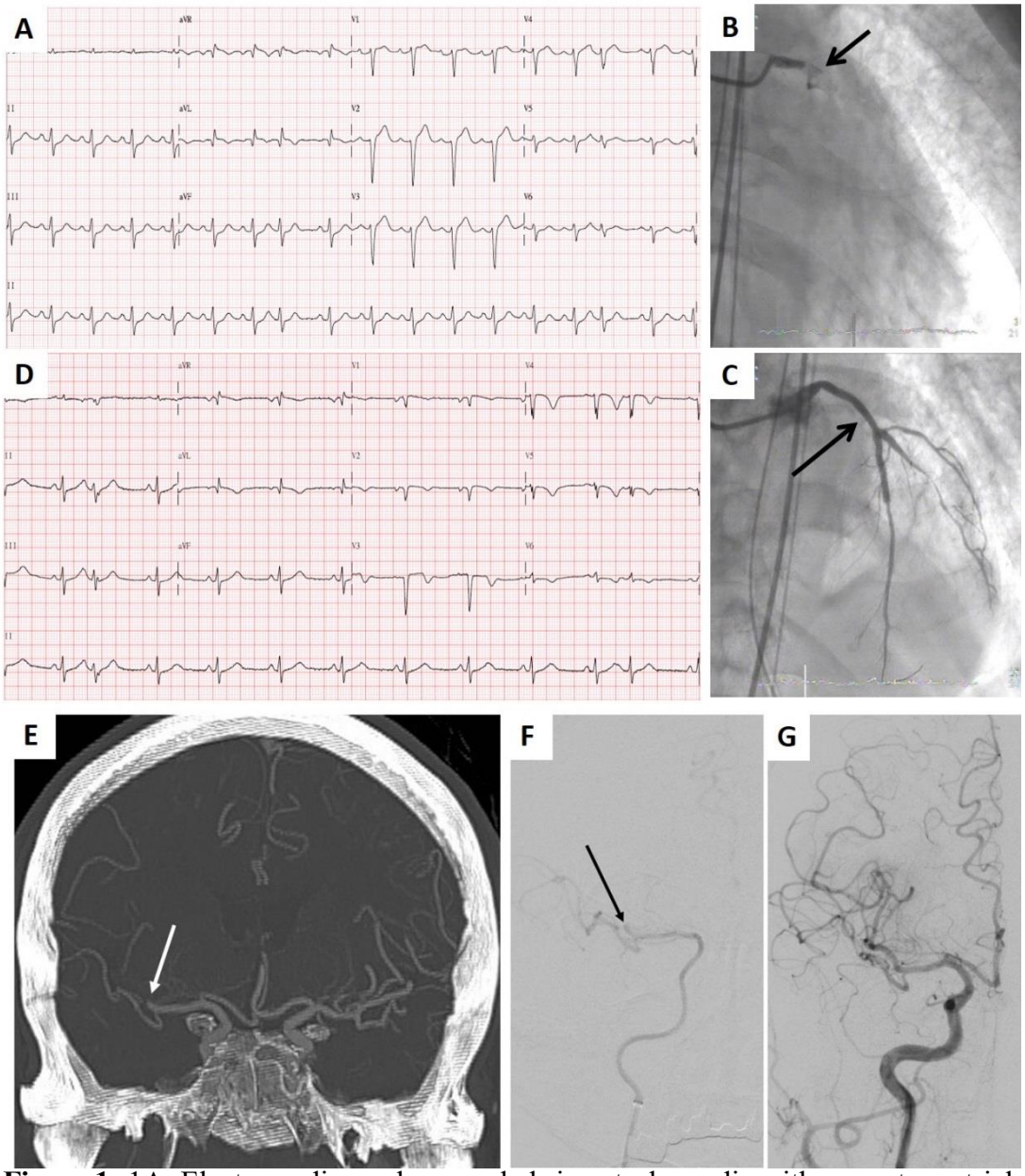

Figure 1. 1A: Electrocardiography revealed sinus tachycardia with premature atrial contraction (PAC), poor R wave progression and ST elevation at V2 to V4 with reciprocal change at inferior leads. 1B: Coronary angiography showed left main (LM) to left anterior descending (LAD) artery and left circumflex (LCX) artery total occlusion as black arrow. 1C: After primary percutaneous coronary intervention (PCI), a bare metal stent was deployed at LM to LAD with revascularization as black arrow. 1D: Post-PCI ECG revealed sinus rhythm with PAC, QS pattern with T wave inversion at precordial leads. 1E: Brain computed tomography revealed right middle cerebral artery (MCA) infarct (M2) as white arrow. 1F: Angiography showed right MCA (M2) total occlusion as black arrow. 1G: Right MCA (M2) occlusion post intra-arterial thrombectomy with revascularization.

