THE EFFECTS OF EMPAGLIFLOZIN ON HEPATIC PARAMETERS AND LIVER FAT CONTENT IN PATIENTS WITH TYPE 2 DIABETES MELLITUS: A SYSTEMATIC REVIEW AND META-ANALYSIS

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by

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS

cov(x,y)	Covariance of x and y.
d.f.	Degrees of freedom.
ε_k	Sampling error
D_k	Cook's distance
I^2	Higgins' and Thompson's I^2 measure of heterogeneity
K	Total number of studies in a meta-analysis
Q	Cochran's Q measure of heterogeneity
s_k^2	Variance of individual study
$\hat{\theta}$	Summary effect
$\hat{\theta}_k$	Individual study's observed effect
w_k	(Inverse-variance) weight of individual study

LIST OF ABBREVIATIONS

ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
CI	Confidence Interval
GGT	Gamma-Glutamyl Transferase
GOSH	Graphical display of study heterogeneity
LFP	Liver Fat Percentage
MD	Mean Difference
NAFLD	Non-Alcoholic Fatty Liver Disease
PI	Prediction Interval
SGLT2	Sodium-Glucose Co-Transporter 2
T2DM	Type 2 Diabetes Mellitus
WSS	Weighted sum of square

KESAN EMPAGLIFLOZIN TERHADAP PARAMETER HATI DAN KANDUNGAN LEMAK HATI DALAM KALANGAN PESAKIT DIABETES MELITUS JENIS 2: TINJAUAN SISTEMATIK DAN ANALISIS META

ABSTRAK

Latar Belakang: Diabetes Mellitus Jenis 2 (T2DM) sering berlaku serentak dengan komplikasi hati, termasuk Penyakit Hati Berlemak Bukan Alkohol (NAFLD), menimbulkan cabaran penting dalam strategi rawatan. Empagliflozin, sebatian Penghalang Pemindahan Natrium-Glukosa Jenis 2 (SGLT2), telah menarik perhatian dalam pengurusan T2DM dan penambahbaikan parameter hati. Walau bagaimanapun, bukti sedia ada mengenai impaknya terhadap hasil hati pada pesakit T2DM masih tiada konklusi dan memerlukan penyelidikan lanjut. Meta-analisis ini bertujuan untuk menilai kesan empagliflozin secara menyeluruh terhadap parameter hati dan peratusan lemak hati (LFP) pada individu dengan T2DM.

Metodologi: Mengikuti garis panduan PRISMA, satu carian menyeluruh dilakukan di pangkalan data seperti PubMed, Scopus, Web of Science, Google Scholar, Cochrane Library, dan ClinicalTrials.gov dari incepsi sehingga Januari 2023. Kriteria penerimaan termasuk pesakit menghidapi T2DM, intervensi adalah rawartan empagliflozin berbanding dengan plasebo atau rawatan standard, dan kajian dengan reka bentuk ujian kawalan rawak. Kriteria pengecualian merangkumi bab buku, ulasan naratif, dan protokol kajian. Penilaian kualiti mengikuti alat risiko penilaian Cochrane untuk ujian rawak (RoB 2). Anggaran saiz kesan diperoleh daripada nilai parameter hati (alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT)) dan LFP, membandingkan kumpulan yang dirawat

dengan empagliflozin dengan kumpulan lain. Analisis statistik dilakukan menggunakan Perisian R versi 4.2.3, melibatkan penentuan kesan rawatan, penilaian *heterogeneity*, dan analisis kepekaan.

Keputusan: Meta-analisis ini merangkumi 6 kajian, dengan jumlah peserta sebanyak 565, dengan 7 anggaran saiz kesan untuk ALT. Bagi AST, 6 anggaran saiz dari 5 kajian telah digabungkan (458 pemerhatian), manakala analisis GGT melibatkan data dari 4 kajian (353 pemerhatian), dan analisis LFP melibatkan 2 kajian (202 pemerhatian). Bagi tahap ALT, perbezaan min purata (MD) dalam tahap ALT tidak signifikan didapati antara individu yang dirawat dengan empagliflozin dan kumpulan kawalan dalam analisis awal (MD = -5.59; 95% CI [-10.98; 1.46]; p = 0.100). Namun, *heterogeneity* yang besar (I^2 = 85%) menunjukkan variasi yang besar di antara kajian-kajian. Penerokaan lanjut menghasilkan ketidaksempurnaan yang signifikan (Q = 41.30, p < 0.001) dan julat *prediction interval* (PI) yang berkisar dari -22.80 hingga 11.62. Apabila menjalankan analisis kepekaan dan mengecualikan kajian yang mempengaruhi, *heterogeneity* turun dari 85.5% kepada 0.0%, tetapi MD tetap tidak signifikan (MD = -2.08; 95% CI [-5.82; 1.65], p = 0.211). Dalam analisis kami terhadap tahap AST, meta-analisis awal menunjukkan tiada perbezaan yang signifikan dalam tahap AST antara individu yang dirawat dengan empagliflozin dengan kumpulan kawalan (MD = -5.44; 95% CI [-11.02 – 0.14]; p = 0.054). Walaupun begitu, terdapat *heterogeneity* yang besar (I^2 = 82%) di antara kajian-kajian, menunjukkan kesan yang pelbagai. Ujian *heterogeneity* lanjut mengesahkan variasi yang signifikan (Q = 27.77, d.f. = 5, p < 0.001), menunjukkan faktor-faktor yang mendasari penyumbangan kepada perbezaan antara kajian-kajian. Julat PI yang luas (-19.49 hingga 8.61) menunjukkan ketidakpastian dalam menilai kesan rawatan yang sebenar.

