2019

Effect of Canagliflozin on liver steatosis in obese non diabetic patients with non-alcoholicfatty liver disease: A double blind randomized controlled trial

Borwonkhun Tontivuthikul
Faculty of Medicine

Effect of Canagliflozin on liver steatosis in obese non diabetic patients with non-alcoholicfatty liver disease: A double blind randomized controlled trial

Mr. Borwonkhun Tontivuthikul

A Thesis Submitted in Partial Fulfillment of the Requirements

for the Degree of Master of Science in Medicine

Department of Medicine

FACULTY OF MEDICINE

Chulalongkorn University

Academic Year 2019

ผลของยาคานากลิโฟลซินต่อไขมันสะสมในตับของผู้ป่วยโรคตับคั่งไขมันที่มีภาวะอ้วนโดยไม่เป็นเบาหวาน: การศึกษาแบบสุ่ม ปกปิดทั้งสองฝ่ายและมีกลุ่มควบคุม

นายบวรคุณ ตันติวุฒิกุล

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาอายุรศาสตร์ ภาควิชาอายุรศาสตร์
คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2562

Thesis Title	Effect of Canagliflozin on liver steatosis in obese non diabetic patients with non-alcoholicfatty liver disease: A double blind randomized controlled trial
By	Mr. Borwonkhun Tontivuthikul
Field of Study	Medicine
Thesis Advisor	WITTHAWAT NAEOWONG, M.D.
Thesis Co Advisor	Professor WEERAPAN KHOVIDHUNKIT, M.D.

Accepted by the FACULTY OF MEDICINE, Chulalongkorn University in Partial Fulfillment of the Requirement for the Master of Science

.....
Dean of the FACULTY OF MEDICINE

(Professor SUTTIPONG WACHARASINDHU, M.D.)

THESIS COMMITTEE

.....
Chairman

(Professor PONLAPAT ROJNCKARIN, M.D.)

.....
Thesis Advisor

(WITTHAWAT NAEOWONG, M.D.)

.....
Thesis Co-Advisor

(Professor WEERAPAN KHOVIDHUNKIT, M.D.)

.....
Examiner

(SARAWUT SIWAMOGSATHAM, M.D.)

.....
External Examiner

(NOPAVUT GERATIKORNSUPUK, M.D.)

บวรคุณ ตันติวุฒิกุล : ผลของยาคานากลิโฟลซินต่อไขมันสะสมในตับของผู้ป่วยโรคตับคั่งไขมันที่มีภาวะอ้วนโดยไม่เป็นเบาหวาน: การศึกษาแบบสุ่ม ปกปิดทั้งสองฝ่ายและมีกลุ่มควบคุม. (Effect of Canagliflozin on liver steatosis in obese non diabetic patients with non-alcoholicfatty liver disease: A double blind randomized controlled trial) อ.ที่ปรึกษาหลัก : อ. นพ.วิทวัส แนววงศ์, อ.ที่ปรึกษาร่วม : ศ. นพ.วีรพันธุ์ โชวิฑูรกิจ

วัตถุประสงค์: เพื่อศึกษาว่าการรักษาโดยใช้ยาคานากลิโฟลซินมีผลต่อไขมันสะสมในตับของผู้ป่วยโรคตับคั่งไขมันที่มีภาวะอ้วนโดยไม่เป็นเบาหวาน แตกต่างจากกลุ่มควบคุมหรือไม่

ระเบียบวิธีวิจัย: ผู้ป่วยโรคตับคั่งไขมันที่มีภาวะอ้วนโดยไม่เป็นเบาหวาน จำนวน 82 รายที่มีระดับดัชนีมวลกาย 25-35 กก./ม.^{2} ถูกสุ่มโดยปกปิดทั้งสองฝ่ายให้รับยาคานากลิโฟลซิน 100 มิลลิกรัมต่อวันร่วมกับการปรับเปลี่ยนอาหารและพฤติกรรมหรือให้รับยาหลอกร่วมกับการปรับเปลี่ยนอาหารและพฤติกรรมเพียงอย่างเดียว (จำกัดพลังงานลดลง 500 กิโลแคลอรี่ต่อวัน ร่วมกับออกกำลังกายแบบแอโรบิก) เป็นระยะเวลา 24 สัปดาห์ และตรวจวัดระดับไขมันสะสมในตับโดยเครื่อง Fibroscan® และวัดการเปลี่ยนแปลงอื่นๆ รวมถึง น้ำหนักตัว ความดันโลหิต ค่าระดับน้ำตาลในเลือด ค่าไขมันในเลือด ค่าการทำงานของตับ และค่าดัชนีตับแข็ง

ผลการศึกษา: ที่สัปดาห์ที่ 24 ค่าไขมันสะสมในตับมีแนวโน้มลดลงในผู้ป่วยที่ได้รับยาคานากลิโฟลซินเมื่อเปรียบเทียบกับกลุ่มควบคุม (CAP -13.8 +/- 40.6 db/m vs -0.6 +/- 42.9 db/m, P = 0.168) และพบว่าน้ำหนักตัวลดลงอย่างมีนัยสำคัญในผู้ป่วยที่ได้รับยาคานากลิโฟลซินเมื่อเปรียบเทียบกับกลุ่มควบคุม (-1.97 +/- 2.14 กก. vs -0.14 +/- 2.57 กก., P = 0.001) การศึกษากลุ่มย่อยยังแสดงให้เห็นว่ามีการลดลงอย่างมีนัยสำคัญของค่าไขมันสะสมในตับในผู้ป่วยกลุ่มที่มีระดับน้ำตาลขณะอดอาหารผิดปกติหรือมีภาวะตับอักเสบ (CAP -40.00 +/- 30.7 vs 16.36 +/- 42.60, P = 0.004 และ -34.75 +/- 40.84 vs 14.86 +/- 38.36, P = 0.031 ตามลำดับ)

สรุปผลการศึกษา: การใช้ยาคานากลิโฟลซินสามารถลดน้ำหนักตัวได้อย่างมีนัยสำคัญและอาจมีผลในการลดไขมันสะสมในตับในของผู้ป่วยโรคตับคั่งไขมันที่มีภาวะอ้วนโดยไม่เป็นเบาหวาน โดยเฉพาะอย่างยิ่งในผู้ป่วยกลุ่มที่มีระดับน้ำตาลขณะอดอาหารผิดปกติ หรือมีภาวะตับอักเสบ

สาขาวิชา อายุรศาสตร์ ลายมือชื่อนิสิต .....

ปีการศึกษา 2562 ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

ลายมือชื่อ อ.ที่ปรึกษาร่วม .....

6174055530 : MAJOR MEDICINE

KEYWORD: Non-alcoholic fatty liver disease, Canagliflozin, Fibroscan, Controlled Attenuation Parameter

Borwonkhun Tontivuthikul : Effect of Canagliflozin on liver steatosis in obese non diabetic patients with non-alcoholicfatty liver disease: A double blind randomized controlled trial. Advisor: WITTHAWAT NAEOWONG, M.D. Co-advisor: Prof. WEERAPAN KHOVIDHUNKIT, M.D.

Objective: To demonstrate whether treatment with canagliflozin had an effect on liver steatosis in obese non-diabetic patients with NAFLD compared with the control group

Methods: 82 nondiabetic patients with BMI 25-35 kg/m^2 underwent 24 weeks of double blind randomized controlled trial with combined canagliflozin 100 mg daily with diet-lifestyle modification or placebo with diet-lifestyle modification (500 calories restriction and moderate intensity aerobic exercise). Primary outcome was the change in Controlled attenuation parameter (CAP) representing hepatic steatosis. Liver stiffness measurement (LSM) representing liver stiffness/fibrosis and other secondary outcomes including changes in body weight, fasting plasma glucose, lipid profile, liver function test and FIB-4 index were also evaluated.

Results: At week 24, the Controlled attenuation parameter (CAP) in the canagliflozin group showed a decreasing trend compared with the control group (-13.8 +/- 40.6 db/m vs -0.6 +/- 42.9 db/m, P = 0.168). Significant weight loss was achieved in the canagliflozin group compared with the control group (-1.97 +/- 2.14 kg vs -0.14 +/- 2.57 kg, P = 0.001). A prespecified subgroup analysis in patients with impaired fasting plasma glucose and in those with hepatitis demonstrated significant reduction in CAP (-40.00 +/- 30.7 vs 16.36 +/- 42.60, P = 0.004 and -34.75 +/- 40.84 vs 14.86 +/- 38.36, P = 0.031, respectively).

Conclusion: Treatment with Canagliflozin resulted in a significant weight reduction and may improve hepatic steatosis in obese non-diabetic patients with NAFLD especially in those with impaired fasting plasma glucose or hepatitis.

Field of Study: Medicine

Academic Year: 2019

Student's Signature .....

Advisor's Signature .....

Co-advisor's Signature .....

ACKNOWLEDGEMENTS

งานวิจัยฉบับนี้ สามารถสำเร็จลุล่วงได้เนื่องจากความกรุณาช่วยเหลือ และคำแนะนำเป็นอย่างดีจาก อาจารย์ นายแพทย์วิทวัส แนววงศ์ อาจารย์ประจำหน่วยต่อมไร้ท่อ และเมตะบอลิสม ภาควิชาอายุรศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ซึ่งเป็นอาจารย์ที่ปรึกษาวิทยานิพนธ์หลัก อาจารย์ได้เสียสละเวลาให้คำปรึกษา ให้ความช่วยเหลือในทุกๆ ด้าน มาโดยตลอด ผู้วิจัยขอกราบขอบพระคุณเป็นอย่างสูงไว้ ณ โอกาสนี้

ขอกราบขอบพระคุณศาสตราจารย์ ดร. นายแพทย์วีรพันธุ์ โชวิฑูรกิจ หัวหน้าหน่วยต่อมไร้ท่อ และเมตะบอลิสม ภาควิชาอายุรศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย อาจารย์ที่ปรึกษาวิทยานิพนธ์ร่วม ที่ช่วยเหลือและให้คำปรึกษาจนงานวิจัยชิ้นนี้สำเร็จ

ขอขอบพระคุณอาจารย์ประจำหน่วยต่อมไร้ท่อ และเมตะบอลิสมทุกท่านที่เมตตาให้โอกาสได้เข้ามาศึกษาต่อ ณ หน่วยต่อมไร้ท่อและเมตะบอลิสม คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัยแห่งนี้

ขอขอบคุณคุณณัฐนิชา ห่วงงาม เจ้าหน้าที่นักวิทยาศาสตร์และนักวิจัยประจำศูนย์ความเป็นเลิศทางการแพทย์ด้านเบาหวาน ฮอร์โมน และเมตะบอลิสม โรงพยาบาลจุฬาลงกรณ์ สภากาชาดไทย ที่ช่วยให้คำปรึกษาตลอดจนช่วยเหลืองานวิจัยชิ้นนี้มาตั้งแต่ต้นด้วยความเอาใจใส่ จนทำให้งานวิจัยสำเร็จลุล่วงเป็นอย่างดี

ขอขอบคุณอาภาภรณ์ การินทร์ เจ้าหน้าที่งานวิจัยทางคลินิกประจำศูนย์ความเป็นเลิศทางการแพทย์ด้านเบาหวาน ฮอร์โมน และเมตะบอลิสม โรงพยาบาลจุฬาลงกรณ์ สภากาชาดไทย ที่ช่วยในการดำเนินงานวิจัยเป็นอย่างดี

ขอขอบพระคุณผู้เข้าร่วมการศึกษา และญาติทุกท่านที่ให้ความไว้วางใจ และเสียสละเวลาอันมีค่าในการเข้าร่วมโครงการวิจัยนี้

วิทยานิพนธ์ฉบับนี้ได้รับเงินทุนสนับสนุนจาก กองทุนวิจัยรัชดาภิเษกสมโภช คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย และ ทุน 90 ปี จุฬาลงกรณ์มหาวิทยาลัย กองทุนรัชดาภิเษกสมโภช

Borwonkhun Tontivuthikul

TABLE OF CONTENTS

Page

List of tables

Page

List of figures

Pages

บทที่ 1

บทนำ

ความสำคัญและที่มาของปัญหาการวิจัย (BACKGROUND AND RATIONALE)

Non-alcoholic fatty liver disease (NAFLD) is diagnosed based on histological or imaging evidences of hepatic steatosis without obvious secondary causes. NAFLD is a spectrum of disease with different severity from hepatic steatosis without hepatocyte injury (nonalcoholic fatty liver, NAFL) to hepatocyte injury with or without fibrosis (nonalcoholic steatohepatitis, NASH).(1) The prevalence of NAFLD diagnosed by imaging is varied among different ethnicities and regions with an average global prevalence between 25.24% - 27.4% in Asian populations.(2)

NAFLD is a great burden not only to the patient's individual health but also to the healthcare system.(3) A 2-fold increase in mortality due to cardiovascular disease and a 10-fold increase in liver related diseases are found in NASH patients especially those with high grade fibrosis.(4) NAFLD differs from other liver diseases since it is much more common. It is believed that the incidence of NAFLD will substantially increase parallel to obesity in the near future.(3) A 10-year burden of NAFLD could increase to an estimated $1.005 trillion in the USA and €334 billion in Europe.(5)

Common comorbidities of NAFLD/NASH are obesity and type 2 diabetes mellitus. The prevalence of obesity and type 2 diabetes mellitus are 51.34% and 81.83% , respectively in NAFLD patients and 22.51% and 43.63%, respectively in patients with NASH.(2) As obesity and insulin resistance, two characteristics of type 2 diabetes, are

the most important pathogenic factors associated with NAFLD, many anti-diabetic drugs have been investigated for treatment of NAFLD.(6)

The American Association for the Study of Liver Disease (AASLD), the Japan Society of Gastroenterology (JSG)–Japan Society of Hepatology (JSH), and the European Association for the Study of the Liver (EASL)–European Association for the Study of Diabetes (EASD)–European Association for the Study of Obesity (EASO) published their own evidence based guidelines for NAFLD/NASH managements.

Due to controversies, there is no standard of care recommendation. Only pioglitazone for patients regardless of diabetic status and vitamin E for non-diabetic patients are suggested as pharmacological agents.(1, 7, 8) Recent studies have shown that SGLT2 inhibitors decrease hepatic fatty accumulation and inflammation in obese rats with NASH.(9, 10) Recent human studies using SGLT2 inhibitors show benefit in treating NAFLD in patients with diabetes.(11, 12)

Effectiveness of canagliflozin in obese non-diabetic patients with NAFLD in terms of decreasing steatosis and fibrosis is unknown. We conducted a 24-week double blind randomized controlled trial using canagliflozin and measured change in CAP, LSM and other metabolic parameters compared with the control group. Our study was the first study in non-diabetic patients and the first study in Thai population.

คำถามของการวิจัย (RESEARCH QUESTION)

คำถามหลัก (PRIMARY RESEARCH QUESTION)

Do obese non-diabetic patients with NAFLD treated with canagliflozin have significant difference in Controlled attenuation parameter (>20 decibels per meter) measured by transient elastography compared with that in the control group?

คำถามรอง (SECONDARY RESEARCH QUESTION)

Do obese non-diabetic patients with NAFLD treated with canagliflozin have significant differences in the following parameters compared with those in the control group?