A case of ST elevation myocardial infarction with concurrent ischemic stroke: etiology and therapy

個案討論：ST 段上升心肌梗塞後缺血性腦梗塞之成因與治療

簡韶甫[1]　張釗監[1,*]

中文摘要

急性心肌梗塞後之缺血性腦梗塞發生率低，但同時合併急性心肌梗塞和缺血性腦梗塞，患者的死亡率會明顯上升。常見的原因包括心肌梗塞後新發生的心房顫動、左心室前壁梗塞引起的心室內血栓或病患本身有凝血異常的疾病。一位 64 歲男性患有 ST 上升型心肌梗塞合併心因性休克而住院，心導管檢查顯示左主冠狀動脈至左前降支和左迴旋支阻塞，接受緊急心導管介入治療，由於生命徵象不穩定，使用強心劑、主動脈氣球幫浦和呼吸器以維持血流動力學，按照現行治療指引使用雙重抗血小板藥物和抗凝劑治療，而後病況逐漸穩定。然而，移除主動脈氣球幫浦後，發生缺血性腦梗塞併有左側偏癱和失語症。病人接受顱內動脈取栓術後恢復良好，美國國家衛生院腦中風評估表(NIHSS)從 14 分下降到 2 分。我們詳細回顧了心肌梗塞後缺血性腦梗塞的病生理機轉，包含左心室功能下降造成血液鬱積、心內膜缺血壞死造成發炎反應、新發生的心房顫動或本身凝血功能異常容易產生血栓。若是和心房顫動相關，按照現行治療指引使用抗血小板藥物和非維他命 K 拮抗劑類口服抗凝血劑治療為主，若為左心室內血栓可以抗凝血劑治療三到六個月後再追蹤，但目前無隨機對照試驗顯示哪一種抗凝血劑效果較好。

關鍵字：ST 段上升心肌梗塞、缺血性腦梗塞、腦梗塞

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Review Article

Adverse Effects of Sodium Glucose Co-Transporter-2 (SGLT2) Inhibitors on the Genitourinary System: An update

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ABSTRACT

Background and Purpose: Sodium glucose co-transporter-2 (SGLT2) inhibitors have proven effective in reducing the risk of major adverse cardiovascular events, heart failure hospitalization, and kidney disease progression in patients with or without diabetes. The latest international consensus has expanded the indications for and prioritized the use of SGLT2 inhibitors. Yet, many patients discontinue these drugs due to genitourinary system side effects. This review intends to help clinicians manage these adverse effects. ***Methods:*** We conducted a search using the keywords "SGLT2 inhibitors," "urinary tract infection (UTI)," and/or "genital mycotic infection (GMI)" in PubMed. We evaluated these papers for their relevance to the incidence, prevention, and treatment of adverse effects. ***Results:*** In clinical trials and cohort studies, SGLT2 inhibitors didn't increase the risk of UTIs in people with diabetes. However, these inhibitors did slightly but significantly raise the UTI risk in non-diabetic individuals. As for GMIs, the trials showed that SGLT2 inhibitors were associated with a 3.5 times higher risk in diabetic patients, who had an infection incidence rate of 5% or more. In non-diabetic individuals, these inhibitors increased the GMI risk 2.4 times, with a 1.4% incidence rate. Risk factors for genital mycotic infections among SGLT2 inhibitor users include a history of these infections, obesity, female gender, and uncircumcised male status. ***Conclusion:*** Before prescribing SGLT2 inhibitors, clinicians should provide comprehensive prevention education to patients at high risk for GMIs. If symptoms arise, prompt treatment is necessary to alleviate discomfort and ultimately improve adherence to the medication.