Analisis kepekaan mengenal pasti satu kajian yang mempengaruhi. Penghapusan kajian ini mengurangkan *heterogeneity* (daripada 82.0% kepada 26.8%), tetapi MD tetap tidak signifikan (MD = -2.54; 95% CI [-6.50; 1.42], $p = 0.150$). Walaupun usaha untuk menangani *heterogeneity*, keputusan yang signifikan secara statistik tidak diperolehi, menunjukkan trend tetapi tidak signifikan secara statistik untuk tahap AST. Dalam analisis kami terhadap tahap GGT, anggaran bersama menunjukkan perbezaan yang signifikan dalam tahap GGT selepas rawatan antara individu yang dirawat dengan empagliflozin dan kumpulan perbandingan (MD = -10.86; 95% CI [-20.18; -1.53], $p = 0.034$), dengan *heterogeneity* di bawah ambang kepentingan ($I^2 = 31\%$). PI untuk tahap GGT merangkumi dari -29.18 hingga 7.47. Tiada kajian yang dikeluarkan sebagai mempengaruhi bagi tahap GGT. Analisis LFP menunjukkan perbezaan min purata (MD -4.903, 95% CI [-9.869; 0.064], $p = 0.0507$). Hanya 2 kajian yang dimasukkan dalam analisis untuk LFP, yang mencegah analisis lanjutan untuk *heterogeneity*, bias penerbitan, analisis kepekaan, dan analisis yang mempengaruhi disebabkan oleh saiz sampel yang terhad.

Kesimpulan: Empagliflozin tidak memberikan kesan signifikan terhadap tahap ALT dan AST pada dos 10 mg. Dos yang lebih tinggi (25 mg) mungkin memberi faedah kepada parameter hati. Penurunan yang signifikan dalam tahap GGT diperhatikan, namun dengan data terhad yang ada. Tiada kesan signifikan terhadap LFP diperhatikan. Penyelidikan lanjut diperlukan untuk meneroka keberkesanan empagliflozin pada dos 25mg untuk meningkatkan fungsi hati.

Kata Kunci: Diabetes Mellitus Jenis 2, Penyakit Hati Berlemak Tanpa Alkohol, empagliflozin, fungsi hati, peratus lemak hati, meta-analisis.

EFFECTS OF EMPAGLIFLOZIN ON HEPATIC PARAMETERS AND LIVER FAT CONTENT AMONG TYPE 2 DIABETES MELLITUS PATIENTS: A SYSTEMATIC REVIEW WITH META-ANALYSIS

ABSTRACT

Background: Type 2 Diabetes Mellitus (T2DM) often coexists with hepatic complications, including Non-Alcoholic Fatty Liver Disease (NAFLD), posing significant challenges in treatment strategies. Empagliflozin, a Sodium-Glucose Co-Transporter 2 (SGLT2) inhibitor, has garnered attention for managing T2DM and improving hepatic parameters. However, existing evidence on its impact on hepatic outcomes in T2DM patients remains inconclusive, necessitating further investigation. This meta-analysis aims to comprehensively evaluate the effect of empagliflozin on hepatic parameters and liver fat percentage (LFP) in individuals with T2DM.

Methods: Following PRISMA guidelines, a thorough search spanned databases such as PubMed, Scopus, Web of Science, Google Scholar, Cochrane Library, and ClinicalTrials.gov from inception to January 2023. The inclusion criteria includes patients with T2DM, interventions of empagliflozin versus placebo or standard treatment, and study with randomized controlled trial design. Exclusion criteria encompass book chapters, narrative reviews, and study protocols. Quality assessment followed the Revised Cochrane risk-of-bias tool for randomized trials (RoB 2). The effect size estimate was derived from post-treatment values of hepatic parameters (alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT)) and LFP, comparing empagliflozin-treated groups with other

groups. Statistical analysis was performed using R Software version 4.2.3, involving determination of treatment effects, assessment of heterogeneity, and sensitivity analysis.

Results: The meta-analysis includes 6 studies, totalling 565 participants, with 7 effect size estimates for ALT. For AST, 6 effect sizes from 5 studies were pooled (458 observations), while GGT analysis included data from 4 studies (353 observations), and LFP analysis involved two studies (202 observations). For ALT level, no significant mean difference (MD) in ALT levels was found between individuals treated with empagliflozin and the control group in the initial analysis (MD = - 5.59; 95% CI [-10.98; 1.46]; p = 0.100). However, substantial heterogeneity (I^2 = 85%) indicated considerable variability among studies. Further exploration revealed significant variability (Q = 41.30, p < 0.001) and wide prediction interval (PI) that ranged from -22.80 to 11.62. Upon sensitivity analysis and removal of influential study, heterogeneity dropped from 85.5% to 0.0%, but the MD remained non-significant (MD = - 2.08; 95% CI [- 5.82; 1.65], p = 0.211). In our analysis of AST levels, the initial meta-analysis showed no significant difference in AST levels between empagliflozin-treated individuals and the control group (MD = -5.44; 95% CI [-11.02 – 0.14]; p = 0.054). Despite this, there was substantial heterogeneity (I^2 = 82%) among the studies, indicating diverse effects. Further heterogeneity tests confirmed significant variability (Q = 27.77, d.f. = 5, p < 0.001), suggesting underlying factors contributing to differences across studies. The wide prediction interval (-19.49 to 8.61) indicates uncertainty in estimating the true treatment effect. Sensitivity analyses identified one influential study. Removing this study reduced heterogeneity (from 82.0% to 26.8%), but the MD remained non-significant (MD = -2.54; 95% CI [-6.50; 1.42], p = 0.150).

Despite efforts to address heterogeneity, statistically significant results were not obtained, indicating a trend but lacking statistical significance for AST levels. In our analysis of GGT levels, the pooled estimate revealed a significant difference in post-treatment GGT levels between individuals treated with empagliflozin and the comparator groups (MD = -10.86; 95% CI [-20.18; -1.53], p = 0.034), with heterogeneity below the threshold of significance (I^2 = 31%). PI for GGT level spanned from -29.18 to 7.47. No study was removed as influential for GGT level. The analysis of LFP showed a non-significant mean difference (MD -4.903, 95% CI [-9.869; 0.064], p = 0.0507). Only two studies were included in the analysis for LFP, precluding subsequent analyses for heterogeneity, publication bias, sensitivity analysis, and influential analysis due to the limited sample size.

Conclusion: Empagliflozin did not significantly affect ALT and AST levels at 10 mg dosage. Higher dosage (25 mg) may benefit liver parameters. Significant reduction in GGT levels was observed, yet with limited data. No significant impact on LFP was noted. Further research is warranted to explore empagliflozin's efficacy at 25mg dosage for improving hepatic outcomes.