- Liver stiffness measurement (LSM) measured by Fibroscan® (transient elastography)
- Fibrosis-4 (FIB-4) Index(13)
- SGPT, SGOT, fasting plasma glucose, total cholesterol, LDL-c, HDL-c, and triglyceride
- Body weight, blood pressure, pulse rate and BMI

We performed prespecified subgroup analysis using the following parameters

- Impaired fasting plasma glucose (FPG 100-125 mg%) or normal fasting plasma glucose (FPG <100 mg%)
- Lower BMI (25 to less than 30 kg/m2) or Higher BMI (30-35 kg/m2)
- Hepatitis (SGPT >40 u/l) or no hepatitis (SGPT <= 40 u/l)
- CAP <= 248 decibels per meter(dB/m), CAP 249-268 dB/m, CAP 269-290 dB/m or CAP >290 dB/m

วัตถุประสงค์ของการวิจัย (OBJECTIVE)

Primary objective

To demonstrate whether there is a significant difference in Controlled attenuation parameter of obese non diabetic patients with NAFLD treated with canagliflozin compared with that in the control group.

Secondary objective

To demonstrate whether there are significant differences in liver stiffness, liver enzyme, lipid profile, body weight, blood pressure, pulse rate and BMI in obese non diabetic patients with NAFLD treated with canagliflozin compared with those in the control group.

To determine associated factors influencing a change in Controlled attenuation parameter/ liver stiffness in obese non diabetic patients with NAFLD treated with canagliflozin compared with the control group

To determine whether canagliflozin have other effects beyond weight loss in Controlled attenuation parameter in obese non diabetic patients with NAFLD

สมมติฐาน (HYPOTHESIS)

Null hypothesis: There is no significant difference in CAP between patients receiving canagliflozin and placebo.

Alternative hypothesis: There is a significant difference in CAP between patients receiving canagliflozin and placebo.

คำสำคัญ (KEY WORDS)

Non-alcoholic fatty liver disease (NAFLD)

Obesity

Canagliflozin

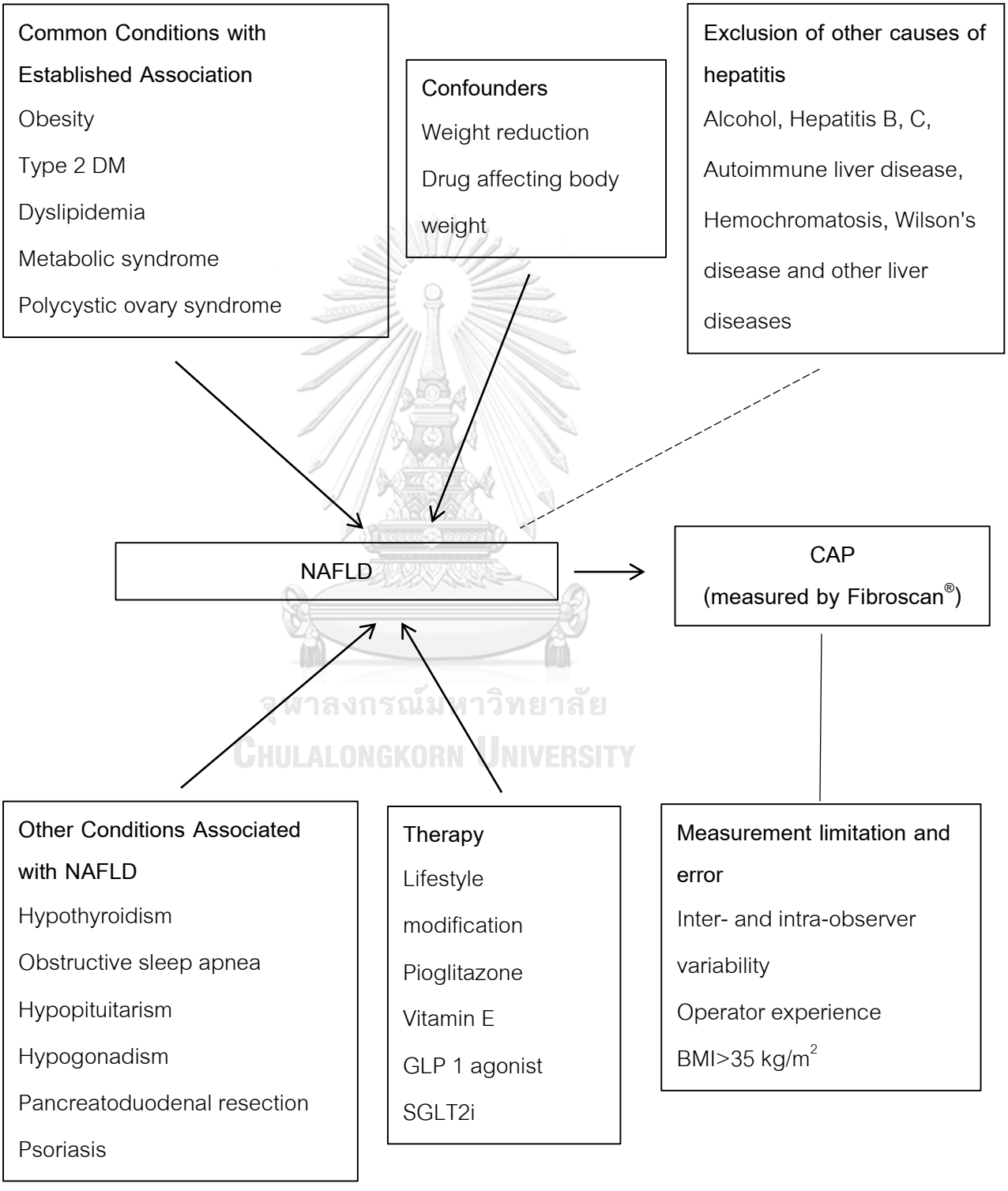
Fibroscan®

Transient elastography

Controlled Attenuation Parameter (CAP)

กรอบแนวความคิดในการวิจัย (CONCEPTUAL FRAMEWORK)

Figure 1 Conceptual framework



ข้อตกลงเบื้องต้น (ASSUMPTION)

- NAFLD is diagnosed based on AASLD and ACG definition.(1)
    - Demonstration of hepatic steatosis by imaging modality (ultrasound, CT or MRI)
    - Exclusion of significant alcohol consumption
    - Exclusion of other causes of hepatic steatosis
    - Absence of coexisting chronic liver diseases

- Type 2 diabetes mellitus is diagnosed based on one of the following ADA 2018 criteria(14)
    - A1C >= 6.5%. The test should be performed in a laboratory using a method that is NGSP certified and standardized to the DCCT assay.
    - FPG >= 126 mg/dL (7.0 mmol/L). Fasting is defined as no caloric intake for at least 8 hours.
    - 2-h PG >= 200 mg/dL (11.1 mmol/L) during an OGTT. The test should be performed as described by the WHO, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.
    - In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose >= 200 mg/dL (11.1 mmol/L).

- Obesity in this study includes patients with BMI 25-35 kg/m^2 according to WHO criteria for obesity in Asian population.(15)

- We excluded patients with BMI>35 kg/m^2 due to limitation of Fibroscan® accuracy in patients with extreme BMI.(16)

การให้คำนิยามเชิงปฏิบัติที่ใช้ในการวิจัย (OPERATIONAL DEFINITION)

- CAP cut-points for steatosis classification in NAFLD (NAS grade) (17)
    - S0 <= 248 decibels per meter (dB/m)
    - S1 249 - 268 decibels per meter (dB/m)
    - S2 269 - 290 decibels per meter (dB/m)
    - S3 > 290 decibels per meter (dB/m)

- Cut-point for Fibrosis classification in NAFLD (18)
    - F0 <4.6 kilopascal (kPa)
    - F1 4.7 - 6.1 kilopascal (kPa)
    - F2 6.2 - 8.8 kilopascal (kPa)
    - F3 8.9 - 12.0 kilopascal (kPa)
    - F4 12.1 - 38.6 kilopascal (kPa)

ข้อจำกัดทางการวิจัย (LIMITATION)

- CAP and LSM are not recognized as gold standard measurements. Results from this study will not be able to compare with other existing standard treatment in terms of efficacy.
- Due to limited accuracy of Fibroscan® in extremely obese patients (BMI>35 kg/m2), we had excluded them form the study even though they might have the greatest benefit from NAFLD treatment.

ผลหรือประโยชน์ที่คาดว่าจะได้รับจากงานวิจัย (EXPECTED BENEFIT AND APPLICATION)

- New insight in efficacy of canagliflozin in liver fat content reduction, weight loss and other metabolic parameters in patients with NAFLD.
- New knowledge about efficacy and adverse reaction of canagliflozin in non-diabetic patients

อุปสรรคที่อาจเกิดขึ้นและมาตรการแก้ไข (OBSTACLES AND STRATEGIES TO SOLVE THE PROBLEMS)

- Canagliflozin may rarely lead to hypoglycemia. Patients with suspicious symptoms of hypoglycemia will be provided with a self-monitoring of blood glucose device and a direct emergency call number of an investigator.
- Canagliflozin may increase a risk of lower limb amputation, therefore, we have excluded the patients at risk from enrollment. Monitoring lower limb infection or ulcer and stop the drug in case these occurs. (This protocol is according to general measurement conducted in patients taking SGLT2 inhibitors—FDA warning)
- Fibroscan® and blood tests will be performed at the beginning and at the end of the study. Adherence to follow up schedule is essential. We will perform telephone call to remind the participants in advance.
- Serious side effects will be reported to Institutional review board (IRB) and Janssen-Cilag according to designated protocol within 24 hours.
- In case of a serious side effect and the patient needs to be hospitalized, the primary physician will be informed about the drug or placebo by the assigned researcher at Endocrine and Metabolism unit.

ปัญหาทางจริยธรรม (ETHICAL CONSIDERATIONS)

หลักการให้ประโยชน์ (Beneficence)

Patients will benefit from a lifestyle modification in both groups as a standard recommended treatment of NAFLD. Treatment group may gain add-on benefit from a medication in terms of weight reduction and decreased hepatic steatosis.

หลักการไม่ก่อให้เกิดอันตราย (Non-maleficence)

A study drug has proven safety in cardiovascular outcome and is approved by FDA for treatment in type 2 diabetics patients.(19) Although it has a recently concern about an increased risk of lower limb amputation, we have excluded susceptible patients from the study. Risk of hypoglycemia will be minimized by patients' education of self-management in case of hypoglycemic episodes. Patients with suspected symptoms of hypoglycemia will be provided with a blood glucose monitor and advocated to check blood glucose whenever symptoms occur and to make a contact with the principle investigator immediately.

หลักความยุติธรรม (Justice)

All patients will be accessible to standard medical care related to their diseases including lifestyle modification and diet management. All related comorbidities including hypertension, dyslipidemia, obesity, and obstructive sleep apnea will also be evaluated and treated. Patients have equal possibility to be randomized into both groups. Patients will be closely monitored during the study.

Janssen-Cilag has role as a drug provider without any access to data, intervention, analysis or report of this study.

หลักการเคารพในสิทธิบุคคล (Autonomy)

Data had been recorded by the investigators without a direct subject identification using data record form. Results were present in general, not as individual data.

บทที่ 2

ทบทวนวรรณกรรมที่เกี่ยวข้อง

NAFLD definition

There are several consensus and guidelines regarding definition of non-alcoholic fatty liver disease (NAFLD). Most of them share required criteria including hepatic steatosis (fat deposition in liver by imaging or histopathology) and excluding other secondary causes of hepatitis. Table 1

Table 1 Diagnostic criteria for non-alcoholic fatty liver disease according to the various guidelines

	EASL(8)	NICE(20)	Asia-Pacific(21)	AISF(7)	AASLD(22)
Required criteria	Steatosis in > 5% of hepatocytes by either imaging or histology	Excessive fat in the liver	Hepatic steatosis by either imaging or histology	Hepatic steatosis on either imaging or histology	Evidence of hepatic steatosis either by imaging or histology
	No other causes of steatosis	No other causes of steatosis	No other causes of steatosis	No other causes of steatosis	No other causes of steatosis

	EASL(8)	NICE(20)	Asia-Pacific(21)	AISF(7)	AASLD(22)
	Insulin resistance	No significant alcohol consumption	No significant alcohol consumption	No significant alcohol consumption	No significant alcohol consumption
					No coexisting chronic liver disease
Alcohol consumption threshold (men)	30 grams/day	30 grams/day	2 standard drinks/day	30 grams/day	21 standard drinks/week
			140 grams/day		294 grams/day
Alcohol consumption threshold (women)	20 grams/day	20 grams/day	1 standard drinks/day	20 grams/day	14 standard drinks/week
			70 grams/week		196 grams/week

EASL: European Association for the Study of the Liver; NICE: National Institute for Health and Care Excellence; AISF: Italian Association for the study of the Liver; AASLD: American Association for the Study of Liver Diseases; MRI: Magnetic resonance imaging.

NAFLD pathogenesis

Obese patients always have excessive adipose tissues. Outgrowth of an adipose tissue causes a hypoxic state of adipocytes leading to production of an inflammatory cytokine (Tumor necrosis factor, TNF). Increased TNF induces adipocyte

lipolysis and decreases adiponectin triggering release of free fatty acid into blood circulation. Malfunction of adipocytes also triggers recruitment of macrophages (via C-C chemokine ligand 2, CCL2) and worsens inflammation of adipose tissue. Inflamed and dying adipocytes promote insulin resistance.(23) Hepatocyte is exposed with free fatty acids and inflammatory cytokines resulted in the first step of hepatic steatosis.(24) Figure 2

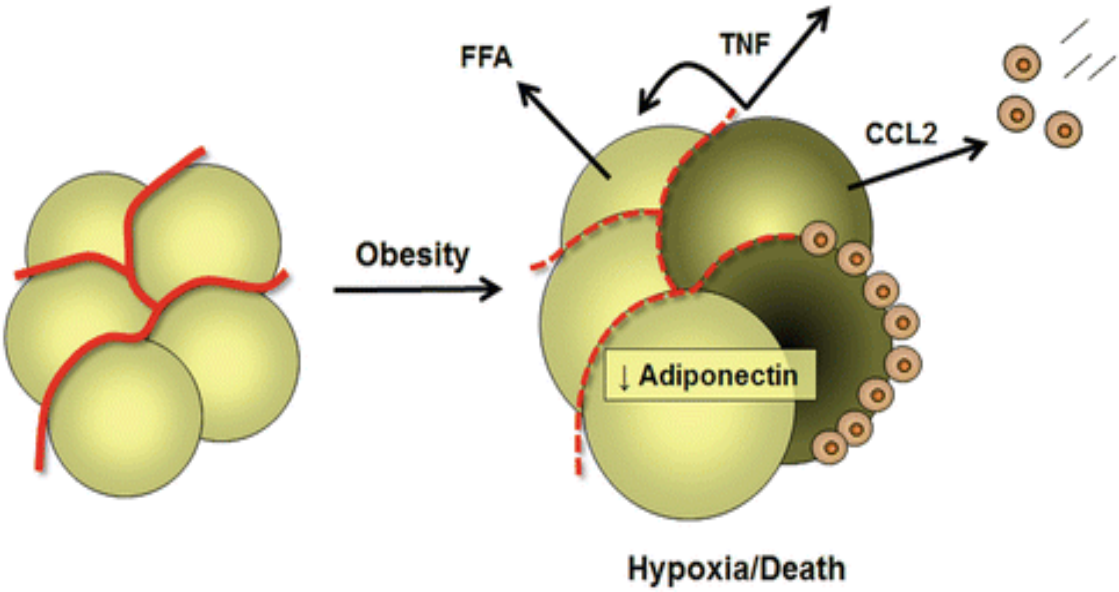


Figure 2 Malfunction of adipose tissue in obese patients

In state of insulin resistance, pancreatic beta cells try to produce more insulin to compensate and lead to hyperinsulinemia. The hepatocyte confronts both free fatty acid and high level of insulin simultaneously. Insulin promotes hepatocyte uptake of free fatty acid (via fatty acid transport protein 5, FATP5 and CD36) and processes to triglyceride which is one of causes of hepatic steatosis. The by-product of triglyceride production especially diacylglycerols can stimulate protein kinase C epsilon (PKCε) and deteriorate hepatic insulin sensitivity.(25) Figure 3a