Keywords: SGLT2 inhibitors, Urinary tract infection, Genital mycotic infection

INTRODUCTION

Since the publication of the EMPA-REG OUTCOME trial in 2015[1], SGLT2 inhibitors have demonstrated the ability to reduce the risk of major adverse cardiovascular events, hospitalization for heart failure, and progression of kidney disease in diverse patient populations with type 2 diabetes. Recent collaborative meta-analysis of placebo-controlled trials clearly shows that the absolute benefits of SGLT2 inhibitors far outweigh the risks of severe adverse effects, such as diabetic ketoacidosis and amputation[2]. In fact, these inhibitors have been conclusively proven to markedly reduce the risk of kidney disease progression by 37%, acute kidney injury by 23%, and cardiovascular death or hospitalization due to heart failure by 23%. Importantly, they provide these protective benefits to an equally significant degree in patients both with and without diabetes. The 2022 Consensus Report by the American Diabetes Association and the European Association for the Study of Diabetes has updated their recommendations on the use of antidiabetic medications in patients with type 2 diabetes[3]. The report no longer considers metformin as the generally first-line therapy for type 2 diabetes and has expanded the indicated patient population for SGLT2 inhibitors as a first-line oral antidiabetic treatment. Despite promising results from clinical trials, meta-analyses of observational studies have revealed that the pooled persistence rate of SGLT2 inhibitor use is only 61.8% (95% confidence interval [CI], 57.8-65.7) at one year and 45.9% (95% CI, 35.5-56.5) at two years[4]. Compared to other antidiabetic medications with proven cardiovascular benefits, a cohort study using Danish nationwide registries identified all first-time users of SGLT2 inhibitors and GLP1-RAs between 2013 and 2021[5]. The study found that over a five-year period, the likelihood of discontinuing therapy was 56% (95% CI, 55–57) for SGLT2 inhibitors, 52% (95% CI, 51–53) for metformin, and 45% (95% CI, 45–46) for GLP1-RA. Interestingly, the persistence of once-daily oral SGLT2 inhibitors is even lower than injection therapies and multiple daily doses of metformin. Thus, understanding why patients discontinue SGLT2 inhibitors is crucial.

The mechanism of SGLT2 inhibitors involves the inhibition of renal glucose reabsorption leading to increased glucose excretion in urine, which raises concerns about the occurrence of urinary tract infections (UTIs) and genital mycotic infections (GMIs) due to augmented glucosuria. GMIs are the most frequent adverse effect of SGLT2 inhibitors[3], often resulting in drug discontinuation in daily practice, despite being described as mild and treatable in clinical trials. On the other hand, while clinical trials did not demonstrate an increased risk of UTIs[6], they remain a theoretical concern, and clinicians may be hesitant to continue prescribing SGLT2 inhibitors if the patient has a UTI. This review aims to tackle these two issues, in order to enhance patient persistence with SGLT2 inhibitors.

MATERIALS AND METHODS

This narrative review was conducted by searching the PubMed database using the following search terms: "SGLT2 inhibitors," "urinary tract infection," and "genital mycotic infections." The search was limited to articles published in the past five years. In addition, relevant articles were identified through a manual search of reference lists. A total of 28 articles were selected for inclusion in this review based on their relevance to the review question.

RESULTS

Most SGLT2 inhibitor studies focus on diabetic patients; hence, the subsequent data, unless otherwise specified, pertain to this group. While non-diabetic patients are also included, their smaller representation warrants separate discussion in distinct paragraphs.

Urinary Tract Infections

In a meta-analysis of 86 randomized controlled trials involving 50,880 patients, there was no significant increase in the risk of UTIs associated with SGLT2 inhibitors. The relative risks of UTIs remained similar when comparing SGLT2 inhibitors to either placebo or active comparators, with values of 1.03 (95% CI, 0.96–1.11) and 1.08 (95% CI, 0.93–1.25), respectively[6]. However, there is a concern that patients in clinical trials may not fully represent those in daily practice, who could be older, frailer, and at a higher risk of developing UTIs.

In a large population-based study of 2 U.S. commercial claim databases compared the incidence of severe and outpatient UTIs in patients with type 2 diabetes starting SGLT2 inhibitors versus DPP-4 inhibitors or GLP-1 agonists[7]. Severe UTIs were defined as a hospitalization for primary UTI, sepsis with UTI, or pyelonephritis. The study included 270,762 patients who started SGLT2 inhibitors and 440,970 who started DPP-4 inhibitors in cohort 1, and 273,617 who started SGLT2 inhibitors and 211,701 who started GLP-1 agonists in cohort 2. The study found that after matching and adjusting for baseline characteristics, SGLT2 inhibitors were not associated with an increased risk of severe UTIs compared to DPP-4

inhibitors (HR 0.98; 95% CI, 0.68-1.41), and were associated with a lower risk of severe UTIs compared to GLP-1 agonists (HR 0.72; 95% CI, 0.53-0.99). Additionally, the use of SGLT-2 inhibitors did not result in a higher risk of outpatient UTIs as compared to DPP-4 inhibitors (HR 0.96; 95% CI, 0.89 - 1.04), and had a lower risk as compared to GLP-1 agonists (HR 0.91; 95% CI, 0.84 - 0.99). To be cautious, high-risk patients (e.g., catheter users, those with hydronephrosis) and those with a previous history of UTIs were excluded from this cohort study.

