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MINIREVIEWS

Uses of knockout, knockdown, and transgenic models in the studies of glucose transporter 4

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Abstract

Currently, glucose transporter 4 (GLUT4) has been considered as the key player for the insulin-stimulated glucose transport in the muscle and adipose tissues. The development of recombinant DNA techniques allows the creations of genetically knockout, knockdown and transgenic animals and cells for the study of GLUT4's physiological functions. Here, we have used key words to search the PubMed and summarized the methods used in *Slc2a4* gene knockout, GLUT4 knockdown and overexpression in the whole body and tissue specific manner. The whole body GLUT4-null mice have growth retardation, but normal glucose tolerance and basal glucose turnover rates. Compared with whole body *Slc2a4* knockout mice, adipose and muscle double knockout mice have impaired insulin tolerance and glucose intolerance. The results of GLUT4 knockdown in 3T3-L1 adipocytes have shown that its expression is needed for lipogenesis after, but not during, differentiation. Transgenic mice with the whole body GLUT4 overexpression have normal body weight and lowered blood glucose level. The adipose tissue specific overexpression of GLUT4 leads to increases in mouse body weight and adipose tissue weight. The insulin-stimulated GLUT4 translocation in the skeletal muscle contributes to the regulation of glucose homeostasis. Data from both transgenic overexpression and tissue specific *Slc2a4* knockout indicate that GLUT4 probably plays a role in the glucose uptake in the fasting state. More studies are warranted to use advanced molecular biology tools to decipher the roles of GLUT4 in the control of glucose homeostasis.

Key Words: Glucose transporter 4; Knockout; Knockdown; Transgene; Overexpression; Insulin

Core Tip: The whole body glucose transporter 4 (GLUT4)-null mice have growth retardation, but normal glucose tolerance and basal glucose turnover rates. The muscle-specific GLUT4 knockout mice have normal body weight and fat pad weight at least before 6 mo of age, whereas the adipose-GLUT4 knockout mice have glucose intolerance. The adipose and muscle GLUT4 double knockout mice develop hyperglycemia in the fasting state, suggesting the role of GLUT4 in fasting state. Compared to the control mice, wholebody GLUT4 transgenic mice have similar growth rate before 10 wk of age, lower blood glucose in the fasting, and lower insulin level in the fed state. The adipose tissue specific GLUT4 overexpression increases body weight, glucose transport rate and adipose tissue weight. Data from both transgenic overexpression and tissue specific knockout of GLUT4 indicate that GLUT4 probably plays a role in the glucose uptake in the fasting state.

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INTRODUCTION

Genes in an organism are codes responsible for genetic traits. In most cases, genes usually exist in the form of nucleotide sequences. In a cell, the DNA sequence of a gene is first transcribed into mRNA, which serves as the template for protein translation. The newly synthesized proteins contribute to biological processes in an organism. To understand the biochemical, biophysical, and genetic functions of a given gene and its protein, recombinant DNA technologies have been developed and used extensively. Since 1970s, the discovery of restriction enzymes has facilitate the development of molecular cloning methods and allowed the manipulation of DNA sequences selectively and specifically to create novel recombinant molecules[1]. DNA fragments are inserted into vectors to form recombinant genetic materials for their replication, studies of gene functions and productions of recombinant proteins. The recombinant DNA technology was first used to study gene functions when the genes responsible for metabolism of galactose in E. coli were fused into the SV40 vector in 1972[2]. For the past few decades, procedures of molecular cloning have been simplified and standardized to construct recombinant DNA with various sizes for different purposes[3]. All these have been applied to generate transgenic organisms and produce recombinant proteins for the use in variety of research and clinical settings.

Glucose enters cells *via* a family of proteins called glucose transporters (GLUTs), which have 14 known members. Glucose transporter 4 (GLUT4) encoded by *SLC2A4* gene in human genome and *Slc2a4* gene in others such as rodent genomes has 409 amino acid residues, and a Km value of 5 mmol/L for glucose[4]. GLUT4 was first identified in a screen for the insulin-stimulated glucose transporter in cell membrane preparations of rat adipocytes using monoclonal antibodies against these membrane proteins[5]. Subsequently, *Slc2a4* gene was cloned from rat adipose tissue, and is homologous with GLUT1, which is encoded by *Slc2a1* gene[6-8]. GLUT4 is expressed in not only adipose and muscle cells, but also other tissues such as the heart and brain[9]. The N- and C- termini of GLUT4 are located in the cytoplasm and responsible for the insulin-mediated translocation from the cytosol to the cell membrane [10]. The current model is that insulin stimulates GLUT4 translocation from the intracellular locations to the plasma membrane, where it facilitates the glucose entry into cells[11]. In addition, exercise also stimulates the expression of *SLC2A4* mRNA in the skeletal muscle and improves insulin sensitivity in human patients[12], which may be mediated by GLUT4[13]. Insulin-stimulated glucose transport is significantly impaired in the skeletal muscle of patients with type 2 diabetes[14]. Therefore, understanding the role of GLUT4 in the regulation of glucose homeostasis is critical for the prevention and treatment of type 2 diabetes.

Here, we summarize the recombinant DNA technologies used to study expression profiles and functions of GLUT4 in tissues and cells. Key words as indicated in the following sections were used to search PubMed. The title and abstracts of the retrieved articles were read by authors. Only the articles that contained descriptions of knockout, transgenic overexpression and knockdown molecular techniques, and confirmed gene or protein expression levels were chosen for further reading. The methods used to manipulate the expression levels of GLUT4 *in vivo* and *in vitro* and reported observations in retrieved studies were summarized here. This review may help researchers who are interested in the physiological functions of GLUT4 to have a clear understanding of the status.

THE COMMON MOLECULAR BIOLOGY TECHNIQUES TO STUDY GENE AND PROTEIN FUNCTIONS

The development of molecular cloning techniques allows isolation, generation, and production of DNA sequence independence of the species and organisms that carry the original sequences. DNA fragments isolated from genomes or created *via* polymerase chain reaction (PCR) are inserted into vectors that can replicate and express in the host cells, and in turn alter the genetic features of the host cells, tissues or organisms[15]. PCR technique quickly produces large numbers of copies of a specific DNA fragment for sequencing analysis and molecular cloning. Cloning of a specific DNA sequence helps to explore the gene's biological functions, and to create large amounts of protein, such as growth hormone, insulin and clotting factors for therapeutic purposes[16]. In addition, a comparison of DNA sequences from different organisms can determine the evolutionary relationship within and between species, and functional domains of a gene. Recombinant DNA technologies can be used in gene therapies to treat diseases such as immunodeficiency diseases and metabolic disorders[17] and diagnosis of genetic diseases[18]. Genetically modified organisms or genetically engineered organisms can be created *via* alterations of the genetic sequences of the chromosome or insertions of the foreign DNA fragments into the genome to alter the phenotypes of the offspring[19].

Genes in plants, animals and microorganisms have been deleted or their expression levels have been knocked down to investigate their functions or treat genetic diseases clinically[20]. Methods are developed to silence or remove the target gene, such as gene silencing, conditional knockout, homologous recombination, and gene editing[20]. Homologous recombination occurs when homologous recombinases (nucleases) recombine two linearized DNA fragments with the same terminal sequences to create a novel fragment for molecular manipulations[21]. This makes accurate gene editing possible and becomes emerging tools in genetics[22]. Zinc finger nucleases, transcription activator-like effector nucleases, and clustered regularly interspaced short palindromic repeat (CRISPR) are developed and shown differences in knockout efficiency, completion time, and off-target efficiency[20]. Each of these techniques uses a nuclease to introduce DNA double-strand breaks at the targeted locations with the guidance of homologous binding proteins or RNA[23]. Gene knockdown methods such as RNA-based RNA interference, small interfering RNA and short hairpin RNA (shRNA), and antisense oligonucleotides have been developed to inhibit protein expression[24]. RNA interference (RNAi) is triggered by double-stranded RNA and causes the sequence-specific mRNA degradation of the single-stranded target RNA[25]. Small non-coding RNA molecules can also act to inhibit RNA translation[26].

In addition to the change of gene expression, tagged proteins or fusion proteins with novel properties can be created using molecular biology tools[27]. Fusion or tagged proteins with two or more domains from different proteins can be easily obtained and purified for their uses in research and clinical treatments, detection of the expression levels, and visualization of the intracellular locations of the expressed proteins[27]. These have been used to create vaccines, multifunctional enzymes, targeted drugs, thrombolytics, antimicrobial peptides, *etc*[28].

MOLECULAR BIOLOGY TECHNIQUES USED IN THE STUDIES OF GLUT4

The identification of GLUT4 and cloning its gene[29] have facilitated the studies of its tissue distribution, functions, the mechanisms responsible for its translocation, and the regulations of its protein and mRNA expressions in different cells. The tagged or fluorescent GLUT4 fusion proteins are used to study its intracellular trafficking. GLUT4 overexpression and knockdown, and *Slc2a4* gene knockout *in vitro* and *in vivo* have been developed to study the insulin-stimulated GLUT4 translocation and glucose homeostasis, which contribute significantly to our understanding of the role of GLUT4[29]. To review the techniques of molecular biology in the study of GLUT4, "GLUT4, molecular biology" and "*SLC2A4*, molecular biology" as keywords were used to search the PubMed database to retrieve relevant articles. We have focused on the techniques used in *Slc2a4* knockout, knockdown and transgenic studies, and results associated with the genetic changes *in vivo* and *in vitro* were analyzed and summarized here. As shown in Figure 1, *Slc2a4* genes have been knocked out and GLUT4 protein has been overexpressed in the whole body and in specific tissues and cells. In addition, GLUT4 protein has been knocked down using shRNA, and its translocation has been studied using fusion or tagged proteins. Various methods such as in situ hybridization, fluorescent microscopy, immunohistochemistry, Western blotting for protein and Northern blot and real-time PCR for mRNA used to determine endogenous or transgenic GLUT4 expressions are also summarized in this review.

Whole-body and tissue specific Slc2a4 knockout studies

Slc2a4 mRNA expression is detected not only in brown and white adipose tissue, skeletal and cardiac muscle, but also in other tissues such as neurons[30]. To study GLUT4 functions, mice with the *Slc2a4* deletion in the whole body or specific tissues or cells have been created. We searched PubMed to retrieve the original articles that initially reported the *Slc2a4* deletions. Table 1 shows the techniques for creating knockouts, experimental animals, methods to confirm the gene deletion and expression, and

Table 1 Methods used to create whole body and tissue specific *Slc2A4* knockout animals, tissues and animals studied, analytic methods included, and observations reported

Methods	Tissues/Animals	Analysis	Observations	Ref.
A construct with a disrupted mouse *Slc2a4* gene was electroporated into WW6/22 ES cells to create deletion, which were microinjected into C57Bl/6 blastocysts	Skeletal muscle/GLUT4-null mice and wild-type control mice	Southern blot for DNA, Northern blot for mRNA, and Western blot for protein measurements	The *Slc2a4-/-*mice have normal glycemia, growth retardation, decreased longevity, cardiac hypertrophy, reduced adipose deposits, postprandial hyperinsulinemia, and lowered insulin sensitivity; The male *Slc2a4-/-*mice have lower and higher blood glucose levels than the controls in fasted and fed states, respectively	[31]
GLUT4-loxP mice were crossed with α-MHC promoter-driven Cre	Heart/Cardiac-selective *Slc2a4-/-*deletion mice (G4H–/– mice) and control mice	Southern blotting and PCR for DNA, and Western blot for GLUT4 levels using various antisera	G4H–/– mice have modest cardiac hypertrophy, normal life span and serum levels of insulin, glucose, FFAs, lactate, and β-hydroxybutyrate, increased basal cardiac glucose transport and GLUT1 expression, and abolished insulin-stimulated cardiac glucose uptake	[33]
GLUT4loxP mice as shown in [33] were crossed with the muscle CK promoter driven Cre transgenic mice to obtain Muscle-G4KO	Skeletal muscle/Muscle-G4KO mice and heterozygous *Slc2a4* deletion mice in the 129SV and C57Bl/6J background	Reverse transcription–PCR for mRNA, and Western blot for GLUT4 protein (anti-GLUT4 AB1346)	Muscle-G4KO mice show a reduction in basal and near-absence of insulin- or contraction-stimulated glucose transport, showing; severe insulin resistance and glucose intolerance from an early age	[34]
GLUT4-null mice were crossed with transgenic mice expressing GLUT 4 driven by MLC promoter[55] to create MLC-GLUT4-null mice	EDL and soleus muscle/MLC-GLUT4-null mice having GLUT4 in the fast-twitch EDL muscle, GLUT4 null mice, and control mice	Western blot for GLUT4 protein (rabbit polyclonal antiserum)	MLC-GLUT4-null mice have less GLUT4 in WAT (females only) and soleus muscle, adipose tissue deposits, adipocyte size, and plasma free fatty acid levels in the fed state than the controls. Glucose uptake in the EDL, but not in the soleus, muscle is restored to normal in male and above normal in female MLC-GLUT4-null mice	[32]
GLUT4–loxP mice were crossed with aP2-driven Cre transgenic mic to obtain G4A-/- mice	Adipose tissue/G4A-/-, and control mice	Western blot for GLUT4 protein in BAT and WAT tissues	G4A-/- mice show impaired insulin-stimulated glucose uptake in adipocytes, glucose intolerance, hyperinsulinemia, and insulin resistance in the muscle and liver	[35]
The G4A-/- mice[35] were crossed with the muscle-G4KO mice[34] to generate AMG4KO mice	Adipose tissue and skeletal muscle/G4A-/-, muscle-G4KO, and AMG4KO mice	Western blot for GLUT4 protein using antibodies from H. Haspel in the Charles River Laboratory	AMG4KO mice develop fasting hyperglycemia and glucose intolerance and are at risk for greater insulin resistance than mice lacking GLUT4 in only one tissue	[37]
The neuron-specific Nestin promoter-driven Cre transgenic mice were crossed with GLUT4-loxP mice (FVB strain) to obtainBG4KO mice	Whole brain/BG4KO and control mice	Western blot for GLUT4 protein in the brain using antibody from Chemicon	BG4KO mice have glucose intolerance, insulin resistance, and impaired glucose sensing, suggesting that the brain GLUT4 may sense and respond to glucose	[36]

α-MHC: α-myosin heavy-chain; AMG4KO: Adipose/muscle-GLUT4 double knockout; BAT: Brown adipose tissue; BG4KO: Brain-specific GLUT4 knockout; Cre: Cre recombinase; CK: Creatine kinase; ES: Embryo stem; EDL: Extensor digitorum longus; GLUT4: Glucose transporter 4; G4A-/-: Adipose tissue-specific GLUT4 knockout; GLUT4-loxP: *Slc2a4* allele with exon 10 flanked by loxP sites; G4KO: GLUT4 knock out; MLC: Myosin light chain; Ref: References; WAT: White adipose tissue.

observations. In the end, seven representative articles that the research groups generated a specific knockout model to study GLUT4 and clearly described the methods of GLUT4 deletion are summarized here as shown in Table 1. The animal models were also used by many other groups.

In1995, the mouse *Slc2a4* locus was disrupted using homologous recombination in embryonic stem cells which generated mice without GLUT4 expression (GLUT4-null) in the whole body[31]. The GLUT4-null mice showed growth retardation, enlarged hearts and complete lack of the white adipose tissue[31]. GLUT4-null mice have normal glucose tolerance and basal glucose turnover rates. However, they are insulin intolerant, suggesting insulin resistance. Later on, the GLUT4-null mice[31] have been used to create mice expressing GLUT4 specifically in the extensor digitorum longus muscle[32].

Tissue specific GLUT4 knockout mice have been created by crossing mice carrying a *Slc2a4* allele with exon 10 flanked by loxP sites with those carrying *Cre* gene expression driven by tissue specific promoters[33-36]. The various phenotypes of these knockout mice help us to understand the roles of GLUT4 in different tissues and glucose metabolism. For example, the muscle-specific GLUT4 knockout mice (muscle-G4KO) were created by breeding mice carrying the *Slc2a4* exon 10 flanked by loxP sites with mice carrying a transgene encoding Cre recombinase under the control of the muscle creatine kinase promoter[34]. Compared with GLUT4-null mice, muscle-G4KO mice have normal body weight and fat pad weight at least before 6 mo of age[34]. The skeletal muscle mass is also normal. The increase in heart weight is consistent with GLUT4-null mice[31] and cardiac-G4KO mice[33]. Compared with the shortened lifespan of GLUT4-null mice, the life span of muscle-G4KO mice is normal. In contrast to GLUT4-null mice and cardiac-G4KO mice, adipose-G4KO mice[35] are similar to muscle-G4KO mice.

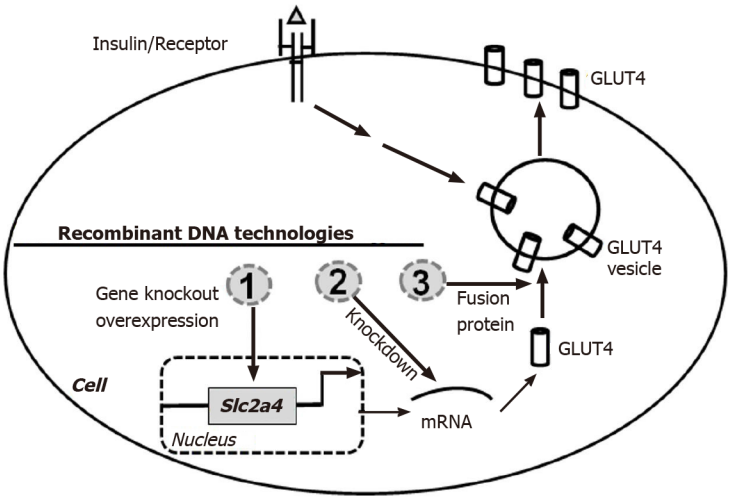

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Figure 1 Recombinant DNA technologies used in the study of glucose transporter 4 functions and its translocation mechanism. *Slc2a4* gene is transcribed into mRNA, which is translated into glucose transporter 4 (GLUT4) protein. The binding of insulin to its receptor leads to the activation of insulin signaling system, which facilitates the movement of GLUT4 from its intracellular location to the cell membrane, and in turn the entry of glucose in the cells. Recombinant DNA technologies have been used to alter gene expression level (1), reduce mRNA translation (2) and tracing intracellular movement of GLUT4 protein (3). 1. *Slc2a4* gene has been deleted in the whole body *via* homologous recombination and in individual tissues or cells *via* Cre-LoxP system driven by tissue specific promoters. In addition, transgenic overexpression of GLUT4 in whole body or specific tissues and cells has been done using mini gene or *SLC2A4* cDNA driven by different promoters, respectively. 2. The GLUT4 protein is knocked down using short-hairpin RNA under the control of different promoters to interfere the translation process. 3. Fusion or tagged GLUT4 has been created to study the insulin-stimulated GLUT4 translocation mechanism using fluorescent microscopy, and immune assays.

However, adipose-G4KO mice have glucose intolerance[35]. Interestingly, unlike other GLUT4 knockout mice, the heart weight of adipose-G4KO mice is normal.

In addition, adipose and muscle *Slc2a4* double knockout (AMG4KO) mice are also created by crossing the respective tissue knockout mice[37]. Interestingly, these AMG4KO mice develop hyperglycemia in the fasting state[37]. It appears that GLUT4 also plays a role in a physiological condition that does not need the insulin-stimulated glucose uptake.

GLUT4 knockdown studies

The deletion of a gene completely stops the genetic information flow. Another way to block the protein expression is to knockdown a gene's expression, which temporarily stops or reduces the expression of the targeted gene. Unlike knockout, gene knockdown involves the methods interfering with RNA molecules (mRNA or non-coding RNA) that bridges DNA and proteins. "GLUT4, knockdown" and "*SLC2A4*, knockdown" as keywords were used to search the PubMed database to retrieve relevant articles. Table 2 summarizes the methods of knockdown and confirmation, cells used, observations of GLUT4 knockdown studies.

Recently, RNAi has emerged as a powerful tool for the study of gene function in mammalian cells [38]. After transfection, the shRNAs molecules are transcribed under promoters in constructs that drive the RNA synthesis within the targeted cells. Oligo nucleotides with sequences of shRNAs may be transfected directly into the cells[38]. In all studies summarized in Table 2, shRNAs method is used to achieve GLUT4 knockdown[39-41], which is delivered *via* recombinant retroviruses. Two of three studies investigated the roles of GLUT4 in 3T3-L1 adipocytes. It appears that GLUT4 expression is needed for the lipogenesis after differentiation in 3T3-L1 cells, but not necessary for lipogenesis during differentiation[41]. In addition, the insulin-regulated aminopeptidase trafficking is not always associated with the GLUT4 movement[40].

Transgenic studies

We have used key words "GLUT4 transgenic" (314 hits) and "GLUT4 overexpression" (609 hit) to search PubMed to retrieve GLUT4 transgenic studies. After going through the titles or abstracts containing "GLUT4 overexpression and GLUT4 transgenic", we found 15 papers that have described their original methods or clearly cited the methods used by them, confirmed the GLUT4 overexpression in mice and provided results. Table 3 summarizes the techniques used to overexpress GLUT4, the methods to confirm the expression and results observed in those animals.

In 1992, a 2.4-kb fragment of 5′ flanking DNA of human *SLC2A4* promoter fused with the bacterial chloramphenicol acetyltransferase (CAT) as a reporter construct was developed and used to show the *SLC2A4* expression profile in mice[42]. In 1993, an 11.5-kb *SLC2A4* mini gene in pHSS6 vector was created and used to overexpress human GLUT4 in the whole body of mice[43]. As shown in Table 3, the

Table 2 Methods used to knockdown glucose transporter 4 and analyze its expression in cell lines, and reported observations

Methods	Cells	Analysis	Observations	Ref.
Recombinant lentivirus was used to express shRNA based on *SLC2A4* sequence (NM_001042.3)	Human head and neck squamous cancer cell lines, HSC-2	Western blot for GLUT4 protein using antibody from Epitomics	The knockdown of GLUT4 expression in HSC-2 cells induced DDX58 and OASL protein expressions, and reduced cell migration in culture	[39]
pSIREN RetroQ system was used to obtain recombinant retroviruses that produce shRNAs corresponding to mouse *Slc2a4* sequence GGTGATTGAACAGAGCTAC (GenBank ID was not provided)	3T3-L1 adipocytes	Immunofluorescence of phase-contrast and epifluorescence images for GLUT4 protein using antibodies (rabbit anti-GLUT4, a gift from Dr. Sam Cushman (National Institutes of Health)	GLUT4 knockdown does not affect IRAP trafficking, showing that IRAP traffics is independent of GLUT4	[40]
Recombinant lentivirus was used to generate shRNA under the control of human H1-RNA promoter using the mouse *Slc2a4* mRNA sequence (GenBank ID not provided)	3T3-L1 adipocytes	Immunofluorescence microscopy and Western blot for GLUT4 using rabbit polyclonal antibody from Chemicon International Inc	GLUT4 knockdown in 3T3-L1 adipocytes reduces insulin-stimulated glucose uptake by 50%-60%, IRAP expression of depending on differentiation stage, and lipogenic capacity of differentiated, but not differentiating cells	[41]

DDX58: DExD/H-Box Helicase 58; GLUT4: Glucose transporter 4; IRAP: Insulin-regulated aminopeptidase; OASL: 2′-5′-Oligoadenylate Synthetase Like; Ref: References; shRNA: Short hairpin RNA.

mouse line containing the 11.5-kb mini gene has been used in seven out of eight papers testing the effects of overexpression of human GLUT4 in the mouse whole body on metabolism[43-49]. CAT activity, *SLC2A4* mRNA and/or GLUT4 protein level in adipose tissue and skeletal muscle and other tissues have been analyzed to confirm the success of transgenic expression[43,44]. In general, the whole-body human GLUT4 overexpression reduces blood glucose in both fasting and fed states and increased glucose uptake in mice, but affects the blood insulin level in wild type mice and diabetic mice differentially[44]. Whole body GLUT4 overexpression does not alter body weight, but can reduce blood glucose level and affect serum insulin level.