Normal physiologic roles of insulin at hepatocytes are inhibition of gluconeogenesis and promotion of lipid storage. Insulin resistance is supposed to decrease hepatic lipid synthesis, in fact, it boosts both lipogenesis (via mammalian target of rapamycin, mTOR) and gluconeogenesis (via forkhead box O1, FOXO1).(26) Figure 3b

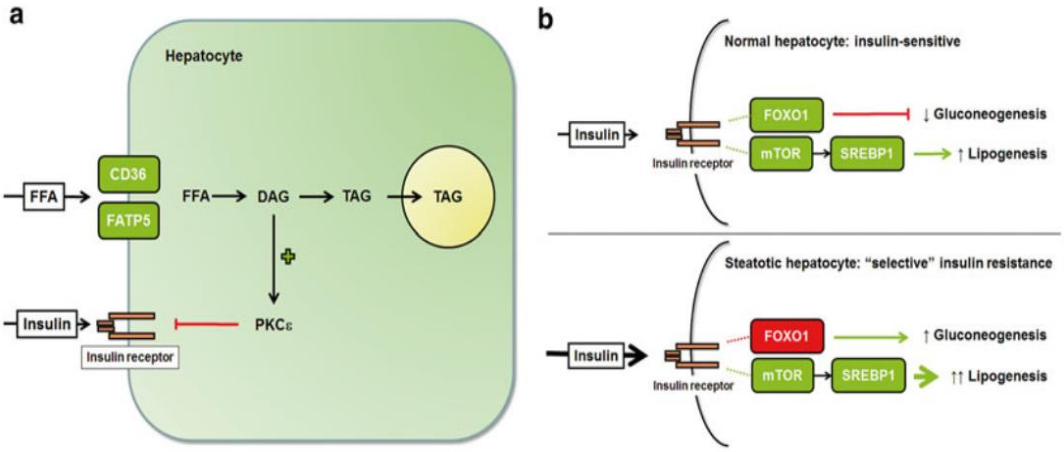


Figure 3 Hepatic storage of free fatty acid (a), intrahepatocyte selective insulin resistance and gluconeogenesis/lipogenesis (b)

(FFA: free fatty acid, DAG: diacylglycerol, TAG: triacylglycerol)

The other pathway of hepatic steatosis arises from a synthesis of new fatty acid from carbohydrate (De novo lipogenesis). This pathway needs insulin regulated transcription factor sterol regulatory element-binding protein-1 (SREBP1) and a glucose regulated carbohydrate response element-binding protein (ChREBP). Both pathways promote the de novo lipogenesis in context of hyperglycemia and hyperinsulinemia.(27) Figure 4

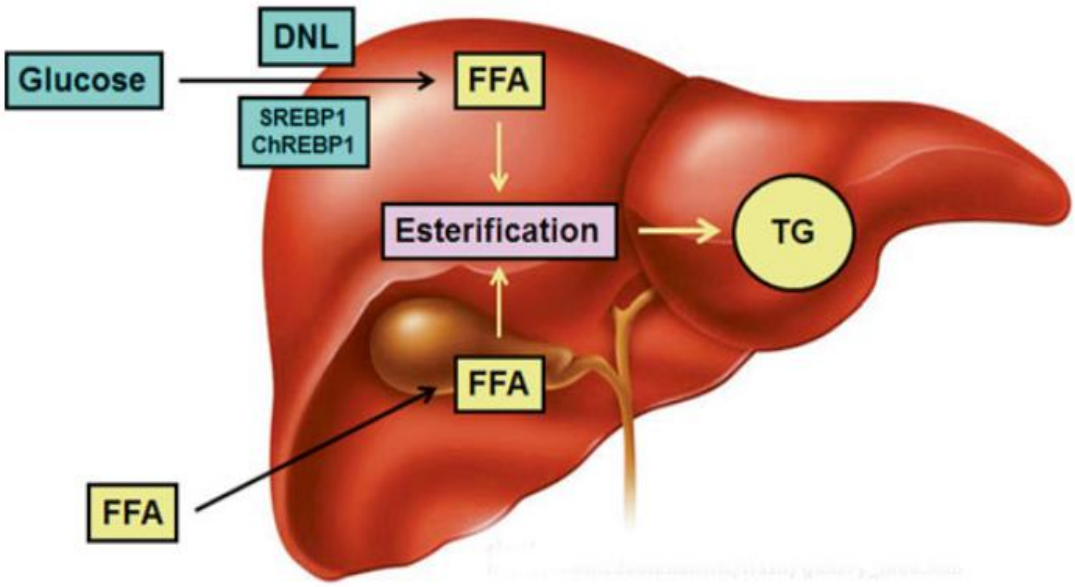


Figure 4 Hepatic steatosis from De novo lipogenesis

(FFA free fatty acid, DNL De novo lipogenesis, TG triglyceride)

Hepatic fat storage causes a stress to endoplasmic reticulum and mitochondrial function. Increased reactive oxygen species (ROS) and unfolded protein response (UPR) encourage inflammation, hepatocyte apoptosis and eventually fibrosis. Multiple genetic and epigenetic factors have influences on hepatic fat storage, response to inflammation and cytokine production.(28) Gut microbiome at intestinal mucosa has an impact on NAFLD pathogenesis in many ways e.g. increased intestinal mucosal permeability of lipopolysaccharide (LPS) activating an inflammatory cascade.(29) Figure 5

Multiple genetic factors, diet and lifestyle contribute progression from hepatic steatosis to inflammation and eventually fibrosis.

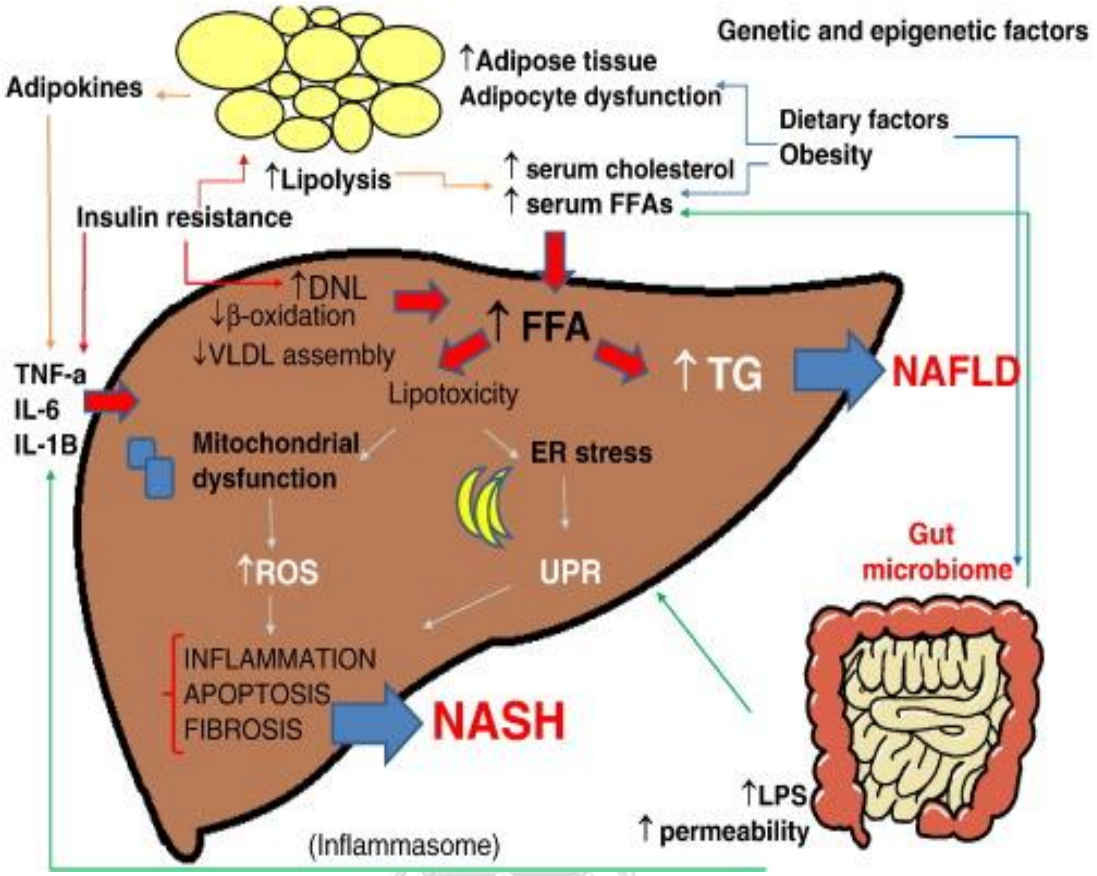


Figure 5 Downstream cascade of NAFLD pathogenesis

FFAs, free fatty acids; DNL, de novo lipogenesis; VLDL, very low density lipoproteins; CH, cholesterol; TNF-α, tumor necrosis factor alpha; IL-6, interleukin 6; TG, triglycerides; ROS, reactive oxygen species; ER, endoplasmic reticulum; UPR, unfolded protein response; LPS, lipopolysaccharide; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis.

Controlled attenuation parameter (CAP) measured by Fibroscan® (transient elastography) as a surrogate maker of hepatic steatosis in NAFLD/NASH

Gold standard diagnoses of NASH requires a liver biopsy. The procedure is also required for monitoring disease activity and evaluating effectiveness of therapies.(30)

The liver biopsy however, is an invasive procedure and not applicable for all NAFLD patients.(31) There are several non-invasive modalities aimed to detect steatosis and early fibrosis.

Fibroscan® (transient elastography) is a device which combines an ultrasonic transducer and a vibrator in one probe. It measures an elastic shear velocity in the soft tissue which is directly associated with tissue stiffness (Liver stiffness measurement, LSM).(32) This tool also evaluates fatty content in the liver referred as controlled attenuation parameter (CAP). This parameter is a representative marker of a hepatic steatosis.(33)

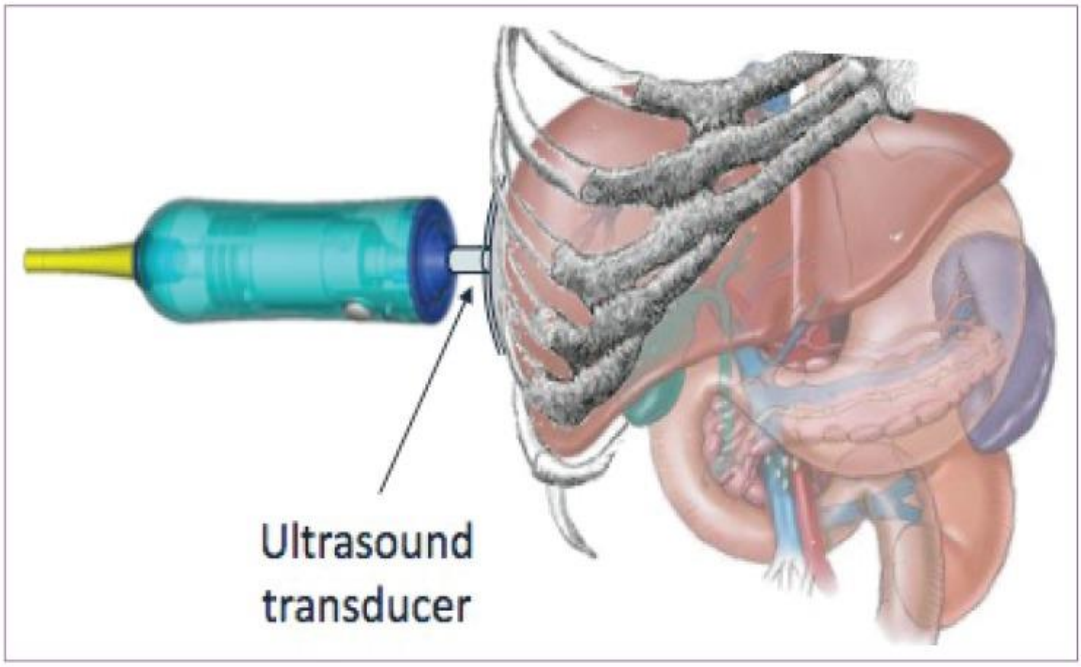


Figure 6 Fibroscan® device of hepatic steatosis and fibrosis

Fibroscan® is one of the most accurate non-invasive tests for diagnosis of steatosis and fibrosis in NAFLD patients.(18) Moreover, several studies have shown a strong correlation between Controlled Attenuation Parameter (CAP) and hepatic

steatosis confirmed by histopathology.(17) LSM is another parameter measured by Fibroscan® representing fibrotic stage with a strong correlation.(18, 34, 35)

Wu found that HBV infected patients treated with entecavir had significant correlation between LSM and liver inflammation defined by liver biopsy (comparing at pretreatment and 72-week after therapy).(36) It is believed that a hepatic steatosis in NAFLD have the same correlation. Therefore, Fibroscan® may be used as an effective tool for monitor treatment's response for NAFLD.

Other two non-invasive well-validated scoring systems: NAFLD fibrosis score and Fibrosis-4 (FIB-4) Index will be also used to predict fibrosis along with Fibroscan®.(13, 37) NAFLD fibrosis score has demonstrated a good diagnostic accuracy for identifying liver fibrosis compared with the gold standard of a liver biopsy (receiver operating characteristic curve of 0.88).(37) FIB-4 index is the other non-invasive evaluation which shows superiority among other non-invasive markers including NAFLD fibrosis score, AST:ALT ratio, AST to platelet ratio index and cirrhosis discriminant score. FIB-4 index with high cut-point of > 2.67 had an excellent negative predictive value (90%) for fibrosis. However, FIB-4 index had a poor performance to detect an early fibrosis with only 50% sensitivity.(38)

MRI can detect liver fat content using characteristic of fat saturation and chemical shifting. MRI has better correlation of microscopic liver fat content compared with conventional ultrasound. Nonetheless, hepatic MRI had a high range of variability (19-40%) influenced by difference of liver fat morphology (microscopic vs macroscopic).(39) The other disadvantage was a high cost of operation and limited availability.