In contrast to previous findings, a retrospective cohort study using the National Health Insurance Database in Korea from 2014 to 2017 assigned patients to DPP-4 inhibitors, SU, and TZD comparator groups based on the propensity score for each comparator among 7,741 people in the assessed SGLT2 inhibitors group[8]. The study found a significantly increased risk of UTIs associated with SGLT2 inhibitors compared to DPP-4 inhibitors (HR 1.57; 95% CI, 1.39-1.77), SU (HR 1.66; 95% CI, 1.47-1.89), and TZD (HR 1.69; 95% CI, 1.33-2.13). However, the authors acknowledged that retrospective cohort studies in Australia[9] and the USA[7], along with a 2017 systematic review and meta-analysis[10], did not show an increased UTI risk with SGLT2 inhibitors.

In a 2022 meta-analysis that focused on non-diabetic patients, four trials were examined which included a total of 8,927 patients with heart failure and chronic kidney disease[11]. The study found that compared to a placebo, SGLT2 inhibitors mildly but significantly increased the risk of urinary tract infections (RR: 1.29; 95% CI, 1.05-1.58). As of now, no specific explanation has been discovered to account for the difference in risk between diabetic and non-diabetic patients.

The prevailing view and consensus suggest that SGLT2 inhibitors do not increase the risk of UTIs in patients with diabetes; however, a mild risk exists in non-diabetic patients. The effects on high-risk UTI patients (e.g., catheter users, abnormal urinary flow, recurrent UTIs) have not been specifically examined. Therefore, it is essential to monitor high-risk UTI and non-diabetic patients closely for infections when prescribing SGLT2 inhibitors, discontinuing the medication if necessary.

Genital Mycotic Infections: Risks and Incidence Rates

A meta-analysis of five cardiovascular outcome trials involving 46,969 participants found that SGLT2 inhibitors increased the risk of GMIs by 3.5 times compared to placebo (HR 3.5; 95% CI, 3.09 - 3.95)[12]. As for specific types of SGLT2 inhibitors, no significant differences in the risk of GMIs were found, even at varying clinical doses[6]. Overall, the adverse effects had an incidence of 5% or more listed in the prescribing information of four FDA-approved SGLT2 inhibitors[13].

The risks of GMIs in the Asian population are heterogeneous. An Indian observational study of 205 patients found a high incidence of GMIs. Within a treatment duration of 7.6 +/- 5.9 months, 25.9% of patients experienced at least one episode of GMIs[14]. In the retrospective cohort study conducted in Korea, which was mentioned in the UTI section, it was found that SGLT-2 inhibitors were associated with a higher risk of GMIs compared to DPP-4 inhibitors (HR 2.39; 95% CI, 2.07–2.76), SU (HR 3.23; 95% CI, 2.73–3.81), and TZD (HR 3.23; 95% CI, 2.35–4.44)[8]. The Korean study aligns with clinical trials, but it found a higher risk of GMIs among patients above 60 years old when comparing SGLT2 inhibitors to DPP-4 inhibitors (HR 4.20; 95% CI, 2.63–6.71), SU (HR 4.27; 95% CI, 2.70-6.75), and TZD (HR 4.11; 95% CI, 2.25-7.50). In contrast to the previous studies, a meta-analysis of 18 randomized controlled trials in Japanese patients showed no significant difference in the risk of GMIs between SGLT2 inhibitors and placebo (HR 1.3; 95% CI, 0.65 - 2.58)[15]. The findings in the Asian population are varied and diverse. The Indian study suggested that the high rate of GMIs among their patient population may be due to poor genital hygiene, hot and humid climate, and the lack of circumcision among males in their communities. The Japanese study suggested that the inconsistency in the results may be due to their short-term follow-ups of up to 24 weeks.