GLUT4 has been overexpressed in a tissue specific manner as shows in Table 4. Majority of the studies have been focused on adipose tissues[50-54]. There is one for the skeletal muscle[55] and one for adipocytes[56]. A 6.3-kb genomic DNA fragment of human *SLC2A4* gene driven by the mouse *ap2*[50] promoter has been used to overexpress GLUT4 in mouse adipose tissues. This method was first published in 1993[50] and was used in many other studies in the genetic settings of wild-type and diabetic mice[51-54]. Like the papers summarized in Table 3, a human *SLC2A4* gene under a tissue-specific promoter was used to overexpress GLUT4. Human *SLC2A4* cDNA was also used to overexpress GLUT4 in adipocytes[56], but mouse *Slc2a4* gene was used to express GLUT4 in the hindlimb muscle [55]. All adipose tissue specific GLUT4 overexpression studies[50-55] tested the *SLC2A4* mRNA or GLUT4 protein level to confirm GLUT4 expression, which is found to be expressed in both brown and white adipose tissues. In conclusion, adipose tissue specific GLUT4 overexpression in mice can cause increases in body and adipose tissue weights[50,51]. The adipose tissue specific GLUT4 transgenic mice also have higher glucose disposal rate, may be caused by increased basal and insulin-stimulated glucose transport rate. It is interesting to find out that the elevated expression of GLUT4 in the adipose tissue only can increase glucose transport rate and adipose tissue weight, which is associated with the significant increase in body weight, suggesting the importance of GLUT4 expression in adipose tissue.

CONCLUSION

Conclusion and future perspectives

As summarized in this review, methods such as whole body and tissue specific gene knockout, recombinant viruses, real-time PCR, immunofluorescence, stable cell line and transgenic animals have been used to study GLUT4 system and insulin action in different target cells and tissues. The advantages of using multiple molecular biology methods allow us to confirm the functions of GLUT4 for insulin-stimulated glucose transport in different cells and tissues, and in the regulation of whole-body glucose homeostasis. Interestingly, GLUT4-null mice which do not have a functional *Slc2a4* gene in the whole genome have normal glucose tolerance and basal glucose turnover rates, but they are insulin-intolerant which suggests insulin resistance[31]. AMG4KO mice (adipose and muscle double knockout) have reduced whole body glucose uptake and hyperglycemia[37]. Compared with GLUT4-null mice, AMG4KO mice have more severe glucose homeostasis defects. Although the explanation for this difference is not clear, differences in genetic background and differences in developmental stages, where GLUT4 is deleted have been proposed. More importantly, the hyperglycemia in these double

Table 3 The transgenic studies using the *SLC2A4* mini gene and its promoter for the whole-body expression in mice

Transgenic constructs	Analysis	Observations	Ref.
A 11.5-kb mini gene of human *SLC2A4* starts with a 5.3-kb fragment upstream of transcription start and terminates within exon 10 of the gene followed by the bacterial CAT in pHSS6 vector	RNase protection assay and Western blot were used for SLC2A4 mRNA and GLUT4 protein in BAT, WAT, heart and skeleton muscle, respectively	The transgene expression was detected in WAT and BAT, heart and skeleton muscle of mice. Female transgenic mice have higher GLUT4 protein in the adipose tissue and less *SLC2A4* mRNA in skeleton muscle than male ones. Transgenic mice have higher GLUT4 protein level in adipose tissue, liver, heart and skeleton muscle than the controls	[43]
The 11.5-kb minigene with the CAT reporter as shown in[43]	Reverse transcription PCR was used to measure *SLC2A4* mRNA in cardiac and hindquarter muscle, BAT and WAT. Immunofluorescent test was for GLUT4 translocation	Transgenic mice gained more weight after 15 wk old of age, and have lower blood glucose in both fasting and fed states, lower insulin level in fasting and higher after refeeding, and higher glycogen contents, GLUT4 translocation in cardiac and skeleton muscle than the control mice	[44]
The 11.5 kb minigene with the CAT reporter as shown in[43]	Western blot was used to detect GLUT4 in gastrocnemius muscles	Transgenic mice have lower serum glucose level in both fasting and fed state, higher insulin level during fasting and lower after fed than the control ones	[45]
The 11.5 kb minigene with the CAT reporter as shown in[43]	Western blot was used to detect GLUT4 in the heart	Transgenic mice have higher glucose uptake, glycolysis and glycogen content, and lower insulin-stimulated glycolysis rate and glycogen synthesis in the heart than the control ones. Glucose and fatty acid oxidation remain the same	[46]
The 11.5 kb minigene with the CAT reporter as shown in[43]	Immunofluorescence was used to detect GLUT4 in cardiac myocytes and adipocytes	Transgenic mice have similar body weight, and epididymal adipose tissue weight and adipocyte size as the controls. Transgenic mice have higher levels of triglycerides, β-hydroxybutyrate and free fatty acids, and parametrial fat weight and lower glucose level after an oral glucose challenge and insulin level after an insulin injection than the controls. The insulin-stimulated glucose uptake is impaired in transgenic mice	[47]
A 2.4-kb of 5′ flanking DNA fragment of human *SLC2A4* promoter fused with the CAT as a reporter construct	CAT activity assay and RNase protection assay were used to detect promoter activation and mRNA, respectively	In transgenic mice, CAT activity can be detected in the tissues that generally express GLUT4, including BAT and WAT, and smooth, skeleton and cardiac muscle, but not the liver	[42]
A 2.4-kb of 5′ flanking DNA of human *SLC2A4* promoter fused to CAT as shown in[42]	Western blot was used to detect GLUT4 in adipose and skeleton muscle tissues	Transgenic mice have slower rise of blood glucose (no difference in glucose and insulin levels) during pentobarbital sodium anesthesia, and higher glucose infusion rate (40% increase) during hyper insulinemic euglycemic clamp than the controls	[48]
A 2.4-kb of 5 flanking DNA of human *SLC2A4* promoter fused to CAT as shown in[42]	Only cited previous publications[42]	Transgenic mice have lower blood glucose, higher lactate and β-hydroxybutyrate levels during both fasting and fed states, and better glucose transport in the soleus muscle when fed a high-fat and high-sugar diet than the controls	[49]

BAT: Brown adipose tissue; CAT: Chloramphenicol acetyltransferase; GLUT4: Glucose transporter 4; Ref: References; WAT: White adipose tissue.

knockout mice develops in the fasting state, rather than fed state[37]. This phenomenon appears to indicate that GLUT4 plays an important role in the control of glucose homeostasis during fasting, a state that insulin level is low. The translational value of these observations is that GLUT4's physiological role from the integrated homeostatic point of view may be extended beyond the insulin-stimulated glucose uptake. Of course, more studies are warranted on this line of research.

On the other hand, the GLUT4 knockdown studies used the shRNAs method and have been done in cell lines to reduce GLUT4 expression. This may be helpful for us to understand the GLUT4 functions and the underlying mechanisms in particular cells. It appears that GLUT4 expression is not necessary for lipogenesis during 3T3-L1 cells differentiation. Apparently, it will be helpful when more GLUT4 knockdown studies are done in animals.

The GLUT4 overexpression in transgenic mice at wholebody level reduces blood glucose in both fasting and fed states and increased glucose uptake, glycolysis and glycogen level[44]. Compared to the control mice, overexpression of GLUT4 in adipose tissue in mice leads to lowered blood glucose in the fasting state, and increase in body weight and adipose tissue weight[50]. The expression of GLUT4 in adipose tissue and skeleton muscle affects the rate of whole-body glucose disposal, which may be caused by increased basal and insulin-stimulated glucose transport rates. This lowered blood glucose level in the transgenic mice also indicates that GLUT4 probably plays a role in the basal glucose uptake.

For the tissue specific GLUT4 knockout, Cre-loxP–mediated gene recombination under the control of promoters has been the main method to delete *Slc2a4* gene. Since the development of CRISPR technology, it has not been used to knockout *Slc2a4* in whole body or tissues, which is a limitation in the field. We have used "GLUT4" and "CRISPR", and "*SLC2A4*" and "CRISPR" as key words to search PubMed, and retrieved eight and two published articles, respectively. However, none of the published articles used the CRISPR methods to knockout *SLC2A4* or *Slc2A4* gene in cells or animals. All of them used CRISPR methods to study the components in the exocytosis process of GLUT4 translocation. As

Table 4 Recombinant DNA techniques to create tissue specific glucose transporter 4 overexpression in animals and cells, analysis performed, and observations reported

Techniques	Tissue/analysis	Observations	Ref.
A 6.3-kb genomic DNA fragment of human *SLC2A4* gene is under the control of a 5.4-kb' DNA fragment of mouse ap2 promoter using Gateway cloning	Adipose-specific overexpression/ Western blot was used to detect GLUT4 in BAT and WAT	Transgenic mice have lower glucose level in the fasting, insulin level in the fed state, higher body weight and body fat at 18 to 21 wk of age, and higher basal and insulin-stimulated glucose transport rates in epididymal, parametrial, and subcutaneous adipocytes than the controls	[50]
Same as in[50]	Adipose-specific overexpression/Only cited previous publications[50]	Transgenic mice have higher body weight, parametrial fat pad weight and adipocyte size, and glucose transport in both fasting and fed states, and lower plasma insulin and glucose levels after a glucose challenge than the controls	[51]
Same as in[50]	Adipose-specific overexpression/Only cited previous publications[50]	Transgenic mice have higher glucose disposal rate in a glucose tolerance test, and palmitic acid-hydroxy stearic acid levels in serum, WAT and BAT than the controls	[52]
Same as in[50]	Adipose-specific overexpression/Western blot was used to detect GLUT4 in BAT and WAT	Transgenic mice fed a high-fat diet have higher glucose disposal rate than those fed a low-fat diet, and stable GLUT4 expression in fat and no increase in body fat	[53]
Same as in[50]	Adipose-specific overexpression/Western blot was used to detect GLUT4 in BAT and WAT	Transgenic mice have higher gonadal adipose weight, basal and maximum insulin stimulated glucose transport in isolated adipocytes, glucose transport rate, triglyceride synthesis and CO_2 production than the controls	[54]
A 4.5-kb DNA fragment of the mouse *Slc4a2* gene is under the control of a 3-kb fragment of the mouse myosin light chain gene promoter	Hindlimb muscle overexpression/Northern blot and Western blot were used to detect *Slc4a2*mRNA and GLUT4 in different tissues respectively	Transgenic mice have higher basal and insulin-stimulated glucose uptake and turnover, higher glycogen content in the skeleton muscle, higher insulin sensitivity, higher levels of free fatty acid and ketone in both fasting and fed state, and lower fasting glucose level than the controls	[55]
The human *SLC2A4* cDNA is driven by the CMV promoter in pCIS2 vector	Rat adipocytes overexpression/Immunofluorescence was used to detect GLUT4 overexpression	Rat adipose cell transfected with the GLUT4 construct had significantly higher antibody binding after insulin stimulation than the control cells	[56]

BAT: Brown adipose tissue; CMV: Cytomegalovirus; GLUT4: Glucose transporter 4; Ref: References; WAT: White adipose tissue.

CRISPR has been developed and used widely, GLUT4 knockout/knockdown through this system may be worth to be done. This may provide us another tool to manipulate the GLUT4 expression in the whole body or in tissue specific manners.

In addition, results of glucose tolerance are different between mice with whole body and tissue specific GLUT4 knockout. Therefore, whether the loss of GLUT4 in a specific tissue (muscle or fat) or the expression of GLUT4 in other tissues without gene deletion plays a role in this difference is worth to be investigated. It is safe to say that more research works are anticipated in the future to precisely define the role of GLUT4 in the control of glucose homeostasis at whole body and tissue levels. In so doing, we develop effective ways to prevent and treat type 2 diabetes mellitus.

FOOTNOTES

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REFERENCES

1 **Nathans D**, Smith HO. Restriction endonucleases in the analysis and restructuring of dna molecules. *Annu Rev Biochem* 1975; **44**: 273-293 [PMID: 166604 DOI: 10.1146/annurev.bi.44.070175.001421]
2 **Jackson DA**, Symons RH, Berg P. Biochemical method for inserting new genetic information into DNA of Simian Virus 40: circular SV40 DNA molecules containing lambda phage genes and the galactose operon of Escherichia coli. *Proc Natl Acad Sci U S A* 1972; **69**: 2904-2909 [PMID: 4342968 DOI: 10.1073/pnas.69.10.2904]
3 **Yoon BK**, Kim JW. How to Begin Molecular Research of Metabolic Diseases. *Endocrinol Metab (Seoul)* 2016; **31**: 505-509 [PMID: 28029022 DOI: 10.3803/EnM.2016.31.4.505]
4 **Huang S**, Czech MP. The GLUT4 glucose transporter. *Cell Metab* 2007; **5**: 237-252 [PMID: 17403369 DOI: 10.1016/j.cmet.2007.03.006]
5 **Elbrink J**, Bihler I. Membrane Transport: Its Relation to Cellular Metabolic Rates: Glucose transport into animal cells is adapted to their metabolic rate and often controls rates of glucose use. *Science* 1975; **188**: 1177-84
6 **Birnbaum MJ**, Identification of a novel gene encoding an insulin-responsive glucose transporter protein. Cell 1989; 57: 305-315 [PMID: 2649253 DOI: 10.1016/0092-8674(89)90968-9]
7 **Bell GI**, Murray JC, Nakamura Y, Kayano T, Eddy RL, Fan YS, Byers MG, Shows TB. Polymorphic human insulin-responsive glucose-transporter gene on chromosome 17p13. *Diabetes* 1989; **38**: 1072-1075 [PMID: 2568955 DOI: 10.2337/diab.38.8.1072]
8 **James DE**, Strube M, Mueckler M. Molecular cloning and characterization of an insulin-regulatable glucose transporter. *Nature* 1989; **338**: 83-87 [PMID: 2645527 DOI: 10.1038/338083a0]
9 **Wang T**, Wang J, Hu X, Huang XJ, Chen GX. Current understanding of glucose transporter 4 expression and functional mechanisms. *World J Biol Chem* 2020; **11**: 76-98 [PMID: 33274014 DOI: 10.4331/wjbc.v11.i3.76]
10 **Vargas E**, Podder V, Carrillo Sepulveda MA. Physiology, Glucose Transporter Type 4. 2021 May 9. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan- [PMID: 30726007]
11 **Thorens B**, Mueckler M. Glucose transporters in the 21st Century. *Am J Physiol Endocrinol Metab* 2010; **298**: E141-E145 [PMID: 20009031 DOI: 10.1152/ajpendo.00712.2009]
12 **Bryant NJ**, Govers R, James DE. Regulated transport of the glucose transporter GLUT4. *Nat Rev Mol Cell Biol* 2002; **3**: 267-277 [PMID: 11994746 DOI: 10.1038/nrm782]
13 **Shepherd PR**, Kahn BB. Glucose transporters and insulin action--implications for insulin resistance and diabetes mellitus. *N Engl J Med* 1999; **341**: 248-257 [PMID: 10413738 DOI: 10.1056/nejm199907223410406]
14 **Andréasson K**, Galuska D, Thörne A, Sonnenfeld T, Wallberg-Henriksson H. Decreased insulin-stimulated 3-0-methylglucose transport in in vitro incubated muscle strips from type II diabetic subjects. *Acta Physiol Scand* 1991; **142**: 255-260 [PMID: 1877373 DOI: 10.1111/j.1748-1716.1991.tb09154.x]
15 **Lee J**, Arun Kumar S, Jhan YY, Bishop CJ. Engineering DNA vaccines against infectious diseases. *Acta Biomater* 2018; **80**: 31-47 [PMID: 30172933 DOI: 10.1016/j.actbio.2018.08.033]
16 **Zhang YP**, Sun J, Ma Y. Biomanufacturing: history and perspective. *J Ind Microbiol Biotechnol* 2017; **44**: 773-784 [PMID: 27837351 DOI: 10.1007/s10295-016-1863-2]
17 **Khan S**, Ullah MW, Siddique R, Nabi G, Manan S, Yousaf M, Hou H. Role of Recombinant DNA Technology to Improve Life. *Int J Genomics* 2016; **2016**: 2405954 [PMID: 28053975 DOI: 10.1155/2016/2405954]
18 **Raymond FL**, Whittaker J, Jenkins L, Lench N, Chitty LS. Molecular prenatal diagnosis: the impact of modern technologies. *Prenat Diagn* 2010; **30**: 674-681 [PMID: 20572117 DOI: 10.1002/pd.2575]
19 **Gordon JW**, Ruddle FH. Integration and stable germ line transmission of genes injected into mouse pronuclei. *Science* 1981; **214**: 1244-1246 [PMID: 6272397 DOI: 10.1126/science.6272397]
20 **Bennett EP**, Petersen BL, Johansen IE, Niu Y, Yang Z, Chamberlain CA, Met Ö, Wandall HH, Frödin M. INDEL detection, the 'Achilles heel' of precise genome editing: a survey of methods for accurate profiling of gene editing induced indels. *Nucleic Acids Res* 2020; **48**: 11958-11981 [PMID: 33170255 DOI: 10.1093/nar/gkaa975]
21 **Jacobus AP**, Gross J. Optimal cloning of PCR fragments by homologous recombination in Escherichia coli. *PLoS One* 2015; **10**: e0119221 [PMID: 25774528 DOI: 10.1371/journal.pone.0119221]
22 **Li H**, Yang Y, Hong W, Huang M, Wu M, Zhao X. Applications of genome editing technology in the targeted therapy of human diseases: mechanisms, advances and prospects. *Signal Transduct Target Ther* 2020; **5**: 1 [PMID: 32296011 DOI: 10.1038/s41392-019-0089-y]
23 **LaFountaine JS**, Fathe K, Smyth HD. Delivery and therapeutic applications of gene editing technologies ZFNs, TALENs, and CRISPR/Cas9. *Int J Pharm* 2015; **494**: 180-194 [PMID: 26278489 DOI: 10.1016/j.ijpharm.2015.08.029]
24 **Behlke M**. Mini-review on current strategies to knockdown long non-coding RNAs. *J Rare Dis Res Treat* 2016; **1**: 66-70 [DOI: 10.29245/2572-9411/2016/3.1066]
25 **Winkle M**, El-Daly SM, Fabbri M, Calin GA. Noncoding RNA therapeutics - challenges and potential solutions. *Nat Rev Drug Discov* 2021; **20**: 629-651 [PMID: 34145432 DOI: 10.1038/s41573-021-00219-z]
26 **Paul S**, Bravo Vázquez LA, Uribe SP, Manzanero Cárdenas LA, Ruíz Aguilar MF, Chakraborty S, Sharma A. Roles of microRNAs in carbohydrate and lipid metabolism disorders and their therapeutic potential. *Biochimie* 2021; **187**: 83-93 [PMID: 34082043 DOI: 10.1016/j.biochi.2021.05.015]

27 **Kant Bhatia S**, Vivek N, Kumar V, Chandel N, Thakur M, Kumar D, Yang YH, Pugazendhi A, Kumar G. Molecular biology interventions for activity improvement and production of industrial enzymes. *Bioresour Technol* 2021; **324**: 124596 [PMID: 33440311 DOI: 10.1016/j.biortech.2020.124596]

28 **Costa S**, Almeida A, Castro A, Domingues L. Fusion tags for protein solubility, purification and immunogenicity in Escherichia coli: the novel Fh8 system. *Front Microbiol* 2014; **5**: 63 [PMID: 24600443 DOI: 10.3389/fmicb.2014.00063]

29 Huang S, Czech MP. The GLUT4 glucose transporter. *Cell Metab* 2007; **5**: 237-252 [PMID: 17403369 DOI: 10.1016/j.cmet.2007.03.006]

30 **Charron MJ**, Katz EB, Olson AL. GLUT4 gene regulation and manipulation. *J Biol Chem* 1999; **274**: 3253-3256 [PMID: 9920861 DOI: 10.1074/jbc.274.6.3253]

31 **Katz EB**, Stenbit AE, Hatton K, DePinho R, Charron MJ. Cardiac and adipose tissue abnormalities but not diabetes in mice deficient in GLUT4. *Nature* 1995; **377**: 151-155 [PMID: 7675081 DOI: 10.1038/377151a0]

32 **Tsao TS**, Stenbit AE, Li J, Houseknecht KL, Zierath JR, Katz EB, Charron MJ. Muscle-specific transgenic complementation of GLUT4-deficient mice. Effects on glucose but not lipid metabolism. *J Clin Invest* 1997; **100**: 671-677 [PMID: 9239415 DOI: 10.1172/jci119579]

33 **Abel ED**, Kaulbach HC, Tian R, Hopkins JC, Duffy J, Doetschman T, Minnemann T, Boers ME, Hadro E, Oberste-Berghaus C, Quist W, Lowell BB, Ingwall JS, Kahn BB. Cardiac hypertrophy with preserved contractile function after selective deletion of GLUT4 from the heart. *J Clin Invest* 1999; **104**: 1703-1714 [PMID: 10606624 DOI: 10.1172/JCI7605]

34 **Zisman A**, Peroni OD, Abel ED, Michael MD, Mauvais-Jarvis F, Lowell BB, Wojtaszewski JF, Hirshman MF, Virkamaki A, Goodyear LJ, Kahn CR, Kahn BB. Targeted disruption of the glucose transporter 4 selectively in muscle causes insulin resistance and glucose intolerance. *Nat Med* 2000; **6**: 924-928 [PMID: 10932232 DOI: 10.1038/78693]

35 **Abel ED**, Peroni O, Kim JK, Kim YB, Boss O, Hadro E, Minnemann T, Shulman GI, Kahn BB. Adipose-selective targeting of the GLUT4 gene impairs insulin action in muscle and liver. *Nature* 2001; **409**: 729-733 [PMID: 11217863 DOI: 10.1038/35055575]

36 **Reno CM**, Puente EC, Sheng Z, Daphna-Iken D, Bree AJ, Routh VH, Kahn BB, Fisher SJ. Brain GLUT4 Knockout Mice Have Impaired Glucose Tolerance, Decreased Insulin Sensitivity, and Impaired Hypoglycemic Counterregulation. *Diabetes* 2017; **66**: 587-597 [PMID: 27797912 DOI: 10.2337/db16-0917]

37 **Kotani K**, Peroni OD, Minokoshi Y, Boss O, Kahn BB. GLUT4 glucose transporter deficiency increases hepatic lipid production and peripheral lipid utilization. *J Clin Invest* 2004; **114**: 1666-1675 [PMID: 15578099 DOI: 10.1172/jci21341]

38 **Moore CB**, Guthrie EH, Huang MT, Taxman DJ. Short hairpin RNA (shRNA): design, delivery, and assessment of gene knockdown. *Methods Mol Biol* 2010; **629**: 141-158 [PMID: 20387148 DOI: 10.1007/978-1-60761-657-3_10]

39 **Chang YC**, Chi LH, Chang WM, Su CY, Lin YF, Chen CL, Chen MH, Chang PM, Wu AT, Hsiao M. Glucose transporter 4 promotes head and neck squamous cell carcinoma metastasis through the TRIM24-DDX58 axis. *J Hematol Oncol* 2017; **10**: 11 [PMID: 28061796 DOI: 10.1186/s13045-016-0372-0]

40 **Jordens I**, Molle D, Xiong W, Keller SR, McGraw TE. Insulin-regulated aminopeptidase is a key regulator of GLUT4 trafficking by controlling the sorting of GLUT4 from endosomes to specialized insulin-regulated vesicles. *Mol Biol Cell* 2010; **21**: 2034-2044 [PMID: 20410133 DOI: 10.1091/mbc.e10-02-0158]

41 **Liao W**, Nguyen MT, Imamura T, Singer O, Verma IM, Olefsky JM. Lentiviral short hairpin ribonucleic acid-mediated knockdown of GLUT4 in 3T3-L1 adipocytes. *Endocrinology* 2006; **147**: 2245-2252 [PMID: 16497797 DOI: 10.1210/en.2005-1638]