Current standard treatments for NAFLD/NASH

Current guidelines focus on providing therapy to susceptible patients with steatohepatitis and early cirrhosis in order to prevent disease progression and mortality.(1, 8) Comprehensive lifestyle modification is accepted as a mainstream therapy associated with improved histology or even NASH remission. It includes calories restriction, macronutrient composition, exercise and physical activity. Table 2

Table 2 Guidance statements about lifestyle interventions

	EASL(8)	NICE(20)	ASIA-PACIFIC(21)	AISF(7)	AASLD(22)
Dietary restrictions	500-1000 kcal deficit; weight loss of 500-1000 g/week with a 7%-10% total weight loss	Main recommendations on diet of NICE's obesity and preventing excess weight gain guidelines	500-1000 kcal deficit	1200-1600 kcal/d; fat-low (< 30% of total calories); carbohydrate-low (< 50% of total calories)	500-1000 kcal deficit
Physical activity	Aerobic and resistance training (150-200 min/week in 3-5 sessions)	Main recommendation of on physical activity of NICE's obesity and preventing excess weight gain guidelines	Aerobic and resistance training	Aerobic and resistance training	Aerobic and resistance training (> 150 min/week)
Gold standard	Low-to-moderate fat	No specific suggestions	All, excluding very low-calorie	Mediterranean diet	No specific suggestions

	EASL(8)	NICE(20)	ASIA-PACIFIC(21)	AISF(7)	AASLD(22)
diet	and moderate-to-high carbohydrate intake		diets		
	Low-carbohydrate ketogenic diets or high-protein				
	Mediterranean diet				

Table 3 Guidance statements about pharmacological treatment of non-alcoholic fatty liver disease

	EASL(7)	NICE(20)	ASIA-PACIFIC (21)	AISF(7)	AASLD(22)
Metformin	Insufficient evidence	Not beneficial	Not beneficial	Not mentioned	Not beneficial
Vitamin E	Insufficient evidence	Consider use regardless of diabetes	Not beneficial	Insufficient evidence	Consider use in non-diabetic, biopsy-proven NASH
PPAR-gamma agonists	Consider use in selected diabetic patients	Consider pioglitazone in adults regardless of diabetes	Insufficient evidence in Asian	Insufficient evidence, potentially useful	Pioglitazone indicated in biopsy-proven NASH (regardless of diabetes)
PUFA	Not beneficial	Insufficient evidence	Not beneficial	Not mentioned	Not beneficial
Pentoxifylline	Insufficient evidence	Not mentioned	Not beneficial	Not mentioned	Not mentioned
GLP-1 analogues	Insufficient evidence, potentially useful	Insufficient evidence	Insufficient evidence in Asian patients	Insufficient evidence, potentially useful	Insufficient evidence
UDCA	Not	Not beneficial	Not mentioned	Not mentioned	Not beneficial

	EASL(7)	NICE(20)	ASIA-PACIFIC (21)	AISF(7)	AASLD(22)
	beneficial				
Obetycolic acid	Scarce evidence	Not mentioned	Waiting for ongoing RCT results	Waiting for ongoing RCT results	Insufficient evidence
Silymarin	Not mentioned	Not mentioned	Insufficient evidence, potentially useful	Not mentioned	Not mentioned
Statins	Safe but not beneficial	Safe but not beneficial	Safe but not beneficial	Safe but not beneficial	Safe but not beneficial

Treatment with some pharmacological products resulted in a histologic improvement with or without fibrosis regression. Belfort(40) found that pioglitazone improved hepatic steatosis but not fibrosis in type 2 diabetic patients.

Aithal(41) confirmed the result with an improvement of both steatosis and fibrosis in non-diabetic patients. In contrast, the PIVENS trial in non-diabetic patients failed to demonstrate an effectiveness of pioglitazone.(42) Armstrong(43) demonstrated significantly better steatotic and fibrotic outcomes in patients treated by liraglutide. Non-diabetic patients were the dominant group in this study. Other anti-diabetic drugs except pioglitazone and liraglutide do not have any evidence to support for treatment of NAFLD.

SGTL2 inhibitors' mechanism and potential for NAFLD therapy

Current established pathogenesis of NASH starts with a fat deposition within a liver, and then propagated by oxidative stress and lipid peroxidation. Finally, it ends up with a transition of simple steatosis to NASH.(44) SGLT2 inhibitors are new anti-diabetic agents reducing renal glucose reabsorption.(45) Consequently, more calories loss and more weight reduction occur. Moreover, SGLT2 inhibitor decreases an oxidative stress parameter such as malondialdehyde and increases a total hepatic anti-oxidative condition.(46) Several animal studies of SGLT2 inhibitors showed promising results in terms of steatotic and fibrotic improvement.(47)

Mice suffering from NASH and be treated with canagliflozin show multiple metabolic improvements in ameliorating hyperglycemia, hyperinsulinemia, and hepatic steatosis. Moreover, the drug also attenuated the development of hepatocellular carcinoma in a mouse model.(48) Figure 7 A human preliminary study (phase II) based on a biopsy proved that canagliflozin had an impact on reduction of NAFLD Activity Score (NAS) and fibrosis score.(49)

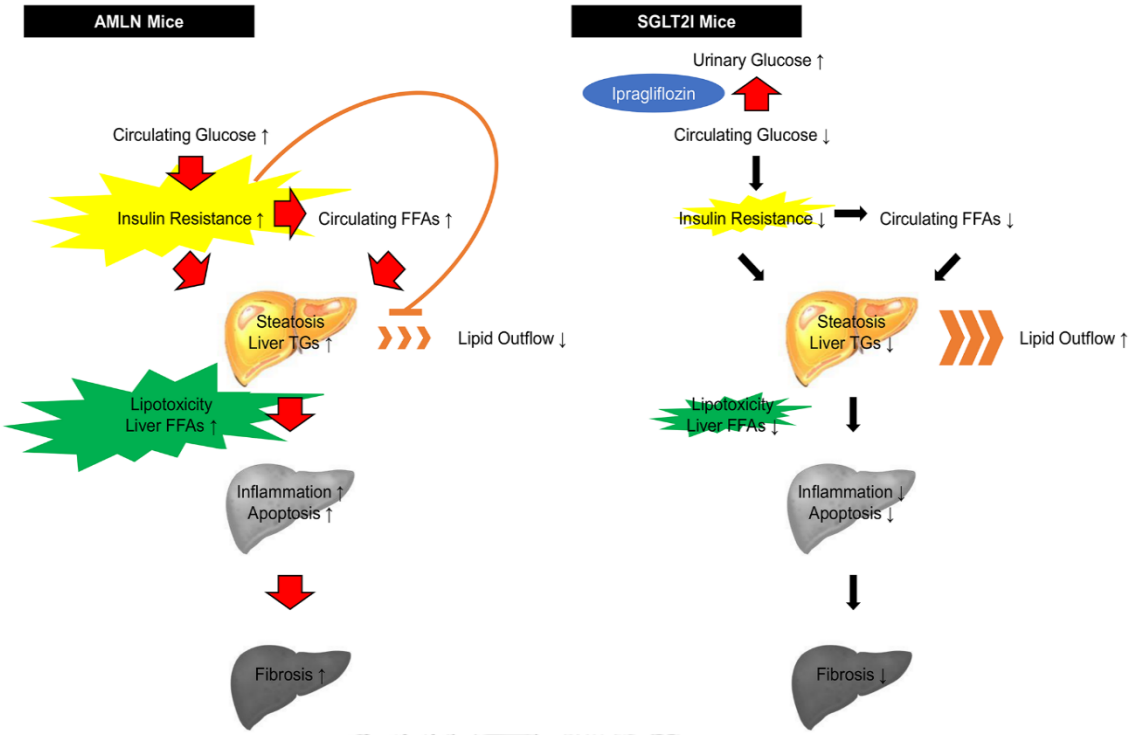


Figure 7 Mechanism of the effect of ipragliflozin on NASH in AMLN and SGLT2I mice

SGLT2 inhibitors have shown safety and efficacy in non-diabetic obese patients. Remogliflozin and sergliflozin show significant increased renal glucose excretion compared with placebo without any hypoglycemic effect.(50) Their efficacy is explained by the same mechanism as diabetic patients which is urine glucose loss and energy excretion.

There are several clinical trials of SGLT2i for NAFLD treatment including 3 studies using empagliflozin, 2 studies using canagliflozin, 2 studies using dapagliflozin, 2 studies using ipragliflozin and 1 early phase II study using licogliflozin. All studies were conducted in patients with type 2 diabetes mellitus.

Post hoc analysis from the cardiovascular outcome trial, EMPA-REG, showed a significant reduction of ALT after 28 - week treatment with empagliflozin (adjusted mean difference of -3.15 U/l). This study showed benefit independent of weight reduction. Patients with higher ALT gained more reduction of ALT with empagliflozin treatment.(51)

The E-lift study also showed a reduction in mean difference of -4.0% of hepatic liver content measured by a MRI proton density fat fraction (MRI-PDFF), again independent of weight loss.(11) A single arm open label study using empagliflozin with histologic outcome showed benefit of empagliflozin in the same line with the other two studies such as weight loss and BMI reduction. The patients receiving empagliflozin had significant improvements of steatotic grading, ballooning and fibrosis.(52)

A 24-week RCT of canagliflozin demonstrated a non-significant reduction of hepatic fat content measured by proton magnetic resonance spectroscopy. This study showed a strong correlation of weight reduction and improvement of hepatic fat content. (53) The other single arm open label study using canagliflozin for 12 weeks showed a significant improvement of ALT, FIB-4 index, FM fibro index, HbA1c and body weight. This study also showed a minimal reduction of CAP with mean difference of -6 dB/m.(54)

A combination of 10 mg dapagliflozin and 4 g Omega-3 (n-3) carboxylic acids significantly reduced liver fat content measured by the MRI proton density fat fraction (PDFF). Dapagliflozin alone decreased body weight and visceral fat volume significantly, however liver fat content reduced without statistical significance.(55) The other 8-week treatment with dapagliflozin demonstrated a significant reduction of PDFF by -3.74%. Surprisingly, this study found no effect of dapagliflozin on insulin sensitivity. On the contrary, several previous studies had strongly confirmed SGLT2is' effect on improvement of insulin sensitivity.(51, 53, 55)

บทที่ 3

วิธีดำเนินการวิจัย

รูปแบบการวิจัย (RESEARCH DESIGN)

Study type: Interventional (Clinical Trial)

Allocation: Randomized

Intervention model: Parallel Assignment

Primary purpose: Treatment

Masking: Double blinding

ระเบียบวิธีการวิจัย (RESEARCH METHODOLOGY)

ประชากร (POPULATION) และตัวอย่าง (SAMPLE)

ประชากร(Population) Obese non-diabetic patients with NAFLD

ประชากรเป้าหมาย (Target population) Obese non-diabetic patients with NAFLD aged 18 to 80 years

ประชากรตัวอย่าง (Sample population)

Obese non-diabetic patients with NAFLD aged 18 - 80 years who sought treatment at outpatient department at King Chulalongkorn Memorial Hospital

วิธีการเข้าถึงอาสาสมัคร (Approach to participant)

- Participants joined the program via direct contact with investigator at designated OPD or were referred by primary care providers to the primary investigator.
- Initial screening was performed by the primary investigator.
- Patients who were interested in the project were invited to have a blood test and were determined whether or not they met the inclusion/exclusion criteria.

กฎเกณฑ์ในการคัดเลือกเข้ามาศึกษา (Inclusion criteria)

- Age 18-80 years old
- BMI 25-35 kg/m2
- Evidence of hepatic steatosis by imaging (Ultrasound, CT or MRI)

กฎเกณฑ์ในการคัดเลือกออกจากการศึกษา (Exclusion criteria)

- Evidence of liver cirrhosis by ultrasound or Fibroscan® (LSM > 12 kPa)
- Estimated GFR < 45 ml/min/1.73m2
- Type2 diabetes mellitus
- SBP < 90 mmHg
- Male patients with alcohol consumption > 30 g/day and female patients with alcohol consumption > 20 g/day(8) using AUDIT, Skinner alcohol question(56)
- Chronic viral hepatitis B or evidence of previous infection, chronic viral hepatitis C, autoimmune liver disease, hemochromatosis, Wilson's disease and other liver diseases
- History of drug induced steatohepatitis within the previous 6 months: Aspirin, Amiodarone, Chemotherapy (5-fluorouracil, tamoxifen, irinotecan, cisplatin, and

asparaginase, irinotecan), Cocaine, Glucocorticoids, Methotrexate, Nucleoside reverse transcriptase inhibitors (NRTI), Tetracycline (Intravenous administration of high doses), Total parenteral nutrition, and Valproic acid(57)

- Weight gain or loss more than 5 kg in the last 6 months
- Current or previous use of following drugs within the past 6 months: SGLT2 inhibitor, pioglitazone, vitamin E, and liraglutide
- Patient underwent bariatric surgery within 5 years
- History of peripheral arterial disease or lower limb amputation
- History of recurrent urinary tract infection
- History of fungal infection in genital area
- Documented genitourinary tract structural abnormality
- Pregnant women or nursing mothers
- Patients did not provide a written informed consent.

<u>เทคนิคในการสุ่มตัวอย่าง (Sample techniques)</u>

Consecutive method was utilized to achieve the determined sample size.

<u>ขนาดตัวอย่าง (Sample size determination)</u>

<u>การคำนวณขนาดตัวอย่างกลุ่มผู้ป่วย</u>

Compare 2 Means: 2-Sample, 2-Sided Equality

Suppose the two groups were 'A' and 'B', and we collected a sample from both groups. We performed a two-sample test to determine whether the mean in group A, μA, was different from the mean in group B, μB. The hypotheses were

H0:μA-μB = 0

H1: μA-μB ≠ 0

where the ratio between the sample sizes of the two groups was **K** = nA/nB

Formulas: This calculator used the following formulas to compute sample size and power, respectively:

$$n_A = \kappa n_B \text{ and } n_B = \left(1 + \frac{1}{\kappa}\right)\left(\sigma \frac{z_{1-\alpha/2} + z_{1-\beta}}{\mu_A - \mu_B}\right)^2$$

$$1 - \beta = \Phi\left(z - z_{1-\alpha/2}\right) + \Phi\left(-z - z_{1-\alpha/2}\right) \quad , \quad z = \frac{\mu_A - \mu_B}{\sigma\sqrt{\frac{1}{n_A} + \frac{1}{n_B}}}$$

where

K = nA/nB was the matching ratio.

σ was standard deviation.

Φ was the standard normal distribution function.

Φ-1 was the standard normal quantile function.

α was Type I error.

β was Type II error, meaning 1-β was power.

Group A mean μA = 20 dB/m. We assumed a clinically significant CAP change of 20 dB/m from Karlas's study.(17) Change of CAP value more than 20 dB/m was an optimum cut-point for steatotic grading change e.g. from S1->S0, S2->S1.

Corresponding with previous study of Dapagliflozin, CAP value had changed 24.3 dB/m in treatment group.(12)

Group B mean μB = 0 dB/m. We assumed that there was no significant change of CAP resulted from comprehensive lifestyle according to the previous study in menopausal woman.(58)

Standard deviation, σ = 30.9 based on SD from previous study(59)

Sampling Ratio, κ = nA/nB

Sample size = 38

Power, 1-β 80%

Type I error rate, α 5%

Expected dropout rate was 10-15%. We aimed to recruit 44 patients in each study arm to have 44 patients per arm at the end of study (total of 88 patients).

การสังเกตและการวัด (OBSERVATION AND MEASUREMENT)

- Ultrasound, CT scan or MRI of liver to identify fatty liver was performed to include patients into study.
- CAP and LSM were measured at starting and ending of trial by using Fibroscan® M probe (EchoSens, Paris, France)
    - Fibroscan® (transient elastography) and Liver stiffness measurement
    - LSM was performed with a Fibroscan® M probe (EchoSens, Paris, France).

- Measurements were performed in the right lobe of the liver through the intercostal spaces, with the patients lying in the dorsal decubitus position with their right arm in a maximal abduction.
- The tip of the probe transducer covered with coupling gel was placed on the skin between the ribs at the level of the right lobe of the liver.
- The operator, assisted by ultrasound time-motion and A-mode images provided by the system, located a portion of the liver that was at least 6 cm thick and free of large vascular structures.
- Once the area of measurement had been located, the operator pressed the probe button to begin the acquisition.
- The measurement depth was between 25 and 45 mm.
- Ten successful acquisitions were performed on each patient. The success rate was calculated as the ratio of the number of successful acquisitions to that of the total number of acquisitions and a success rate of at least 60% or the interquartile range (IQR) <30% were considered reliable. The median value was determined as representative of the liver stiffness.

- Controlled Attenuation Parameter (CAP)
  - CAP measured ultrasonic attenuation in the liver at 3.5 MHz using signal acquired by Fibroscan® M probe.
  - The CAP was measured only on validated measurements according to the same criteria used for LSM and on the same signal.
  - The final CAP value which ranged between 100 to 400 decibels per meter (dB/m) was the median of individual measurement.

