Review

Bioconversion, Pharmacokinetics, and Therapeutic Mechanisms of Ginsenoside Compound K and Its Analogues for Treating Metabolic Diseases

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Bioconversion, Pharmacokinetics, and Therapeutic Mechanisms of Ginsenoside Compound K and Its Analogues for Treating Metabolic Diseases

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Abstract: Rare ginsenoside compound K (CK), is an intestinal microbial metabolite with a low natural abundance that is primarily produced by physicochemical processing, side chain modification, or metabolic transformation in the gut. Moreover, CK exhibits potent biological activity compared to primary ginsenosides, which has raised concerns in the field of ginseng research and development as well as ginsenosides-related dietary supplements and natural products. Ginsenosides Rb1, Rb2, and Rc are generally used as a substrate to generate CK via several bio-conversion processes. Current research shows that CK has a wide range of pharmacological actions including boosting osteogenesis, lipid and glucose metabolism, lipid oxidation, insulin resistance, anti-inflammatory, and anti-apoptosis properties. Further research on the bioavailability and toxicology of CK can advance its medicinal application. The purpose of this review is to lay the groundwork for future clinical studies and the development of CK as a therapy for metabolic disorders. Furthermore, the toxicology and pharmacology of CK are investigated as well in this review. The findings indicate that CK primarily modulates signaling pathways associated with AMPK, SIRT1, PPARs, WNTs, and NF-kB. It also demonstrates a positive therapeutic effect of CK on nonalcoholic fatty liver disease, obesity, hyperlipidemia, diabetes, and its complications, as well as osteoporosis. Additionally, the analogues of CK showed more bioavailability, less toxicity, and more efficacy against disease states. Enhancing bioavailability and regulating hazardous variables are crucial for its use in clinical trials.

Keywords: ginsenoside compound K; metabolic disease; obesity; NAFLD; diabetes; osteoporosis

1. Introduction

In recent years, metabolic disorders become a global health concern because of their rapidly increasing prevalence [1]. Global human health is severely challenged by the increasing incidence of metabolic diseases, which include type 2 diabetes (T2D), obesity, nonalcoholic fatty liver disease (NAFLD), gout, osteoporosis, hypothyroidism and hyperthyroidism [2]. The International Diabetes Federation (IDF) [3] reports that 537 million individuals worldwide had diabetes in 2021, with more than 90% of cases being type 2 diabetes. By 2045, the figure is predicted to rise to 783 million. In addition, obesity has emerged as the biggest global problem of concern for public health, with the incidence of overweight and obesity rising sharply in recent years. In 2016, there were over 650 million and over 1.9 billion obese and overweight adults worldwide, respectively, making up around 39% of the world's population [4]. According to estimations in 2019, the worldwide incidence of gout was 0.1%–0.3% [5] and the prevalence of non-alcoholic fatty liver disease (NAFLD) was 29.62% in Asia [5]. These findings demonstrate that metabolic disorders represent a substantial challenge in

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human society due to the associated high mortality and morbidity; thus, understanding the pathophysiology and therapies of metabolic diseases is critical.

The pharmacological properties of Korean ginseng (*Panax ginseng* Meyer), which has been revered as one of the most renowned traditional Chinese herbal medicines for over two hundred years, are largely attributed to its bioactive triterpenoid saponins. These triterpenoid saponins, called ginsenosides, are divided into three categories: panaxadiol (PPD), panaxatriol (PPT), and oleanic acid [6]. More than 218 ginsenosides have been determined from multiple parts of the ginseng plant (roots, flowers, berries, and leaves) and these byproducts have become popular for research [7]. However, Ginsenoside compound K (CK) is one of the most significant among these ginsenosides due to its high uptake and absorption rate into the human gastrointestinal tract and ultimately the systemic circulation [8]. Compared to other ginsenosides, CK has superior membrane permeability and a lower molecular weight, which contribute to its increased bioavailability [9]. Major Ginsenosides undergo the transition to produce CK, which is rarely present in natural ginseng. Human gut bacteria and endophytes have been reported to use deglycosylated processes to bio-convert CK products. In the past year, yeasts have been metabolically altered, and produced by enzymes have become viable alternatives for producing CK [10]. According to current research, CK possesses pharmacological properties that include hepatoprotective, anti-inflammatory, anti-carcinogenic, anti-diabetic, anti-allergic, neuro-protective, and anti-aging activities [11].