Keywords: Type 2 Diabetes Mellitus, Non-Alcoholic Fatty Liver Disease, empagliflozin, liver function, liver fat percentage, meta-analysis.

CHAPTER 1: INTRODUCTION

1.1 Background

Type 2 Diabetes Mellitus (T2DM) represents a global health concern with an alarming increase in prevalence, posing a substantial burden on healthcare systems worldwide (Khan et al., 2020). The World Health Organization (WHO) estimates that over 400 million individuals worldwide are affected by T2DM, with projections indicating a continuous upward trajectory in the coming years ("Diabetes Facets and Figures| International Diabetes Federation," 2021). This rise in prevalence is largely attributed to various factors, including sedentary lifestyles, obesity, unhealthy dietary patterns, genetic predisposition, and an aging population (Galicia-Garcia et al., 2020).

The impact of T2DM extends beyond its immediate metabolic effects, significantly affecting various organs and systems within the human body (Henson et al., 2023). Among these organs, the liver plays a pivotal role and is particularly vulnerable to the consequences of T2DM. The intricate interplay between T2DM and hepatic health is well-documented, with T2DM exerting profound effects on liver function. Patients with T2DM often experience hepatic complications, ranging from simple hepatic steatosis to more severe conditions such as non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), cirrhosis, and hepatocellular carcinoma (HCC) (Tanase et al., 2020a).

Non-alcoholic fatty liver disease (NAFLD) is strongly associated with T2DM, with its prevalence reaching 55.5% among T2DM patients globally (Younossi et al., 2019). The liver's central role in glucose metabolism, lipid regulation, and insulin sensitivity renders it susceptible to the disturbances caused by insulin resistance, a hallmark feature of T2DM (Mohamed et al., 2016). Insulin resistance leads to

increased hepatic gluconeogenesis, dysregulated lipid metabolism, and augmented hepatic fat accumulation, eventually contributing to the development and progression of hepatic complications in T2DM patients (Titchenell et al., 2017). Furthermore, the bidirectional relationship between T2DM and liver diseases accentuates the need for effective management strategies targeting hepatic complications among T2DM patients (Tanase et al., 2020a).

Managing hepatic complications in T2DM patients assumes paramount importance in mitigating the progression of liver-related conditions and preventing associated adverse outcomes. Early identification and intervention to manage hepatic complications are critical in averting the development of advanced liver diseases and their associated morbidity and mortality. Addressing hepatic complications in T2DM patients not only improves liver health but also plays a crucial role in enhancing overall health outcomes and reducing the burden on healthcare systems (Cusi, 2020).

This background underscores the urgency of understanding the escalating prevalence of T2DM, recognizing its impact on hepatic health, and emphasizing the necessity for managing hepatic complications in T2DM patients. Such awareness is fundamental in shaping effective interventions and healthcare strategies aimed at ameliorating hepatic outcomes and improving the overall well-being of individuals living with T2DM.

1.2 Problem statement

T2DM represents a multifaceted metabolic disorder, often accompanied by hepatic complications such as NAFLD that pose significant challenges in current treatment paradigms. While existing therapeutic approaches for T2DM encompass

various pharmacological interventions, limitations persist in adequately addressing hepatic complications associated with this disease.

The management of T2DM-related hepatic complications remains a critical area of concern due to the insufficient efficacy of current treatments in mitigating hepatic manifestations. Despite advancements in pharmacotherapy, there exists an unmet need to address the intricate interplay between T2DM and hepatic dysfunction comprehensively. Therapy targeting NAFLD to delay its progression has become a global focus, in which the management of co-morbidities that are interlinked with NAFLD remains the cornerstone of treatment for NAFLD (Androutsakos et al., 2022).

Empagliflozin, a sodium-glucose co-transporter 2 (SGLT2) inhibitor, has emerged as a promising alternative intervention for T2DM management. Its mechanism of action involves promoting urinary glucose excretion, resulting in improved glycemic control. Beyond its established glucose-lowering effects, emerging evidence suggests that empagliflozin might exert favorable impacts on hepatic parameters among individuals with T2DM (Androutsakos et al., 2022). However, the existing body of evidence regarding the effect of empagliflozin on hepatic parameters remains contentious, with conflicting findings reported in published meta-analyses (Coelho et al., 2021; Simental-Mendía et al., 2021; Wei et al., 2021; Xing et al., 2020; Y. Zhang et al., n.d.).

The discrepancy in the reported outcomes emphasizes the need for a comprehensive and systematic assessment via meta-analysis to ascertain the precise impact of empagliflozin on hepatic parameters and liver fat content among individuals diagnosed with T2DM. By collating and analyzing available data, this study aims to provide a comprehensive understanding of the potential impact of empagliflozin as a therapeutic intervention specifically targeting T2DM-related hepatic complications.

1.3 Significance of the study

Given the intricate association between T2DM and NAFLD, therapeutic interventions addressing both conditions garner significant interest. Managing NAFLD becomes crucial within the context of T2DM treatment, considering that hepatic dysfunction can exacerbate the advancement of T2DM. Currently, no specific pharmaceutical agent is targeting NAFLD among T2DM patients. Hence, an extensive review of empagliflozin's potential in ameliorating hepatic parameters is imperative to elucidate its precise role as a liver-protective agent among individuals with T2DM.

This meta-analysis aims to review the impact of empagliflozin on improving hepatic parameters that serve as prognosticators for NAFLD progression, thereby shedding light on the additional pharmacological potential of empagliflozin. Furthermore, this meta-analysis seeks to fill the existing void in understanding the exact role of empagliflozin on hepatic parameters in T2DM patients. Through a rigorous analysis of available evidence, this study intends to contribute valuable insights that could potentially inform clinical practice and guide future research directions in addressing hepatic complications associated with T2DM.

1.4 Research question

What is the effect of empagliflozin on hepatic parameters and liver fat content among T2DM patients based on systematic review and meta-analysis?

1.5 Research Objectives

1.5.1 General objective

To determine the pooled mean difference estimates of liver function and liver fat between T2DM patients receiving empagliflozin and control/those not receiving empagliflozin.