- Anthropometric data including body weight (kg), BMI (kg/m2), a wrist circumference (cm) were collected at the start, at 12 weeks and the end of study (24 weeks).
- Blood test were collected at the start and the end of study including:
    - Liver enzyme (AST, ALT, ALP)
    - Fasting plasma glucose
    - Lipid profile (Total cholesterol, LDL-c, HDL-c, Triglyceride)
- Possible reported ADRs during trial were monitored. (detail in case record form)

ขั้นตอนในการดำเนินการวิจัย

- Target population were informed about risks, benefits of trial and invited to join the program. Potential patients were sought at the outpatient clinic of medical department of KCMH.
- Participant's medical history was reviewed according to the inclusion and exclusion criteria.
- Patients with high positive predictive value of ultrasound positive NAFLD defined by HSI> 36. HSI was calculated by following formula.

Hepatic steatosis index (HSI) = 8 × (ALT/AST ratio) + BMI (+2, if female; +2, if diabetes mellitus)

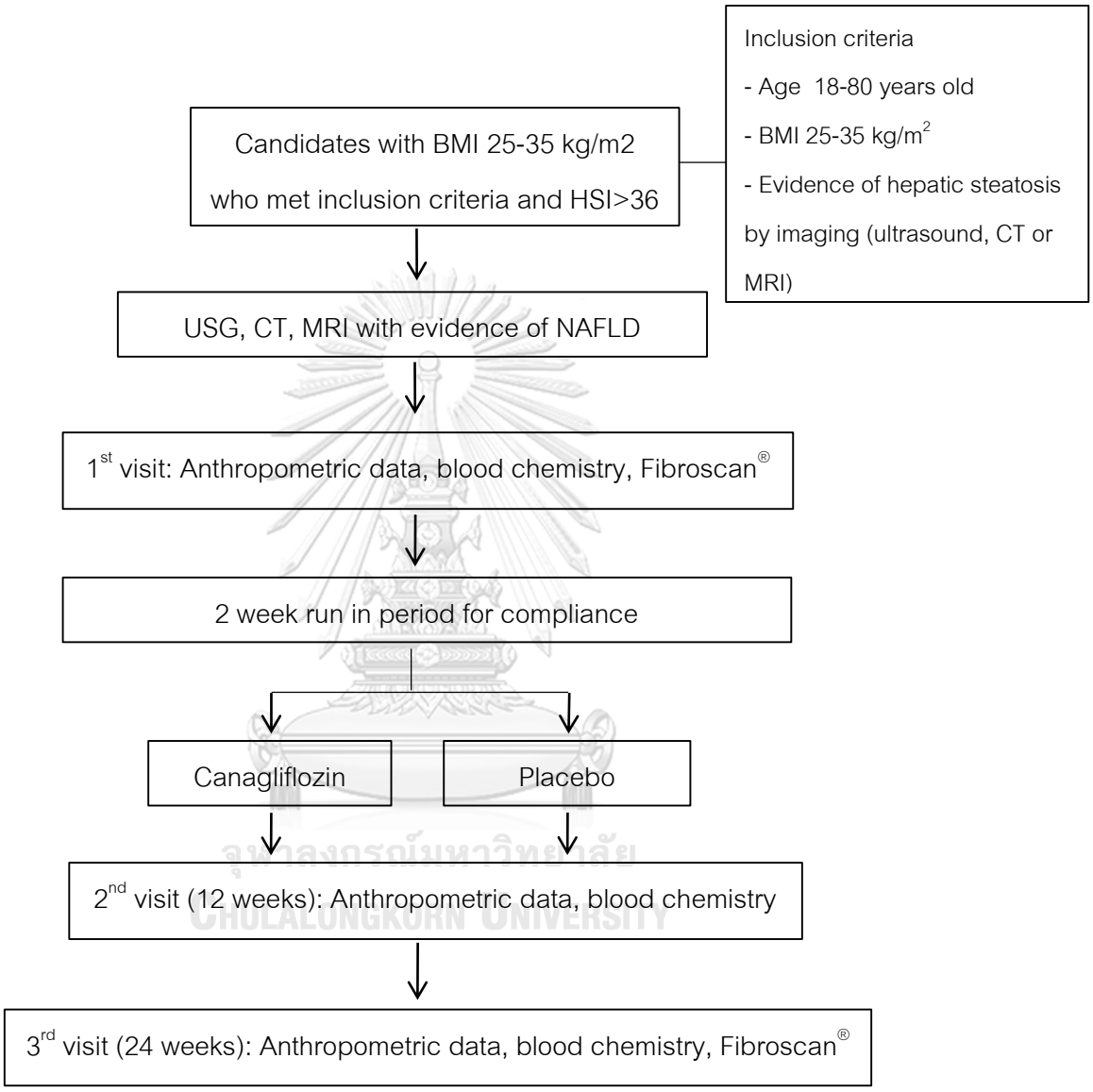
- Patients with hepatic steatosis index > 36 underwent imaging of liver to identify hepatic steatosis, by ultrasonography, CT or MRI unless they had imaging done within 3 months before recruitment.
- Patients' anthropometric data were measured and blood samples were collected for baseline evaluation. Baseline CAP and LSM were done at the beginning of the study.

- Patients were randomized into treatment or control group at 1:1 ratio. Randomization was stratified according to initial CAP value (248-280 dB/m vs > 280 dB/m), transaminitis or not and the initial fasting plasma glucose.
- Baseline data of the patients were sent to a third-party person via web link at clinical statistic research center. The center performed the randomization process and sent back the randomization number to the investigators via a web link. The investigators were blinded to randomization process throughout the study.
- Both groups were prescribed with 1 tab of placebo once daily for two weeks. (run in period) The patients were evaluated for possibility of long term adherence. Patients with poor compliance or unwillingness to continue medication were excluded from the study before randomization.
- Patients were prescribed with 1 tab of canagliflozin (100 mg) or placebo once daily for 24 weeks. Placebo tablets were manufactured by Faculty of Pharmaceutical Sciences, Chulalongkorn University. The placebo's film coated pills had the same external characteristic compared with the original drug. The original drugs were unpacked from original package and repacked with new package which were used for both studied drug and placebo. A pharmacist assigned for drug dispensation was also blinded. Only a few assigned pharmacists at Faculty of Pharmaceutical sciences, Chulalongkorn University and an assigned researcher at Endocrine and metabolism unit who was not involved in any study process were acknowledged of randomization group.
- Patients were followed up at a designated outpatient clinic every 12 weeks; the visit data visit were collected at each visit including anthropometric data and blood chemistry.
- Patients in both groups were encouraged to have lifestyle modification including:
    - Calories restriction: 500-1000 calories from daily intake
    - Caloric count and diet education for all patients were conducted by experienced clinical dietitians.

- Intake of saturated fat to < 7% of total calories, reducing trans-fat intake, and maintaining dietary cholesterol intake at < 200 mg/day and total fat at 25% to 35% of total calories
- Moderate intensity aerobic exercise at least 150 minutes per week. Moderate intensity was defined as target heart 50-70% of maximal heart rate during exercise. Maximal heart rate was calculated by 220 – age. In the other word, moderate intensity exercise was aerobic exercise which each patient could talk, but only a few words without a complete sentence.

- At 24 weeks, all patients received anthropometric data measurement and blood collection at the end of study evaluation. CAP and LSM were done at the end of study.
- We did not conduct any interim analysis during the study.
- All patients were advised to continue lifestyle modification after the end of study.

Figure 8 Study framework



* Hepatic steatosis index (HSI) = 8 × (ALT/AST ratio) + BMI (+2, if female; +2, if diabetes mellitus)

การรวบรวมข้อมูล (DATA COLLECTION)

Patients' data were collected from electronic medical records of KCMH. Anthropometric data were collected by trained medical personnel at outpatient department. Blood chemistry tests were analyzed at central laboratory of KCMH.

การวิเคราะห์ข้อมูล (DATA ANALYSIS)

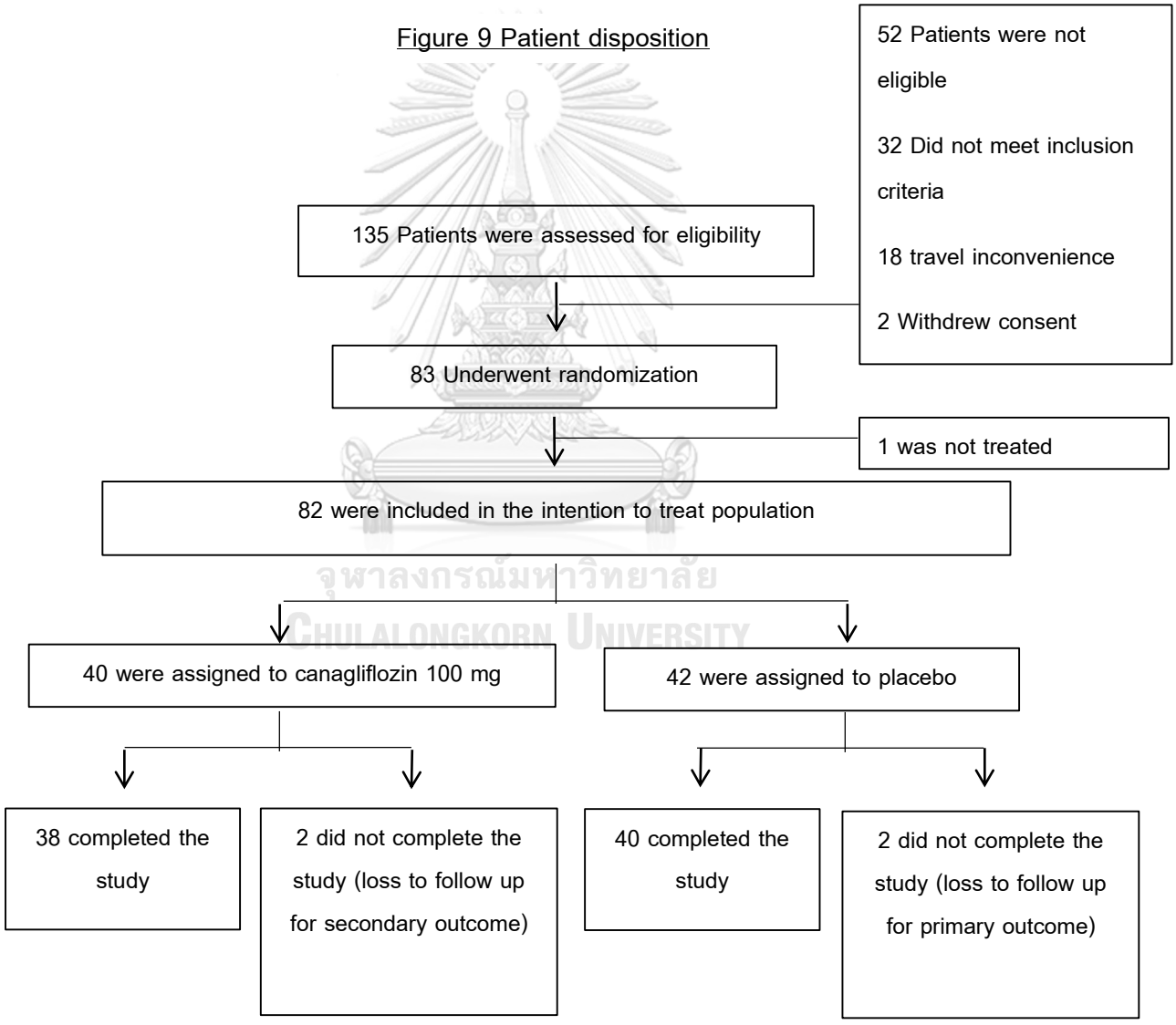
Statistical analyses followed the intention-to-treat paradigm. SPSS version 22, IBM® was used for statistical analysis. Analysis of covariance (ANCOVA) was used for comparison of mean CAP change. (primary outcome)

Other secondary outcomes which were continuous data were also compared by Student's T test. Association between changes in CAP and other clinical variables including body weight, BMI, steatotic grading were investigated using multiple logistic regression analysis (univariate and multivariate analysis).

บทที่ 4

ผลการวิเคราะห์ข้อมูล

Figure 9 Patient disposition



Baseline characteristics

The study was conducted during July 2019 to March 2020. 78 out of 82 patients completed the study. There was no dropout during the study. 4 patients lost to follow at the last visit for primary and secondary outcomes and were excluded from data analysis. Those patients denied any adverse event by telephone follow up call.

The mean age of patients was 54 years old in the canagliflozin group and 50 years old in the control group with female gender predominant. The mean BMI was 30 kg/m2 in the canagliflozin group and 29 kg/m2 in the control group. The patients' mean blood pressure was in normal range in both groups. Most patients had normal fasting plasma glucose. 20 patients (25%) had impaired plasma glucose. (9 patients in canagliflozin group and 11 patients in control group) The patients' lipid profiles were in low risk range for people without established cardiovascular diseases. Most patients had normal liver function test. 15 patients (19%) had hepatitis with elevated SPGT more than 40 u/l. The mean initial glomerular filtration rate (GFR) calculated by CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) equation was 93 ml/min/1.73m2 in the canagliflozin group and 96 ml/min/1.73m2 in the control group.

The pre-treatment Controlled Attenuation Parameter (CAP) was in range of severe steatosis (304 db/m in canagliflozin group and 295 db/m in control group). Most patients had mild fibrosis with transient elastography (TE) of 5.4 kPa in the canagliflozin group and 5.6 kPa in the control group. Corresponding with Fibrosis-4 (FIB-4) Index of 0.86 in the canagliflozin group and 0.76 in the control group, most patients had high negative predictive value for advanced fibrosis. There was no significant difference in any parameter of baseline characteristics between the two groups as shown in **Table 4**

Table 4 Baseline characteristics

	Canagliflozin and lifestyle modification mean (SD) N = 38	Placebo with lifestyle modification mean (SD) N = 40	P- value*
Age (years)	53.97 (11.41)	49.98 (13.83)	0.169
Gender: male (%)	18 (47.37%)	15 (37.5%)	0.492
Weight (kg)	78.06 (13.02)	78.02 (12.89)	0.989
BMI (kg/m2)	30.01 (3.49)	29.60 (3.30)	0.415
SBP (mmHg)	134.13 (19.38)	132.28 (17.73)	0.660
DBP (mmHg)	79.79 (9.82)	81.80 (11.79)	0.417
PR (/min)	74.37 (12.40)	73.85 (10.76)	0.518
Fasting plasma glucose (mg/dl)	93.92 (9.87)	95.25 (10.88)	0.574
Total cholesterol (mg/dl)	209.66 (41.77)	205.70 (30.76)	0.634
HDL-c (mg/dl)	50.76 (10.78)	49.60 (12.35)	0.660
Triglyceride (mg/dl)	154.87 (77.60)	139.13 (58.65)	0.314
LDL-c (mg/dl)	127.42 (33.80)	127.30 (31.15)	0.987
SGOT (AST) (u/l)	23.13 (9.32)	25.43 (15.78)	0.440
SGPT (ALT) (u/l)	32.76 (22.49)	38.25 (37.48)	0.438
GFR (ml/min/1.73m2)	93.27 (17.40)	96.62 (20.43)	0.634
CAP (dB/m)	304.53 (47.63)	295.10 (32.82)	0.310
TE (kPa)	5.45 (1.15)	5.62 (2.58)	0.710
Fibrosis-4 (FIB-4) Index	0.91 (0.45)	0.85 (0.43)	0.305

* P values for continuous variables were calculated by Student's t-test, and P values for categorical variables were calculated by the chi-square test.

Primary outcome and secondary outcomes

Primary outcome at 24 weeks demonstrated a trend of hepatic steatosis improvement with a reduction in CAP of 13.8 dB/m in the canagliflozin group compared with 0.6 dB/m in the control group (P = 0.168). For secondary outcomes, there were no significant differences between the two groups in terms of hepatic fibrosis (TE and FIB-4 index). This study highlighted the effect of canagliflozin on weight loss with 1.97 kg in the canagliflozin group compared with 0.14 kg in the control group as shown in Figure 10. Change in other parameters were shown in Table 5.