In the 2022 meta-analysis focusing on non-diabetic patients, the risk of GMIs was found to be significantly heightened (RR 2.44; 95% CI, 1.14-5.25) compared to the placebo group[11]. Nevertheless, it's essential to recognize that the baseline prevalence of GMIs in non-diabetic patients is low, which implies that even though there is an increased risk, the absolute incidence remains relatively modest compared to diabetic patients. Specifically, the incidence is 1.4% among non-diabetic patients treated with SGLT2 inhibitors, compared to 0.5% in the placebo group.

Genital Mycotic Infections: Risk factors

Prior to the introduction of SGLT2 inhibitors, diabetic patients were predisposed to GMIs due to several traditional risk factors, such as poorly controlled glycemia, antibiotic and corticosteroid use, immunosuppression, and atopy. In men, particularly uncircumcised ones, poor hygiene may promote yeast growth in the moist, warm space beneath the foreskin. Women may be at increased risk due to pregnancy, estrogen or oral contraceptive use, and specific sexual

behaviors, such as orogenital sex[16].

A retrospective cohort study examined the link between baseline characteristics and GMIs in a UK primary care database of 21,004 people with type 2 diabetes initiating SGLT2 inhibitors and 55,471 controls initiating DPP-4 inhibitors[17]. It found that the incidence of GMIs varied greatly. Individuals with a history of prior infection had a higher one-year absolute risk of GMIs (females 23.7%, males 12.1%) compared to those without (females 10.8%, males 2.7%). GMIs within the first month of SGLT2 inhibitor treatment were linked to a higher adjusted risk of medication discontinuation at one year (31.8% vs. 19.4% without infections), with an adjusted HR of 1.48 (95% CI, 1.21-1.81). Multivariable models showed that higher BMI (>30kg/m^2) was independently associated with an increased risk of GMIs in patients treated with both SGLT2 inhibitors and DPP-4 inhibitors, while higher HbA1c, corticosteroid usage, or estrogen usage was only associated with increased infection risk in patients using DPP-4 inhibitors, not SGLT2 inhibitors. The author postulated that the association between glycosuria and GMIs reaches a saturation point after using SGLT2 inhibitors. Therefore, traditional risk factors increase the incidence of GMIs in people taking DPP-4 inhibitors but not in those taking SGLT2 inhibitors.

In a meta-analysis of five randomized controlled trials involving 1111 participants using a DPP-4 and SGLT2 inhibitors combination and 1128 using SGLT2 inhibitors alone, it was discovered that the combination therapy significantly reduced the risk of GMIs[18]. The calculated risk ratio of 0.51 (95% CI, 0.28-0.92) favored this combination therapy. Moreover, the frequency of GMIs did not significantly differ when SGLT2 inhibitors were combined with glimepiride, metformin, or exenatide, compared to the use of SGLT2 inhibitors alone.

In a case-based observational study using data from Truven Health MarketScan in the USA, 23,276 patients newly initiating both SGLT2 inhibitors and an antifungal agent were included, with a follow-up period extending up to 365 days[19]. The study found an increased risk of GMIs across all time periods after starting SGLT2 inhibitors, with the strongest effect observed during the first 90-day interval.

Genital Mycotic Infections: Prevention

Proper genital hygiene and sufficient water intake are essential for both men and women taking SGLT2 inhibitors. For men, to prevent and treat GMIs, it is suggested to retract the foreskin, thoroughly wash the genitals daily, and keep the foreskin retracted until the glans penis is dry[20].

For women experiencing symptoms, general advice includes avoiding local irritants like perfumed soaps or wipes. For those with recurrent GMIs, it is recommended to avoid wearing ill-fitting clothing made from non-breathable fabric, refrain from using daily panty liners, and abstain from vaginal douching[21].

Genital Mycotic Infections: Treatment in male

For men dealing with GMIs, the 2022 European guideline on balanoposthitis recommends specific treatments[20]. Clotrimazole cream 1% should be applied twice daily for 7-14 days as the primary topical treatment. In cases of severe symptoms, a single 150 mg dose of fluconazole is suggested. If there is a suspected resistance or allergy to imidazoles, Nystatin cream 100,000 units/g can be used. When significant inflammation is present, a topical imidazole combined with 1% hydrocortisone is recommended. The guideline highlights increasing fluconazole resistance in serious candidal infections among men without offering further management suggestions. A small prospective non-compared study evaluated oral itraconazole as an alternative treatment for Candida balanitis[22]. The study involved 26 patients, with Candida albicans predominant in 69.2% of cases, C. glabrata in 15.38%, and C. krusei in 3.84%. A single 400 mg dose of itraconazole effectively treated 84.6% of cases, curing both C. albicans and C. non-albicans species within 3 days and preventing recurrence after 15 days. Itraconazole presents a viable therapeutic option for Candida balanitis amid growing resistance concerns.