1.5.2 Specific objectives

a) To determine the pooled mean difference estimates of liver function (based on alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyl transpeptidase (GGT)) between T2DM patients receiving empagliflozin and control/those not receiving empagliflozin.
b) To determine the pooled mean difference estimates of liver fat percentage (LFP) between T2DM patients receiving empagliflozin and control/those not receiving empagliflozin.

1.6 Research hypothesis

a) There is a significant mean difference in hepatic parameters (ALT, AST, GGT) between empagliflozin treatment and other groups (placebo or other treatment).
b) There is a significant mean difference in liver fat percentage between empagliflozin treatment and other groups (placebo or other treatment).

CHAPTER 2: LITERATURE REVIEW

PART A: EMPAGLIFLOZIN AND T2DM

2.1 Introduction to the literature review

This meta-analysis intends to review the effect of empagliflozin on hepatic parameters. There has been conflicting evidence from previous meta-analyses conducted, in which there is still a debate on empagliflozin as a potential agent to ameliorate hepatic parameters among T2DM patients. This literature review intended to describe the interlink between T2DM and NAFLD, understand the pathological changes that lead to the progression of the diseases, and review the current treatment and challenges in the treatment and prevention of hepatic disorders among T2DM patients. Subsequently, the current role of empagliflozin as a potential agent in ameliorating hepatic parameters will be discussed in this review.

The literature review has been conducted by using the search terms “T2DM AND (NAFLD OR hepatic OR liver)”, “empagliflozin AND (hepatic OR liver)” in PubMed. Articles that were deemed to be relevant to the current review of “T2DM and liver” or “empagliflozin and liver” were selected from the search results for this review.

Table 2.1 Number of articles related to the research topic

Search term	**Database**	**Number of articles**
T2DM AND (NAFLD OR liver OR hepatic)	PubMed	3,696
Empagliflozin AND (NAFLD OR liver OR hepatic)	PubMed	180

2.2 T2DM and Treatment Approaches

T2DM has emerged as a significant global health concern, affecting approximately 1 in 11 adults and a staggering 463 million individuals worldwide (Akhtar et al., 2022). The multifaceted nature of this metabolic disorder arises from a combination of factors, including impaired insulin secretion, insulin resistance, or a confluence of both. Initially, compensatory mechanisms drive increased insulin secretion to counteract the hyperglycaemic state. However, as T2DM progresses, beta-cell dysfunction ensues, leading to impaired insulin secretion and exacerbating the condition (Galicia-Garcia et al., 2020). Notably, individuals with T2DM commonly present with obesity and elevated body fat percentages. Adipose tissue, through various inflammatory mechanisms, contributes significantly to insulin resistance by augmenting the release of free fatty acids and disrupting adipokine regulation. This intricate interplay culminates in the persistent hyperglycaemic state observed in T2DM patients (Henson et al., 2023). If left unmanaged, chronic hyperglycaemia poses a substantial risk of inflicting damage upon multiple organs, categorized broadly into microvascular complications (such as retinopathy, nephropathy, and neuropathy) and macrovascular complications, increasing the susceptibility to cardiovascular diseases (Mansour et al., 2023).

Historically, the treatment landscape for T2DM has evolved significantly, witnessing key milestones and shifts in therapeutic strategies. The cornerstone of management has traditionally revolved around lifestyle modifications, including dietary adjustments and regular physical exercise. However, as the disease progresses or when lifestyle interventions prove inadequate in achieving optimal glycaemic control, pharmacological interventions become essential ("Type 2 Diabetes: Symptoms, Causes, Diagnosis, and Treatment," n.d.).

Metformin, an oral anti-hyperglycaemic agent, has served as the first-line therapy for T2DM for several decades owing to its efficacy in improving insulin sensitivity and reducing hepatic glucose production. Other available therapies are oral sulfonylureas, dipeptidyl-4 (DPP-4) inhibitors, glucagon-like peptide-1 (GLP-1) receptor agonists, SGLT2 inhibitors, alpha-glucosidase inhibitors, and insulin (Weinberg Sibony et al., 2023). The choice of these agents is tailored based on individualized patient profiles, considering factors such as glycaemic control, comorbidities, and potential side effects (Williams et al., 2022).

Limitations persist despite the array of available treatments, particularly concerning the hepatic complications associated with T2DM. NAFLD is intricately linked with T2DM and significantly impacts overall health outcomes. Currently, there exists no specific pharmacological therapy targeting NAFLD in the realm of T2DM management. Consequently, ongoing research endeavours aim to explore potential therapeutic agents capable of ameliorating hepatic parameters, seeking to address this crucial aspect of the disease continuum. Novel hypoglycaemic agents such as DPP-4 inhibitors, GLP-1 receptor agonists, and SGLT2 inhibitors have been shown to be beneficial among T2DM patients with NAFLD. These medications were shown to exert potentially favourable effects on glucose and lipid metabolism, improve insulin

sensitivity, decrease free fatty acid concentrations, and reduce hepatic fat content. However, the hepatic-protective role of these agents has not been established to date (Zou et al., 2023).

While substantial progress has been achieved in the treatment landscape of T2DM, there remain unmet needs, particularly in addressing hepatic complications and refining therapeutic approaches to mitigate the multifaceted aspects of this complex metabolic disorder. Ongoing research and development endeavours continue to explore novel avenues to enhance treatment modalities and improve outcomes for individuals affected by T2DM.

2.3 Liver Complications in Type 2 Diabetes

T2DM and NAFLD represent interconnected metabolic disorders sharing intricate pathophysiological mechanisms that contribute to their coexistence and exacerbate disease progression. The prevalence of NAFLD among T2DM patients was shown to be around 70% in one cross-sectional study involving 2839 T2DM patients (Targher et al., 2021a). Central to the pathophysiological link between T2DM and NAFLD is insulin resistance. Insulin resistance, a hallmark of T2DM, disrupts the balance of hepatic glucose and lipid metabolism (Targher et al., 2021b). Insulin is an anabolic hormone that mediates glucose metabolism by enhancing glucose uptake by adipose and hepatic tissue, meanwhile suppressing hepatic gluconeogenesis. In T2DM, insulin clearance is compromised and this is correlated with the severity of disease progression. Insulin exhibits both anti-inflammatory and proinflammatory properties. The presence of insulin resistance will coupled with inflammation, both promoting each other and fastening the progression of NAFLD (Tanase et al., 2020b).