CK is an active molecule that can reduce blood lipids and control glucose consumption [12]. Notably, CK is a regulator of PPARγ [13], and AMPK [14]. It has been demonstrated that AMPK increases glucose utilization, mobilizes lipid storage, and promotes autophagy to turnover macromolecular routes, so promoting the breakdown of biomolecules for the creation of energy [15]. PPARγ influences lipid metabolism, which enhances sensitivity to insulin and glucose metabolism [16]. AMPK and PPARγ are the fundamental targets in metabolic disorders, including nonalcoholic fatty liver disease, diabetes, osteoporosis, NAFLD, and obesity [17,18]. In addition, CK down-regulates PPAR, leptin, aP2, and C/EBP adipogenic markers, which cause obesity, diabetes, and other metabolic diseases [19]. Moreover, the Deregulation of metabolic processes associated with TP53 results in various human pathologies, such as obesity, diabetes, liver, and cardiovascular illnesses [20]. CK significantly regulates the TP53 expression in different disease states [21].

As a result, it is hypothesized that CK may be involved in metabolic illnesses by controlling inflammation and energy. However, due to the shortage of adequate information on CK regarding its use for the cure of metabolic illness, the cytotoxicity is well documented, which prevents further development of the drug. This study includes an in-depth assessment of the use of CK to treat metabolic illnesses and the signaling pathways involved, as well as an analysis of its usual negative effects and pharmacokinetics. To give direction and evidence for CK research on metabolic conditions, we investigated the Google Scholar, Web of Science, PubMed, and CNKI databases up to December 2023.

2. Physical and chemical properties

CK (20-*O*-β-D-glucopyranosyl-20(*S*)-protopanaxadiol) is a minor tetra-cyclic triterpenoid that is rarely found in natural ginseng. CK is isolated via various biotransformation processes from the protopanaxadiol saponins including ginsenoside RB1, Rb2, and Rc [22]. Several approaches for CK synthesis have been described in detail (**Biotransformation of CK section**). CK is a white crystalline powder with a molecular weight of 622.9 g/mol, molecular formula $C_{36}H_{62}O_8$ [23], and PubChem CID 9852086. The physical and chemical properties of CK are shown in Table 1 (data collected from https://www.chemicalbook.com/).

Table 1. Physical and chemical properties of CK.

Name	Compound K
Alias	20-*O*-β-D-glucopyranosyl-20(*S*)-protopanaxadiol
CAS number	39262-14-1

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Pubchem CID	9852086
Compound type	tetra-cyclic tri-terpenoid
Molecular formula	$C_{36}H_{62}O_8$
Molecular weight	622.9 g/mol
Form	powder
color	White
Solubility	DMF: 10 mg/ml; DMSO: 10 mg/ml; DMSO: PBS (pH 7.2) (1:1): 0.5 mg/ml
Density	1.19
pka	12.94±0.70(Predicted)
Melting point	181 ~ 183℃
Boiling point	723.1±60.0 °C
LogP	5.500
Stability	Hygroscopic