At week 12, there was a significant reduction only in body weight in the canagliflozin group compared with the control group (-1.85 kg vs -0.09 kg, P < 0.001). Table 6

Figure 10 Weight change (kg) over 24 weeks

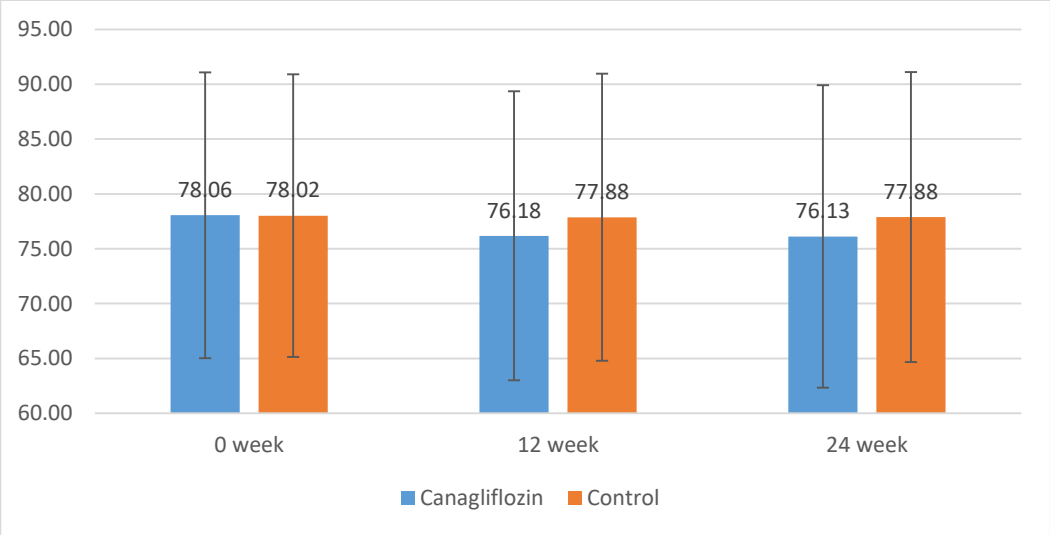


Table 5 Change of outcomes at 24 weeks

Change at 24 weeks compared with baseline	Canagliflozin with lifestyle modification mean (SD) N = 38	Placebo with lifestyle modification mean (SD) N = 40	Mean difference 95% CI	P- value*
Primary outcome CAP change (db/m)	-13.8 (40.6)	-0.6 (42.9)	-24.81 to 17.34	0.168
Secondary outcomes TE change (kPa)	-0.4 (0.9)	0 (1.1)	-1.38 to 0.31	0.114
Weight change (kg)	-1.97 (2.14)	-0.14 (2.57)	-2.87 to -0.73	0.001***
SBP change (mmHg)	-10 (18)	-3 (18)	-15.06 to 0.90	0.081
DBP change (mmHg)	-7 (10)	-8 (13)	-3.78 to 6.92	0.560
PR change (/min)	0 (14)	1 (11)	-6.30 to 4.74	0.780
Fasting plasma glucose change (mg/dl)	0.5 (9.2)	1.3 (8.0)	-4.78 to 3.02	0.656
Total cholesterol change (mg/dl)	-10.6 (32.5)	1.68 (35.3)	-27.55 to 3.10	0.116
HDL-c change (mg/dl)	0.6 (7.0)	0.9 (5.6)	-3.13 to 2.60	0.852
Triglyceride change (mg/dl)	-19.4 (67.6)	1.2 (38.2)	-45.24 to 4.00	0.099
LDL-c change (mg/dl)	-5.9 (33.2)	-0.6 (32.7)	-22.26 to 7.47	0.325
SGOT change (AST) (u/l)	-0.6 (6.8)	1.0 (14.5)	-6.80 to 3.55	0.533
SGPT change (ALT) (u/l)	-5.0 (15.0)	-2.2 (20.0)	-10.83 to 5.18	0.485
GFR change (ml/min/1.73m2)	-2.10 (7.16)	-3.45 (6.92)	-1.84 to 4.52	0.403

Fibrosis-4 (FIB-4) Index change	0.05 (0.19)	0.08 (0.30)	-0.14 to 0.08	0.585

* P values for continuous variables were calculated by Student's t-test or the Wilcoxon rank-sum test.

Table 6 Change of outcomes at 12 weeks

Change at 12 weeks compared with baseline	Canagliflozin with lifestyle modification mean (SD) N = 38	Placebo with lifestyle modification mean (SD) N =40	Mean difference 95% CI	P- value*
Weight change (kg)	-1.85 (1.77)	-0.09 (1.32)	-2.46 to -1.04	<0.001***
SBP change (mmHg)	-8 (16)	-2 (18)	-14.18 to 1.34	0.103
DBP change (mmHg)	-4 (8)	-3 (11)	-5.20 to 3.57	0.713
PR change (/min)	-1 (13)	0 (8)	-6.58 to 3.80	0.595
Fasting plasma glucose change (mg/dl)	-0.4 (7.2)	1.1 (8.4)	-5.01 to 2.07	0.410
Total cholesterol change (mg/dl)	-4.11 (36.58)	9.80 (23.87)	-27.76 to -0.05	0.490
HDL-c change (mg/dl)	0.5 (5.2)	0.7 (6.5)	-2.90 to 2.40	0.850
Triglyceride change (mg/dl)	-8.4 (77.8)	5.3 (39.1)	-41.20 to 13.91	0.327
LDL-c change (mg/dl)	-2.9 (30.4)	9.1 (24.6)	-24.44 to 0.45	0.059
SGOT change (AST) (u/l)	1.8 (5.6)	0.2 (11.2)	-2.45 to 5.63	0.436
SGPT change (ALT) (u/l)	-1.6 (8.6)	-3.4 (20.3)	-5.25 to 8.95	0.606
GFR change	-1.44 (6.02)	-1.66 (7.29)	-2.81 to 3.24	0.886

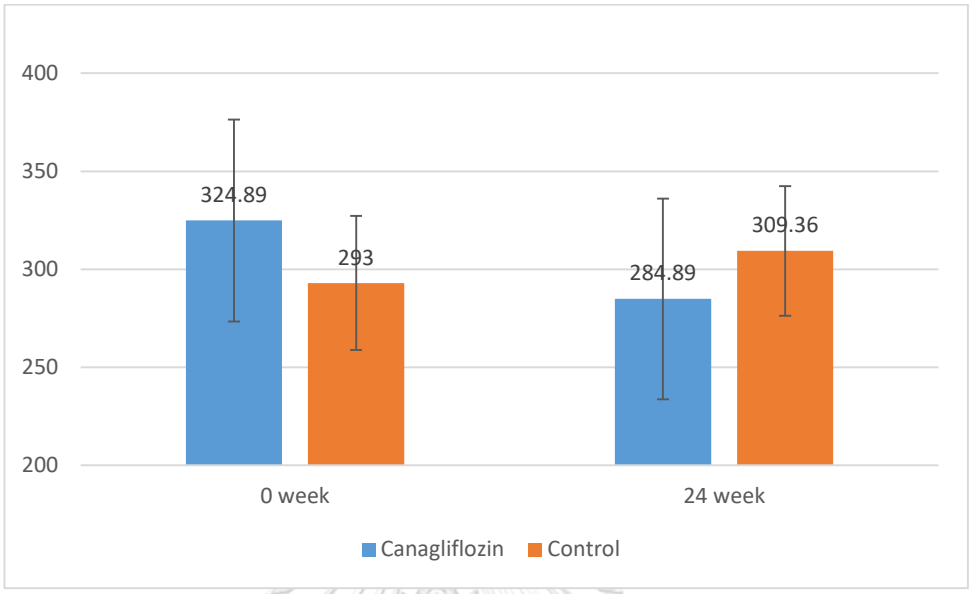
(ml/min/1.73m2)				

* P values for continuous variables were calculated by Student's t-test or the Wilcoxon rank-sum test.

Prespecified subgroup analysis

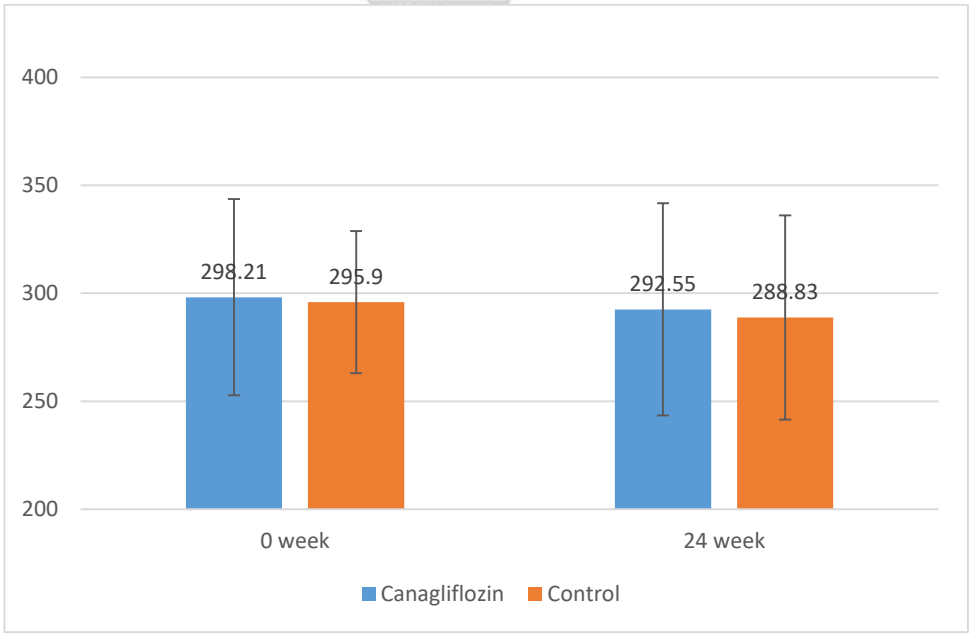
Patients with normal fasting plasma glucose had less CAP change than patients with impaired fasting plasma glucose. Patients with impaired fasting plasma glucose demonstrated a statistically significant reduction of CAP by comparison with normal fasting plasma glucose (-40.00 +/- 30.70 vs 16.36 +/- 42.60, P = 0.004) as shown in Figure 11. Patients with hepatitis also demonstrated a statistically significant reduction of CAP compared with no hepatitis (-34.75 +/- 40.84 vs 14.86 +/- 38.36, P = 0.031) as shown in Figure 14. Other prespecified subgroup analysis did not show any significant difference of CAP change as shown in Table 7.

Figure 11 CAP change (db/m) over 24 weeks in patients with impaired fasting plasma glucose



P = 0.004 at 24 weeks*

Figure 12 CAP change (db/m) over 24 weeks in patients with normal fasting plasma glucose



P = 0.896 at 24 weeks

Table 7 Prespecified subgroup analysis for primary outcome (CAP)

Change of CAP at 24 weeks compared with baseline (db/m)	Canagliflozin with lifestyle modification mean (SD)	Placebo with lifestyle modification mean (SD)	Mean difference 95% CI P value*	P- value for interaction **
Normal fasting plasma glucose (N=58)	-5.66 (40.30) (N = 29)	-7.07 (41.87) (N = 29)	-20.20 to 23.03 0.896	0.007***
Impaired fasting plasma glucose (N=20)	-40.00 (30.70) (N = 9)	16.36 (42.60) (N = 11)	-92.04 to -20.69 0.004***	
Lower BMI (25-30 kg/m2) (N = 44)	-15.90 (38.31) (N = 21)	-8.60 (35.18) (N = 23)	-29.65 to 15.06 0.514	0.464
Higher BMI (30-35 kg/m2) (N= 34)	-11.18 (44.40) (N = 17)	10.18 (50.59) (N = 17)	-54.61 to 11.90 0.200	
No hepatitis (SGPT</=40 u/l) (N = 63)	-8.20 (39.38) (N = 30)	-3.91 (43.58) (N = 33)	-25.29 to 16.71 0.684	0.061
Hepatitis (SGPT>40 u/l) (N=15)	-34.75 (40.84) (N = 8)	14.86 (38.36) (N = 7)	-94.02 to -5.20 0.031***	
CAP <248 db/m (N = 8)	0.50 (0.47) (N = 4)	34.75 (60.37) (N = 4)	-115.78 to 47.28 0.344	0.356
CAP 249-268 db/m (N=13)	-13.29 (31.22) (N = 7)	19.17 (41.25) (N= 6)	-76.69 to 11.78 0.135	
CAP 269-290 db/m (N=9)	14.25 (22.88) (N = 4)	-12.00 (16.96) (N = 5)	-5.02 to 57.52 0.088	
CAP > 290 db/m (N=48)	-21.30 (45.75) (N = 23)	-8.76 (41.52) (N = 25)	-37.90 to 12.81 0.324	

* P values for continuous variables were calculated by Student's t-test or the Wilcoxon rank-sum test.