Increased circulating free fatty acids, stemming from adipose tissue lipolysis due to insulin resistance, contribute to ectopic fat deposition in the liver, leading to hepatic steatosis—a characteristic feature of NAFLD (Utzschneider and Kahn, 2006). Dysregulated hepatic lipogenesis, characterized by increased de novo lipogenesis and impaired fatty acid oxidation, contributes to the accumulation of triglycerides within hepatocytes. Simultaneously, impaired lipolysis and secretion of triglyceride-rich very-low-density lipoproteins contribute to the retention of lipid droplets within the liver, further exacerbating hepatic steatosis (Perla et al., 2017; Targher et al., 2021b).

Chronic low-grade inflammation and oxidative stress play pivotal roles in the progression from simple steatosis to non-alcoholic steatohepatitis (NASH) in NAFLD. Adipose tissue dysfunction and the release of pro-inflammatory cytokines, along with increased hepatic production of reactive oxygen species (ROS), contribute to hepatocyte injury, inflammation, and fibrosis (Ma et al., 2021).

Dysregulated adipokines, such as adiponectin and leptin, influence insulin sensitivity, lipid metabolism, and inflammation, contributing to the development and progression of both T2DM and NAFLD (Targher et al., 2021b). Alterations in gut microbiota composition and increased intestinal permeability also play a role in the pathophysiology of both conditions, contributing to metabolic dysregulation and systemic inflammation (H. J. Tsai et al., 2021).

The intricate pathophysiological links between T2DM and NAFLD involve shared mechanisms encompassing insulin resistance, dysregulated lipid metabolism, chronic inflammation, and gut dysbiosis. Understanding these interconnected pathways is crucial in elucidating the complex interplay between these metabolic

disorders and developing targeted interventions aimed at halting disease progression and improving clinical outcomes in individuals affected by T2DM and NAFLD.

Most patients with NAFLD are asymptomatic and typically detected as incidental findings during routine laboratory assessment. (Bhatt and Smith, 2015). Liver enzymes such as ALT, AST, and GGT are biochemical markers of liver dysfunction. The commonly observed pattern in hepatic steatosis due to NAFLD is an increased level of transaminases, with ALT level exceeding AST level. GGT level may increase modestly with NAFLD progression (Sattar et al., 2014). Improvement in these parameters can be an indicator of positive hepatic outcomes. The hallmark of NAFLD is the accumulation of hepatic fat, which occurs when fatty acid uptake and de novo synthesis exceed oxidation and secretion (E. Zhang et al., 2021).

Based on a study that was conducted on 70 T2DM patients and 70 nondiabetic subjects, T2DM patients were shown to have having higher percentage of liver fat than the nondiabetic subjects. The percentage increase in liver fat correlates with the BMI and waist circumference of the subjects, in which T2DM patients were shown to have approximately 80% more liver fat than the nondiabetic subjects at a BMI of 40 kg/m^2 (Kotronen et al., 2008). In T2DM, the impaired suppression of adipose tissue lipolysis by insulin results in increased free fatty acids delivery to the liver. This supply of fatty acid will result in the synthesis of excess triglyceride in the liver and the accumulation of hepatic fat is further stimulated by diminished hepatic fatty acid oxidation due to insulin resistance (Bhatt and Smith, 2015).

Several antidiabetic medications have been studied for the treatment of NAFLD, such as metformin, thiazolidinedione, and glucagon-like-peptide-1 analogues. These agents were found to ameliorate the progression and development of

NAFLD (Hüttl et al., 2021). Pioglitazone, a thiazolidinedione and liraglutide, GLP-1 analogues have been shown to ameliorate liver fat among NASH patients with or without T2DM (Armstrong et al., 2016; Belfort et al., 2006). However, currently, no specific pharmacological agent is targeting NAFLD among T2DM.

2.4 Empagliflozin and Its Role in T2DM Management

Empagliflozin is a pleiotropic SGLT-2 inhibitor that acts as a novel hypoglycemic agent, which was approved by the United States Food and Drug Administration (US FDA) in 2014. It can be deployed as a single therapy or as part of a combination therapy with other antidiabetic medications. It showed a dose-dependent reduction in HbA1c, with a reduction of 0.929% with 10 mg and 1.064% with 25 mg as compared to baseline (Haider et al., 2019). The overall therapeutic effect of empagliflozin has been extensively studied since its emergence, in which empagliflozin was established with cardiovascular benefits and also shown to be beneficial among patients with chronic kidney diseases ("Empagliflozin in Patients with Chronic Kidney Disease," 2023; "FDA Approves Treatment for Wider Range of Patients with Heart Failure | FDA," n.d.). For patients with cardiovascular disease, empagliflozin was shown to reduce hospitalizations for heart failure and cardiovascular-related death. The new indication as a cardio-protective agent of empagliflozin was approved in 2016 by the FDA (Sizar et al., 2023).

Empagliflozin inhibits the sodium-glucose co-transporter-2 that is found in the proximal tubules of kidneys. Through this inhibition, it reduces renal reabsorption of glucose and increases glucose excretion in urine (Sizar et al., 2023). The hypoglycaemic effect of empagliflozin is independent of insulin and pancreatic beta-cell function, thus it is a suitable agent in the advanced stage of T2DM with impaired beta-cells function and depletion of insulin (Habtemariam, 2019).

Apart from hypoglycaemic and cardio-protective effects, empagliflozin was also shown to be associated with weight loss. This is probably due to diuresis and the loss of calories in urine (Gallo et al., 2015). Weight loss with empagliflozin is mostly due to fat mass reduction, accompanied by reductions in both abdominal visceral and subcutaneous adipose tissue (Ridderstråle et al., 2014). In addition to the improvements in other adiposity indices, this lead to the hypothesis of the favourable effect of empagliflozin on liver fat (Neeland et al., 2016). Some studies revealed that empagliflozin may alleviate NAFLD. A study conducted by Kahl et al. revealed that empagliflozin significantly reduces liver fat among well-controlled T2DM patients. The study concluded that empagliflozin is a potential agent targeting early treatment of NAFLD among T2DM patients (Kahl et al., 2020). From a secondary analysis of the EMPA-REG OUTCOME study, empagliflozin was shown to reduce aminotransferases among T2DM patients. Higher reduction in ALT levels was observed as compared to the changes in AST levels, and this is potentially consistent with the reduction of hepatic fat (Sattar et al., 2018b).