3. Pharmacokinetics, safety, and toxicological studies of CK

The bioavailability rate of ginsenosides without conversion or modification indicates limited intestinal absorption [24]. Following oral ingestion, ginsenosides undergo several biological changes in the digestive system that result in the conversion of these molecules into deglycosylated metabolites that have more biological activity than their precursor components [25]. According to other research, some microorganisms and gut bacteria or soil fungi around ginseng roots hydrolyze ginsenosides to produce CK. It is essential to research the metabolic processes that control intestinal microbiota since it plays a crucial role in the biological transformation and therapeutic effects of CK [24]. Based on recent research on human metabolism, a high-fat diet greatly speeds up and increases the digestion of CK, and women have higher concentrations of CK than men [26]. After delivering Korean ginseng extract to ten healthy males for 36 hours, the drug levels in their blood samples were reported in additional pharmacokinetic investigation on CK [27]. The mean time to achieve the Cmax (Tmax) of CK was greater compared to Rb1 (12.20±1.81 vs. 8.70±2.63 h), and the average highest plasma concentration (Cmax) of CK was substantially greater than the mean concentration of Rb1 (8.35±3.19 vs. 3.94±1.97 ng/mL). Intestinal microflora probably converts Rb1 to CK because of the delay in CK absorption. Compared to Rb1, CK had a plasma half-life ($t_{1/2}$) that was seven times shorter. The findings of this study suggest that there is a notable distinction in the pharmacokinetics of CK and Rb1. In a different study, 76 participants were given either a placebo or CK in seven individual doses taken orally (25, 50, 100, 200, 400, 600, and 800 mg) while they were fasting; the exposure to CK grew linearly between 100 and 400 mg, and the time range to attain Tmax was 1.5–6.0 h. After the seventh administration, the steady state was reached, and there were no serious adverse effects (AEs) reported. Watery stool (diarrhea) and stomachache were the most commonly reported AEs. All AEs were mild to severe, and the majority of them were cured quickly without any intervention. These findings demonstrated that CK was both safe and well-tolerated for the course of the treatment [26,28].

According to toxicity tests, CK was applied on 3T3L1 pre-adipocyte cells in a dose-dependent manner. The maximum concentration of 40μM did not affect the viability of the cells [29]. Fang et al examined Ginsenoside CK for cytotoxicity at various concentrations (0.2-10.0 μM). They observed that CK concentrations below 10 μM had no discernible impact on the survival rate of HaCaT keratinocyte cells [30]. The osteoblastic cell line MC3T3-E1 was exposed to CK at various concentrations (0.1–10 μM) and did not exhibit any appreciable toxicity [31]. When ginsenoside CK was evaluated on HepG2 cells, substantial cytotoxic effects were detected with increasing concentrations of ginsenoside CK up to 30 M compared to the control group [32]. Additionally, different CK doses (5-40 μM) were applied to HepG2 cells for a 24-hours to assess the cellular toxicity of CK. Even at dosages of 40 μM, CK did not exhibit any cellular damage [33]. However, the administration of a higher dose of CK (10 μM) significantly boosted the development of HT22

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hippocampus neuron cells [34]. After the treatment with 1.25-10 μM of CK, the rate of survival of the L02 cells climbed dramatically. CK showed no toxicity at any test concentration on L02 cells [35]. Human fibroblast-like synoviocytes RA-FLS cells and murine macrophage cells were treated at the same concentrations of 0.1-5 μM for the treatment of rheumatoid arthritis. The results showed that these cells were not affected by CK at a concentration of ≤5 μM [36]. Gu et al evaluated the possible cytotoxicity of CK on MIN6 mouse pancreatic β-cell at various doses (2-32μM). CK showed minimal effect at 16 μM and decreased the cell viability at 32 μM [37]. CK has a time- and concentration-dependent mild to moderate cytotoxic impact on cancer cells. The most susceptible to CK exposure were Hk-1 cells (a cell line used to study Nasopharyngeal Carcinoma), as 41.1–88.0% of cell mortality was seen at low levels (10–20 μM) [38]. Boopathi et al reported that CK exhibits negligible cytotoxic effect on A549 lung cancer cells, Caco-2 colorectal cancer cells, and MCF-7 breast cancer cells at 12.5 μg/mL, whereas, normal cell Raw 264.7 demonstrated less toxicity at 6.25μg/ml [39]. At concentrations ranging from 8 to 64 μmol/l, compound K inhibited the growth of HT-29 cells in a dose-dependent manner; the dosage that produced 50% inhibition of growth (IC50) was 32 μmol/l [40]. Oral CK delivery to rats and mice in a toxicity trial did not result in toxicity or death at the maximal doses of 8 and 10 g/kg respectively [41]. During a beagle toxicity investigation, dogs in the 36 mg/kg group experienced considerable weight loss and reversible hepatotoxicity. There was no discernible toxicity in the animals in the 4 and 12 mg/kg groups [42]. Table 2 elucidated the cytotoxicity of CK on different cell lines.

Table 2. Cytotoxic studies of CK in several cell lines.