Patients on SGLT2 inhibitor therapy need not discontinue treatment if balanoposthitis occurs. However, if the condition is resistant to treatment or recurs frequently, it may be due to resistance or other pathogens causing balanoposthitis, such as Streptococci, Staphylococci, anaerobic bacteria, Trichomonas vaginalis, Mycoplasma genitalis, or Herpes simplex virus. In some cases, balanoposthitis may not be due to infectious pathogens, but rather to inflammatory dermatoses. Thus, if symptoms are resistant to initial treatment or recur, discontinuing SGLT2 inhibitors and considering a referral to a urologist becomes necessary.

Genital Mycotic Infections: Treatment in Female

For women dealing with GMIs, the CDC's Sexually Transmitted Infections Treatment Guidelines from 2021 provide the management of vulvovaginal candidiasis (VVC)[23]. VVC is classified as uncomplicated or complicated, with the latter including women with diabetes, recurrent

VVC, severe VVC, or non-albicans Candida infections. Uncomplicated VVC can be effectively treated with short-course topical azole formulations (1-3 days), while complicated VVC, such as in women with diabetes, requires a more extended treatment (7-14 days). Recurrent VVC, defined as three or more symptomatic episodes within a year, is mostly caused by C. albicans, with C. glabrata and other non-albicans Candida species found in 10-20% of cases. To maintain clinical and mycologic control, a longer initial therapy (7-14 days of topical treatment or 100-300 mg oral fluconazole every third day for three doses) is recommended, followed by a six-month oral fluconazole maintenance regimen (100-200 mg weekly). For non-albicans VVC, the optimal treatment remains unknown, but a 7–14-day non-fluconazole azole regimen (oral or topical) is recommended. In case of recurrence, a three-week course of 600 mg boric acid administered vaginally daily is recommended, with a 70% success rate; however, boric acid suppositories are seldom utilized in primary care due to their limited availability in community pharmacies.

While C. albicans is the main pathogen in women without diabetes, in diabetic women, C. glabrata and other non-albicans species are more common. Non-albicans species, such as glabrata, often exhibit increased resistance to antifungal agents[16]. A study comparing topical nystatin and standard oral fluconazole for recurrent VVC treatment found mycological cure rates of 64.3% (27/42) in the nystatin group and 12.5% (2/16) in the fluconazole group for C. glabrata infections. This suggests that nystatin may be more effective for C. glabrata or fluconazole-resistant Candida[24]. For patients who develop VVC after initiating SGLT2 inhibitors, a combined treatment approach using oral fluconazole to cover C. albicans and topical nystatin for non-albicans coverage may be a reasonable strategy.

If a patient has a poor response to initial treatment, it could be due to resistance, other pathogens (e.g., Trichomoniasis, Bacterial Vaginosis), or different conditions (e.g., Lichen sclerosus, Contact dermatitis, Chronic lichen simplex, Vulvodynia). In such cases, stopping SGLT2 inhibitors and referring the patient to a gynecologist for further treatment is advisable.

DISCUSSION

To enhance patient adherence to SGLT2 inhibitors and maximize their benefits, clinicians should trust evidence that shows no increased UTI risk and reassure patients who experience UTIs. Additionally, clinicians must inform patients about GMI symptoms and teach preventive hygiene techniques before prescribing the medication. Given limited time and diabetic education resources, clinicians need to identify high-risk patients for GMIs and provide more thorough preventive education. If a patient develops a GMI, clinicians should confidently initiate treatment for quick relief, potentially increasing SGLT2 inhibitor adherence. The suggested management strategy is illustrated in Figure 1. However, as medical doctors, we often lack the time and instruments to conduct a comprehensive examination of a patient's genital organs during routine outpatient visits. Instead, we typically prescribe empirical antifungal treatment based on the patient's subjective symptoms while using SGLT2 inhibitors. Therefore, if symptoms persist despite initial treatment, it is crucial to discontinue the medication and refer the patient to a specialist.

Even though the data on non-diabetic patients are relatively limited, most adverse effects such as GMIs appear to be minor, neutral (for instance, lower limb amputation), or even non-existent (like DKA) in these patients when compared to those with diabetes. The exception to this is the UTI risk, which is elevated in non-diabetic patients and not in diabetic ones. These comparisons are presented in Table 1. No specific theory yet explains the discrepancy in UTI and GMI occurrences between diabetic and non-diabetic patients. Possible explanations could include different glucose thresholds for urinary bacterial or fungal diseases, or the limited number of non-diabetic patients, which could skew the results.