** P value for interaction were assessed by multivariate regression model.

Figure 13 CAP change of subgroup analysis

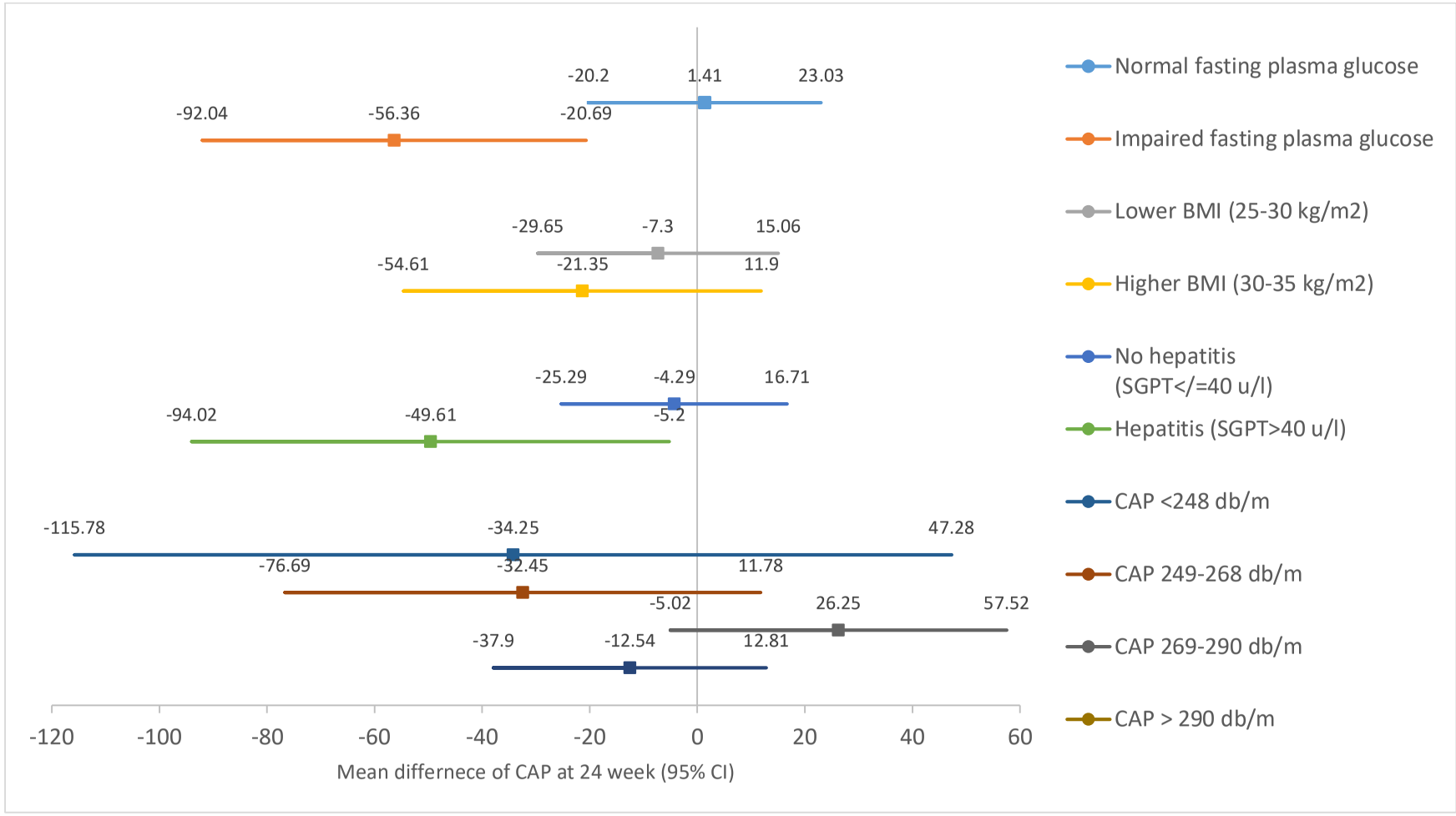
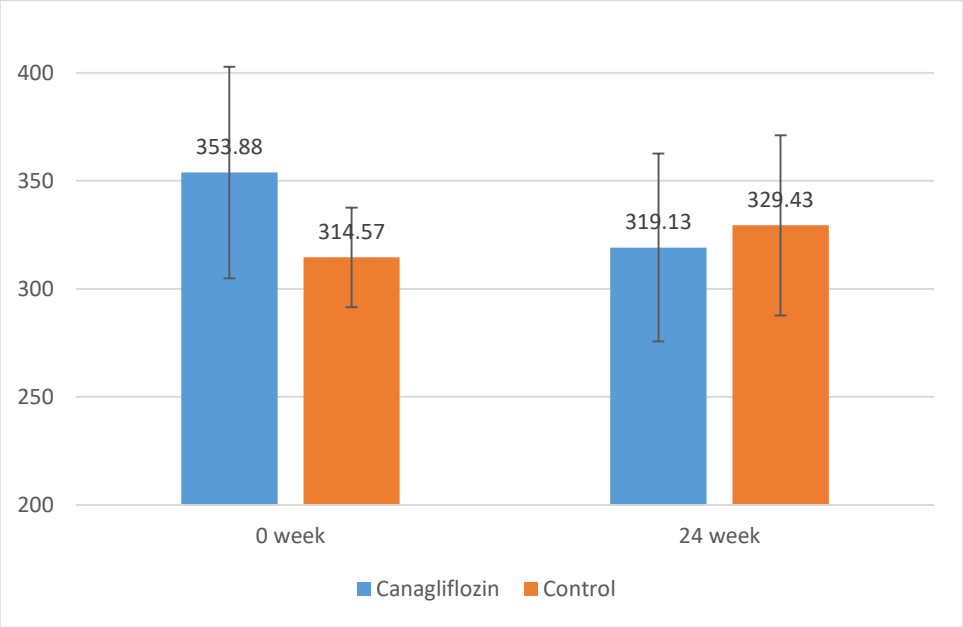
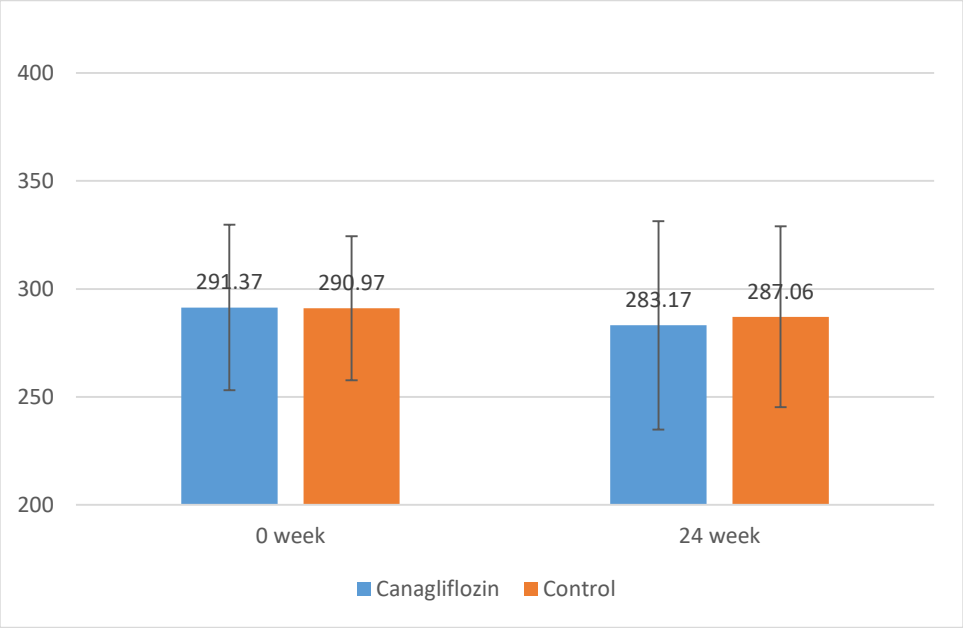


Figure 14 CAP change (db/m) over 24 weeks in patients with hepatitis



P = 0.031 at 24 week*

Figure 15 CAP change (db/m) over 24 weeks in patients without hepatitis



P = 0.684 at 24 week

Weight change showed a significant reduction in almost all subgroups except for patients with hepatitis, initial CAP < 248 db/m or CAP between 269 – 290 db/m as shown in Table 8.

We also analyzed an effect of weight loss on CAP change. CAP change in patients with weight loss were -18.81 +/- 41.45 db/m in the canagliflozin group vs -15.85 +/- 40.30 db/m in the control group (P 0.801). CAP change in patients without weight loss were 13.00 +/- 23.39 db/m in the canagliflozin group vs 14.6 +/- 40.71 db/m in the control group (P 0.928).

Table 8 Prespecified subgroup analysis for weight change

Change of body weight at 24 weeks compared with baseline (kg)	Canagliflozin with lifestyle modification mean (SD)	Placebo with lifestyle modification mean (SD)	P- value*
Normal fasting plasma glucose (N=58)	-1.5 (1.78)	0.00 (2.76)	0.015***
Impaired fasting plasma glucose (N=20)	-3.28 (2.74)	-0.52 (2.02)	0.018***
Lower BMI (25-30 kg/m2) (N = 44)	-1.95 (2.33)	-0.22 (2.34)	0.019***
Higher BMI (30-35 kg/m2) (N= 34)	-1.92 (1.95)	-0.03 (2.92)	0.033***
No hepatitis (SGPT<40) (N = 63)	-1.92 (2.31)	-0.17 (2.55)	0.006***
Hepatitis (SGPT>40) (N=15)	-2.00 (1.48)	0.01 (2.84)	0.102
CAP <248 db/m (N = 8)	-3.00 (3.28)	0.35 (1.73)	0.121
CAP 249-268 db/m (N=13)	-1.83 (2.25)	1.07 (2.25)	0.041***

CAP 269-290 db/m (N=9)	-1.60 (1.68)	-0.22 (1.74)	0.270
CAP > 290 db/m (N=48)	-1.84 (2.06)	-0.49 (2.87)	0.069

* P values for continuous variables were calculated by Student's t-test or the Wilcoxon rank-sum test.

Adverse events and management

There were five reported adverse events including one female case of a genitourinary tract fungal infection in the canagliflozin group, one female case of an acute urticarial rash in the canagliflozin group and three cases (2 males and 1 female) of dizziness in the control group as shown in Table 9.

The first patient was treated with topical antifungal agent and encouraged to practice better perineal hygienic care. She did not withhold study medication during fungal infection treatment.

The second patient developed erythematous rash on chest, back and extremities without mucosal or respiratory system involvement. She was suspended from the study drug for one week and treated with oral antihistamine and topical steroid. She restarted the study medication without any subsequent adverse events.

Three patients with dizziness had spontaneous recovery after increasing daily oral fluid intake more than 1.5 liters.

2 patients in the canagliflozin group had attained the final visit for Fibroscan® (primary outcome) and anthropometric measurement, however, they were absent for final follow up for blood test (secondary outcomes). On the contrary, 2 patients in the control group had the blood test done, but not Fibroscan®. These 4 patients had incomplete data and were excluded from the study data analysis.

Table 9 Adverse events

Events	Canagliflozin with lifestyle modification N = 38 (%)	Placebo with lifestyle modification N = 40 (%)
Dizziness or lightheadedness	0	3 (7.5%)
Urinary tract infections	0	0
Allergic reaction including rash or hives	1 (2.63%)	0
Constipation	0	0
Vaginal yeast infections and vaginal itching - symptoms	1 (2.63%)	0
Yeast infection at the head of the penis	0	0
Bone fractures	0	0
Amputation of the toes	0	0

บทที่ 5

อภิปรายผลการวิจัย

This is the first study using a SGLT2 inhibitor for treatment of NAFLD in obese non-diabetic patients. This study demonstrated a trend of hepatic steatosis improvement in patients receiving canagliflozin compared with placebo. Our result is consistent with the previous studies using SGLT2 inhibitors in diabetic patients with NAFLD.

Kuchay's study(11) using empagliflozin 10 mg per day for 24 weeks showed a significant reduction of liver fat measured by MRI-PDFF compared with placebo. This work had different patients' characteristics from our study. All patients were diagnosed as type 2 diabetes with average duration of 6 years and mean higher baseline ALT of 65 U/l vs 32 U/l.

Type 2 diabetic patients with NAFLD treated with dapagliflozin 5 mg per day for 24 weeks in Shimizu's study(12) showed significant CAP reduction of 24 db/m. This was in a good agreement with our subgroup analysis of patients with impaired fasting plasma glucose which demonstrated significant CAP reduction of 34 db/m.

Canagliflozin 300 mg for 24 weeks had exhibited an efficacy in decreasing intrahepatic triglyceride accumulation measured by a proton magnetic resonance spectroscopy in Cusi's work.(53) This study further highlighted an effect of canagliflozin on improvement of hepatic insulin sensitivity in patients with NAFLD and diabetes. In contrast, a 8 week study of dapagliflozin did not change tissue insulin sensitivity measured using a [18F]-fluorodeoxyglucose and positron emission tomography during hyperinsulinemic-euglycemic clamp in diabetic patients.(60)

In diabetic patients with NAFLD, SGLT2is induce glucosuria and energy loss leading to weight loss and intrahepatic triglyceride reduction from beta oxidation stimulation in both adipose tissue and liver.(61) Due to improvement of insulin sensitivity, less adipose tissue produces free fatty acid into bloodstream, less hepatic fat delivery and accumulation.(62) This mechanism via improvement of insulin sensitivity is the most well-known rationale in using SGLT2is for NAFLD treatment. Apart from weight loss, SGLT2is promote intrahepatic fatty acid oxidation and ketogenesis leading to reduction of hepatic de novo lipogenesis.(52) This could be an alternative mechanism which reverses NAFLD pathogenesis.

There are other clinical trials using antidiabetic medications which act on insulin sensitivity in non-diabetic patients including pioglitazone, rosiglitazone and metformin.(63) Aithal et al(41) reported on a histological improvement in 12-month treatment of pioglitazone in non-diabetic patients. The patients in the treatment arm had significantly decreased C-peptide, which correlated favorably well with improving insulin sensitivity. Garinis et al(64) confirmed support of insulin sensitivity theory by a significant decrease of homeostasis model assessment of insulin resistance (HOMA-IR) in metformin treated non-diabetic patients with NAFLD.

The significant reduction of hepatic steatosis in subgroup analysis of patients with impaired fasting plasma glucose has further strengthened the important role of insulin resistance. This may be a plausible explanation for non-significant hepatic fat reduction in our study. Most patients in our study were euglycemia with only a few with impaired fasting plasma glucose. This result further supports the proposed mechanism of NAFLD in non-diabetic patients sharing the same pathogenesis but with earlier onset or less severity.(65)

The patients in canagliflozin group had a significant reduction of body weight (2.5%). This is consistent with previous studies(53), Canagliflozin resulted in weight loss

more than 5% at 24 weeks corresponding with meta-analysis of SGLT2i s positive effects on BMI change.(66) SGLT2 inhibitors had shown efficacy in non-diabetic patients with significantly increased renal glucose excretion compared with placebo without any hypoglycemic effect.(50) Nevertheless, effect of glucosuria and energy loss were less in non-diabetic patients and less magnitude of weight loss could be expected. Inconsistent effect of weight loss among patients with or without hepatitis and patients with different CAP value could be due to a small number of patients in each subgroup analysis.

Prespecified subgroup analysis found a significant reduction of CAP only in patients with hepatitis. This concurs well with prior studies with pioglitazone, vitamin E and also confirmed previous findings in the FLIRT study.(42, 67) SGLT2is have shown efficacy in increasing adiponectin and improvement of insulin resistance which significantly correlated with the loss of liver steatosis. A higher pretreatment level of liver enzyme was a strong predictor of good responder.(68)

There are a number of limitations in our study. Firstly, we did not measure any parameter regarding insulin sensitivity other than fasting plasma glucose. Secondly, we did not perform liver biopsy which is considered as a gold standard for diagnosis. Lastly, 24 weeks might be too short to evaluate hepatic fibrotic changes.

To the best of our knowledge, our study was the first to investigate the effect of canagliflozin on NAFLD in obese non-diabetic patients. Another ongoing study with Licogliflozin(69) shared the same targeted population with our study. The main weakness in their study is that they measure ALT change at 12 weeks as a primary outcome which is not a good clinical surrogate endpoint.

In conclusion, we have provided an evidence that canagliflozin may have beneficial effects on hepatic steatosis in patients with NAFLD and impaired fasting plasma glucose. Further studies with a longer duration of treatment and exclusive

selection of patients with impaired fasting plasma glucose or hepatitis may be arrange to confirm our findings.

REFERENCES

1. Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, et al. The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Gastroenterological Association, American Association for the Study of Liver Diseases, and American College of Gastroenterology. Gastroenterology. 2012;142(7):1592-609.
2. Global epidemiology of nonalcoholic fatty liver disease—Meta-analytic assessment of prevalence, incidence, and outcomes - Younossi - 2016 - Hepatology - Wiley Online Library.
3. Younossi Z, Anstee QM, Marietti M, Hardy T, Henry L, Eslam M, et al. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. Nature Reviews Gastroenterology & Hepatology. 2018;15(1):11-20.
4. Ekstedt M, Franzén LE, Mathiesen UL, Thorelius L, Holmqvist M, Bodemar G, et al. Long-term follow-up of patients with NAFLD and elevated liver enzymes. Hepatology. 2006;44(4):865-73.
5. Younossi ZM, Blissett D, Blissett R, Henry L, Stepanova M, Younossi Y, et al. The economic and clinical burden of nonalcoholic fatty liver disease in the United States and Europe. Hepatology. 2016;64(5):1577-86.
6. Hu M, Phan F, Bourron O, Ferré P, Foufelle F. Steatosis and NASH in type 2 diabetes. Biochimie. 2017;143:37-41.
7. AISF position paper on nonalcoholic fatty liver disease (NAFLD): Updates and future directions | Elsevier Enhanced Reader.
8. Liver otEAftS, Diabetes oEAftS, Obesity oEAftS. EASL-EASD-EASO Clinical Practice Guidelines for the Management of Non-Alcoholic Fatty Liver Disease. OFA. 2016;9(2):65-90.
9. Hayashizaki-Someya Y, Kurosaki E, Takasu T, Mitori H, Yamazaki S, Koide K, et al. Ipragliflozin, an SGLT2 inhibitor, exhibits a prophylactic effect on hepatic steatosis and fibrosis induced by choline-deficient l-amino acid-defined diet in rats. European Journal of Pharmacology. 2015;754:19-24.
10. Yokono M, Takasu T, Hayashizaki Y, Mitsuoka K, Kihara R, Muramatsu Y, et al. SGLT2 selective inhibitor ipragliflozin reduces body fat mass by increasing fatty acid oxidation in high-fat diet-induced obese rats. European Journal of Pharmacology. 2014;727:66-74.
11. Kuchay MS, Krishan S, Mishra SK, Farooqui KJ, Singh MK, Wasir JS, et al. Effect of Empagliflozin on Liver Fat in Patients With Type 2 Diabetes and Nonalcoholic Fatty Liver Disease: A Randomized Controlled Trial (E-LIFT Trial). Diabetes Care. 2018;41(8):1801-8.
12. Shimizu M, Suzuki K, Kato K, Jojima T, Iijima T, Murohisa T, et al. Evaluation of the effects of dapagliflozin, a sodium-glucose co-transporter-2 inhibitor, on hepatic steatosis and fibrosis using transient elastography in patients with type 2 diabetes and non-alcoholic fatty liver disease. Diabetes Obes Metab. 2018.
13. Sterling RK, Lissen E, Clumeck N, Sola R, Correa MC, Montaner J, et al. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. Hepatology. 2006;43(6):1317-25.
14. Association AD. 2. Classification and Diagnosis of Diabetes. Diabetes Care. 2015;38(Supplement 1):S8-S16.
15. The Asia-Pacific perspective : redefining obesity and its treatment.