Study model	Concentrations	Method of detection	Duration of experiment	Result	Ref.
3T3-L1 preadipocyte cell lines	(0, 10, 20, 30, 40 μM)	MTS assay	24 h	A high dose of CK (40 μM) did not affect cell viability	[29]
HaCaT keratinocytes cells	0.2, 0.4, 0.6, 0.8, 1.0, and 10.0 μM	MTT assay	24 h	The survival of HaCaT cells was not significantly affected by CK doses below 10 μM.	[30]
MC3T3-E1 osteoblastic cell line	0.01, 0.1, 1, 10 μM	MTT assay	48h	CK at various doses did not exhibit any appreciable toxicity.	[43]
HepG2	1, 2, 5, 10, 15, 20, and 30 μM	MTT assay	24 h	As ginsenoside CK concentration increased to 30 μM, notable cytotoxic effects were seen.	[32]
HepG2	5–40 μM	CellTiter 96 AQueous One Solution	24h	Up to 40 μM doses, CK did not exhibit any cellular toxicity.	[33]

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		Cell Proliferation Assay kit			
HT22 mouse hippocampal neuron cell	2.5, 5, and 10 μM	MTT assay	24 h	Ginsenoside CK can increase the survival of HT22 cells.	[34]
L02 Human liver cell line	0.625, 1.25, 2.5, 5, 10 and 20 μM	MTT assay	24 h	The viability of the L02 cells appeared dramatically after treatment with CK at dosages of 1.25, 2.5, 5, or 10 μM.	[35]
RA-FLS and Raw 264.7	0.1, 0.5, 2.5, and 5 μM	MTT assay	48 h	The ability to survive RA-FLS and RAW264.7 cells was not significantly impacted by CK at doses of ≤5μM.	[36]
MIN6 cell line	2, 4, 8, 16, and 32 μM	MTT assay	24 h	At 16 μM CK showed little toxicity on MIN6 cell	[37]
Hk-1 Nasopharyngeal Carcinoma cells,	1–20μM	MTT assay	24 h	The IC50 of CK was 11.5 on HK-1 cells	[38]
A549 lung cancer cells, MCF7 breast cancer cells, Caco-2 human colorectal adenocarcinoma cells, and normal RAW 264.7 cells	0,3.125,6.25,12.5, 25 μg/mL	MTT assay	24 h	At 12.5 μg/mL concentration, CK showed considerable cytotoxic effect on A549 cells, MCF-7 cells, and Caco-2 cells growth. However, at 6.25 μg/mL Raw 264.7	[39]

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				cells showed less toxicitry.	
HT-29 Human colon cancer cells	8, 16, 32 and 64 μmol/l	MTT assay	24 h	CK inhibited the growth of HT-29 cells in a dose-dependent manner.	[40]
HL-60 human myeloid leukemia cell line	10, 20, 30, 50 μM	MTT assay	72–96 h	24.3 μM was needed to achieve 50% growth inhibition (IC50) at 96 hours.	[44]
U937, Jurkat, CEM-CM3, Molt4, and H9 leukemia cell lines	Did not mention	MTT assay	96 h	The IC50 values of Compound K were as follows: 20μg/mL for U937, 26μg/mL for Jurkat, 36μg/mL for CEM-CM3, μg/mL for Molt 4, and 64μg/mL for H9.	[45]
Rat and mice	8 and 10 mg/kg Respectively	Acute oral repeated dose	26-week	There were no indications of unusual clinical harm or death after 14 days. There were a few notable variations observed in this shift at weeks 9, 10, 12, 15, 17, 21–24, and 26. As a result, CK had a minimally negative impact on the animal's body weight.	[41]

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Beagle dogs	4, 12, or 36 mg/kg	oral doses	26 week	No obvious toxicity was shown by the animals in the 4 and 12 mg/kg groups. The 36 mg/kg group showed elevated plasma enzyme levels, localized liver necrosis, and a decrease in body weight.	[46]