It should be noted that even before the introduction of SGLT2 inhibitors, recurrent vulvovaginal candidiasis was already a significant global burden, affecting approximately 138 million women worldwide annually, with a global prevalence of 3871 per 100,000 women[25]. The total annual lost productivity due to recurrent vulvovaginal candidiasis was estimated to be $14.39 billion in 2010. Another pressing issue is the growing resistance to antifungal drugs, which presents a serious risk to human health and food security[26]. The limited number of available systemic antifungals and their extensive use have fostered resistance among many pathogenic fungi. This can notably constrain treatment options and could render certain fungal infections untreatable. With SGLT2 inhibitors proving effective in both diabetic and non-diabetic patients, their prescription rates are poised to surge. This rise could inevitably result in more cases of GMIs, potentially exacerbating an already prevalent fungal disease. As a consequence, more patients would require treatment for GMIs, potentially intensifying antifungal resistance. As we tackle these interconnected

issues, we must recognize the potential dangers that we have yet to fully understand – the long-term impacts of widespread fungal diseases and escalating antifungal resistance could be significant.

CONCLUSION

SGLT2 inhibitors have proven to be highly beneficial for both diabetic and non-diabetic patients, offering significant advantages in terms of organ protection. To ensure the optimal use of these medications, enhancing physicians' confidence in the evidence related to urinary tract and genital tract infections is vital, as this can lead to improved persistence rates and positively impact a greater number of individuals who could benefit from SGLT2 inhibitors. However, it's crucial to address the heightened risk of genital mycotic infections associated with these drugs, as it may undermine patients' trust in their long-term safety and effectiveness. Further research is needed to explore preventive measures and treatment options for genital mycotic infections.

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TABLE

Table1. Adverse Effects of SGLT2 Inhibitors in Diabetic vs. Non-Diabetic Patients (Relative Risk to Placebo in Randomized Controlled Trials)

Adverse Effect	Diabetic Patients	Non-Diabetic Patients
UTIs	RR 1.03 (95% CI, 0.96–1.11)[a]	RR 1.29 (95% CI, 1.05-1.58)[b]
GMIs	RR 3.5 (95% CI, 3.09 - 3.95)[c]	RR 2.44 (95% CI, 1.14-5.25)[b]
DKA	RR 2·12 (95% CI, 1·49–3·04)[d]	One event in 30,000 patient-years [d]
Lower Limb Amputation	RR 1·15 (95% CI, 1·02–1·30)[d]	RR 1·06 (95% CI, 0·93–1·21)[d]

Abbreviation: UTI (Urinary Tract Infection), GMI (Genital Mycotic Infection), DKA (Diabetic Ketoacidosis), RR (Relative Risk), Randomized Controlled Trial (RCT)

[a] Meta-analysis by Puckrin et al., which included 72 RCTs involving 37,116 patients[6].

[b] Meta-analysis by Tsai et al., which included 4 RCTs involving 8,927 patients[11].

[c] Meta-analysis by Marilly at al., which included 5 RCTs involving 46,969 patients[12].

[d] Meta-analysis by Baigent at al., which included 13 RCTs involving 90,413 patients[2].