42 **Liu ML**, Olson AL, Moye-Rowley WS, Buse JB, Bell GI, Pessin JE. Expression and regulation of the human GLUT4/muscle-fat facilitative glucose transporter gene in transgenic mice. *J Biol Chem* 1992; **267**: 11673-11676 [PMID: 1601840]

43 **Olson AL**, Liu ML, Moye-Rowley WS, Buse JB, Bell GI, Pessin JE. Hormonal/metabolic regulation of the human GLUT4/muscle-fat facilitative glucose transporter gene in transgenic mice. *J Biol Chem* 1993; **268**: 9839-9846 [PMID: 8486663]

44 **Gibbs EM**, Stock JL, McCoid SC, Stukenbrok HA, Pessin JE, Stevenson RW, Milici AJ, McNeish JD. Glycemic improvement in diabetic db/db mice by overexpression of the human insulin-regulatable glucose transporter (GLUT4). *J Clin Invest* 1995; **95**: 1512-1518 [PMID: 7706456 DOI: 10.1172/jci117823]

45 **Brozinick JT Jr**, McCoid SC, Reynolds TH, Wilson CM, Stevenson RW, Cushman SW, Gibbs EM. Regulation of cell surface GLUT4 in skeletal muscle of transgenic mice. *Biochem J* 1997; **321** (Pt 1): 75-81 [PMID: 9003403 DOI: 10.1042/bj3210075]

46 **Belke DD**, Larsen TS, Gibbs EM, Severson DL. Glucose metabolism in perfused mouse hearts overexpressing human GLUT-4 glucose transporter. *Am J Physiol Endocrinol Metab* 2001; **280**: E420-E427 [PMID: 11171596 DOI: 10.1152/ajpendo.2001.280.3.E420]

47 **Liu ML**, Gibbs EM, McCoid SC, Milici AJ, Stukenbrok HA, McPherson RK, Treadway JL, Pessin JE. Transgenic mice expressing the human GLUT4/muscle-fat facilitative glucose transporter protein exhibit efficient glycemic control. *Proc Natl Acad Sci U S A* 1993; **90**: 11346-11350 [PMID: 8248251 DOI: 10.1073/pnas.90.23.11346]

48 **Marshall BA**, Mueckler MM. Differential effects of GLUT-1 or GLUT-4 overexpression on insulin responsiveness in transgenic mice. *Am J Physiol* 1994; **267**: E738-E744 [PMID: 7977725 DOI: 10.1152/ajpendo.1994.267.5.E738]

49 **Marshall BA**, Hansen PA, Ensor NJ, Ogden MA, Mueckler M. GLUT-1 or GLUT-4 transgenes in obese mice improve glucose tolerance but do not prevent insulin resistance. *Am J Physiol* 1999; **276**: E390-E400 [PMID: 9950801 DOI: 10.1152/ajpendo.1999.276.2.E390]

50 **Shepherd PR**, Gnudi L, Tozzo E, Yang H, Leach F, Kahn BB. Adipose cell hyperplasia and enhanced glucose disposal in transgenic mice overexpressing GLUT4 selectively in adipose tissue. *J Biol Chem* 1993; **268**: 22243-22246 [PMID: 8226728]

51 **Gnudi L**, Jensen DR, Tozzo E, Eckel RH, Kahn BB. Adipose-specific overexpression of GLUT-4 in transgenic mice alters lipoprotein lipase activity. *Am J Physiol* 1996; **270**: R785-R792 [PMID: 8967408 DOI: 10.1152/ajpregu.1996.270.4.R785]

52 **Yore MM**, Syed I, Moraes-Vieira PM, Zhang T, Herman MA, Homan EA, Patel RT, Lee J, Chen S, Peroni OD, Dhaneshwar AS, Hammarstedt A, Smith U, McGraw TE, Saghatelian A, Kahn BB. Discovery of a class of endogenous

mammalian lipids with anti-diabetic and anti-inflammatory effects. *Cell* 2014; **159**: 318-332 [PMID: 25303528 DOI: 10.1016/j.cell.2014.09.035]

53 **Gnudi L**, Tozzo E, Shepherd PR, Bliss JL, Kahn BB. High level overexpression of glucose transporter-4 driven by an adipose-specific promoter is maintained in transgenic mice on a high fat diet, but does not prevent impaired glucose tolerance. *Endocrinology* 1995; **136**: 995-1002 [PMID: 7867610 DOI: 10.1210/endo.136.3.7867610]

54 **Tozzo E**, Shepherd PR, Gnudi L, Kahn BB. Transgenic GLUT-4 overexpression in fat enhances glucose metabolism: preferential effect on fatty acid synthesis. *Am J Physiol* 1995; **268**: E956-E964 [PMID: 7762651 DOI: 10.1152/ajpendo.1995.268.5.E956]

55 **Tsao TS**, Burcelin R, Katz EB, Huang L, Charron MJ. Enhanced insulin action due to targeted GLUT4 overexpression exclusively in muscle. *Diabetes* 1996; **45**: 28-36 [PMID: 8522056 DOI: 10.2337/diab.45.1.28]

56 **Quon MJ**, Guerre-Millo M, Zarnowski MJ, Butte AJ, Em M, Cushman SW, Taylor SI. Tyrosine kinase-deficient mutant human insulin receptors (Met1153-->Ile) overexpressed in transfected rat adipose cells fail to mediate translocation of epitope-tagged GLUT4. *Proc Natl Acad Sci U S A* 1994; **91**: 5587-5591 [PMID: 8202531 DOI: 10.1073/pnas.91.12.5587]

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META-ANALYSIS

Hepatitis C virus among blood donors and general population in Middle East and North Africa: Meta-analyses and meta-regressions

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Abstract

BACKGROUND

Despite the Middle East and North Africa (MENA) Region reported to have the highest prevalence of hepatitis C virus (HCV) globally, HCV infection levels in the majority of MENA countries remain inadequately characterized. Blood donor data have been previously used as a proxy to assess levels and trends of HCV in the general population, however, it is unclear how comparable these populations are in MENA and whether blood donors provide an appropriate proxy.

AIM

To delineate HCV epidemiology among blood donors and the general population in the MENA.

METHODS

The data source was the systematically gathered MENA HCV Epidemiology Synthesis Project Database. Random-effects meta-analyses and meta-regressions were conducted. For comparison, analyses were conducted for Europe, utilizing the Hepatitis C Prevalence Database of the European Centre for Disease Prevention and Control.

RESULTS

One thousand two hundred and thirteen HCV antibody prevalence measures and 84 viremic rate measures were analyzed for MENA. Three hundred and seventy-seven antibody prevalence measures were analyzed for Europe. In MENA, pooled mean prevalence was 1.58% [95% confidence interval (CI): 1.48%–1.69%] among blood donors and 4.49% (95%CI: 4.10%–4.90%) in the general population. In Europe, pooled prevalence was 0.11% (95%CI: 0.10%–0.13%) among blood donors and 1.59% (95%CI: 1.25%–1.97%) in the general population. Prevalence in the

general population was 1.72-fold (95%CI: 1.50–1.97) higher than that in blood donors in MENA, but it was 15.10-fold (95%CI: 11.48–19.86) higher in Europe. Prevalence was declining at a rate of 4% per year in both MENA and Europe [adjusted risk ratio: 0.96 (95%CI: 0.95–0.97) in MENA and 0.96 (95%CI: 0.92–0.99) in Europe]. Pooled mean viremic rate in MENA was 76.29% (95%CI: 67.64%–84.02%) among blood donors and 65.73% (95%CI: 61.03%–70.29%) in the general population.

CONCLUSION

Blood donor data provide a useful proxy for HCV infection in the wider population in MENA, but not Europe, and could improve HCV burden estimations and assess progress toward HCV elimination by 2030.

Key Words: Hepatitis C virus; Viral hepatitis; Blood donors; General population; Middle East and North Africa; Meta-analysis; Meta-regression

Core tip: We investigated hepatitis C virus (HCV) epidemiology among blood donors and the wider general population in Middle East and North Africa (MENA). For comparison, similar analyses were performed for Europe. Our results indicated that HCV antibody prevalence in the population of MENA and Europe appears to be declining by 4% per year. Blood donor data in MENA (but not in Europe) were found to provide a useful proxy for HCV infection levels and trends in the general population. Thus, the data can be utilized in HCV estimates and to assess, track and validate progress towards World Health Organization elimination goals for HCV.

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INTRODUCTION

In the Middle East and North Africa (MENA) an estimated 15 million individuals are chronically infected with hepatitis C virus (HCV), making it the global region most affected by HCV infection[1]. Left untreated, chronic HCV infection may lead to several morbidities, including liver cancer, fibrosis, and cirrhosis[2]. Prompted by development of highly efficacious direct-acting antivirals (DAAs), the World Health Organization (WHO) has set a global target to eliminate HCV as a public health problem by 2030[3].

Despite disproportionally high HCV infection levels in specific MENA countries, *e.g.*, Egypt[4-7] and Pakistan[8-11], relative to global levels[1,12], only three countries in this region have conducted nationally representative population-based surveys[13-15]. HCV infection levels in the remaining countries remain inadequately characterized[1].

Blood donors have been used as a proxy population to provide a crude estimate of HCV infection levels in the general population[16,17]. However, in developed countries, such as the United States[18] and countries of the European Union[16], blood donors are not considered representative of the wider general population. In these countries, strict donor selection and blood safety regulations[19] have resulted in a large disparity in HCV infection levels between blood donors and the general populations. This raises two questions: How comparable are HCV infection levels between blood donors and the general population in MENA? Are blood donor data, which are readily available, thanks to blood screening, an appropriate proxy for the general population in this region?

In this context, objectives of this study were to delineate HCV epidemiology in blood donors and general populations in MENA, and to assess how representative blood donor data are of HCV antibody (Ab) prevalence in the general population of this region. The study was also conducted to infer programmatic implications on blood safety in the region. These objectives were accomplished through analyses of a large, systematically gathered database, including 2622 HCV Ab prevalence measures on 49.8 million individuals by: (1) Estimating the pooled mean prevalence among blood donors and in general populations (henceforth the general population); and (2) Identifying predictors and trends of prevalence in these populations and sources of between-study heterogeneity. We further conducted

similar analyses for Europe, a region in which stringent donor selection and blood safety processes have been implemented[19], for comparison. We did so by utilizing a large systematically gathered database including 419 HCV Ab prevalence measures for 25.7 million individuals[20], to compare outcomes with results for MENA.

MATERIALS AND METHODS

Data sources

This study was conducted as part of the MENA HCV Epidemiology Synthesis Project[1], an ongoing project with the aim of delineating HCV epidemiology and informing key public health research, policy, and programming priorities in MENA. The source of data was the MENA HCV Epidemiology Synthesis Project Database[1]. The database included 685 HCV Ab prevalence measures on 46 634 214 blood donors and 528 measures on 2 358 944 individuals of the general population, such as pregnant women, healthy adults, and children. The database also included eight HCV viremic rate measures on 58 986 blood donors and 76 measures on 14 936 individuals of the general population. HCV viremic rate was defined as the proportion of those who had tested Ab positive that are subsequently confirmed to be chronically infected by testing positive for HCV RNA – the proportion of those HCV RNA positive among HCV Ab-positive individuals[21,22].

The database was populated through a series of systematic reviews for HCV infection across MENA that were previously conducted as part of this project[5,6,8,23-28]. All reviews followed a standardized methodology, and specific details such as literature search strategy, databases searched, and eligibility criteria can be found in each of these reviews[5,6,8,23-28]. The methodology used for these reviews was informed by the Cochrane Collaboration Handbook[29], and all findings were reported using the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA)[30]. Literature searches were conducted to identify primary data on HCV measures in international and national/regional databases, the MENA HIV/AIDS Epidemiology Synthesis Project Database[31,32], abstract archives of international conferences, and grey literature, including public health reports and routine data reporting. Literature searches were broad, with no language restrictions to ensure inclusiveness. All records reporting HCV measures after 1989, the year in which the virus was officially identified[33], were included in the reviews[5,6,8,23-28].

Blood donors are typically a diverse group with different rates of HCV Ab prevalence depending on the rigor of the donor selection process[19]. The vast majority of HCV Ab prevalence studies in MENA did not specify the type of blood donors, and therefore the term blood donors in the present analysis encompassed the different blood donor types, including regular voluntary nonremunerated donors, one-time voluntary nonremunerated donors, family replacement donors, and paid donors.

For the MENA HCV Epidemiology Synthesis Project, the MENA region was defined to include 24 countries (Figure 1). Given the distinctive nature of the HCV epidemics in Egypt[4-7] and Pakistan[8-11], relative to other MENA countries, separate analyses were performed for each of these countries.

HCV measures in blood donors and the general population were also analyzed for Europe, a region in which stringent donor selection and blood safety regulations have been implemented for decades [19], for comparison with MENA outcomes. Europe's HCV Ab prevalence measures were retrieved from the Hepatitis C Prevalence Database of the European Centre for Disease Prevention and Control [20]. The database was populated through a systematic review[16] and multiple reports[34,35]. The database included 257 HCV Ab prevalence measures on 25 232 790 blood donors and 120 measures on 410 444 individuals of the general population, such as pregnant women and healthy adults.

Pooled mean HCV Ab prevalence and viremic rate

Meta-analyses for countries and subregions were performed to pool HCV Ab prevalence in blood donors and the general population, whenever three or more measures were available, and a minimum sample size of 25 participants was met. Random-effects meta-analyses were performed using the DerSimonian–Laird method[36], with inverse-variance weighting to pool measures[36]. Freeman–Tukey type arcsine square-root transformation was used to stabilize the variance of each measure, factoring knowledge regarding the applicability of this transformation[37,38]. Heterogeneity was formally assessed[36]. Forest plots were generated and examined visually, and Cochran's Q-test was conducted. Statistical significance of heterogeneity was indicated whenever P was < 0.10[36,39]. The I^2 and its confidence interval (CI) were calculated to assess the percentage of variance that is explained by true differences in prevalence across studies, rather than chance[36]. Prediction intervals were also determined to describe the distribution of prevalence around the pooled mean estimate[36]. Meta-analyses were also used to estimate the pooled mean HCV viremic rate among blood donors and in the general population. Meta-analyses and forest plots were generated using R version 3.4.3.

Predictors, trends, and sources of between-study heterogeneity

Univariable and multivariable random-effects meta-regressions were conducted following established methodology[29]. A priori relevant independent variables in meta-regressions included subpopulation

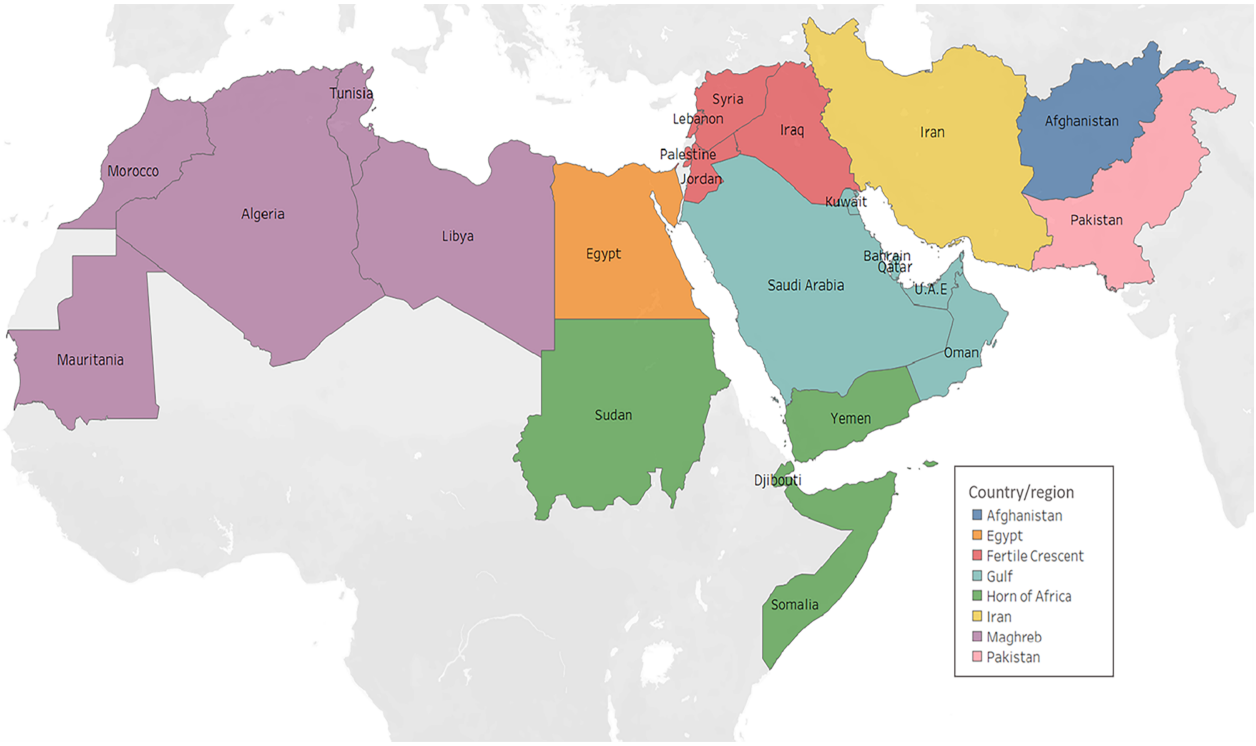

Figure 1 Map of the countries and subregions included in the Middle East and North Africa Region.

(blood donors *vs* the general population), country/subregion, and year of data collection. Factors associated with HCV Ab prevalence at $P \leq 0.20$ in the univariable analysis qualified for inclusion in the multivariable analysis. Here, an adjusted relative risk (ARR) $P \leq 0.05$ was considered to indicate strong evidence for an association.

In studies in which the year of data collection variable was missing, the variable was imputed. This was done by first subtracting the year of data collection (when available) from the year of publication for each study, and using the median of these values in imputing the year of data collection. Sensitivity analysis was performed without the imputed observations to determine the impact of the imputation on the results, confirming the results of the original meta-regression. Meta-regressions were performed on STATA version 13 using the *metan* command.

RESULTS

HCV Ab prevalence among blood donors and the general population in MENA

Studies on HCV Ab prevalence among blood donors and the general population in MENA are listed in Supplementary Tables 1 and 2. HCV Ab prevalence data were available for 23 of the 24 MENA countries. The largest number of data points were retrieved from Egypt, followed by the Gulf and Fertile Crescent Subregions. HCV Ab prevalence in blood donors ranged from 0 to 38.20%, with a median of 0.86% (Table 1). Studies reporting the highest HCV Ab prevalence were reported from parts of Egypt in the 1990s, a period during which HCV infection was widespread following the parenteral antischistosomal therapy (PAT) campaigns that contributed in a major way to the HCV epidemic in Egypt[5-7,40]. The pooled mean prevalence was 1.58% (95%CI: 1.48%–1.69%). It was lowest in the Fertile Crescent Subregion at 0.21% (95%CI: 0.18%–0.25%) and highest in Egypt at 10.40% (95%CI: 9.59%–11.23%), followed by Pakistan at 3.47% (95%CI: 2.96%–4.02%). HCV Ab prevalence in the general population ranged from 0 to 73.38%, with a median of 3.14%. The pooled mean prevalence was 4.49% (95%CI: 4.10%–4.90%). It was lowest in Iran at 0.33% (95%CI: 0.21%–0.47%) and highest in Egypt at 13.08% (95%CI: 11.51%–14.73%), followed by Pakistan at 8.81% (95%CI: 7.62%–10.06%). All outlier high HCV Ab prevalence measures were investigated and found to reflect blood donors or general populations in specific settings that were affected by high exposure to the virus, such as specific villages in the Nile delta in Egypt following the PAT era[5-7,40]. There was strong evidence for heterogeneity in HCV Ab prevalence in all meta-analyses ($P < 0.01$), with almost all variation being attributed to true variation in prevalence across studies rather than chance ($I^2 > 99.4\%$). Heterogeneity was also confirmed by the estimated prediction intervals (Table 1).

Table 1 Results of meta-analyses on studies reporting HCV Ab prevalence among blood donors and in the general population in Middle East and North Africa

Population	Studies	Samples	HCV Ab prevalence		Pooled HCV Ab prevalence		Heterogeneity measures		
	Total (*n*)	Total (*n*)	Range (%)	Median (%)	Mean (%)	95%CI	Q (*P* value)[1]	I^2 (confidence limits)[2]	Prediction interval (%)[3]
Blood donors									
Afghanistan	40	737407	0.00-7.19	0.60	0.75	0.57-0.96	3046.03 (*P* < 0.01)	98.7% (98.6%-98.9%)	0.02-2.41
Egypt	116	1566669	0.00-38.20	10.97	10.40	9.59-11.23	24513.7 (*P* < 0.01)	99.5% (99.5%-99.6%)	3.64-19.96
Fertile Crescent[4]	157	3488952	0.00-3.95	0.27	0.21	0.18-0.25	3674.2 (*P* < 0.01)	95.8% (95.4%-96.1%)	0.00-0.67
Gulf[5]	156	20891379	0.00-27.19	0.89	0.78	0.71-0.86	29882.0 (*P* < 0.01)	99.5% (99.5%-99.5%)	0.20-1.69
Horn of Africa[6]	22	48076	0.0-6.03	1.0	0.97	0.57-1.45	327.8 (*P* < 0.01)	93.6% (91.5%-95.2%)	0.00-3.78
Iran	73	15971802	0.00-3.13	0.24	0.31	0.22-0.40	55740.9 (*P* < 0.01)	99.9% (99.9%-99.9%)	0.00-1.43
Maghreb[7]	49	2145044	0.11-6.58	0.65	0.68	0.49-0.91	13475.9 (*P* < 0.01)	99.6% (99.6%-99.7%)	0.00-2.82
Pakistan	73	1797644	0.01-20.79	3.00	3.47	2.96-4.02	24753.7 (*P* < 0.01)	99.7% (99.7%-99.7%)	0.38-9.32
All countries/subregions	686	46646973	0.00-38.2	0.86	1.58	1.48-1.69	481819.0 (*P* < 0.01)	99.9% (99.9%-99.9%)	0.01-5.18
General population									
Afghanistan	6	12048	0.00-4.03	0.88	0.61	0.20-1.18	21.7 (*P* < 0.01)	76.9% (48.5%-89.6%)	0.00-2.68
Egypt	147	110603	0.00-54.10	11.82	13.08	11.51-14.73	8457.0 (*P* < 0.01)	98.3% (98.1-98.4%)	0.62-36.45
Fertile Crescent[4]	64	189456	0.00-8.87	0.19	0.42	0.24-0.64	1117.8 (*P* < 0.01)	94.4% (93.4%-95.2%)	0.00-2.39
Gulf[5]	85	222829	0.00-22.54	0.83	1.41	1.0-1.88	5343.3 (*P* < 0.01)	98.4% (98.3%-98.6%)	0.00-7.66
Horn of Africa[6]	27	29552	0.00-8.50	1.53	1.86	1.26-2.57	262.0 (*P* < 0.01)	90.1% (86.8%-92.6%)	0.00-6.13
Iran	50	101677	0.00-2.35	0.45	0.33	0.21-0.47	206.9 (*P* < 0.01)	76.3% (69.0%-81.9%)	0.00-1.25
Maghreb[7]	42	1378206	0.00-6.16	0.62	0.87	0.55-1.26	7595.3 (*P* < 0.01)	99.5% (99.5%-99.5%)	0.00-4.38
Pakistan	106	301814	0.44-73.38	6.82	8.81	7.62-10.06	13619.0 (*P* < 0.01)	99.2% (99.2%-99.3%)	0.60-24.62
All countries/subregions	527	2346185	0.00-73.38	3.14	4.49	4.10-4.90	83750.3 (*P* < 0.01)	99.4% (99.4%-99.4%)	0.00-16.88

[1]Q: the Cochran's Q statistic is a measure assessing the existence of heterogeneity in effect size (here, HCV Ab prevalence) across studies.
[2]I^2: A measure assessing the magnitude of between-study variation that is due to true differences in effect size (here, HCV Ab prevalence) across studies rather than chance.
[3]Prediction interval: a measure estimating the 95% interval of the distribution of true effect sizes (here, HCV Ab prevalence).
[4]Countries include Iraq, Jordan, Lebanon, Palestine, and Syria.
[5]Countries include Bahrain, Kuwait, Oman, Qatar, Saudi Arabia, and United Arab Emirates.
[6]Countries include Djibouti, Somalia, Sudan, and Yemen.
[7]Countries include Algeria, Libya, Mauritania, Morocco, and Tunisia. Ab: Antibody; CI: Confidence interval; HCV: Hepatitis C virus.