It has been demonstrated that CK is safe and well-tolerated in both human and animal subjects. These preclinical findings imply that CK may be harmful to the liver. Although the relative weight of the kidney was high, there was no histological change, but nephrotoxicity should be noted. Abdominal pain and diarrhea were CK-related adverse events observed in clinical trials. Both clinical trials and data on AEs associated with CK are scarce. Thus, more research is required to determine the processes underlying CK-induced gastrointestinal tract irritation and CK-induced damage, particularly hepatotoxicity. We studied ADMET analysis of CK there mentioned the drug likeliness and toxicity of CK **(Supporting information 1).**

4. Biotransformation of CK

It has been demonstrated that the naturally occurring ginseng plant does not provide significant amounts of the minor ginsenoside CK. Therefore, several studies have concentrated on using various techniques, including hydrolysis, enzymatic biotransformation, microbial transformation, etc., to convert major ginsenosides to CK. Additionally, endophyte biotransformation is an effective technique within microbial transformation because of its low cost, excellent selectivity, accuracy, and environmental safety [10]. Chemical hydrolysis conditions led to the nonspecific cleavage of glycone moieties at position 20, which in turn caused side reactions of hydroxylation, hydration, and epimerization. Furthermore, these methods proved to increase environmental contamination [47,48]. In contrast, owing to their notable selectivity, mild reaction conditions, and environmental compatibility, enzymatic or microbial conversion modalities have emerged as the most popular ones. Figure 1 demonstrates the biotransformation of major ginsenosides to CK via several pathways using enzymatic and microbial conversion methods.

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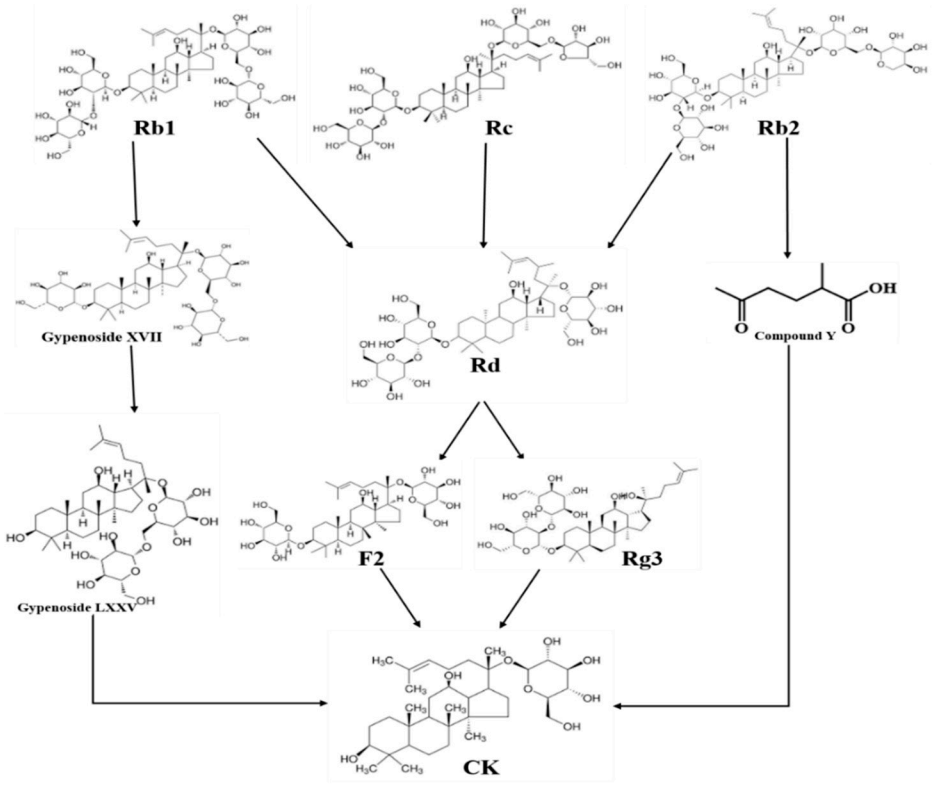

Figure 1. Biotransformation of major ginsenosides to CK via different pathways using Enzymatic and microbial conversion methods.

4.1. Enzymatically synthesis

One possible approach could be highly region-specific enzymatic transformation. Enzymatic techniques have been employed to produce CK from ginseng root extract, employing β-glucosidase (β-glu), β-glycosidase, and bi-composites of β-glucosidase (Table 3).