Previous meta-analyses that had been conducted to study the effect of SGLT-2 inhibitor on hepatic outcomes revealed a significant reduction of ALT, AST, and GGT levels among T2DM patients with or without NAFLD (Coelho et al., 2021; Simental-Mendia et al., 2021). However, there is no meta-analysis being conducted to study the individual effect of empagliflozin on liver parameters.

2.5 Empagliflozin and its role in the management of T2DM with NAFLD

The favourable effect of empagliflozin on hepatic parameters was reported in several studies (Chehrehgosha et al., 2021; Elhini et al., 2022; Kuchay et al., n.d.). It

was reported from animal studies in which empagliflozin was found to attenuate NAFLD progression by promoting autophagy, reducing endoplasmic reticulum (ER) stress and inhibiting hepatic apoptosis (Nasiri-Ansari et al., 2021). Enhancement of the hepatic macrophage autophagy was shown via the Adenosine Monophosphate-Activated Protein Kinase (AMPK)/ Mammalian Target of Rapamycin (mTOR) signaling pathway, thereby slowing NAFLD-related liver injury by inhibiting the expression levels of Interleukin-17/Interleukin-23 axis-related molecules (Meng et al., 2021).

Few meta-analyses have revealed the role of empagliflozin among NAFLD patients, with conflicting evidence on the effects of empagliflozin on hepatic outcomes. A meta-analysis by (Tang et al., 2022) concluded that empagliflozin may not provide a positive outcome among patients with NAFLD, with no significant change in hepatic enzymes post-treatment. From this meta-analysis, only 3 RCTs with a total of 212 patients were included. The study included NAFLD patients, regardless of their underlying diabetic status. A similar meta-analysis revealed that empagliflozin significantly reduced AST levels but not ALT levels among NAFLD patients (Y. Zhang et al., n.d.). In this study, 4 RCTs were included with a total number of 244 patients.

Two meta-analyses were conducted to study the effect of SGLT-2 inhibitors among T2DM patients with NAFLD. One meta-analysis revealed that there is a significant reduction in ALT but no significant decrease in AST level after the intervention (Xing et al., 2020). This meta-analysis only included one study of empagliflozin out of six studies included for analysis. Another more recent meta-analysis concluded that SGLT-2 inhibitors significantly reduce ALT and AST levels

post-treatment (Wei et al., 2021). In this meta-analysis, empagliflozin contributed to two out of ten studies included for data synthesis.

Since the evidence of the effect of empagliflozin on hepatic parameters is controversial, this meta-analysis intended to study comprehensively the effect of empagliflozin among T2DM patients with or without NAFLD.

2.6 Methodological Approaches in Previous Studies

Previous meta-analyses conducted varied on the type of intervention and study population. Two meta-analyses studied the effect of SGLT2 inhibitors on hepatic outcomes among T2DM patients (Coelho et al., 2021; Simental-Mendía et al., 2021). In these meta-analyses, the individual effect of empagliflozin on the hepatic parameters was not addressed.

Two meta-analyses conducted by Tang et al and Zhang et al deployed NAFLD patients as the study population, in which they investigated the effect of empagliflozin on hepatic parameters (Tang et al., 2022; Y. Zhang et al., n.d.). Of note, only a total of 3 and 4 studies respectively were included for analysis. Another two meta-analyses were conducted to study T2DM patients with NAFLD (Wei et al., 2021; Xing et al., 2020). For meta-analyses that specifically select T2DM patients with NAFLD, the study population is more homogenous as compared to selecting all patients with either T2DM or NAFLD. However, this will further restrict the selection of studies for data synthesis. To overcome the limitation, SGLT-2 inhibitors were selected as the intervention to provide greater insight into the role of this pharmacological drug class. However, the individual effect of empagliflozin cannot be ascertained since the data was pooled with other SGLT-2 inhibitors for analysis. Nevertheless, these meta-

analyses shared the common limitation of a small sample size, in which the study outcomes might not be precise enough to reach any conclusion. There are difficulties in investigating the diversity within the analysis. Furthermore, it is challenging to conduct any sensitivity analysis as it is unlikely to detect any outlying or influencing studies when the studies included for analysis are limited and of small sample size. Some of the previous meta-analyses revealed the presence of heterogeneity, however, the source of heterogeneity was not properly explored and addressed.

The current meta-analysis intended to broaden the scope of the study population, in which T2DM patients were selected regardless of their underlying NAFLD status. This meta-analysis will only focus on the effect of empagliflozin on hepatic parameters. More studies will be anticipated by including T2DM patients without NAFLD, which might provide different insights into the roles of empagliflozin on hepatic parameters. Since T2DM and NAFLD often co-exist, it is rationale to include all T2DM patients as these patients might have underlying progressive liver disease that is still undiagnosed. With the prospect of the inclusion of more studies, there is a potential to explore the heterogeneity of the included studies during the analysis.

2.7 Gaps in the Current Knowledge

While empagliflozin has demonstrated promising results in reducing cardiovascular events and improving renal outcomes in individuals with T2DM, its effects on hepatic outcomes remain an area with gaps in current knowledge. Research on the direct influence of empagliflozin specifically on hepatic outcomes such as liver fat content and markers of liver injury in individuals with T2DM is relatively sparse.

The conflicting evidence of the effect of empagliflozin on hepatic parameters from previous meta-analyses may be due to the limited sample size of the studies that were conducted among NAFLD patients. For meta-analysis that includes all T2DM patients as the study population, all SGLT-2 inhibitors were pooled for analysis and hence the individual effect of empagliflozin on hepatic parameters cannot be ascertained.

Due to the bidirectional relationship between NAFLD and T2DM, the current review intended to investigate the potential role of empagliflozin in ameliorating hepatic parameters and hepatic fat among T2DM patients. Selecting T2DM as the population can also provide insight into the role of empagliflozin in the early treatment or prevention of NAFLD among T2DM patients.