HCV Ab prevalence among blood donors and the general population in Europe

HCV Ab prevalence data were available for 30 countries in Europe. HCV Ab prevalence in blood donors ranged from 0 to 3.28%, with a median of 0.06% (Table 2). The pooled mean prevalence was 0.11%

Table 2 Results of meta-analyses on studies reporting HCV Ab prevalence among blood donors and in the general population in Europe

Subpopulation	Studies	Samples	HCV Ab prevalence		Pooled HCV Ab prevalence		Heterogeneity measures		
	Total (*n*)	Total (*n*)	Range (%)	Median (%)	Mean (%)	95%CI	Q (*P* value)[1]	I^2 (confidence limits)[2]	Prediction interval (%)[3]
Blood donors	257	25232790	0.0-3.28	0.06	0.11	0.10-0.13	35657.5 ($P < 0.01$)	99.3 (99.3-99.3)	0.00-0.51
The general population	120	410444	0.0-16.83	1.11	1.59	1.25-1.97	9176.9 ($P < 0.01$)	98.7 (98.6-98.8)	0.0-7.57

[1]Q: the Cochran's Q statistic is a measure assessing the existence of heterogeneity in effect size (here, HCV Ab prevalence) across studies.
[2]I^2: a measure assessing the magnitude of between-study variation that is due to true differences in effect size (here, HCV Ab prevalence) across studies rather than chance.
[3]Prediction interval: a measure estimating the 95% interval of the distribution of true effect sizes (here, HCV Ab prevalence). Ab: Antibody; CI: Confidence interval; HCV: Hepatitis C virus.

(95%CI: 0.10%–0.13%). Prevalence in the general population ranged from 0 to 16.83%, with a median of 1.11%. The pooled mean prevalence was 1.59% (95%CI: 1.25%–1.97%). There was strong evidence for heterogeneity in HCV Ab prevalence ($P < 0.01$), with the majority of variation being attributed to true variation in prevalence across studies rather than chance ($I^2 > 98.7\%$).

HCV viremic rate of blood donors and the general population

The HCV viremic rate of blood donors in different MENA countries ranged from 61.84% to 93.33%, with a median of 70.78% (Supplementary Table 3). The pooled mean for the entire MENA region was 76.29% (95%CI: 67.64%–84.02%), indicating that approximately three-quarters of Ab-positive blood donors are chronically infected. The viremic rate ranged from 22.73% to 100% in the general population in different MENA countries, with a median of 68.27% (Supplementary Table 3). The pooled mean for the entire MENA region was 65.73% (95%CI: 61.03%–70.29%). There was strong evidence for heterogeneity in the viremic rates ($P < 0.01$), with most variation being attributed to true variation in the viremic rate across studies rather than chance ($I^2 > 77.4\%$).

Predictors and trends of HCV Ab prevalence in MENA

The meta-regressions for MENA indicated that HCV Ab prevalence in the general population was 1.72-fold (95%CI: 1.50–1.97) higher than that in blood donors (Table 3). They also indicated substantial variation in prevalence by country and subregion with Egypt and Pakistan having a higher prevalence than the rest of MENA countries. Importantly, the analyses indicated that HCV Ab prevalence has been declining over the last three decades at an average rate of 4% per year (ARR of 0.96; 95%CI: 0.95–0.97). Subgroup analyses were conducted on the above results. The same regressions were repeated, but for Egypt, Pakistan and other MENA countries individually (Table 4). These analyses indicated that HCV Ab prevalence in the general population was 1.30-fold (95%CI: 1.07–1.59) higher than that among blood donors in Egypt, 2.52-fold (95%CI: 1.89–3.36) higher in Pakistan, and 1.73-fold (95%CI: 1.42–2.11) higher in the remaining MENA countries. The analyses also confirmed the same rate of decline for prevalence at 4% in the rest of MENA countries. The rate of decline was slightly higher in Egypt at 6%. There was no evidence for a decline in prevalence, however, in Pakistan.

In a sensitivity analysis, the same regressions were also repeated, but excluding all blood donor data (not shown). The analyses indicated that HCV Ab prevalence in MENA is declining at a rate of 5% per year (ARR of 0.95; 95%CI: 0.93–0.97), indicating a marginally higher rate of decline in the general population.

Predictors and trends of HCV Ab prevalence in Europe

The meta-regressions for Europe indicated that HCV Ab prevalence in the general population is 15.10-fold (95%CI: 11.48–19.86) higher than that in blood donors (Table 5). The analyses indicated further that HCV Ab prevalence has been declining over the last three decades at a similar rate to that of MENA, at 4% per year (ARR of 0.96; 95%CI: 0.92–0.99).

A sensitivity analysis was conducted on the above results. The same regressions were repeated, but excluding all blood donor data (not shown). The analyses indicated that HCV Ab prevalence in Europe is declining at a rate of 10% per year (ARR of 0.90; 95%CI: 0.85–0.96), higher than that in MENA.

Table 3 Univariable and multivariable meta-regression models for HCV Ab prevalence among blood donors and the general population in Middle East and North Africa

	Studies	Samples	Univariable analysis				Multivariable analysis[b]	
	Total (*n*)	Total (*n*)	RR (95%CI)	*P* value	F *P* value[a]	Variance explained R^2 (%)	ARR (95%CI)	*P* value
Subpopulations								
Blood donors	686	46646973	1	-			1	-
The general population	527	2346185	2.92 (2.41-3.55)	< 0.001	< 0.001	10.73	1.72 (1.50-1.97)	< 0.001
Country/subregion								
Afghanistan	46	749455	1	-			1	-
Egypt	263	1677272	14.89 (10.2-21.8)	< 0.001			9.48 (6.54-13.75)	< 0.001
Fertile Crescent[1]	221	3678408	0.52 (0.35-0.77)	< 0.001			0.49 (0.34-0.72)	< 0.001
Gulf[2]	241	21114208	1.24 (0.84-1.82)	0.280			0.82 (0.56-1.19)	0.398
Horn of Africa[3]	49	77628	2.05 (1.25-3.37)	0.005			1.33 (0.82-2.15)	0.244
Iran	123	16073479	0.50 (0.33-0.77)	< 0.001			0.42 (0.28-0.63)	< 0.001
Maghreb[4]	91	3523250	1.02 (0.66-1.56)	0.936			0.77 (0.51-1.16)	0.207
Pakistan	179	2099458	6.96 (4.71-10.29)	< 0.001	< 0.001	58.39	5.44 (3.73-7.93)	< 0.001
Year of data collection	1213	48993158	0.95 (0.94-0.97)	< 0.001	< 0.001	3.71	0.96 (0.95-0.97)	< 0.001

[1]Countries include Iraq, Jordan, Lebanon, Palestine, and Syria.
[2]Countries include Bahrain, Kuwait, Oman, Qatar, Saudi Arabia, and United Arab Emirates.
[3]Countries include Djibouti, Somalia, Sudan, and Yemen.
[4]Countries include Algeria, Libya, Mauritania, Morocco, and Tunisia.
[a]Variables with $P \leq 0.2$ were eligible for inclusion in the multivariable analysis.
[b]The adjusted R-squared for the full model was 62.65%.
Ab: Antibody; ARR: Adjusted relative risk; CI: Confidence interval; RR: Relative risk.

DISCUSSION

Levels and trends of HCV Ab prevalence in blood donors and in the general population of MENA were assessed using a large standardized database. There was large variability in HCV Ab prevalence by country and subregion, with Egypt and Pakistan, the largest countries in MENA by population size, having several fold higher prevalence than the rest of MENA countries. HCV Ab prevalence in the remaining MENA countries was at about 1% or less, similar to that in Europe and most other countries globally[12,41]. These results confirm our understanding of HCV epidemiology across MENA countries and subregions[4-11,21-28,42-49].

Strikingly, HCV Ab prevalence is declining rapidly in both MENA and Europe, and at a similar rate of about 4% per year. The exception to this downward trend was Pakistan where there was no evidence for a decline. These declines may be reflective, in part, of the declining incidence of HCV infection within these regions through improvements to infection control following the discovery of this virus three decades ago, and scale-up of HCV treatment worldwide[3]. They also may reflect the progressive improvement in effective blood donor selection, such as by motivating and retaining voluntary nonremunerated donors to donate regularly[19].

A major finding of this study is that HCV Ab prevalence in blood donors in MENA was similar to HCV Ab prevalence in the general population; unlike the situation in Europe. While HCV Ab prevalence in the general population was almost twofold higher than that of HCV Ab prevalence in blood donors in MENA, it was 15-fold higher in Europe (Table 3 *vs* Table 5). HCV Ab prevalence in blood donors in MENA appears to closely reflect the background prevalence in the wider population. Of note that HCV Ab prevalence in blood donors is a function of not only the prevalence in the general

Table 4 Subgroup analyses: Univariable and multivariable meta-regression models for HCV Ab prevalence among blood donors and the general population in Egypt, Pakistan, and rest of Middle East and North Africa countries

	Studies	Samples	Univariable analysis				Multivariable analysis[b]	
	Total (*n*)	Total (*n*)	RR (95%CI)	*P* value	F *P* value[a]	Variance explained R^2 (%)	ARR (95%CI)	*P* value
Egypt								
Subpopulations								
Blood donors	116	1566669	1	-			1	-
The general population	147	110603	1.25 (1.00-1.57)	0.049	0.087	2.05	1.30 (1.07-1.59)	0.008
Year of data collection	263	1677272	0.94 (0.93-0.95)	< 0.001	< 0.001	24.48	0.94 (0.93-0.95)	< 0.001
Pakistan								
Subpopulations								
Blood donors	73	1797644	1	-			-	-
The general population	106	301814	2.52 (1.89-3.36)	< 0.001	< 0.001	19.03	-	-
Year of data collection	179	2099458	1.00 (0.98-1.03)	0.648	0.648	0.00	-	-
Rest of MENA countries								
Subpopulations								
Blood donors	497	43282660	1	-	-		1	-
The general population	274	1933768	1.80 (1.47-2.21)	< 0.001	< 0.001	5.44	1.73 (1.42-2.11)	< 0.001
Country/subregion								
Afghanistan	46	749455	1	-	-		1	-
Fertile Crescent[1]	221	3678408	0.53 (0.35-0.81)	0.003			0.50 (0.33-0.75)	0.001
Gulf[2]	241	21114208	1.26 (0.83-1.91)	0.273			0.86 (0.56-1.30)	0.462
Horn of Africa[3]	49	77628	2.08 (1.22-3.54)	0.007			1.37 (0.81-2.32)	0.247
Iran	123	16073479	0.51 (0.33-0.81)	0.004			0.43 (0.28-0.67)	< 0.001
Maghreb[4]	91	3523250	1.04 (0.65-1.64)	0.883	< 0.001	11.48	0.79 (0.50-1.24)	0.298
Year of data collection	771	45216428	0.95 (0.94-0.96)	< 0.001	< 0.001	6.22	0.96 (0.95-0.98)	< 0.001

[1]Countries include Iraq, Jordan, Lebanon, Palestine, and Syria.
[2]Countries include Bahrain, Kuwait, Oman, Qatar, Saudi Arabia, and United Arab Emirates.
[3]Countries include Djibouti, Somalia, Sudan, and Yemen.
[4]Countries include Algeria, Libya, Mauritania, Morocco, and Tunisia.
[a]Variables with a *P* value ≤ 0.2 were eligible for inclusion in the multivariable analysis. No multivariable analysis was conducted for Pakistan, as only one variable qualified for inclusion in the model.
[b]The adjusted R-squared was 27.35% for the multivariable model for Egypt and 17.92% for the multivariable model for rest of MENA countries. Ab: Antibody; ARR: Adjusted relative risk; CI: Confidence interval; RR: Relative risk.

population, but also of the effectiveness of blood donation programs to collect blood from regular voluntary nonremunerated blood donors[19]. This finding suggests that risk reduction strategies through selection and retention of safer blood donors (regular voluntary nonremunerated blood donors) are not yet effectively implemented widely in MENA as in Europe[19], where the source of blood largely comes from such donors. Indeed regulatory framework (including legislation, regulation, policies and standards) and a functioning regulatory authority to enforce the regulatory framework is largely at its infancy in MENA[19], where, as of 2016, only 55% of MENA countries had legislation covering safety and quality of blood transfusions, and only two countries had achieved 100% voluntary nonremunerated blood donations[19,50]. Furthermore, HCV Ab prevalence in blood donors may be reflective of people in the general population unaware of their HCV infection status, in the context of which an individual aware of their positive HCV infection status would not donate blood.

Nevertheless, contingent on the quality of blood donor management systems implemented within countries of MENA, this finding indicates that HCV Ab prevalence in blood donors in MENA (unlike in North America[18] and Europe[16]) can be used for the time being as a proxy to estimate infection levels in the general population. This outcome has important consequences. With the lack of nationally repres-

Table 5 Univariable and multivariable meta-regression models for HCV Ab prevalence among blood donors and the general population in Europe

	Studies	Samples	Univariable analysis				Multivariable analysis[b]	
	Total (*n*)	Total (*n*)	RR (95%CI)	*P* value	F *P* value[a]	Variance explained R^2 (%)	ARR (95%CI)	*P* value
Subpopulations								
Blood donors	257	25232790	1				1	
The general population	120	410444	15.57 (11.83-20.49)	< 0.001	< 0.001	53.62	15.10 (11.48-19.86)	< 0.001
Year of data collection	377	25643234	0.93 (0.88-0.98)	0.004	0.005	2.17	0.96 (0.92-0.99)	0.020

[a]Variables with $P \leq 0.2$ were eligible for inclusion in the multivariable analysis.
[b]The adjusted R^2 for the full model was 54.27%.
Ab: Antibody; ARR: Adjusted relative risk; CI: Confidence interval; RR: Relative risk.

entative population-based surveys in most countries of this region, blood donor data, which are readily available, can be easily used to assess levels and trends of this infection in the wider population. They can also be used to generate other estimates, such as those related to the disease burden of HCV sequelae, and can be leveraged to assess, track and validate progress toward the WHO elimination goals for this infection[3]. The present study also provides adjustment factors to improve use of blood donor data (Table 2), so that they better reflect HCV levels in the wider population. These adjustment factors can be used at a regional level, or can be fine-tuned so as to be specific for individual countries.

This study had several limitations. Availability of data varied across MENA, with HCV Ab prevalence data being unavailable for Bahrain. The majority of HCV viremia data were collected at times before the launch of DAA treatment programs (Supplementary Table 3); thus, they may not represent the current viremic rate in blood donors and in the general population. Analysis for the different blood donor types was not conducted as the specification of blood donor type was not available for the vast majority of HCV Ab prevalence measures. The sample size of blood donors was larger than that of the general population; however, the sample size in the general population was still substantial at 2.3 million. Despite these limitations, an immense volume of data was acquired, allowing various analyses and an array of consequential inferences to be drawn. While high heterogeneity was found, most (63%) of it was subsequently explained in meta-regression analyses in terms of prevalence variation by country and subregion within MENA.

CONCLUSION

HCV Ab prevalence in the wider population of MENA and Europe appears to be rapidly declining by 4% per year. Blood donor data in MENA (but not in Europe) provide a useful proxy for HCV infection levels and trends in the general population; at least in countries where effective blood donor selection and blood donor management programs are not in place. Thus, the data can be utilized in HCV infection and disease burden estimates and to assess, track and validate progress toward WHO elimination goals for this infection. While these findings are specific for MENA, they may also apply to resource-limited countries of other regions.

ARTICLE HIGHLIGHTS

Research background

The Middle East and North Africa (MENA) Region is the most affected by hepatitis C virus (HCV) infection, with approximately 20% of the global chronically infected individuals residing in this region. Despite this, only three countries conducted national population-based surveys to delineate HCV infection levels in the general population.

Research motivation

HCV infection in blood donors have been used as a proxy for HCV infection levels in the general population. However, it is unclear how comparable blood donors are to the general population in countries in MENA and whether they are a suitable proxy population.

Research objectives
To delineate HCV epidemiology in blood donors and in the general population in MENA.

Research methods
The MENA HCV Epidemiology Synthesis Project Database was used as a data source. Studies reporting HCV in blood donors and in the general population were retrieved, and random-effects meta-analyses and random-effects meta-regressions were performed. For regional comparison, similar analyses were performed for countries in Europe, using the Hepatitis C Prevalence Database from the European Centre for Disease Prevention and Control (ECDC).

Research results
A total of 1213 HCV Ab prevalence measures and 84 viremic rate measures were retrieved from the MENA HCV Epidemiology Synthesis Project, and 377 HCV Ab prevalence measures were retrieved from the ECDC. The pooled mean prevalence in MENA was 1.58% [95% confidence interval (CI): 1.48%–1.69%] in blood donors and 4.49% (95%CI: 4.10%–4.90%) in the general population, and in Europe was 0.11% (95%CI: 0.10%–0.13%) among blood donors and 1.59% (95%CI: 1.25%–1.97%) in the general population. In MENA, the prevalence in the general population was 1.72-fold (95%CI: 1.50–1.97) higher than that in blood donors, and in Europe it was 15.10-fold (95%CI: 11.48–19.86) higher. HCV prevalence appeared to be declining by 4% annually in both MENA and Europe.

Research conclusions
Blood donor data in MENA (but not in Europe) appears to be comparable with that in the general population and therefore can be used as a useful proxy for HCV infection levels and trends in the general population, at least in countries where effective blood donor selection and blood donor management programs are not yet firmly in place. Blood donor data may be used to estimate HCV infection and disease burden and to assess, track, and validate progress toward World Health Organization elimination goals for this infection.

Research perspectives
With the lack of nationally representative population-based surveys in most countries in MENA and beyond, blood donor data, which are readily available, can be easily used to assess levels and trends of this infection in the wider population. The study rationalizes and facilitates generation of estimates at low costs and demands for resources, even in resource-limited settings where population-level data are most scarce. While these findings are specific for MENA, they may also apply and be of relevance to other global regions.

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FOOTNOTES

Author contributions: Mahmud S conducted data extraction and analysis, and wrote the first draft of the paper; Abu-Raddad L conceived and led the design of the study, analyses, and drafting of the article; All authors contributed to data collection and acquisition, and/or database development, and/or discussion and interpretation of the results, and to the writing of the manuscript.

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REFERENCES

1 **World Health Organization**. Epidemiology of hepatitis C virus in the WHO Eastern Mediterranean Region: implications for strategic action. [cited 20 May 2021]. Available from: https://apps.who.int/iris/bitstream/handle/10665/336174/9789290222866-eng.pdf
2 **Lauer GM**, Walker BD. Hepatitis C virus infection. *N Engl J Med* 2001; **345**: 41-52 [PMID: 11439948 DOI: 10.1056/NEJM200107053450107]
3 **World Health Organization**. Global health sector strategy on viral hepatitis 2016-2021. Towards ending viral hepatitis. [cited 20 May 2021]. Available from: https://apps.who.int/iris/bitstream/handle/10665/246177/WHO-HIV-2016.06-eng.pdf
4 **Ayoub HH**, Abu-Raddad LJ. Impact of treatment on hepatitis C virus transmission and incidence in Egypt: A case for treatment as prevention. *J Viral Hepat* 2017; **24**: 486-495 [PMID: 28039923 DOI: 10.1111/jvh.12671]
5 **Kouyoumjian SP**, Chemaitelly H, Abu-Raddad LJ. Characterizing hepatitis C virus epidemiology in Egypt: Systematic reviews, meta-analyses, and meta-regressions. *Sci Rep* 2018; **8**: 1661 [PMID: 29374178 DOI: 10.1038/s41598-017-17936-4]
6 **Mohamoud YA**, Mumtaz GR, Riome S, Miller D, Abu-Raddad LJ. The epidemiology of hepatitis C virus in Egypt: A systematic review and data synthesis. *BMC Infect Dis* 2013; **13**: 288 [PMID: 23799878 DOI: 10.1186/1471-2334-13-288]
7 **Ayoub HH**, Chemaitelly H, Kouyoumjian SP, Abu-Raddad LJ. Characterizing the historical role of parenteral antischistosomal therapy in hepatitis C virus transmission in Egypt. *Int J Epidemiol* 2020; **49**: 798-809 [PMID: 32357208 DOI: 10.1093/ije/dyaa052]
8 **Al Kanaani Z**, Mahmud S, Kouyoumjian SP, Abu-Raddad LJ. The epidemiology of hepatitis C virus in Pakistan: Systematic review and meta-analyses. *R Soc Open Sci* 2018; **5**: 180257 [PMID: 29765698 DOI: 10.1098/rsos.180257]
9 **Ayoub HH**, Abu-Raddad LJ. Treatment as prevention for hepatitis C virus in Pakistan: mathematical modelling projections. *BMJ Open* 2019; **9**: e026600 [PMID: 31133586 DOI: 10.1136/bmjopen-2018-026600]
10 **Mahmud S**, Al Kanaani Z, Abu-Raddad LJ. Characterization of the hepatitis C virus epidemic in Pakistan. *BMC Infect Dis* 2019; **19**: 809 [PMID: 31521121 DOI: 10.1186/s12879-019-4403-7]
11 **Ayoub HH**, Al Kanaani Z, Abu-Raddad LJ. Characterizing the temporal evolution of the hepatitis C virus epidemic in Pakistan. *J Viral Hepat* 2018; **25**: 670-679 [PMID: 29345847 DOI: 10.1111/jvh.12864]
12 **Gower E**, Estes C, Blach S, Razavi-Shearer K, Razavi H. Global epidemiology and genotype distribution of the hepatitis C virus infection. *J Hepatol* 2014; **61**: S45-S57 [PMID: 25086286 DOI: 10.1016/j.jhep.2014.07.027]
13 **Qureshi H**, Bile KM, Jooma R, Alam SE, Afridi HU. Prevalence of hepatitis B and C viral infections in Pakistan: findings of a national survey appealing for effective prevention and control measures. *East Mediterr Health J* 2010; **16** Suppl: S15-S23 [PMID: 21495584]
14 **El-Zanaty FH**, Way AA. Egypt demographic and health survey. 5th ed. Cairo: Ministry of Health and Population, 2005
15 **Daw MA**, El-Bouzedi A; In association with Libyan Study Group of Hepatitis & HIV. Prevalence of hepatitis B and hepatitis C infection in Libya: results from a national population based survey. *BMC Infect Dis* 2014; **14**: 17 [PMID: 24405790 DOI: 10.1186/1471-2334-14-17]
16 **Hofstraat SHI**, Falla AM, Duffell EF, Hahné SJM, Amato-Gauci AJ, Veldhuijzen IK, Tavoschi L. Current prevalence of chronic hepatitis B and C virus infection in the general population, blood donors and pregnant women in the EU/EEA: a systematic review. *Epidemiol Infect* 2017; **145**: 2873-2885 [PMID: 28891457 DOI: 10.1017/S0950268817001947]
17 **Tognon F**, Sevalie S, Gassimu J, Sesay J, Hann K, Sheku M, Bearse E, Di Gennaro F, Marotta C, Pellizzer G, Putoto G, Lado M, Franke MF, Dibba Y, Gevao S, Beynon F, Mesman AW. Seroprevalence of hepatitis B and hepatitis C among blood donors in Sierra Leone: A multi-year retrospective study. *Int J Infect Dis* 2020; **99**: 102-107 [PMID: 32717396 DOI: 10.1016/j.ijid.2020.07.030]
18 **Denniston MM**, Jiles RB, Drobeniuc J, Klevens RM, Ward JW, McQuillan GM, Holmberg SD. Chronic hepatitis C virus infection in the United States, National Health and Nutrition Examination Survey 2003 to 2010. *Ann Intern Med* 2014; **160**: 293-300 [PMID: 24737271 DOI: 10.7326/M13-1133]
19 **World Health Organization**. Global status report on blood safety and availability 2016. [cited 15 May 2021]. Available from: https://apps.who.int/iris/bitstream/handle/10665/254987/9789241565431-eng.pdf
20 **European Centre for Disease Prevention and Control**. Hepatitis C Prevalence Database. [cited 15 March 2021]. Available from: https://www.ecdc.europa.eu/en/all-topics-z/hepatitis-c/tools/hepatitis-c-prevalence-database
21 **Harfouche M**, Chemaitelly H, Kouyoumjian SP, Mahmud S, Chaabna K, Al-Kanaani Z, Abu-Raddad LJ. Hepatitis C virus

viremic rate in the Middle East and North Africa: Systematic synthesis, meta-analyses, and meta-regressions. *PLoS One* 2017; **12**: e0187177 [PMID: 29088252 DOI: 10.1371/journal.pone.0187177]