Table 3. Enzymatic biotransformation method for the development of CK production.

Enzymes	Transformation pathway	Optimum condition	Remarks	Ref.
β-glu from *Paecilomyces Bainier* sp. 229	Rb1→Rd→F2→CK	PH=3.5, Temp=55° C, Time=48h	84.3% ginsenoside Rb1 was converted to CK	[49]
β-glu from *Terrabacter ginsenosidimutans*	Rb1→gypenoside XVII→ gypenoside LXXV→CK	pH 7.0 37°C	Complete conversion of CK from Rb1 via intermediate metabolites gypenosides XVII and LXXV.	[50]
β-glu from *Lactobacillus brevis*	Rb1→gypenoside XVII→ gypenoside LXXV→CK	pH =6.0 Temp=30°	89% molar conversion	[51]

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		C Time=6 h		
β-glycosidase from *S. solfataricus*	Rb1/Rb2→Rd→F2→CK Rc →compound Mc → CK	PH=5.5, Temp=85° C, Time=12h	Although good specificity, the conversion rate is low.	[52]
β-glucosidase from *Pyrococcus furiosus*	Rb1/Rb2→Rd→F2→CK	PH=5.5, Temp=95° C, Time=6h	After five hours, aglycone PPD was produced by hydrolyzing the CK.	[53]
β-glucosidase from *Microbacterium esteraromaticum*	Rb2→Compound Y→CK	PH=7, Temp=40° C,	Ginsenoside Rb2 (0.74 mg/ml) changes after 12 hours into compound Y (0.27 mg/ml) and CK (0.1 mg/ml).	[54]
β-glu@Cu(PTA) biocomposite	Rb1→Rd→F2→CK	PH=3, Temp=45° C, Time=24h	In these conditions, the conversion of the rare ginsenoside CK achieved 49.15%.	[55]
β-Glu&SN@Zn-BTC (β-Glu and snailase were co-immobilized on Zn-BTC) biocomposite	Rb1→Rd→F2→CK	PH =4.5 Temp =50°C Time =48h	The CK conversion rate achieved 53.5%.	[56]
Sna&β-Glu@H-Cu-BDC biocomposite	Rb1→Rd→F2→CK	PH =4.5 Temp =50°C Time =48h	The average conversion efficiency was about 60.12%, and the concentration of CK was roughly 0.94 mg mL−1.	[57]

The bioconversion of ginsenoside Rb1 to CK through β-glu is an effective production method in industry [55]. Microbes isolated from the soils of ginseng farms, soybeans, tea, the gastrointestinal tract of humans, kimchi, and other fermented items can be a source of β-glu [58]. Heat-resistant β-glu yields the highest amount of CK from protopanaxadiol-type ginsenosides [59]. Qin et al. used chromatography to purify a new ginsenoside-hydrolyzing β-glu from *Paecilomyces Bainier* sp. 229 to increase the conversion rate of Rb1 into CK. At 45 °C and pH 3.5, the ginsenosides Rb1 and the enzyme exhibited the highest level of activity. The pathway Rb1 → Rd → F2 → CK converted roughly 84.3% of the ginsenoside Rb1 to CK one day after the incubation [49]. Furthermore, it has been observed that recombinant β-glu enzymes found in *Terrabacter ginsenosidimutans* sp. [50] and *Lactobacillus brevis* [51] can convert Rb1 into CK.

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β-glycosidases are a substitute for β-glucosidases and are frequently employed in the hydrolysis of ginsenosides [60]. Particularly, PPD-type ginsenosides are hydrolyzed by β-glycosidases [61]. Noh et al [52]documented synthesizing CK from ginseng root extract by employing β-glycosidases derived from *Sulfolobus solfataricus*. Two transformation pathways were described by them to turn Rb1, Rb2, Rc, or Rd into CK: (1) Rb1 or Rb2 → Rd → F2 → compound K, and (2) Rc → compound Mc → compound K. Despite the strong specificity of this approach, the ginsenoside to CK conversion rate was low. Thus, recombinant β-glycosidase derived from *Pyrococcus furiosus* [53]and *Microbacterium esteraromaticum* [62] has been created to convert significant ginsenosides into minor ginsenosides. *Pyrococcus furiosus* was highly productive in turning Rd into CK, yielding an 83% conversion rate [53].