For this study, a systematic review with meta-analysis approach was used for the analysis of the post-intervention level of hepatic enzymes and liver fat percentage. Together with qualitative data from the systematic review, the quantitative data from statistical analysis can provide a clearer insight into the role of empagliflozin on hepatic parameters.

2.8 Conclusion of the Literature Review

Based on the literature review, SGLT-2 inhibitors seem to exert beneficial effects on hepatic parameters among T2DM patients. However, studies conducted on empagliflozin alone showed conflicting evidence among NAFLD patients due to the limited sample size. Thus, the effect of empagliflozin on hepatic outcomes is still ambiguous in the current literature. Thus, this meta-analysis intended to comprehensively review the potential role of empagliflozin in ameliorating hepatic parameters among T2DM patients.

2.9 Theoretical framework

The current meta-analysis aims to investigate the potential role of empagliflozin in ameliorating hepatic parameters. The mechanism underlying it is the inhibition of overwhelmed de novo lipogenesis and reactivation of inhibited fatty acid oxidation to move lipids out of the liver, in addition to attenuation of abnormal oxidative and inflammatory responses through their protective roles in inhibiting hepatocyte death (E. Zhang et al., 2021). Thus, this leads to the hypothesis of the favorable effect of empagliflozin on hepatic parameters and liver fat content.

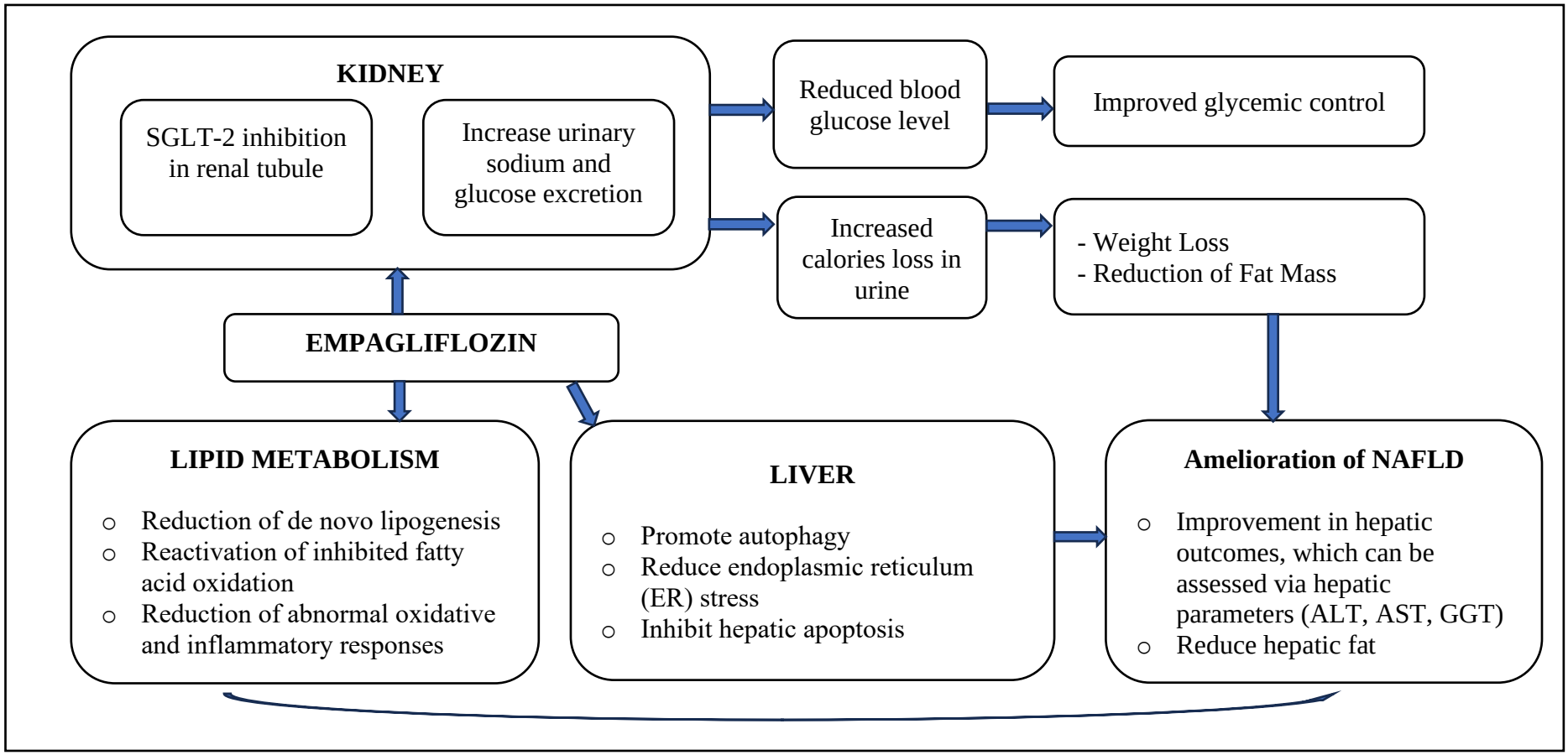

Figure 2.1 Schematic diagram of mechanism of action of empagliflozin

2.10 Operational definition

2.10.1 Hepatic parameters

Hepatic parameters such as ALT, AST, and GGT levels pertain to their quantitative measurement in the blood, serving as biomarkers indicative of liver health and potential liver injury or dysfunction associated with NAFLD (Lee et al., 2023).

Elevated ALT levels often serve as a key biomarker indicating liver injury or inflammation. Increased ALT levels, typically quantified in units per litre (U/L) through blood tests, are frequently observed in NAFLD patients, signalling liver damage due to fat accumulation in the liver cells (Hadizadeh et al., 2017).

Elevated AST levels can also be present in NAFLD, although they might be less specific to liver-related issues than ALT. AST levels, measured in units per litre (U/L) via blood tests, can indicate liver damage or inflammation associated with NAFLD but are also found in other conditions affecting the liver, heart, or muscles (Hadizadeh et al., 2017; Ndrepepa, 2021).