22 **Ayoub HH**, Chemaitelly H, Omori R, Abu-Raddad LJ. Hepatitis C virus infection spontaneous clearance: Has it been underestimated? *Int J Infect Dis* 2018; **75**: 60-66 [PMID: 30031139 DOI: 10.1016/j.ijid.2018.07.013]

23 **Chemaitelly H**, Chaabna K, Abu-Raddad LJ. The Epidemiology of Hepatitis C Virus in the Fertile Crescent: Systematic Review and Meta-Analysis. *PLoS One* 2015; **10**: e0135281 [PMID: 26296200 DOI: 10.1371/journal.pone.0135281]

24 **Chemaitelly H**, Mahmud S, Rahmani AM, Abu-Raddad LJ. The epidemiology of hepatitis C virus in Afghanistan: systematic review and meta-analysis. *Int J Infect Dis* 2015; **40**: 54-63 [PMID: 26417880 DOI: 10.1016/j.ijid.2015.09.011]

25 **Fadlalla FA**, Mohamoud YA, Mumtaz GR, Abu-Raddad LJ. The epidemiology of hepatitis C virus in the Maghreb region: systematic review and meta-analyses. *PLoS One* 2015; **10**: e0121873 [PMID: 25803848 DOI: 10.1371/journal.pone.0121873]

26 **Chaabna K**, Kouyoumjian SP, Abu-Raddad LJ. Hepatitis C Virus Epidemiology in Djibouti, Somalia, Sudan, and Yemen: Systematic Review and Meta-Analysis. *PLoS One* 2016; **11**: e0149966 [PMID: 26900839 DOI: 10.1371/journal.pone.0149966]

27 **Mohamoud YA**, Riome S, Abu-Raddad LJ. Epidemiology of hepatitis C virus in the Arabian Gulf countries: Systematic review and meta-analysis of prevalence. *Int J Infect Dis* 2016; **46**: 116-125 [PMID: 26996460 DOI: 10.1016/j.ijid.2016.03.012]

28 **Mahmud S**, Akbarzadeh V, Abu-Raddad LJ. The epidemiology of hepatitis C virus in Iran: Systematic review and meta-analyses. *Sci Rep* 2018; **8**: 150 [PMID: 29317673 DOI: 10.1038/s41598-017-18296-9]

29 **Higgins J**, Green S. Cochrane handbook for systematic reviews of interventions. John Wiley & Sons, 2011

30 **Moher D**, Liberati A, Tetzlaff J, Altman DG; PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med* 2009; **6**: e1000097 [PMID: 19621072 DOI: 10.1371/journal.pmed.1000097]

31 **Abu-Raddad L AF**, Semini I, Reidner G, Wilson D, Tavil O. Characterizing the HIV/AIDS epidemic in the Middle East and North Africa: Time for strategic action. Washington DC: The World Bank Press, 2010

32 **Abu-Raddad LJ**, Hilmi N, Mumtaz G, Benkirane M, Akala FA, Riedner G, Tawil O, Wilson D. Epidemiology of HIV infection in the Middle East and North Africa. *AIDS* 2010; **24** Suppl 2: S5-23 [PMID: 20610949 DOI: 10.1097/01.aids.0000386729.56683.33]

33 **Kuo G**, Choo QL, Alter HJ, Gitnick GL, Redeker AG, Purcell RH, Miyamura T, Dienstag JL, Alter MJ, Stevens CE. An assay for circulating antibodies to a major etiologic virus of human non-A, non-B hepatitis. *Science* 1989; **244**: 362-364 [PMID: 2496467 DOI: 10.1126/science.2496467]

34 **European Centre for Disease Prevention Control**. Epidemiological assessment of hepatitis B and C among migrants in the EU/EEA. [cited 13 April 2021]. Available from: https://www.ecdc.europa.eu/sites/portal/files/media/en/publications/Publications/epidemiological-assessment-hepatitis-B-and-C-among-migrants-EU-EEA.pdf

35 **European Directorate for the Quality of Medicines & Healthcare (EDQM)**. Report on the Collection, Testing and Use of Blood and Blood Components in Europe. [cited 13 April 2021]. Available from: https://www.edqm.eu/en/blood-transfusion-reports-70.html

36 **Borenstein M**, Hedges LV, Higgins JP, Rothstein HR. Introduction to meta-analysis. John Wiley & Sons, 2011

37 **Freeman MF**, Tukey JW. Transformations related to the angular and the square root. *Ann Math Stat* 1950; **21**: 607-611 [DOI: 10.1214/aoms/1177729756]

38 **Schwarzer G**, Chemaitelly H, Abu-Raddad LJ, Rücker G. Seriously misleading results using inverse of Freeman-Tukey double arcsine transformation in meta-analysis of single proportions. *Res Synth Methods* 2019; **10**: 476-483 [PMID: 30945438 DOI: 10.1002/jrsm.1348]

39 **Higgins JP**, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ* 2003; **327**: 557-560 [PMID: 12958120 DOI: 10.1136/bmj.327.7414.557]

40 **Frank C**, Mohamed MK, Strickland GT, Lavanchy D, Arthur RR, Magder LS, El Khoby T, Abdel-Wahab Y, Aly Ohn ES, Anwar W, Sallam I. The role of parenteral antischistosomal therapy in the spread of hepatitis C virus in Egypt. *Lancet* 2000; **355**: 887-891 [PMID: 10752705 DOI: 10.1016/s0140-6736(99)06527-7]

41 **Messina JP**, Humphreys I, Flaxman A, Brown A, Cooke GS, Pybus OG, Barnes E. Global distribution and prevalence of hepatitis C virus genotypes. *Hepatology* 2015; **61**: 77-87 [PMID: 25069599 DOI: 10.1002/hep.27259]

42 **Chemaitelly H**, Mahmud S, Kouyoumjian SP, Al-Kanaani Z, Hermez JG, Abu-Raddad LJ. Who to Test for Hepatitis C Virus in the Middle East and North Africa? *Hepatol Commun* 2019; **3**: 325-339 [PMID: 30859146 DOI: 10.1002/hep4.1310]

43 **Chemaitelly H**, Mahmud S, Mumtaz GR, Benova L, Ayoub HH, Kouyoumjian SP. Hepatitis C Virus in the Middle East and North Africa. In: Handbook of Healthcare in the Arab World. Laher I, editor. Cham: Springer International Publishing, 2019: 1-27.

44 **Harfouche M**, Chemaitelly H, Mahmud S, Chaabna K, Kouyoumjian SP, Al Kanaani Z, Abu-Raddad LJ. Epidemiology of hepatitis C virus among hemodialysis patients in the Middle East and North Africa: systematic syntheses, meta-analyses, and meta-regressions. *Epidemiol Infect* 2017; **145**: 3243-3263 [PMID: 28988562 DOI: 10.1017/S0950268817002242]

45 **Mahmud S**, Al-Kanaani Z, Chemaitelly H, Chaabna K, Kouyoumjian SP, Abu-Raddad LJ. Hepatitis C virus genotypes in the Middle East and North Africa: Distribution, diversity, and patterns. *J Med Virol* 2018; **90**: 131-141 [PMID: 28842995 DOI: 10.1002/jmv.24921]

46 **Mahmud S**, Chemaitelly HS, Kouyoumjian SP, Al Kanaani Z, Abu-Raddad LJ. Key associations for hepatitis C virus genotypes in the Middle East and North Africa. *J Med Virol* 2020; **92**: 386-393 [PMID: 31663611 DOI: 10.1002/jmv.25614]

47 **Mahmud S**, Kouyoumjian SP, Al Kanaani Z, Chemaitelly H, Abu-Raddad LJ. Individual-level key associations and modes of exposure for hepatitis C virus infection in the Middle East and North Africa: a systematic synthesis. *Ann Epidemiol* 2018; **28**: 452-461 [PMID: 29661680 DOI: 10.1016/j.annepidem.2018.03.007]

48 **Mahmud S**, Chemaitelly H, Al Kanaani Z, Kouyoumjian SP, Abu-Raddad LJ. Hepatitis C Virus Infection in Populations

With Liver-Related Diseases in the Middle East and North Africa. *Hepatol Commun* 2020; **4**: 577-587 [PMID: 32258952 DOI: 10.1002/hep4.1491]

49 **Mahmud S**, Mumtaz GR, Chemaitelly H, Al Kanaani Z, Kouyoumjian SP, Hermez JG, Abu-Raddad LJ. The status of hepatitis C virus infection among people who inject drugs in the Middle East and North Africa. *Addiction* 2020; **115**: 1244-1262 [PMID: 32009283 DOI: 10.1111/add.14944]

50 **World Health Organization**. Global Database on Blood Safety. GDBS Summary Report 2011. [cited 15 March 2021]. Available from: https://www.who.int/bloodsafety/global_database/GDBS_Summary_Report_2011.pdf

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META-ANALYSIS

Effects of dexmedetomidine on cardioprotection and other postoperative complications in elderly patients after cardiac and non-cardiac surgerie

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Abstract

BACKGROUND

After cardiac and non-cardiac surgeries, elderly patients have a high probability of developing cardiac complications and postoperative delirium. Although several clinical trials have investigated whether perioperative intravenous dexmedetomidine can protect the heart and reduce postoperative complications such as delirium in elderly patients, the obtained results have been inconsistent. We conducted a meta-analysis to investigate the effects of dexmedetomidine on cardioprotection and other postoperative complications in elderly patients undergoing cardiac or non-cardiac surgery.

AIM

To investigate the effects of dexmedetomidine on cardiac complications and delirium in elderly patients undergoing cardiac or non-cardiac surgery.

METHODS

The PubMed, Cochrane Library, web of science, and other sources were comprehensively searched for all randomized controlled trials published before May 2021 that investigated the efficacy of dexmedetomidine in the prevention of cardiac and postoperative delirium (POD).

RESULTS

In total, 18 studies involving 1025 patients were included in the meta-analysis. Intravenous dexmedetomidine significantly reduced cardiac troponin I (cTnI) and the inflammatory factor tumor necrosis factor-α (TNF-α) was comparable to the control group. Dexmedetomidine also reduced the POD and mortality rates. However, patients in the dexmedetomidine group were more likely to have a

decreased heart rate (within the normal range) and hypotension during dexmedetomidine administration than those in the control group. There was no difference in the occurrence of myocardial infarction, bradycardia, or stroke between the two groups. Dexmedetomidine significantly shortened the time to extubate; however, it did not shorten the length of stay in the intensive care unit.

CONCLUSION

The administration of dexmedetomidine during cardiac and non-cardiac surgeries can provide myocardial protection by inhibiting inflammation and cTnI, which may be beneficial for the rapid recovery of patients. Meanwhile, the administration of dexmedetomidine reduced the incidence of POD and decreased mortality (in-hospital).

Key Words: Dexmedetomidine; Cardioprotection; Postoperative delirium; Complication; Meta-analysis

Core Tip: After cardiac and non-cardiac surgeries, elderly patients have a high probability of developing cardiac complications and postoperative delirium. Although several clinical trials have investigated whether perioperative intravenous dexmedetomidine can protect the heart and reduce postoperative complications such as delirium in elderly patients, the obtained results have been inconsistent. We conducted a meta-analysis to investigate the effects of dexmedetomidine on cardioprotection and other postoperative complications in elderly patients undergoing cardiac or non-cardiac surgery.

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INTRODUCTION

Elderly patients (> 65 years old) have decreased organ functions and are prone to hemodynamic fluctuations and increased cardiac oxygen consumption, which induces or aggravates myocardial ischemia and hypoxia, leading to severe adverse cardiac events[1]. Postoperative delirium (POD) in elderly patients undergoing major operations, including heart surgery, is a relatively common and serious complication, which is associated with higher morbidity and mortality, cognitive dysfunction, increased length of hospital stay, and increased medical costs[2]. In addition, POD, infection, acute renal failure, major adverse cardiac events, and neurological complications, including permanent or transient stroke, coma, perioperative myocardial infarction (MI), heart block, and cardiac arrest, are major complications [3,4]. However, POD is the most common surgical complication in elderly patients aged 65 years and older[5,6]. The occurrence of delirium may significantly extend the length of hospitalization, delayed recovery, delayed cognitive dysfunction, and increased mortality[4]. Dexmedetomidine can reduce the occurrences of POD postoperative pain, nausea, and vomiting[4,7]. Meanwhile, dexmedetomidine can provide rapid and stable recovery and early extubation after surgery by maintaining the patient's hemodynamics[8].

Dexmedetomidine, a derivative of medetomidine, is a highly selective α2 adrenergic receptor agonist [9] that can inhibit the sympathetic nervous system and act on the noradrenergic glands in the locus coeruleus of the pons to inhibit the release of norepinephrine[6,10]. The dorsal vagus nucleus and suspicious nucleus are the human parasympathetic nerve center, and the central area of the parasympathetic nerve is directly controlled by the nucleus tractus solitarius. Because the nucleus tractus solitarius is abundant in α2 adrenergic receptors, the combination of dexmedetomidine and the nucleus tractus solitarius α2 adrenergic receptors can increase the activity of the vagus nerve and reduce the myocardial cAMP production and L-type calcium channel current, which slows down the heart rate, increases coronary blood flow, and plays a role in myocardial protection[1]. In addition, the anxiolytic, sedative/hypnotic, and analgesic effects of dexmedetomidine[2,11], including the intraoperative hemostatic effect, also enhance cardioprotection[12]. Regarding inflammation, dexmedetomidine can block the accumulation of inflammatory cells in the nervous system[10], reduce neuronal damage caused by immune responses, and reduce surgical complications such as stress response and postoperative cognitive impairment to exert its neuronal protection[9,10]. However, high-dose dexmedetomidine can cause adverse reactions such as hypertension, decreased reflex heart rate,

decreased cardiac output, and decreased drug tolerance in elderly patients, requiring special attention to drug dosage[1].

MATERIALS AND METHODS

This meta-analysis was reported according to the statement of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)[13]. In addition, this study was not registered with the PROSPERO.

Search strategy

We performed this meta-analysis according to PRISMA guidelines. PRISMA is the minimum set of evidence-based items used for reporting in systematic reviews and meta-analyses, and can be adopted as the basis for systematic reviews for reporting different types of research. We searched 1025 studies from PubMed, Cochrane Library, and Web of Science that were published before May 2021; other search systems focused on 10 studies (Google) to confirm further eligible studies.

We manually screened whether the studies we included met our final research criteria, and then selected 18 of them for research. The basic search strategy included the following words: ("dexmedetomidine"[MeSH Terms] OR"dexmedetomidine"[All Fields]) AND ("cardiac"[MeSH Terms] OR"cardiac"[All Fields]) AND ("aged"[MeSH Terms] OR"aged"[All Fields] OR"elderly"[All Fields]). No language restrictions were applied during the search.

Bradycardia, hypertension, and hypotension were defined as HR < 60 bpm, SBP > 160 mmHg or 20% of baseline, and SBP < 90 mmHg or 20% of baseline, respectively.

Eligibility criteria

We included the following standard studies: (1) Elderly patients (> 65 years old) undergoing cardiac or non-cardiac surgery; (2) We selected a dexmedetomidine group combined with normal saline or other anesthetics, regardless of the initial administration time, dose, duration, or dose; (3) The research content type was randomized controlled clinical trial (RCT); and (4) The research results included hypertension, hypotension, heart rate, cardiac status, complications, POD, stroke, mortality, extubation time, intensive care unit (ICU) time, and cardiac enzyme markers.

Exclusion criteria

Non-randomized controlled trials, case reports, meeting abstracts, and comments were excluded.

Including non-elderly surgery and no results of our study were also excluded.

Study selection and data collection process

Two authors (Yang and Hu) independently conducted qualified research selection and data extraction. Disagreements between the two authors were discussed with the third author (Duan) to arrive at the final solution. The extracted data were as follows: first author, annual publication, type of surgery, number of patients, dose of dexmedetomidine, method of anesthesia, hypotension, hypertension, heart rate, myocardial infarction, bradycardia, delirium, stroke, mortality, extubation time, ICU duration, and myocardial enzyme markers.

Risk of bias in individual studies

Two examiners (Yang and Hu) independently used version 2 of the Cochrane tool to assess RCT deviation risk for methodological quality assessment. When the two examiners disagreed, the disagremments were resolved *via* discussions with a third examiner (Duan).

Statistical analysis

We employed a Review Manager 5.0 software (Cochrane Collaboration Company) for rigorous statistical analysis. Risk ratios (RRs) with 95% confidence intervals (CIs) and the Mantel-Haenszel method (fixed or random model) were adopted to analyze dichotomous data. For continuous outcomes, the mean differences (MD) or standardized mean differences (SMD) with 95%CIs were calculated. If significant heterogeneity existed (I^2 > 50%), sensitivity analysis was performed, each study was ignored separately, and a random effects model was selected.

RESULTS

As illustrated in Figure 1. We initially identified 1035 studies by searching the database. After screening out duplicate studies, 486 studies entered the next step of screening: 443 studies were excluded because the focused on non-elderly patients, were non-RCT, were unable to obtain full-text qualifications or not published, *etc.* (Google search); 25 excluded studies did not comply with the statistical data in this

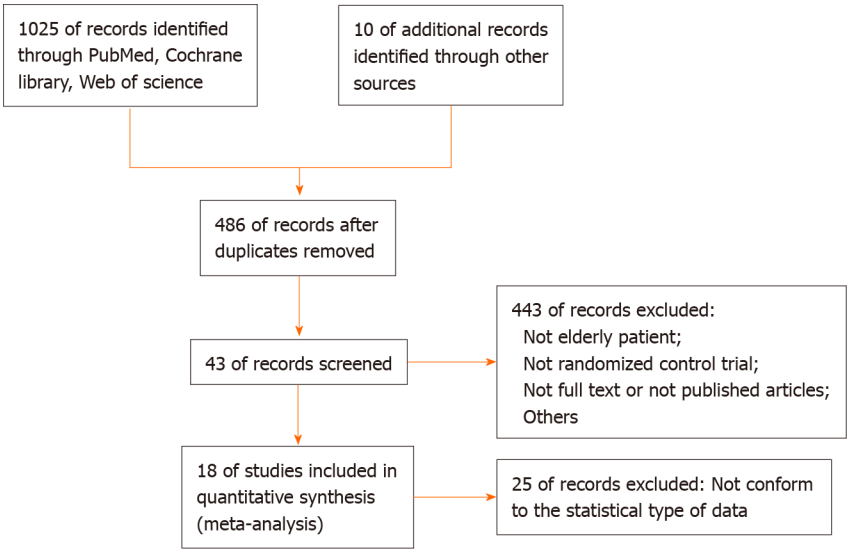

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Figure 1 Flow chart of study selection.

article. Finally, we included 18 studies that met all eligibility criteria (Figure 1). The basic characteristics of the enrolled studies are presented in Table 1. A summary of the risk of bias is presented in Figure 2. Regarding the blindness of patients, researchers, and evaluators, 11 trials were rated as double-blind low-risk trials, and seven trials were rated as high-risk or unclear-risk trials because related articles were not clear about blindness.

Meta-analysis of intraoperative data

We determined that there was no significant difference in hypertension between the dexmedetomidine and control groups (Figure 3, RR: 0.99, 95%CI: 0.78-1.25, $P = 0.92$, $I^2 = 21\%$). However, 426 patients had hypotension among the 2025 patients, of whom 265 and 161 were in the dexmedetomidine and control groups, respectively. Therefore, it could be inferred that dexmedetomidine had a significant effect on lowering blood pressure (Figure 3, RR: 1.63, 95%CI: 1.40-1.90, $P < 0.001$, $I^2 = 2\%$).

HR was significantly lower in the dexmedetomidine group than in the control group (Figure 4, MD: -8.46, 95%CI: -12.56 to -4.36, $P < 0.001$). However, heterogeneity I^2 was as high as 87%. Further sensitivity analysis indicated that the heterogeneity decreased to 45% after excluding Shokri and Ali 2020[7], Tosun *et al*[12] 2013, and Zhou *et al*[14] 2019. This study was relatively unstable, and the number of included studies needs to be increased in the future.

Meta-analysis of other cardiac complications

In total, six studies investigated the occurrence of MI. In these studies, 26 patients developed MI in the dexmedetomidine group (Figure 5, RR: 0.74, 95%CI: 0.49-1.13, $P = 0.16$, $I^2 = 0\%$). However, there was no statistically significant difference between the dexmedetomidine and control groups. In addition, five studies participated in the study of bradycardia, and there was no significant statistical difference between the dexmedetomidine and control groups (Figure 5, RR: 1.51, 95%CI: 0.79-2.89, $P = 0.21$, $I^2 = 63\%$). However, we performed a sensitivity analysis by excluding Turan 2020 and Zhang 2020, and the heterogeneity decreased to zero ($P = 0.004$).

Meta-analysis of cardiac troponin I and tumor necrosis Factor-α

The level of cardiac troponin I (cTnI) in elderly patients after surgery was analyzed. The obtained results indicated that the level of cTnI in the dexmedetomidine group was lower than that in the control group after surgery (Figure 6, SMD: -3.14, 95%CI: -5.16 to -1.11, $P = 0.002$, $I^2 = 97\%$). Statistical heterogeneity was absent when the studies conducted by Elgebaly 2020 and Shen 2017 were excluded, and the obtained results were statistically significant (SMD: -1.00, 95%CI: -1.37 to -0.63, $P < 0.001$, $I^2 = 0\%$).

As illustrated in Figure 6, the level of Factor-α (TNF-α) in the dexmedetomidine group was lower (SMD: -0.72, 95%CI: -1.30 to -0.13, $P = 0.02$, $I^2 = 52\%$) after surgery than that of the control group. Sensitivity analysis and subgroup analysis failed to eliminate the heterogeneity; therefore, a random-effects model was adopted.