16. de Lédinghen V, Vergniol J, Capdepont M, Chermak F, Hiriart J-B, Cassinotto C, et al. Controlled attenuation parameter (CAP) for the diagnosis of steatosis: a prospective study of 5323 examinations. Journal of Hepatology. 2014;60(5):1026-31.
17. Karlas T, Petroff D, Sasso M, Fan J-G, Mi Y-Q, de Lédinghen V, et al. Individual patient data meta-analysis of controlled attenuation parameter (CAP) technology for assessing steatosis. Journal of Hepatology. 2017;66(5):1022-30.
18. Boursier J, Vergniol J, Guillet A, Hiriart J-B, Lannes A, Le Bail B, et al. Diagnostic accuracy and prognostic significance of blood fibrosis tests and liver stiffness measurement by FibroScan in non-alcoholic fatty liver disease. Journal of Hepatology. 2016;65(3):570-8.
19. Neal B, Perkovic V, Mahaffey KW, de Zeeuw D, Fulcher G, Erondu N, et al. Canagliflozin and Cardiovascular and Renal Events in Type 2 Diabetes. N Engl J Med. 2017;377(7):644-57.
20. nonalcoholic-fatty-liver-disease-nafld-assessment-and-management-pdf-1837461227461.pdf.
21. Wong VW-S, Chan W-K, Chitturi S, Chawla Y, Dan YY, Duseja A, et al. Asia–Pacific Working Party on Non-alcoholic Fatty Liver Disease guidelines 2017—Part 1: Definition, risk factors and assessment. Journal of Gastroenterology and Hepatology. 2018;33(1):70-85.
22. The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases - Chalasani - 2018 - Hepatology - Wiley Online Library.
23. Alkhouri N, Gornicka A, Berk MP, Thapaliya S, Dixon LJ, Kashyap S, et al. Adipocyte Apoptosis, a Link between Obesity, Insulin Resistance, and Hepatic Steatosis. J Biol Chem. 2010;285(5):3428-38.
24. Endo M, Masaki T, Seike M, Yoshimatsu H. TNF-α Induces Hepatic Steatosis in Mice by Enhancing Gene Expression of Sterol Regulatory Element Binding Protein-1c (SREBP-1c). Exp Biol Med (Maywood). 2007;232(5):614-21.
25. Samuel VT, Liu Z-X, Qu X, Elder BD, Bilz S, Befroy D, et al. Mechanism of Hepatic Insulin Resistance in Non-alcoholic Fatty Liver Disease. J Biol Chem. 2004;279(31):32345-53.
26. Brown MS, Goldstein JL. Selective versus Total Insulin Resistance: A Pathogenic Paradox. Cell Metabolism. 2008;7(2):95-6.
27. Postic C, Girard J. Contribution of de novo fatty acid synthesis to hepatic steatosis and insulin resistance: lessons from genetically engineered mice. J Clin Invest. 2008;118(3):829-38.
28. Anstee QM, Daly AK, Day CP. Genetics of Alcoholic and Nonalcoholic Fatty Liver Disease. Semin Liver Dis. 2011;31(02):128-46.
29. Rivera CA, Adegboyega P, van Rooijen N, Tagalicud A, Allman M, Wallace M. Toll-like receptor-4 signaling and Kupffer cells play pivotal roles in the pathogenesis of non-alcoholic steatohepatitis. Journal of Hepatology. 2007;47(4):571-9.
30. Sanyal AJ, Brunt EM, Kleiner DE, Kowdley KV, Chalasani N, Lavine JE, et al. Endpoints and clinical trial design for nonalcoholic steatohepatitis. Hepatology.54(1):344-53.
31. Sumida Y, Nakajima A, Itoh Y. Limitations of liver biopsy and non-invasive diagnostic tests for the diagnosis of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. World J Gastroenterol. 2014;20(2):475-85.
32. Sandrin L, Tanter M, Gennisson JL, Catheline S, Fink M. Shear elasticity probe for soft tissues with 1-D transient elastography. IEEE Transactions on Ultrasonics, Ferroelectrics, and Frequency Control. 2002;49(4):436-46.

33. Afdhal NH. Fibroscan (Transient Elastography) for the Measurement of Liver Fibrosis. Gastroenterol Hepatol (N Y). 2012;8(9):605-7.
34. Shah AG, Lydecker A, Murray K, Tetri BN, Contos MJ, Sanyal AJ. Comparison of Noninvasive Markers of Fibrosis in Patients With Nonalcoholic Fatty Liver Disease. Clinical Gastroenterology and Hepatology. 2009;7(10):1104-12.
35. Yoneda M, Yoneda M, Mawatari H, Fujita K, Endo H, Iida H, et al. Noninvasive assessment of liver fibrosis by measurement of stiffness in patients with nonalcoholic fatty liver disease (NAFLD). Dig Liver Dis. 2008;40(5):371-8.
36. Wu S-D, Liu L-L, Cheng J-L, Liu Y, Cheng L-S, Wang S-Q, et al. Longitudinal monitoring of liver fibrosis status by transient elastography in chronic hepatitis B patients during long-term entecavir treatment. Clin Exp Med. 2018;18(3):433-43.
37. Angulo P, Hui JM, Marchesini G, Bugianesi E, George J, Farrell GC, et al. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. Hepatology. 2007;45(4):846-54.
38. Shah AG, Lydecker A, Murray K, Tetri BN, Contos MJ, Sanyal AJ. USE OF THE FIB4 INDEX FOR NON-INVASIVE EVALUATION OF FIBROSIS IN NONALCOHOLIC FATTY LIVER DISEASE. Clin Gastroenterol Hepatol. 2009;7(10):1104-12.
39. Fishbein M, Castro F, Cheruku S, Jain S, Webb B, Gleason T, et al. Hepatic MRI for Fat Quantitation: Its Relationship to Fat Morphology, Diagnosis, and Ultrasound. Journal of Clinical Gastroenterology. 2005;39(7):619–25.
40. Belfort R, Harrison SA, Brown K, Darland C, Finch J, Hardies J, et al. A placebo-controlled trial of pioglitazone in subjects with nonalcoholic steatohepatitis. N Engl J Med. 2006;355(22):2297-307.
41. Aithal GP, Thomas JA, Kaye PV, Lawson A, Ryder SD, Spendlove I, et al. Randomized, placebo-controlled trial of pioglitazone in nondiabetic subjects with nonalcoholic steatohepatitis. Gastroenterology. 2008;135(4):1176-84.
42. Sanyal AJ, Chalasani N, Kowdley KV, McCullough A, Diehl AM, Bass NM, et al. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. N Engl J Med. 2010;362(18):1675-85.
43. Armstrong MJ, Gaunt P, Aithal GP, Barton D, Hull D, Parker R, et al. Liraglutide safety and efficacy in patients with non-alcoholic steatohepatitis (LEAN): a multicentre, double-blind, randomised, placebo-controlled phase 2 study. Lancet. 2016;387(10019):679-90.
44. Alcoholic and Non-Alcoholic Fatty Liver Disease: Bench to Bedside. Chalasani N, Szabo G, editors2016 2016.
45. Gallo LA, Wright EM, Vallon V. Probing SGLT2 as a therapeutic target for diabetes: basic physiology and consequences. Diabetes and Vascular Disease Research. 2015;12(2):78–89.
46. Kabil SL, Mahmoud NM. Canagliflozin protects against non-alcoholic steatohepatitis in type-2 diabetic rats through zinc alpha-2 glycoprotein up-regulation. European Journal of Pharmacology. 2018;828:135-45.
47. Nakano S, Katsuno K, Isaji M, Nagasawa T, Buehrer B, Walker S, et al. Remogliflozin Etabonate Improves Fatty Liver Disease in Diet-Induced Obese Male Mice. J Clin Exp Hepatol. 2015;5(3):190-8.
48. Seko Y, Sumida Y, Sasaki K, Itoh Y, Iijima H, Hashimoto T, et al. Effects of canagliflozin, an SGLT2 inhibitor, on hepatic function in Japanese patients with type 2 diabetes mellitus:

pooled and subgroup analyses of clinical trials. Journal of Gastroenterology. 2018;53(1):140-51.
49. Akuta N, Watanabe C, Kawamura Y, Arase Y, Saitoh S, Fujiyama S, et al. Effects of a sodium-glucose cotransporter 2 inhibitor in nonalcoholic fatty liver disease complicated by diabetes mellitus: Preliminary prospective study based on serial liver biopsies. Hepatology Communications. 2017;1(1):46-52.
50. Exploring glycosuria as a mechanism for weight and fat mass reduction. A pilot study with remogliflozin etabonate and sergliflozin etabonate in healthy obese subjects- ClinicalKey.
51. Sattar N, Fitchett D, Hantel S, George JT, Zinman B. Empagliflozin is associated with improvements in liver enzymes potentially consistent with reductions in liver fat: results from randomised trials including the EMPA-REG OUTCOME® trial. Diabetologia. 2018;61(10):2155-63.
52. Lai L-L, Vethakkan SR, Nik Mustapha NR, Mahadeva S, Chan W-K. Empagliflozin for the Treatment of Nonalcoholic Steatohepatitis in Patients with Type 2 Diabetes Mellitus. Dig Dis Sci. 2020;65(2):623-31.
53. Cusi K, Bril F, Barb D, Polidori D, Sha S, Ghosh A, et al. Effect of canagliflozin treatment on hepatic triglyceride content and glucose metabolism in patients with type 2 diabetes. Diabetes, Obesity and Metabolism. 2019;21(4):812-21.
54. Seko Y, Nishikawa T, Umemura A, Yamaguchi K, Moriguchi M, Yasui K, et al. Efficacy and safety of canagliflozin in type 2 diabetes mellitus patients with biopsy-proven nonalcoholic steatohepatitis classified as stage 1–3 fibrosis. Diabetes Metab Syndr Obes. 2018;11:835-43.
55. Eriksson JW, Lundkvist P, Jansson P-A, Johansson L, Kvarnström M, Moris L, et al. Effects of dapagliflozin and n-3 carboxylic acids on non-alcoholic fatty liver disease in people with type 2 diabetes: a double-blind randomised placebo-controlled study. Diabetologia. 2018;61(9):1923-34.
56. WHO_MSD_MSB_01.6a_tha.pdf.
57. Rabinowich L, Shibolet O. Drug Induced Steatohepatitis: An Uncommon Culprit of a Common Disease. Biomed Res Int. 2015;2015:168905.
58. Rezende REF, Duarte SMB, Stefano JT, Roschel H, Gualano B, de Sá Pinto AL, et al. Randomized clinical trial: benefits of aerobic physical activity for 24 weeks in postmenopausal women with nonalcoholic fatty liver disease. Menopause. 2016;23(8):876-83.
59. Lee Y-h, Kim JH, Kim SR, Jin HY, Rhee E-J, Cho YM, et al. Lobeglitazone, a Novel Thiazolidinedione, Improves Non-Alcoholic Fatty Liver Disease in Type 2 Diabetes: Its Efficacy and Predictive Factors Related to Responsiveness. J Korean Med Sci. 2017;32(1):60-9.
60. Latva-Rasku A, Honka M-J, Kullberg J, Mononen N, Lehtimäki T, Saltevo J, et al. The SGLT2 Inhibitor Dapagliflozin Reduces Liver Fat but Does Not Affect Tissue Insulin Sensitivity: A Randomized, Double-Blind, Placebo-Controlled Study With 8-Week Treatment in Type 2 Diabetes Patients. Diabetes Care. 2019;42(5):931-7.
61. Shibuya T, Fushimi N, Kawai M, Yoshida Y, Hachiya H, Ito S, et al. Luseogliflozin improves liver fat deposition compared to metformin in type 2 diabetes patients with non-alcoholic fatty liver disease: A prospective randomized controlled pilot study. Diabetes, Obesity and Metabolism. 2018;20(2):438-42.
62. Marchisello S, Pino AD, Scicali R, Urbano F, Piro S, Purrello F, et al. Pathophysiological, Molecular and Therapeutic Issues of Nonalcoholic Fatty Liver Disease: An Overview. International Journal of Molecular Sciences. 2019;20(8).
63. Parikh P, Ingle M, Patel J, Bhate P, Pandey V, Sawant P. An open-label randomized

control study to compare the efficacy of vitamin e versus ursodeoxycholic acid in nondiabetic and noncirrhotic Indian NAFLD patients. Saudi Journal of Gastroenterology. 2016;22(3):192.
64. Garinis GA, Fruci B, Mazza A, Siena MD, Abenavoli S, Gulletta E, et al. Metformin versus dietary treatment in nonalcoholic hepatic steatosis: a randomized study. Int J Obes. 2010;34(8):1255-64.
65. Gastaldelli A, Harrison SA, Belfort-Aguilar R, Hardies LJ, Balas B, Schenker S, et al. Importance of changes in adipose tissue insulin resistance to histological response during thiazolidinedione treatment of patients with nonalcoholic steatohepatitis. Hepatology. 2009;50(4):1087-93.
66. Raj H, Durgia H, Palui R, Kamalanathan S, Selvarajan S, Kar SS, et al. SGLT-2 inhibitors in non-alcoholic fatty liver disease patients with type 2 diabetes mellitus: A systematic review. World J Diabetes. 2019;10(2):114-32.
67. Long-term efficacy of rosiglitazone in nonalcoholic steatohepatitis: Results of the fatty liver improvement by rosiglitazone therapy (FLIRT 2) extension trial - Ratziu - 2010 - Hepatology - Wiley Online Library.
68. Rosiglitazone treatment in nondiabetic subjects with nonalcoholic fatty liver disease. - PubMed - NCBI.
69. Effect of LIK066 on Reduction of Fatty Content in Livers of Obese Patients - Full Text View - ClinicalTrials.gov.

VITA

NAME	Borwonkhun Tontivuthikul
DATE OF BIRTH	25 April 1990
PLACE OF BIRTH	Bangkok
INSTITUTIONS ATTENDED	Faculty of Medicine, Chulalongkorn University
HOME ADDRESS	78/50, Senanivet Village, Soi Senanikom 1 (110), Phahonyothin Road, Jorakaebua subdistrict, Ladprao district, Bangkok, Thailand, 10230