GGT levels, measured in units per liter (U/L) through blood tests, might also be elevated in NAFLD cases, indicating liver or bile duct damage. While GGT levels can be associated with NAFLD, they are less specific to this condition and can be influenced by various factors like alcohol consumption or other liver-related disorders ("Gamma-Glutamyl Transferase (GGT) Test: What It Is & Results," n.d.).

In the context of NAFLD, monitoring ALT, AST, and GGT levels serves as surrogate markers in assessing liver health and diagnosing liver injury or inflammation associated with fat accumulation in the liver (Sanyal et al., 2015). Elevated levels of these hepatic parameters can prompt further investigation and management strategies aimed at addressing NAFLD and its potential complications.

Hepatic parameters will be represented by the change in concentration of liver enzymes (e.g., ALT, AST, GGT) as an indicator of hepatic function for this review. Amelioration of hepatic parameters by empagliflozin will be indicative of its favourable effect on the liver.

2.10.2 Liver fat content

Liver fat content refers to the quantification or measurement of the amount of fat present in the liver tissue. It involves assessing the proportion or concentration of fat within the liver, typically expressed as a percentage of total liver weight or as the fraction of fat relative to other liver components. It is represented by the percentage change in liver fat for this review.

Practically, the operational definition of liver fat content involves employing various imaging techniques or biomarkers to quantify the volume or concentration of fat in the liver tissue. These methods may include imaging modalities such as Magnetic Resonance Imaging (MRI), proton magnetic resonance spectroscopy, computed tomography (CT), ultrasound-based techniques like controlled attenuation parameter (CAP), and various MRI sequences such as Dixon or chemical shift imaging. These imaging methods allow for the visualization and quantification of fat accumulation within the liver (Lv et al., 2018).

The operational definition of liver fat content thus involves employing these techniques or biomarkers to measure and quantify the amount of fat within the liver, providing clinicians and researchers with quantitative data to assess liver health, diagnose conditions like non-alcoholic fatty liver disease (NAFLD), and monitor the progression or regression of hepatic fat accumulation in various clinical settings.

CHAPTER 2

LITERATURE REVIEW

PART B: META-ANALYSIS

2.12 Introduction to Meta-Analysis

Meta-analysis stands as a pivotal methodology within the realm of scientific research, offering a comprehensive means to synthesize and interpret findings across diverse studies. Unresolved medical issues or clinical questions are usually studied more than once, in these studies might differ in study scale, study population, intervention, comparator, or study outcomes. Most of the time, this will yield various studies with conflicting evidence and make clinical judgement often difficult. As the landscape of academic inquiry continues to expand, the utilization of meta-analysis has gained prominence for its ability to distil vast bodies of research into cohesive narratives, providing nuanced insights that transcend the limitations of individual studies (Ab, 2010).

Meta-analysis is typically based on randomized clinical trials, offering a notably refined estimation of treatment effects or disease risk factors when compared to any individual study that contributes to the pooled analysis. Through the conduct of a meta-analysis, the exploration of heterogeneity sources among study outcomes can significantly improve understanding regarding the influence of various study factors on treatment outcomes. This comprehensive understanding not only enriches the interpretation of treatment effects but also augments the applicability of study outcomes to specific populations, thereby advancing the generalizability of the findings (Ab, 2010).

Meta-analyses fall within the scope of systematic reviews, constituting a subset of this broader study type. Both approaches share similar methodologies aimed at addressing particular research questions. This comes with a clearly stated objective with pre-set eligibility criteria for the selection of studies, reproducible methodology, systematic search to select studies that fit into the predefined eligibility criteria, assessment of the validity of studies via risk of bias assessment, and lastly the systematic presentation and synthesis of findings from included studies. Meta-analysis deployed additional statistical analyses to pool quantitative data from selected studies, providing a point estimate of an effect and the measures of precision of that estimate. By conducting a meta-analysis, the strength of evidence of a particular treatment or risk factor of diseases can be examined. Meta-analysis is crucial in the medical field in better understanding of unresolved medical issues, supported by evidence that was systematically sought from numerous similar studies across the world (Ab, 2010).

2.13 Basic Concepts and Terminology

Meta-analysis synthesizes data from multiple studies to estimate effect sizes and explore heterogeneity among study outcomes (Harrer et al., 2022). This review aims to delve into the concepts of effect size estimation, sources of heterogeneity, and their implications within meta-analyses.

Effect size estimate in meta-analysis refers to the magnitude or strength of an observed effect, typically quantified through standardized mean differences (SMD), risk ratios (RR), odds ratios (OR), or correlation coefficients. It provides a quantitative measure of the treatment effect or relationship between variables across diverse studies, facilitating comparison and interpretation of findings (Harrer et al., 2022).

Heterogeneity within a meta-analysis signifies variability in study outcomes beyond what might be expected due to chance. Sources of heterogeneity encompass diverse factors, including methodological differences, variations in study populations, interventions, outcomes, and measurement tools. Statistical measures such as Cochran's Q test and I^2 statistic aid in quantifying heterogeneity and assessing its significance. Understanding and addressing heterogeneity are crucial in meta-analysis as they impact the interpretation of results and influence the choice of statistical models. Substantial heterogeneity may prompt sensitivity analyses, subgroup analyses, or the utilization of random-effects models to account for between-study variability. Moreover, identifying sources of heterogeneity can guide researchers in exploring underlying reasons for divergent study findings. Several strategies are employed to manage heterogeneity in meta-analysis, including subgroup analyses, meta-regression, sensitivity analyses, and the exclusion of outliers (Harrer et al., 2022).

Effect size estimation and addressing heterogeneity are fundamental components of meta-analysis, influencing the robustness and interpretability of pooled results. While effect size provides a quantitative measure of the magnitude of an effect, managing heterogeneity is pivotal in ensuring the validity and reliability of meta-analytical findings. Careful consideration of effect size estimation methods and exploration of heterogeneity sources contribute to enhancing the utility and impact of meta-analysis in evidence synthesis.

2.14 Conducting a Meta-Analysis

Meta-analysis, a systematic approach to synthesizing evidence from multiple studies, heavily relies on a comprehensive literature search and precise selection criteria to ensure the validity and reliability of its findings. The effectiveness and