Meta-analysis of other complications

Seven studies have demonstrated that the use of dexmedetomidine use is associated with POD. The occurrence of POD was reported in all the RCTs. Notably, the occurrence of POD in the

Table 1 Study characteristics

Ref.	No. of patients in study		DEX dose		Surgical procedure	Anesthesia	Outcome parameters
	DEX	Control	Loading dose (μg.kg^{-1})	Infusion rate (μg.kg^{-1}.hr^{-1})			
Aouad *et al*[26], 2019	48	50	1	N/A	Elective surgery	GA	Hypotension; HR
Cheng *et al*[25], 2016	222	283	N/A	0.24-0.6	Cardiac Surgery	GA	Delirium; MI; Mortality; Stroke
Chi *et al* [21], 2016	34	33	1	0.6	Off-pump coronary artery bypass graft surgery	GA	Hypertension; ICU time
Elgebaly *et al*[8], 2020	30	30	N/A	0.4	Open-heart surgery	GA	HR; cTnI; ET; Length of ICU stay
Ji *et al*[3], 2013	568	566	N/A	0.24-0.6	CABG or valve surgery or CABG or valve surgery combined with other procedures. patients excluded were those undergoing emergency surgery, off-pump or robotic surgery, surgery requiring deep hypothermic circulatory arrest, or surgery involving the thoracic aorta	GA	Delirium; Mortality stroke; MI
Lee *et al* [18], 2016	20	20	1	0.5	Orthopedic surgery in supine position	GA	HR; Hypertension
Lee *et al* [2], 2018	95	109	1	0.2-0.7	Laparoscopic major non-cardiac surgery	GA	Incidence of delirium
Li *et al* [20], 2020	309	310	0.6	0.5	Major non-cardiac surgery	GA	Delirium: Length of ICU stay (h)
Ríha *et al* [11], 2012	17	21	1	0.5-1.5	Elective CABG procedures with the use of cardiopulmonary bypass to treat coronary artery disease	GA	Length of ICU stay; MI; ET
Shen *et al* [1], 2017	30	30	0.5	0.5	Coronary heart disease and underwent gastric cancer operation	GA	Hypotension; cTnI; MI; Bradycardia
Shokri *et al*[7], 2020	144	142	N/A	0.7-1.2	Coronary artery bypass grafting	GA	Delirium; Hypotension; HR; Bradycardia; ET; Mortality
Soliman *et al*[27], 2016	75	75	1	0.3	Aortic vascular surgery	GA	Hypertension; HR; MI; Bradycardia; Mortality
Sun *et al* [5], 2019	281	276	N/A	0.1	Major elective noncardiac surgery	GA	Delirium; Hypotension; Hypertension; Mortality
Tosun *et al* [12], 2013	18	20	0.5	0.5	Coronary artery bypass graft surgery	GA	HR
Turan *et al* [15], 2020	398	396	0.1	0.2	Cardiac surgery	GA	Hypotension; MI; Bradycardia; mortality; ICU time; Stroke
Zhang *et al*[16], 2020	120	120	0.5	0.3	Hip Fracture Operation	GA	Delirium; Hypotension; Hypertension; Bradycardia
Zhou *et al* [22], 2019	14	14	0.5	0.5	Valve replacement surgery	GA	cTnI
Zhou *et al* [14], 2019	53	47	N/A	0.2-0.7	Cardiac surgery	GA	HR; ET; cTnI

CABG: Coronary artery bypass grafting; cTnI: Cardiac troponin I; ET: Extubation time; N/A: Not applicable; DEX: Dexmedetomidine; GA: General Anesthesia; MI: Myocardial infarction; ICU: Intensive care unit.

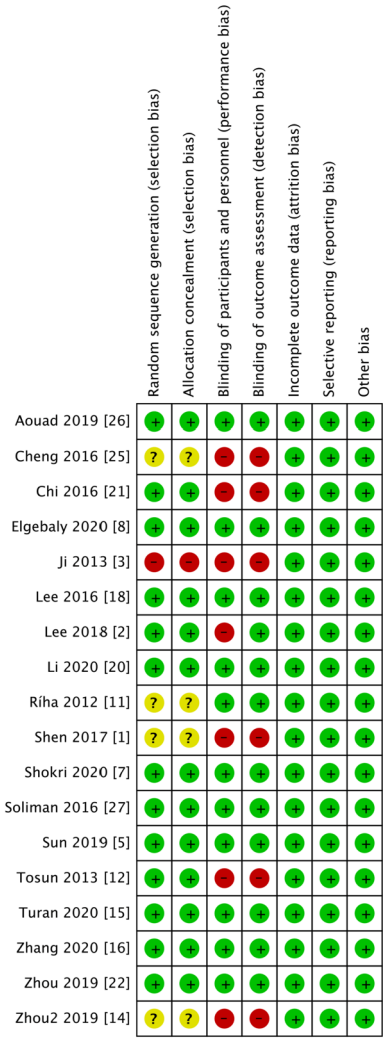

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Figure 2 Risk of bias assessment according to cochrane risk of bias methods.

dexmedetomidine group was significantly lower than that in the control group (Figure 7, RR: 0.63, 95%CI: 0.51-0.76, $P < 0.001$, $I^2 = 0\%$). Low heterogeneity was observed, which we rated as high-quality evidence. We conclude that dexmedetomidine may significantly reduce delirium.

Among the 18 high-quality studies, three studies demonstrated that there was no correlation between the use of dexmedetomidine and postoperative stroke (Figure 7, RR: 1.19, 95%CI: 0.59-2.40, $P = 0.62$, $I^2 = 0\%$). In addition, regarding mortality, the results indicated that the dexmedetomidine group had lower postoperative mortality than the control group (Figure 7, RR: 0.32, 95%CI: 0.18-0.58, $P = 0.0002$). There was low heterogeneity among the studies regarding mortality outcomes ($I^2 = 0$).

Meta-analysis of extubation time and ICU length of stay

Extubation time (ET) was recorded in 4 studies including 484 patients. The results demonstrated that the use of dexmedetomidine in the perioperative period can shorten ET in patients compared to control patients (Figure 8, MD: -2.03, 95%CI: -2.87 to -1.18, $P < 0.001$). With low heterogeneity ($I^2 = 38\%$), it can be deduced that dexmedetomidine shortens extubation time after surgery without subgroup analysis. However, regarding ICU length of stay, there was no statistical difference between the dexmedetomidine and control groups (Figure 8, MD: 0.05, 95%CI: -9.11 to -9.21, $P = 0.99$), and heterogeneity I^2 was also higher at 98%.

DISCUSSION

Dexmedetomidine is a highly selective and effective α2-adrenergic receptor agonist that can provide dose-dependent sedation, anti-anxiety, and moderate analgesia[15], with minimal inhibition of respiratory functions. The central sympathetic nervous system reduces the systemic inflammatory response after surgery and regulates the immune system[5,16,17].

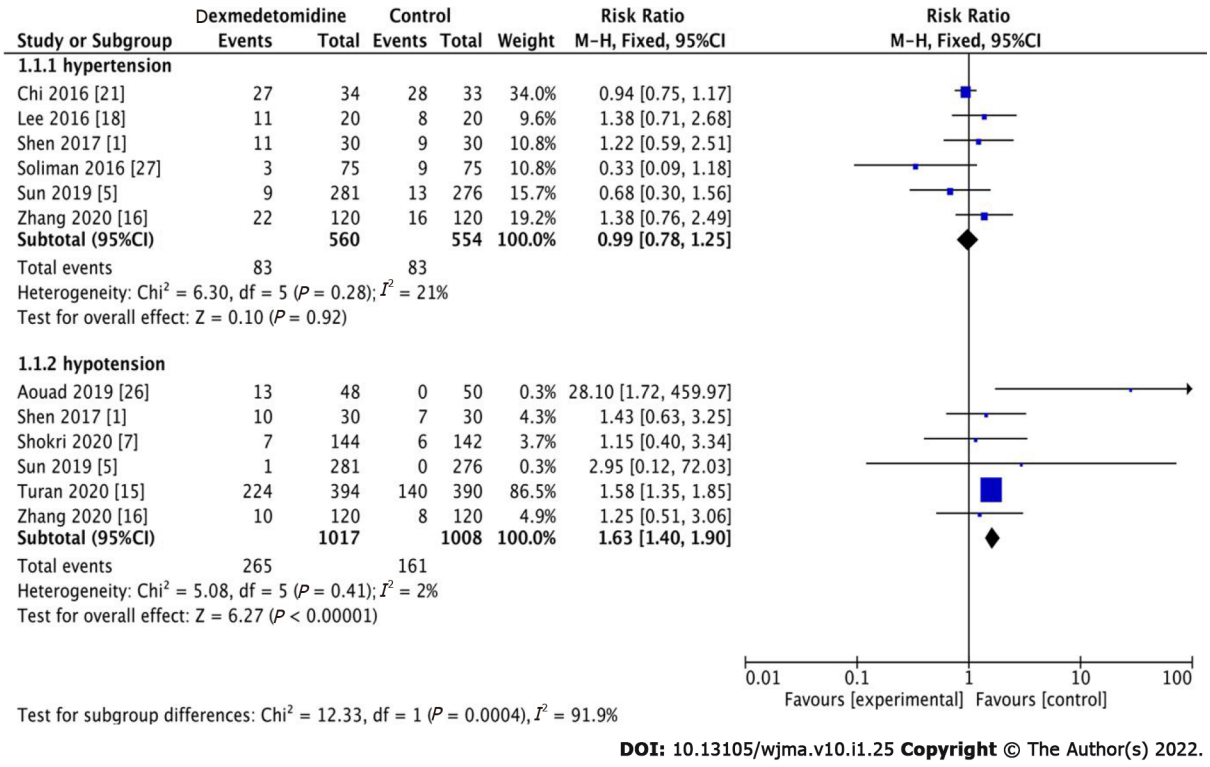

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Figure 3 Meta-analysis of the incidence of hypertension and hypotension during the operation.

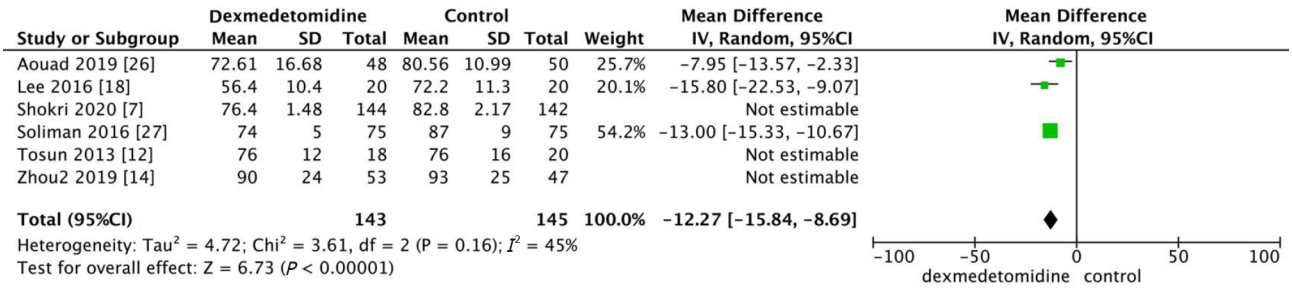

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Figure 4 Meta-analysis of heart rate.

First, we compared the occurrence of intraoperative hypertension and hypotension, and determined that dexmedetomidine could increase the risk of intraoperative hypotension; however, it could also lower than the HR within the normal range. This was attributed to the fact that dexmedetomidine stimulates vascular smooth muscle α2 receptors, thereby resulting in a transient increase in blood pressure and a decrease in HR[18]. Studies have shown that dexmedetomidine has an effective sedative effect in heart and vascular surgeries, minimizes the variability of heart rate and blood pressure, and reduces the response of tachycardia to painful stimulation[6,8,19].

Second, we explored cardiac complications and inferred that dexmedetomidine did not increase the occurrence of myocardial infarction and bradycardia[20]; however, it reduced the expression of cTnI and improved the protection of the myocardium. Studies have demonstrated that more bradycardia was observed in dexmedetomidine, which was the expected result of α2-adrenergic receptor agonists; however, considering that the occurrence of bradycardia was transient, intervention was required, and the proportion of patients was low[21]. In clinical practice, postoperative administration of small doses of dexmedetomidine might be an acceptable and safe strategy for patients in general surgery wards[5].

cTnI is a highly sensitive and specific marker that is adopted as the gold standard diagnostic marker for myocardial infarction in coronary artery bypass grafting (CABG); cTnI is also a significant predictor of the prognosis of patients with heart diseases[14]. The low postoperative cardiac biomarker cTnI exhibited cardioprotective effects of dexmedetomidine[8]. Regarding inflammation, our study determined that dexmedetomidine reduced the expression of TNF-α compared to the control group. Dexmedetomidine provides end-organ protection *via* anti-inflammatory, antioxidant, and anti-apoptotic effects[15]. With several basic effects such as surgical intervention, systemic inflammation, and oxidative

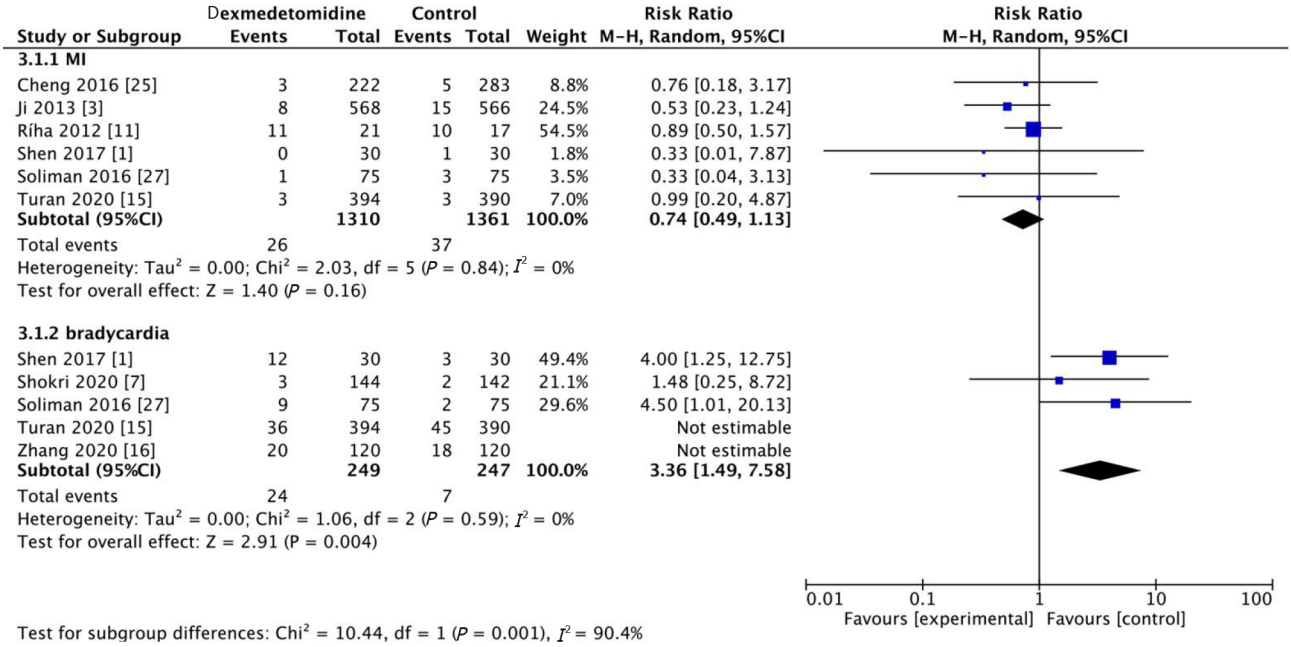

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Figure 5 Meta-analysis of cardiac complications (myocardial infarction and bradycardia). MI: Myocardial infarction.

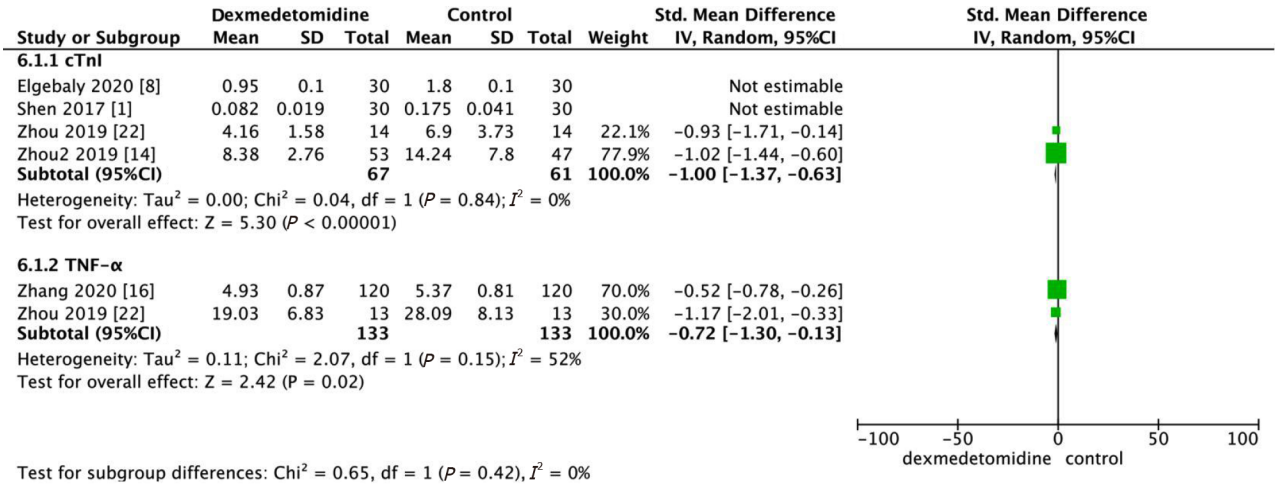

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Figure 6 Meta-analysis of cardiac troponin I (cTnI) (mg/L) and TNF-α (tumor necrosis factor-α) (μl/L).

stress, reperfusion after myocardial ischemia increases myocardial injury and leads to myocardial cell apoptosis[22]. The most well-known mechanisms by which dexmedetomidine reduces inflammation include the NF-kB pathway, Toll-like receptors, and several inflammatory mediators[22], which in turn reduce the demand for opioids and benzodiazepines[15]. Several studies have shown that postoperative inflammation may be the cause of POD[2,15]. We also determined that the occurrence of POD reduced more significantly in the dexmedetomidine group than in control group after surgery. In addition, POD occurs within 3 d after the operation and is affected by memory loss and impaired comprehension[6,23]. Delirium is a frequent postoperative complication in elderly patients after non-cardiac surgery that caused by several stressors, including neurotransmitter imbalance (especially cholinergic deficiency), inflammation, and electrolyte or metabolic disorders[7,15,24]. It has been reported that the incidence of POD in patients undergoing cardiac surgery is 20%-50%, especially in elderly patients admitted to the ICU and patients undergoing orthopedic surgery. This is associated with higher morbidity and mortality, while the longer length of hospital stay is related to ICU time, increased economic burden, and hospital-acquired complications[6,23]. This study also demonstrated that although the use of dexmedetomidine could reduce the postoperative mortality of patients, it did not reduce the risk of postoperative stroke. Studies have also shown that the mortality rate of patients receiving dexmedetomidine in the hospital, 30 d and 1 year later, was significantly reduced[3]. Stroke is a

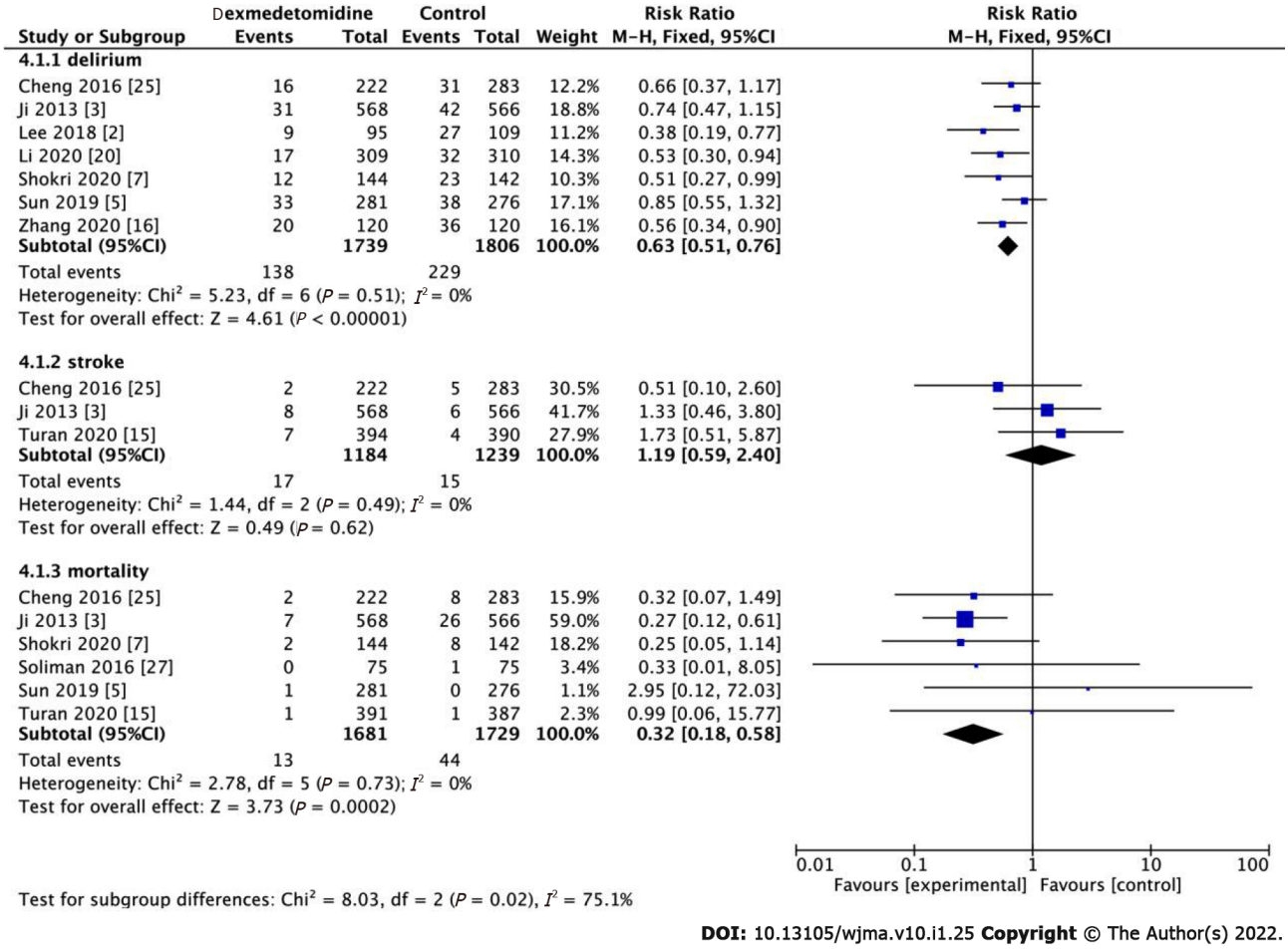

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Figure 7 Meta-analysis of postoperative delirium, stroke, and mortality (In-hospital). POD: Postoperative delirium.

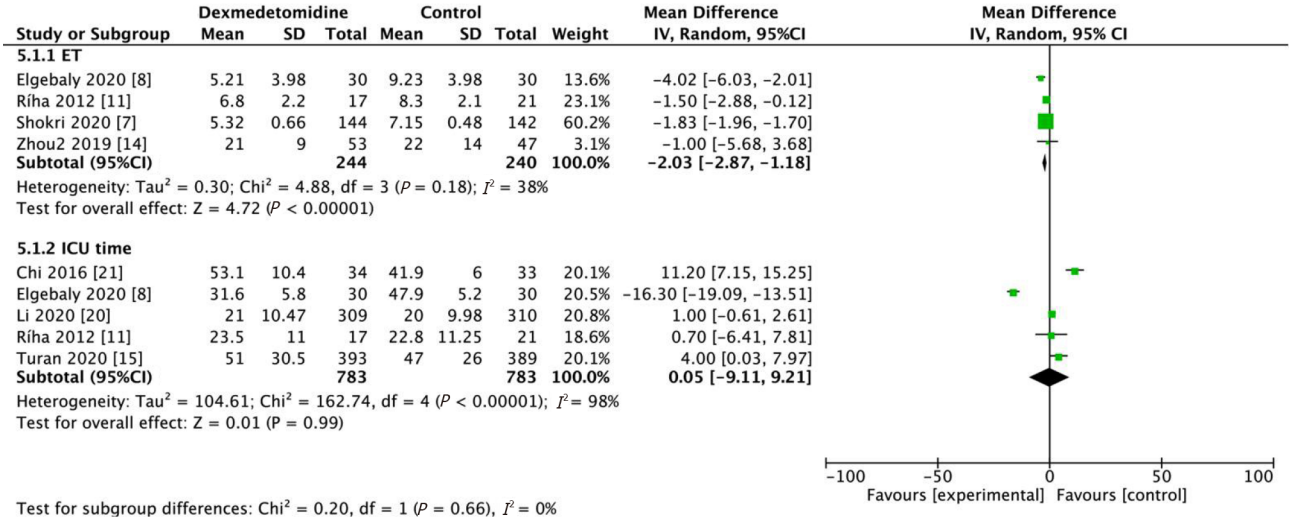

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Figure 8 Meta-analysis of extubation time (h) and intensive care unit length of stay (h). ET: Extubation time; ICU: Intensive care unit.

devastating complication of cardiac surgery. Among patients undergoing different cardiac surgeries, the prevalence of stroke is 1.6%-5.25%[25]; however, the exact cause of postoperative stroke remains unclear. Finally, we found that although the patients receiving dexmedetomidine had a significantly shorter postoperative extubation time than control patients, dexmedetomidine did not significantly shorten the time spent in the ICU. Dexmedetomidine can shorten the time of extubation, which can promote the rapid recovery of heart function in patients[8,9,25-27], reduce the use of psychotropic

drugs, and facilitate recovery from activities as soon as possible.