FIGURE AND FIGURE LEGEND

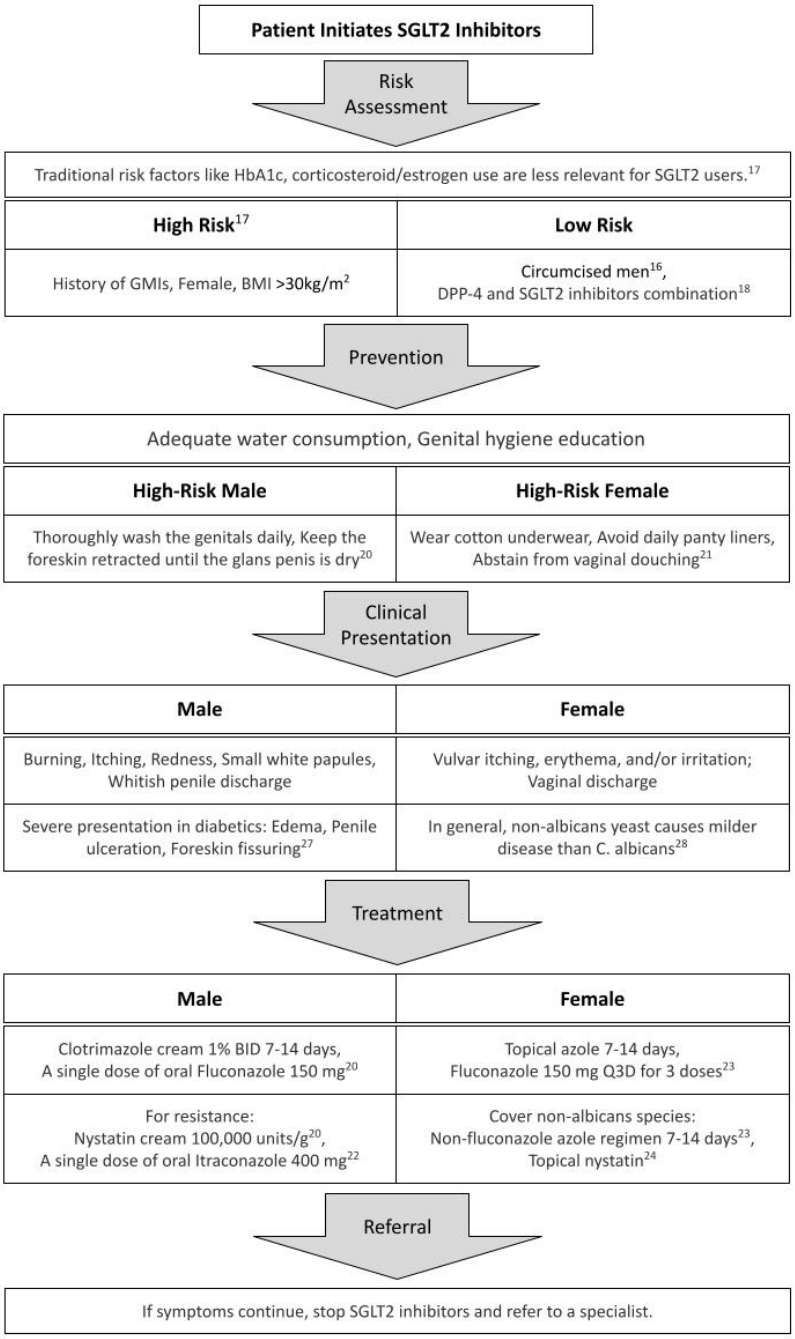

Figure 1. Management strategy for Genital Mycotic Infections (GMIs) in patients with type 2 diabetes using SGLT2 inhibitors.

Adverse Effects of Sodium Glucose Co-Transporter-2 (SGLT2) Inhibitors on the Genitourinary System: An update

鈉-葡萄糖協同轉運蛋白 2（SGLT2）抑制劑對泌尿生殖系統之不良影響：最新進展

張維倫[1]　林慶齡[2]　龔信宗[2,*]

中文摘要

SGLT2 抑制劑經過臨床試驗證實，對糖尿病與非糖尿病患者皆能明顯降低主要心血管事件、心臟衰竭住院以及腎病進展的風險。2022 年的美國糖尿病學會（ADA）和歐洲糖尿病研究學會（EASD）共識，將 SGLT2 抑制劑在藥物治療選擇的位階提高，並擴大了其適用範疇。SGLT2 抑制劑最常見的副作用為泌尿生殖系統的感染，臨床上許多病患因無法耐受這類副作用而選擇停藥。本研究透過回顧相關文獻，探討泌尿生殖系統副作用的發生率、風險因子、預防與治療策略，以協助臨床醫師管理這些副作用，提升病患對藥物的依從性，使病患得到最大的器官保護效果。

關鍵字：鈉-葡萄糖協同轉運蛋白 2（SGLT2）抑制劑、泌尿道感染、生殖道黴菌感染

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The Fu-Jen Journal of Medicine (FJJM) is a peer-reviewed journal which aims to enhance research quality of staffs in the College of Medicine, Fu Jen Catholic University. The journal publishes original investigations across a wide range of medical disciplines including original research articles in basic and clinical sciences, case reports, review articles, brief reports, and letter to the editor. FJJM is now issued by the Center of Medical Education in the College of Medicine, Fu Jen Catholic University. To promote journal quality, the manuscript submitted to FJJM after August first 2015 has to be prepared in English to meet the international standards.

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