CONCLUSION

The administration of dexmedetomidine during cardiac and non-cardiac surgeries can provide myocardial protection by inhibiting inflammation and cTnI, which could be beneficial to the rapid recovery of patients. Furthermore, intravenous dexmedetomidine reduces POD and mortality in patients.

ARTICLE HIGHLIGHTS

Research background

There was also a consistent conclusion on whether dexmedetomidine had a protective effect on the heart and improved postoperative complications, for which we conducted a meta-analysis.

Research motivation

It has guiding significance for clinical application of dexmedetomidine on the heart and postoperative complications in elderly patients undergoing cardiac or non-cardiac surgery.

Research objectives

Our main goal is to investigate the effects of dexmedetomidine on cardiac complications and delirium in elderly patients undergoing cardiac or non-cardiac surgery.

Research methods

We collected references to randomized controlled trials examining the efficacy of dexmedetomidine in the treatment of cardiac and postoperative complications.

Research results

Dexmedetomidine significantly reduced the cardiac troponin I and he inflammatory factor tumor necrosis factor-α, and reduced the extubation time, postoperative delirium and mortality. It really brings benefits to patients.

Research conclusions

Dexmedetomidine has cardioprotective effects and improves patient postoperative complications.

Research perspectives

In clinical practice, we will study the effect of dexmedetomidine at an appropriate dose on the heart and postoperative complications which will have certain guiding significance.

FOOTNOTES

Author contributions: Yang YL conceived and designed the study; Yi J, Hu BJ and Duan HW collected the data and performed the literature search; Yang YL was involved in the writing of the manuscript; Pan MZ and Xie PC contributed equally to this work; all authors have read and approved the final manuscript.

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REFERENCES

1 **Shen J**, Sun Y, Han D, Zhu K, Zhao W. [Effects of dexmedetomidine on perioperative cardiac adverse events in elderly patients with coronary heart disease]. *Zhong Nan Da Xue Xue Bao Yi Xue Ban* 2017; **42**: 553-557 [PMID: 28626102 DOI: 10.11817/j.issn.1672-7347.2017.05.012]
2 **Lee C**, Lee CH, Lee G, Lee M, Hwang J. The effect of the timing and dose of dexmedetomidine on postoperative delirium in elderly patients after laparoscopic major non-cardiac surgery: A double blind randomized controlled study. *J Clin Anesth* 2018; **47**: 27-32 [PMID: 29549829 DOI: 10.1016/j.jclinane.2018.03.007]
3 **Ji F**, Li Z, Nguyen H, Young N, Shi P, Fleming N, Liu H. Perioperative dexmedetomidine improves outcomes of cardiac surgery. *Circulation* 2013; **127**: 1576-1584 [PMID: 23513068 DOI: 10.1161/CIRCULATIONAHA.112.000936]
4 **Park JW**, Kim EK, Lee HT, Park S, Do SH. The Effects of Propofol or Dexmedetomidine Sedation on Postoperative Recovery in Elderly Patients Receiving Lower Limb Surgery under Spinal Anesthesia: A Retrospective Propensity Score-Matched Analysis. *J Clin Med* 2021; **10** [PMID: 33401537 DOI: 10.3390/jcm10010135]
5 **Sun Y**, Jiang M, Ji Y, Sun Y, Liu Y, Shen W. Impact of postoperative dexmedetomidine infusion on incidence of delirium in elderly patients undergoing major elective noncardiac surgery: a randomized clinical trial. *Drug Des Devel Ther* 2019; **13**: 2911-2922 [PMID: 31695323 DOI: 10.2147/DDDT.S208703]
6 **Bi X**, Wei J, Zhang X. Effects of dexmedetomidine on neurocognitive disturbance after elective non-cardiac surgery in senile patients: a systematic review and meta-analysis. *J Int Med Res* 2021; **49**: 3000605211014294 [PMID: 33983077 DOI: 10.1177/03000605211014294]
7 **Shokri H**, Ali I. A randomized control trial comparing prophylactic dexmedetomidine versus clonidine on rates and duration of delirium in older adult patients undergoing coronary artery bypass grafting. *J Clin Anesth* 2020; **61**: 109622 [PMID: 31668468 DOI: 10.1016/j.jclinane.2019.09.016]
8 **Elgebaly AS**, Fathy SM, Sallam AA, et al Cardioprotective effects of propofol-dexmedetomidine in open-heart surgery: A prospective double-blind study [J]. *Ann Card Anaesth* 2020; **23**: 134-141 [DOI: 10.4103/aca.ACA_168_18]
9 **Chen M**, Li X, Mu G. Myocardial protective and anti-inflammatory effects of dexmedetomidine in patients undergoing cardiovascular surgery with cardiopulmonary bypass: a systematic review and meta-analysis. *J Anesth* 2021 [PMID: 34342722 DOI: 10.1007/s00540-021-02982-0]
10 **Lu S**, Chen X, Chen Q, Cahilog Z, Hu L, Chen Y, Cao J, Ning J, Yi B, Lu K, Gu J. Effects of dexmedetomidine on the function of distal organs and oxidative stress after lower limb ischaemia-reperfusion in elderly patients undergoing unilateral knee arthroplasty. *Br J Clin Pharmacol* 2021; **87**: 4212-4220 [PMID: 33754371 DOI: 10.1111/bcp.14830]
11 **Říha H**, Kotulák T, Březina A, Hess L, Kramář P, Szárszoi O, Netuka I, Pirk J. Comparison of the effects of ketamine-dexmedetomidine and sevoflurane-sufentanil anesthesia on cardiac biomarkers after cardiac surgery: an observational study. *Physiol Res* 2012; **61**: 63-72 [PMID: 22188112 DOI: 10.33549/physiolres.932224]
12 **Tosun Z**, Baktir M, Kahraman HC, Baskol G, Guler G, Boyaci A. Does dexmedetomidine provide cardioprotection in coronary artery bypass grafting with cardiopulmonary bypass? *J Cardiothorac Vasc Anesth* 2013; **27**: 710-715 [PMID: 23725681 DOI: 10.1053/j.jvca.2012.12.013]
13 **Moher D**, Liberati A, Tetzlaff J, Altman DG; PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med* 2009; **6**: e1000097 [PMID: 19621072 DOI: 10.1371/journal.pmed.1000097]
14 **Zhou HM**, Ling XY, Ni YJ, Wu C, Zhu ZP. Pre-cardiopulmonary bypass administration of dexmedetomidine decreases cardiac troponin I level following cardiac surgery with sevoflurane postconditioning. *J Int Med Res* 2019; **47**: 3623-3635 [PMID: 31234690 DOI: 10.1177/0300060519856750]
15 **Turan A**, Duncan A, Leung S, Karimi N, Fang J, Mao G, Hargrave J, Gillinov M, Trombetta C, Ayad S, Hassan M, Feider A, Howard-Quijano K, Ruetzler K, Sessler DI; DECADE Study Group. Dexmedetomidine for reduction of atrial fibrillation and delirium after cardiac surgery (DECADE): a randomised placebo-controlled trial. *Lancet* 2020; **396**: 177-185 [PMID: 32682483 DOI: 10.1016/S0140-6736(20)30631-0]
16 **Zhang W**, Wang T, Wang G, Yang M, Zhou Y, Yuan Y. Effects of Dexmedetomidine on Postoperative Delirium and Expression of IL-1β, IL-6, and TNF-α in Elderly Patients After Hip Fracture Operation. *Front Pharmacol* 2020; **11**: 678 [PMID: 32477139 DOI: 10.3389/fphar.2020.00678]
17 **Sun W**, Zhao J, Li C. Dexmedetomidine Provides Protection Against Hippocampal Neuron Apoptosis and Cognitive Impairment in Mice with Alzheimer's Disease by Mediating the miR-129/YAP1/JAG1 Axis. *Mol Neurobiol* 2020; **57**: 5044-5055 [PMID: 32839917 DOI: 10.1007/s12035-020-02069-z]
18 **Lee SH**, Na S, Kim N, Ban MG, Shin SE, Oh YJ. The Effects of Dexmedetomidine on Myocardial Function Assessed by Tissue Doppler Echocardiography During General Anesthesia in Patients With Diastolic Dysfunction: A CONSORT-Prospective, Randomized, Controlled Trial. *Medicine (Baltimore)* 2016; **95**: e2805 [PMID: 26871847 DOI: 10.1097/MD.0000000000002805]
19 **Klinger RY**, White WD, Hale B, Habib AS, Bennett-Guerrero E. Hemodynamic impact of dexmedetomidine administration in 15,656 noncardiac surgical cases. *J Clin Anesth* 2012; **24**: 212-220 [PMID: 22484025 DOI: 10.1016/j.jclinane.2011.07.016]
20 **Li CJ**, Wang BJ, Mu DL, Hu J, Guo C, Li XY, Ma D, Wang DX. Randomized clinical trial of intraoperative

dexmedetomidine to prevent delirium in the elderly undergoing major non-cardiac surgery. *Br J Surg* 2020; **107**: e123-e132 [PMID: 31903588 DOI: 10.1002/bjs.11354]

21 **Chi X**, Liao M, Chen X, Zhao Y, Yang L, Luo A, Yang H. Dexmedetomidine Attenuates Myocardial Injury in Off-Pump Coronary Artery Bypass Graft Surgery. *J Cardiothorac Vasc Anesth* 2016; **30**: 44-50 [PMID: 26429360 DOI: 10.1053/j.jvca.2015.06.026]

22 **Zhou H**, Zhou D, Lu J, Wu C, Zhu Z. Effects of Pre-Cardiopulmonary Bypass Administration of Dexmedetomidine on Cardiac Injuries and the Inflammatory Response in Valve Replacement Surgery With a Sevoflurane Postconditioning Protocol: A Pilot Study. *J Cardiovasc Pharmacol* 2019; **74**: 91-97 [PMID: 31356535 DOI: 10.1097/FJC.0000000000000698]

23 **Shen QH**, Li HF, Zhou XY, Yuan XZ. Dexmedetomidine in the prevention of postoperative delirium in elderly patients following non-cardiac surgery: A systematic review and meta-analysis. *Clin Exp Pharmacol Physiol* 2020; **47**: 1333-1341 [PMID: 32215933 DOI: 10.1111/1440-1681.13312]

24 **Pan H**, Liu C, Ma X, Xu Y, Zhang M, Wang Y. Perioperative dexmedetomidine reduces delirium in elderly patients after non-cardiac surgery: a systematic review and meta-analysis of randomized-controlled trials. *Can J Anaesth* 2019; **66**: 1489-1500 [PMID: 31264195 DOI: 10.1007/s12630-019-01440-6]

25 **Cheng H**, Li Z, Young N, Boyd D, Atkins Z, Ji F, Liu H. The Effect of Dexmedetomidine on Outcomes of Cardiac Surgery in Elderly Patients. *J Cardiothorac Vasc Anesth* 2016; **30**: 1502-1508 [PMID: 27435836 DOI: 10.1053/j.jvca.2016.02.026]

26 **Aouad MT**, Zeeni C, Al Nawwar R, Siddik-Sayyid SM, Barakat HB, Elias S, Yazbeck Karam VG. Dexmedetomidine for Improved Quality of Emergence From General Anesthesia: A Dose-Finding Study. *Anesth Analg* 2019; **129**: 1504-1511 [PMID: 31743169 DOI: 10.1213/ANE.0000000000002763]

27 **Soliman R**, Zohry G. The myocardial protective effect of dexmedetomidine in high-risk patients undergoing aortic vascular surgery. *Ann Card Anaesth* 2016; **19**: 606-613 [PMID: 27716690 DOI: 10.4103/0971-9784.191570]

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META-ANALYSIS

Leptin levels in women with unexplained infertility: A systematic review and meta-analysis

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Abstract

BACKGROUND

Unexplained infertility (UI) is usually used for any heterosexual couple who failed to have a successful clinical pregnancy without identifying clear causes after they undergo all standard fertility tests. Evidence shows that leptin is one of the most accurate biomarkers for UI. Nevertheless, conflicting results regarding leptin levels in women with UI have been reported.

AIM

To find the serum leptin levels in women with UI.

METHODS

All studies written in English and conducted before April 30, 2021 from PubMed/MEDLINE, Embase, Cochrane Central Register of Controlled Trials, ClinicalTrials.gov, Google Scholar, OpenGrey, OATD, and the infertility conference abstract were included. Studies were found eligible if they provided the mean and standard deviation of leptin for the case group and control group. The quality assessment of individual studies was evaluated using the Joanna Briggs Institute Quality Assessment Tool. Data synthesis and statistical analysis were done using STATA software version 16.

RESULTS

A total of 378 studies were reviewed, and just six studies that fulfilled the eligibility criteria were included in this meta-analysis. The pooled result showed that leptin levels were significantly higher in women with UI compared to fertile women, with a standardized mean difference of 0.97 (95% confidence interval: -0.49-2.43). However, heterogeneity across studies was highly significant ($P < 0.00001$; $I^2 = 98.8\%$).

CONCLUSION

The results of this study suggest that leptin levels are elevated in women with UI compared with fertile women; hence, leptin could be a potential biomarker for UI in women, and it may be useful for identifying women with a high risk of infertility.

Key Words: Leptin; Meta-analysis; Serum level; Unexplained infertility; Women

Core Tip: A comprehensive systematic review and meta-analysis was conducted to find the serum leptin levels in women with unexplained infertility. Six studies were included in this meta-analysis, after passing all quality checkups. The pooled result showed that leptin levels were significantly higher in women with unexplained infertility compared to fertile women, with a standardized mean difference of 0.97. Leptin could be a potential biomarker for unexplained infertility in women, and it may be useful for identifying women with a high risk of infertility.

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INTRODUCTION

Reproductive medicine has made many breakthroughs in the field of infertility diagnosis and treatment. Following this breakthrough, many untreated infertility cases now can be treated using one of the advanced reproduction methods such as assisted reproductive technologies[1]. Despite this, some infertility cases cannot still be diagnosed or treated using the current methods, especially in developing countries[2]. This type of infertility is widely known as unexplained infertility (UI)[3].

UI is usually used for any heterosexual couple who failed to have a successful clinical pregnancy without clear causes after they undergo all standard fertility tests[4]. Worldwide, the prevalence of UI ranges from 10% to 37%[2,5-7], and females are found to be responsible for at least 50%. The complexity of the reproductive process especially in females[8], makes it very hard to identify the source of UI. Despite this, many studies have been done to find the pathophysiologic basis and the possible relationship between obesity, endocrinological imbalance, genetic factors, immunological factors, and UI[7,9,10], but unfortunately, till now, the pathophysiology and exact role of these factors and how they contribute to UI have not been fully understood. To overcome this diagnostic issue, many serum biomarkers have been used as predictive markers for UI in females. Out of these biomarkers, leptin was found to be one of the most accurate biomarkers[10,11].

Leptin is an adipokinetic hormone that plays a key role in energy homeostasis and body weight regulation, and acts as a neuroendocrine mediator in different body systems, including the reproductive system[12-15]. Leptin is secreted mainly from adipocyte cells in the white adipose tissues, and altered with obesity[16]. Also, it can be produced from other cells related to the reproductive system in both males and females, such as placental syncytiotrophoblast cells, hypothalamus cells, and pituitary cells [12]. Evidence shows that leptin receptors can be found along the hypothalamic-pituitary-ovarian (HPO) axis of females. Therefore, leptin has direct regulatory effects, both inhibitory and stimulatory depending on its concentration, on all parts of the HPO axis, and all stages of the reproductive process starting from puberty, menstrual cycle, pregnancy, early embryo development, and lactations[13,17].

In the context of reproduction, several scholars have tried to find a relationship between adipose tissue hormones (adipokines) and the female reproductive system in general, and especially between leptin and ovarian functions. Findings have shown that high leptin concentration inhibited ovarian steroidogenesis, folliculogenesis, and oogenesis. The high level of leptin is associated with low levels of ovarian hormones, estradiol, and progesterone, and the poor quality of produced ova[18-20]. Therefore, it may be considered that the concentration of leptin is related to female infertility, and it could explain some cases of female UI. Meanwhile, studies on the association between leptin and UI reported

conflicting results. Some studies showed that serum leptin levels were higher in women with UI compared with the fertile women[11,19-21], whereas other studies showed that there was no significant difference in leptin in women with UI compared to fertile women[22,23]. Thus, this study was aimed to find serum leptin levels in women with UI by conducting a systematic review and meta-analysis in order to quantitatively pool all findings from the relevant studies.

MATERIALS AND METHODS

Inclusion and exclusion criteria

All studies that defined UI based on the World Health Organization standard definition and reported the plasma level of leptin in women with UI and fertile women were eligible for this study. Studies were not eligible for this study if: (1) They were reviews, letters, editorials, or studies using animals or cell lines; (2) No healthy control group was included; or (3) The study enrolled participants who had diseases other than UI, and/or were on any kind of medication.

Information sources

The current study was performed according to PRISMA guidelines (Preferred Reporting Items for Systematic Reviews and Meta-Analyses)[24]. The information was retrieved from electronic and non-electronic database sources. Electronic sources included: PubMed/MEDLINE, Embase, Cochrane Central Register of Controlled Trials, and ClinicalTrials.gov. Non-electronic sources included: Direct Google search, Google Scholar, OpenGrey, OATD, WorldCat.org, American Society for Reproductive Medicine and Canadian Fertility and Andrology Society (ASRM/CFAS) Conjoint Annual Meeting, Abstracts of the Scientific Oral and Poster Sessions, and European Society of Human Reproduction and Embryology (ESHRE) Annual Meeting, Abstracts of the Scientific Oral and Poster Sessions. All those databases were searched from their inception to April 30, 2021, for human studies published in English.

Search strategy

The terms AND, OR, and NOT were used as Boolean search terms to develop the research strategy, and the final search strategy included the use of Title/Abstract related to (Women with Unexplained Infertility) AND (Leptin) taken from the study questions. Non-electronic sources were used combined with direct Google search, Google Scholar, OpenGrey, OATD, and WorldCat.org, American Society for Reproductive Medicine and Canadian Fertility and Andrology Society (ASRM/CFAS) Conjoint Annual Meeting, Abstracts of the Scientific Oral and Poster Sessions, and ESHRE Annual Meeting, Abstracts of the Scientific Oral and Poster Sessions. In addition, a manual search by the investigators was done for the grey literature and unpublished thesis/papers.

Selection process

The selection of the studies was done following these steps: (1) All retrieved studies were exported to the EndNote X9 citation manager, to check for duplication, and then the duplicated articles were removed; (2) Three authors (AA, MA, and SO) screened and evaluated the remaining studies independently by carefully reading the title and abstract, and all studies that mentioned the outcomes of the review [(Women with Unexplained Infertility)/Leptin] in their titles and abstracts were considered for further evaluation based on the objectives, methods, participants, and the key findings, serum levels of leptin in women with UI; (3) Two authors (AA and MA) independently evaluated the quality of the relevant studies against the checklist; and (4) Any discrepancy was resolved by discussion between the two authors (AA and MA), or by asking a third reviewer if consensus could not be reached. The selection process of the studies is presented using PRISMA statement flow diagram (Figure 1).

Data collection process

After the selection of all appropriate articles for this study, the relevant data were extracted by two investigators independently (AA and MA) using a data extraction template and presented using Microsoft Word 2016. The investigators contacted the authors of any study who did not report the aforementioned data (*via* email) to obtain the original data and after the expiration of the 2-wk timeline, the studies with the missing data that could not be obtained were removed. The extraction template contained author name, year of publication, study design, method of serum leptin measurement, body mass index (BMI), age, sample size, leptin concentration, and the LH/FSH ratio (Table 1). The data extraction accuracy was verified by comparing the data extraction results from the two investigators (AA and MA), who independently extracted the data in a randomly selected subset of papers (30% of the total).

Data items

The main outcome of this study was the serum leptin levels in women with UI, and it was measured by the direct report from the individual studies. To quantify the outcome "serum leptin levels in women

Table 1 Main characteristics of studies included in the meta-analysis

Ref.	Region	Study design	Method	BMI (kg/m²)		Age, years		Sample size		Leptin (mean + SD), ng/mL		LH/FSH ratio
				UI	Controls	UI	Controls	UI	Controls	UI	Controls	
Tafvizi and Masomi[20], 2016	Iran	Case–control	EIA	23.6 ± 0.27	23.66 ± 0.33	29.3 ± 4.2	31.03 ± 3.76	40	30	31.20 ± 2.83	24.89 ± 2.94	0.85
Al-Fartosy *et al* [21], 2019	Iraq	RCT	EIA	22.3 ± 3	21.7 ± 1.4	29.45 ± 6.38	30.92 ± 5.90	63	33	28.4 ± 1.3	27.2 ± 2.3	0.61
Kamyabi and Gholamalizad [22], 2015	Iran	Case-control	EIA	24.84	22.26	30	29	30	30	27.83 ± 25.29	31.27 ± 11.02	NR
Kumari *et al*[11][1], 2017	Indian	Case-control	EIA	24.1 ± 3.9	24.31 ± 3.19	29.53 ± 4.43	29.58 ± 4.01	120	109	68.9 ± 2.46	37.5 ± 1.84	0.85
Baig *et al*[23], 2019	Pakistan	Case-control	RIA	NR	NR	NR	NR	235	205	35.32 ± 0.9	37.11 ± 1.19	2.16
Demir *et al*[19], 2007	Turkey	PCS	RIA	25	24.5	29.3	28.9	27	30	7 ± 1	3.4 ± 1	1.17

[1]The serum leptin level in the original study was measured in pg, and converted to ng using the standard method.

BMI: Body mass index; EIA: Enzyme-linked immunosorbent assay; FSH: Follicle-stimulating hormone; LH: Luteinizing hormone; NR: Not reported; PCS: Prospective comparable controlled study; RIA: Radioimmunoassay; RCT: Randomized control trial; SD: Standard deviation; UI: Unexplained infertility.

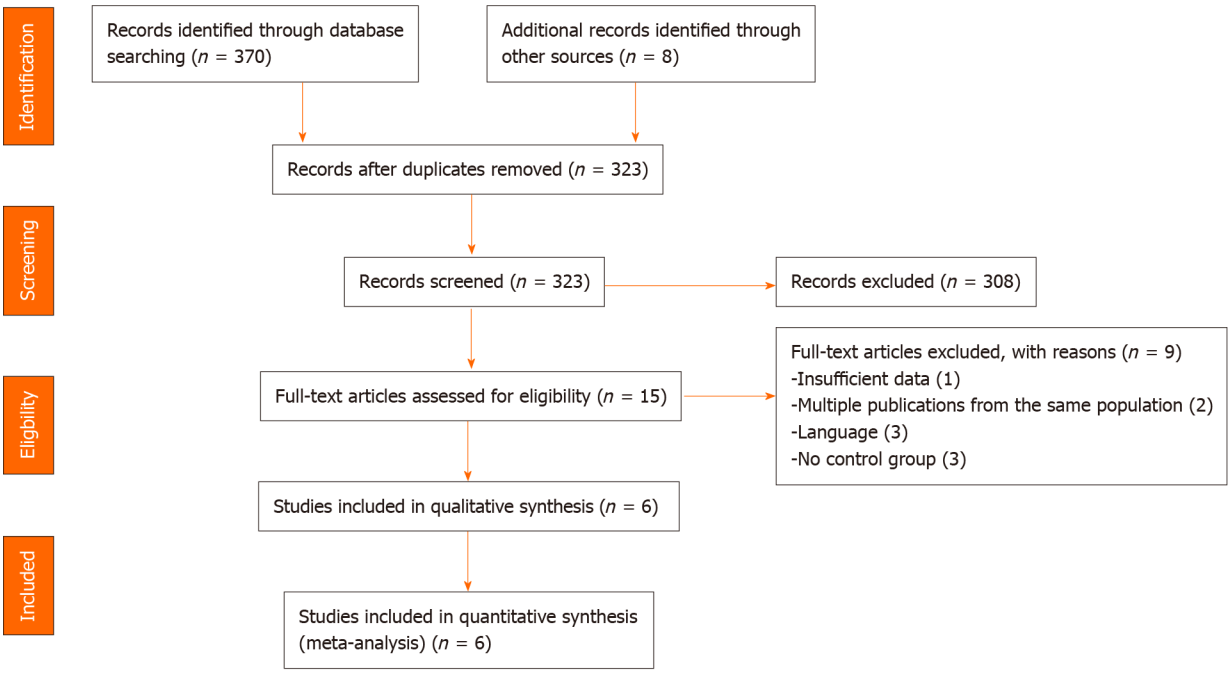

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Figure 1 Flow diagram of the studies included in this meta-analysis.

with UI", the investigators considered studies that reported serum leptin levels in their results. The result is expressed as the mean and standard deviation (SD).

Study risk of bias assessment

The inclusion criteria were appraised for all retrieved articles by using their title and abstract first, and then, the full text was screened to check the quality of each study before the final selection. The following was the quality assessment criteria for the studies in the current review: (1) The diagnosis of female UI occurred after performing all available fertility tests; (2) The sample was representative for the cases and controls; (3) The controls enrolled were taken from the same population; (4) The controls had no any past history of UI; (5) The cases and controls were matched for age or BMI or the two of them together; and (6) The methods which were used to check the serum level of leptin were the same for

cases and controls for each individual study.

A comprehensive search (electronic/database search, manual search, grey literature search, and unpublished studies search) was done to minimize the risk of bias. The risk of bias from the included studies was individually appraised by three investigators (AA, MA, and SO) using a critical appraisal tool (Joanna Briggs Institute Quality Assessment Tool)[25]. The publication bias for the included studies was checked by visual inspection of the funnel plot and checking the statistical symmetry of the funnel plot using Egger's regression test.

Effect measures

In light of the study objectives, the mean difference was used to synthesize and present the results for the analysis.

Synthesis methods

STATA software version 16 was used to synthesize and analyze the meta-analysis data. The recommendations of the I^2 statistic described by Higgins *et al*[26] (I^2 of 75/100% and above suggesting considerable heterogeneity) were used to perform this meta-analysis. The standardized mean difference (SMD) and 95% confidence interval (CI) were calculated for each study, based on the sample size, study mean, and SD of serum leptin levels in the case and control groups.

The potential publication bias was checked using a funnel plot and Egger's regression test, and it was assumed to be significant if P values were less than 0.10. To identify the sources of heterogeneity, meta-regression was performed to evaluate the between-study heterogeneity and to assess the influence of different study features, such as sample size, test method, BMI, and LH/FSH ratio.

The studies were excluded from the final review if: (1) They had missing data; and (2) They had a high risk of bias. The study results were reported according to the PRISMA guidelines and the findings of the included studies are first presented using a narrative synthesis, followed by a meta-analysis chart.

RESULTS

Study selection

As shown in Figure 1, a total of 378 articles were identified through the major electronic and non-electronic databases, and other relevant sources. A total of 55 studies were removed due to duplication, and the remaining 323 studies were kept for further critical screening. From the 323 studies which were kept in the first phase, 308 were excluded after they went through a very careful screening according to their titles and abstracts. From the remaining 15 articles, 9 studies were excluded due to inconsistency with the study inclusion criteria. Finally, 6 studies that fulfilled the eligibility criteria, involving 515 women with UI and 437 controls, were included for the systematic review and meta-analysis.

Study characteristics

The circulating leptin levels in patients with UI *vs* controls were evaluated in all 6 studies. In 4 studies, serum leptin levels were measured by enzyme-linked immunosorbent assay and by radioimmunoassay in the remaining two studies. Two studies were done in Iran, and one in each in Pakistan, Iraq, India, and Turkey. All studies were matched using both BMI and age. However, one study did not report them, but it stated that "the study was matched using BMI and age". In all included studies, the mean range was between 21.7 and 24.8 for BMI, and between 29 and 31 years for age. In addition, some missing information in the original studies was retrieved from the corresponding authors. Table 1 shows the detailed characteristics of all included studies.

Results of synthesis

Figure 2 shows the result of this meta-analysis. The pooled estimate of the included studies showed that leptin levels were significantly higher in UI cases than in controls, with an SMD of 0.97 (95%CI: -0.49 to 2.43). The heterogeneity across studies was highly significant ($P < 0.00001$; $I^2 = 98.8\%$), hence the random effect model was employed for the analysis.

Sensitivity and meta-regression analysis

A sensitivity analysis was conducted by sequentially excluding studies from the meta-analysis to further investigate the possible sources of the heterogeneity among the studies, and the results suggested that the meta-analysis result was stable. In addition, multivariate meta-regression was performed to evaluate the influence of several factors that may modify the association between serum leptin levels and UI, including test method, BMI, sample size, and LH/FSH ratio (adjusted $P = 0.177$, 0.208, 0.997, and 0.15, respectively). However, the results showed that these confounding factors did not substantially affect the heterogeneity. For the test of publication bias, the funnel plots (Figure 3) were visually symmetric and the Egger's test for publication bias yielded a P value of 0.279, hence these results did not provide evidence of a significant effect.

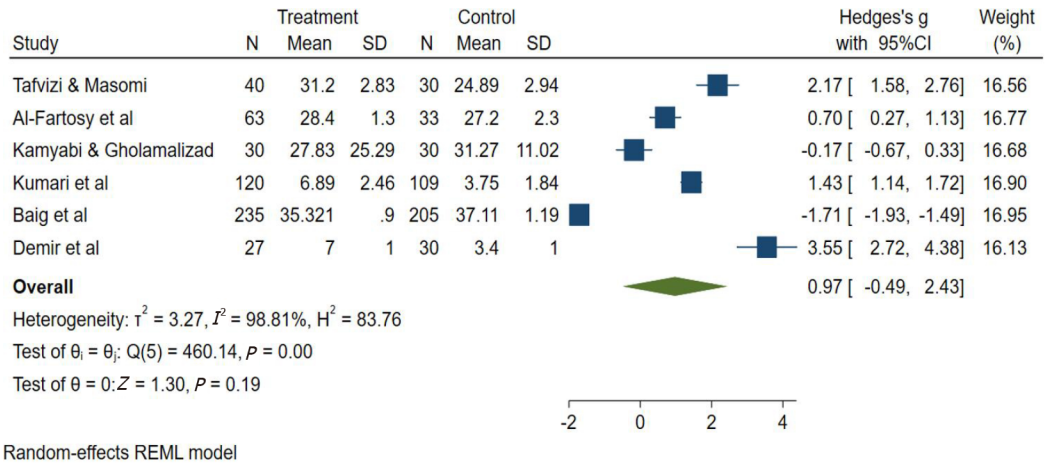

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Figure 2 Forest plot (random-effects model) for circulating leptin levels in women with unexplained infertility.

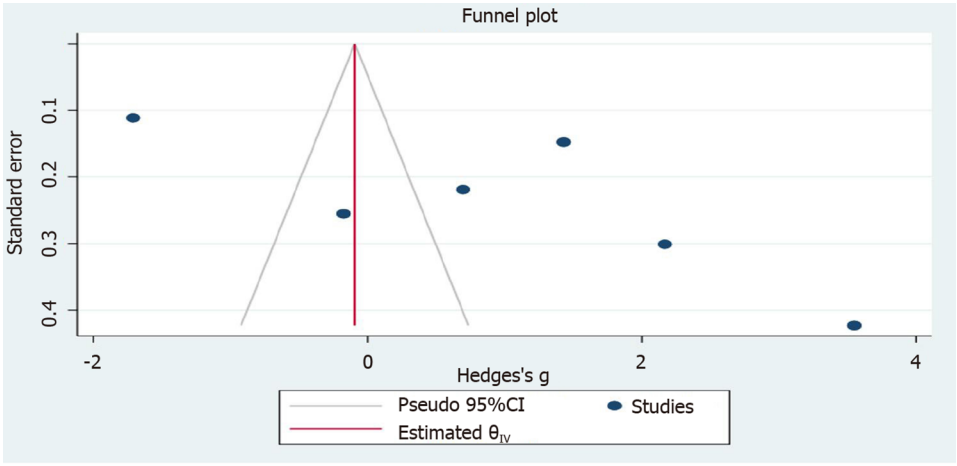

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Figure 3 Funnel plot for publication bias.

DISCUSSION

The term UI emerged due to the fact that the current knowledge on assessment and treatment of the reproductive system is still inadequate[3]. Many studies have been carried out to find the exact cause of UI and the best diagnostic biomarker. However, leptin has been found as one of the best biomarkers. Leptin displays biological activities by binding and activating specific leptin receptors, which are found in many organs including the hypothalamus-pituitary-ovarian axis (HPO axis) in females. Leptin plays a role in the function of the HPO axis by stimulating the release of gonadotrophin-releasing hormone, gonadotrophins, and aromatase enzymes from the hypothalamus, pituitary gland, and ovaries, respectively[13,15,17,27,28]. Also, the available evidence indicated that a high level of leptin has negative effects on female reproduction due to its inhibitory effect on the HPO axis, ovarian physiology, folliculogenesis, steroidogenesis, and the production and release of oxytocin and prostaglandin[29-32]. In line with available evidence, the current study showed that leptin level was significantly higher in women with UI compared with the control group. Similar results were found when leptin concentrations were pooled in endometriosis and polycystic ovary syndrome (PCOS) cases, pathological conditions with a strong correlation with infertility. In these studies, leptin levels were higher in women with endometriosis and PCOS groups compared with control groups[33,34].

Studies have suggested that factors like age and BMI have effects on leptin levels, and usually have a positive correlation with female infertility[35,36]. However, in the present study, all the included studies were matched by both age and BMI, and because of that, the effect of age and BMI on leptin level was not examined.

This is the first comprehensive quantitative meta-analysis summarizing available evidence to determine the serum leptin level in women with UI.

CONCLUSION

The results of this meta-analysis suggest the presence of elevated levels of leptin levels in women with UI compared with fertile women. Hence, leptin could be a potential biomarker for UI in women, and it may be useful for identifying women at a high risk of infertility. However, further investigations need to be carried out in order to clarify the exact association between leptin levels and UI.

ARTICLE HIGHLIGHTS

Research background

Despite many breakthroughs in the field of infertility diagnosis and treatment, there are still some infertility cases with unknown causes (unexplained infertility). To overcome this diagnostic issue, many serum biomarkers have been used as predictive markers for unexplained infertility in females.

Research motivation

Leptin is one of the most accurate biomarkers for unexplained infertility. Nevertheless, conflicting results regarding leptin levels in women with unexplained infertility have been reported.

Research objectives

The objective of this study was to conduct a systematic review and meta-analysis to find the serum leptin levels in women with unexplained infertility.

Research methods

A systematic literature search was conducted before April 30, 2021 from PubMed/MEDLINE, Embase, Cochrane Central Register of Controlled Trials, ClinicalTrials.gov, Google Scholar, OpenGrey, OATD, and the infertility conferences abstract.

Research results

A total of six articles were included in this meta-analysis after 15 articles had been subjected to full-text evaluations.

Research conclusions

Leptin could be a potential biomarker for unexplained infertility in women.

Research perspectives

The effect of other adipokines should be evaluated in future studies to find their possible relation with female infertility.

FOOTNOTES

Author contributions: Abdullah AA, Ahmed M, and Oladokun A conceived and designed the review, developed the search strings, screened and selected the studies, and drafted the manuscript, and Oladokun A rigorously reviewed the manuscript; Abdullah AA is the guarantor of the review; Abdullah AA and Ahmed M extracted the data, evaluated the quality of the studies, and carried out analysis and interpretation.

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REFERENCES

1 **Patrizi G**, Manna C, Moscatelli C, Nieddu L. Pattern recognition methods in human-assisted reproduction. *Int Trans Oper Res* 2004; **11**: 365-379 [DOI: 10.1111/j.1475-3995.2004.00464.x]
2 **Abebe MS**, Afework M, Abaynew Y. Primary and secondary infertility in Africa: systematic review with meta-analysis. *Fertil Res Pract* 2020; **6**: 20 [PMID: 33292584 DOI: 10.1186/s40738-020-00090-3]
3 **Mol BW**, Tjon-Kon-Fat R, Kamphuis E, van Wely M. Unexplained infertility: Is it over-diagnosed and over-treated? *Best Pract Res Clin Obstet Gynaecol* 2018; **53**: 20-29 [PMID: 30518485 DOI: 10.1016/j.bpobgyn.2018.09.006]
4 **Abdallah KS**, Hunt S, Abdullah SA, Mol BWJ, Youssef MA. How and Why to Define Unexplained Infertility? *Semin Reprod Med* 2020; **38**: 55-60 [PMID: 33058088 DOI: 10.1055/s-0040-1718709]
5 **World health Organization**. Infertility 2021 [April 29, 2021]. Available from: https://www.who.int/reproductivehealth/topics/infertility/keyissues/en/
6 **Abdullah AA**, Ahmed M, Oladokun A. Prevalence of infertility in Sudan: A systematic review and meta-analysis. *Qatar Med J* 2021; **2021**: 47 [PMID: 34650907 DOI: 10.5339/qmj.2021.47]
7 **Abdelazim IA**, Purohit P, Farag RH, Zhurabekova G. Unexplained infertility: prevalence, possible causes and treatment options. A review of the literature. *J Obstet Gynecol Investig* 2018; **1** 1: e17-e22 [DOI: 10.5114/jogi.2018.74250]
8 **Christensen A**, Bentley GE, Cabrera R, Ortega HH, Perfito N, Wu TJ, Micevych P. Hormonal regulation of female reproduction. *Horm Metab Res* 2012; **44**: 587-591 [PMID: 22438212 DOI: 10.1055/s-0032-1306301]
9 **Ehsani M**, Mohammadnia-Afrouzi M, Mirzakhani M, Esmaeilzadeh S, Shahbazi M. Female Unexplained Infertility: A Disease with Imbalanced Adaptive Immunity. *J Hum Reprod Sci* 2019; **12**: 274-282 [PMID: 32038075 DOI: 10.4103/jhrs.JHRS_30_19]
10 **Qu T**, Yan M, Shen WJ, Li L, Zhu P, Li Z, Huang J, Han T, Hu W, Zhou R, Li P, Xu L, Huang T, Zhong Y, Gu J. Predictive serum markers for unexplained infertility in child-bearing aged women. *Am J Reprod Immunol* 2020; **83**: e13194 [PMID: 31585484 DOI: 10.1111/aji.13194]
11 **Kumari P**, Jaiswar SP, Shankhwar P, Deo S, Ahmad K, Iqbal B, Mahdi AA. Leptin as a Predictive Marker in Unexplained Infertility in North Indian Population. *J Clin Diagn Res* 2017; **11**: QC28-QC31 [PMID: 28511457 DOI: 10.7860/JCDR/2017/22444.9567]
12 **Herrid M**, Palanisamy SK, Ciller UA, Fan R, Moens P, Smart NA, McFarlane JR. An updated view of leptin on implantation and pregnancy: a review. *Physiol Res* 2014; **63**: 543-557 [PMID: 24908087 DOI: 10.33549/physiolres.932674]
13 **Mantzoros CS**, Magkos F, Brinkoetter M, Sienkiewicz E, Dardeno TA, Kim SY, Hamnvik OP, Koniaris A. Leptin in human physiology and pathophysiology. *Am J Physiol Endocrinol Metab* 2011; **301**: E567-E584 [PMID: 21791620 DOI: 10.1152/ajpendo.00315.2011]
14 **Blüher S**, Mantzoros CS. Leptin in reproduction. *Curr Opin Endocrinol Diabetes Obes* 2007; **14**: 458-464
15 **Pérez-Pérez A**, Sánchez-Jiménez F, Maymó J, Dueñas JL, Varone C, Sánchez-Margalet V. Role of leptin in female reproduction. *Clin Chem Lab Med* 2015; **53**: 15-28 [PMID: 25014521 DOI: 10.1515/cclm-2014-0387]
16 **Mitchell M**, Armstrong DT, Robker RL, Norman RJ. Adipokines: implications for female fertility and obesity. *Reproduction* 2005; **130**: 583-597 [PMID: 16264089 DOI: 10.1530/rep.1.00521]
17 **Khan SM**, Hamnvik OP, Brinkoetter M, Mantzoros CS. Leptin as a modulator of neuroendocrine function in humans. *Yonsei Med J* 2012; **53**: 671-679 [PMID: 22665330 DOI: 10.3349/ymj.2012.53.4.671]
18 **Fornari MC**, Sarto A, Berardi VE, Martínez MA, Rocha MG, Pasqualini S, Diez RA. Effect of ovaric hyper-stimulation on blood lymphocyte subpopulations, cytokines, leptin and nitrite among patients with unexplained infertility. *Am J Reprod Immunol* 2002; **48**: 394-403 [PMID: 12607776 DOI: 10.1034/j.1600-0897.2002.01128.x]
19 **Demir B**, Guven S, Guven ES, Atamer Y, Gunalp GS, Gul T. Serum leptin level in women with unexplained infertility. *J Reprod Immunol* 2007; **75**: 145-149 [PMID: 17485120 DOI: 10.1016/j.jri.2007.04.001]
20 **Tafvizi F**, Masomi M. Comparison of Serum Leptin Level in Women with Unexplained Infertility and Fertile Women in Iran. *J Obstet Gynaecol India* 2016; **66**: 466-470 [PMID: 27651647 DOI: 10.1007/s13224-016-0843-9]
21 **Al-Fartosy AJM**, Awad NA, Mahmood RA. A comparative study of leptin, oxidant/antioxidant status and some trace elements in women of healthy control and unexplained infertility in Basrah-Iraq. *Indones Biomed J* 2019; **11**: 327-337 [DOI: 10.18585/inabj.v11i3.915]
22 **Kamyabi Z**, Gholamalizade T. A Comparative Study of Serum and Follicular Fluid Leptin Concentrations among Explained Infertile, Unexplained Infertile and Fertile Women. *Int J Fertil Steril* 2015; **9**: 150-156 [PMID: 26246872 DOI: 10.22074/ijfs.2015.4235]
23 **Baig M**, Azhar A, Rehman R, Syed H, Tariq S, Gazzaz ZJ. Relationship of Serum Leptin and Reproductive Hormones in Unexplained Infertile and Fertile Females. *Cureus* 2019; **11**: e6524 [PMID: 32025443 DOI: 10.7759/cureus.6524]
24 **Page MJ**, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, Shamseer L, Tetzlaff JM, Akl EA, Brennan SE, Chou R, Glanville J, Grimshaw JM, Hróbjartsson A, Lalu MM, Li T, Loder EW, Mayo-Wilson E, McDonald S, McGuinness LA, Stewart LA, Thomas J, Tricco AC, Welch VA, Whiting P, Moher D. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ* 2021; **372**: n71 [PMID: 33782057 DOI: 10.1136/bmj.n71]
25 **Jordan Z**, Lockwood C, Munn Z, Aromataris E. The updated Joanna Briggs Institute Model of Evidence-Based Healthcare. *Int J Evid Based Healthc* 2019; **17**: 58-71 [PMID: 30256247 DOI: 10.1097/XEB.0000000000000155]

26 **Higgins JP**, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ* 2003; **327**: 557-560 [PMID: 12958120 DOI: 10.1136/bmj.327.7414.557]

27 **Catalano S**, Marsico S, Giordano C, Mauro L, Rizza P, Panno ML, Andò S. Leptin enhances, *via* AP-1, expression of aromatase in the MCF-7 cell line. *J Biol Chem* 2003; **278**: 28668-28676 [PMID: 12734209 DOI: 10.1074/jbc.m301695200]

28 **Panwar S**, Herrid M, Kauter KG, McFarlane JR. Effect of passive immunization against leptin on ovarian follicular development in prepubertal mice. *J Reprod Immunol* 2012; **96**: 19-24 [PMID: 22989496 DOI: 10.1016/j.jri.2012.07.004]

29 **Masarwi M**, Shamir R, Phillip M, Gat-Yablonski G. Leptin stimulates aromatase in the growth plate: limiting catch-up growth efficiency. *J Endocrinol* 2018; **237**: 229-242 [PMID: 29615477 DOI: 10.1530/JOE-18-0028]

30 **Ashrafi Mahabadi S**, Tafvizi F. Reduction of Soluble Leptin Receptor Levels in Women with Unexplained Infertility and the Effect of Leptin Receptor Gln223Arg Polymorphism on its Serum Level. *J Obstet Gynecol Cancer Res* 2020; **5**: 149-158 [DOI: 10.30699/jogcr.5.4.149]

31 **Vilariño-García T**, Pérez-Pérez A, Santamaría-López E, Prados N, Fernández-Sánchez M, Sánchez-Margalet V. Sam68 mediates leptin signaling and action in human granulosa cells: possible role in leptin resistance in PCOS. *Endocr Connect* 2020; **9**: 479-488 [PMID: 32375121 DOI: 10.1530/EC-20-0062]

32 **Landry DA**, Sormany F, Haché J, Roumaud P, Martin LJ. Steroidogenic genes expressions are repressed by high levels of leptin and the JAK/STAT signaling pathway in MA-10 Leydig cells. *Mol Cell Biochem* 2017; **433**: 79-95 [PMID: 28343310 DOI: 10.1007/s11010-017-3017-x]

33 **Kalaitzopoulos DR**, Lempesis IG, Samartzis N, Kolovos G, Dedes I, Daniilidis A, Nirgianakis K, Leeners B, Goulis DG, Samartzis EP. Leptin concentrations in endometriosis: A systematic review and meta-analysis. *J Reprod Immunol* 2021; **146**: 103338 [PMID: 34126469 DOI: 10.1016/j.jri.2021.103338]

34 **Zheng SH**, Du DF, Li XL. Leptin Levels in Women With Polycystic Ovary Syndrome: A Systematic Review and a Meta-Analysis. *Reprod Sci* 2017; **24**: 656-670 [PMID: 27798245 DOI: 10.1177/1933719116670265]

35 **Daghestani MH**, Daghestani M, Daghistani M, El-Mazny A, Bjørklund G, Chirumbolo S, Al Saggaf SH, Warsy A. A study of ghrelin and leptin levels and their relationship to metabolic profiles in obese and lean Saudi women with polycystic ovary syndrome (PCOS). *Lipids Health Dis* 2018; **17**: 195 [PMID: 30131073 DOI: 10.1186/s12944-018-0839-9]

36 **Van Zyl S**, Van der Merwe LJ, Van Rooyen FC, Joubert G, Walsh CM. The relationship between obesity, leptin, adiponectin and the components of metabolic syndrome in urban African women, Free State, South Africa. *South Afr J Clin Nutr* 2017; **30**: 8-13 [DOI: 10.1080/16070658.2017.1267380]

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