However, there was inadequate stability of free β-Glu, which hindered circulation and recovery, unable to recycle β-Glu and snailase, self-digestion rate, and long reaction time. This limitation can be overcome by enzymatic immobilization technology, which will also make it easier to use β-Glu in commercial production β-Glu@Cu(PTA) biocomposite reached a 49.15% conversion rate of Rb1 to CK [55]. Green synthesis of Zn-BTC co-immobilized snailase and β-glucosidase (β-Glu) resulted in the formation of β-Glu&SN@Zn-BTC biocomposite, which reached the CK conversion rate of 53.5% in 48 hours at pH 4.5. The CK concentration was 1.07 mg/mL, and 83% of all products were made up of CK [56]. Using Sna&β-Glu@H-Cu-BDC (large-sized snailase&β-glucosidase@hollow-Cu-H2BDC) biocomposite for the synthesis of the CK. Cau et al [57] reported that the total amount of CK was about 0.94 mg mL−1, and the average conversion rate of CK was around 60.12% after growing the conversion system.

4.2. Biotransformation of CK by human gut microbiota

Microbial transformation is generally regarded as a key technique for producing CK [63]. It involves using crude enzymes from *Fusarium sacchari* [64], *Lactobassillus paralimentarius* [65], *Microbacterium esteraromaticum* [54] *Caulobacter leidyia*, and *Acremonium strictum* [66]. For instance, Hasegawa et al. [67] thoroughly examined the metabolic conversion of CK from ginsenosides Rb1, Rb2, and Rc by gut flora. Ginsenosides Rb1, Rb2, and Rc were transformed into CK through anaerobic incubation with human gut microbiota. Among them, bacterial strains obtained from human intestinal feces, including *Bacteroides sp*., *Bifidobacterium sp*., and *Eubacterium sp*., successfully converted Rc into CK [68]. The composition of the gut microbiota determined the primary metabolic pathway used by intestinal bacteria to break down ginsenoside Rb1 into CK.

Compared to bacteria, fungi are easily cultured and can biotransform to replace human intestinal bacteria as a source of CK [64]. Zho et al. [69] used fungal biotransformation to efficiently manufacture CK from *Panax notoginseng* (PNG) saponins at a reasonable cost. The same group also showed that the fungus *Paecilomyces bainier* sp. 229 could efficiently convert PNG saponins into CK; this resulted in a substantially higher conversion rate of CNS to CK (82.6% vs. 35.4%) than before [70]. Furthermore, fungi that were produced from ginseng-cultivated soil, such as *Fusarium moniliforme* [71], *A. strictum* [72], *A. niger* [73], and *F. sacchari* [64], demonstrated good biotransformation of major ginsenosides into minor bioactives. Cumulative Generation of Bioactive CK from Fermented Black Ginseng using a novel *Aspergillus niger KHNT-1* Strain Obtained from Korean staple food kimchi [74]. *Leuconostoc* strains were also isolated from kimchi, which showed good conversion of PPD-type ginsenosides to CK [75]. Microorganisms have frequently been used in the biotransformation of major ginsenosides into minor bioactive. Furthermore, a synthetic biology approach has been utilized for transformation. PPD ginsenosides could be easily converted into CK by the metabolically modified yeast expressing the heterologous UGTPg1 gene [76].

5. Mechanism of CK against metabolic diseases

The pathophysiology of metabolic disease is highly complex and is caused by multiple factors. Numerous studies have demonstrated what beneficial impacts of CK on metabolic disorders. After reviewing researches, we concluded that CK is a useful medicinal substance for treating osteoporosis,

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hyperlipidemia, obesity, hepatocyte steatosis, NAFLD, and diabetes and its consequences. Table 4 displays the pharmacological molecular pathways of CK in the treatment of metabolic disorders.

Table 4. Pharmacological action of CK in treating metabolic disorders and its underlying molecular processes.

Disease	Experimental models	Dosage form	Doses of administrations	Mechanism	Ref.
Obesity	C57BL/6J mice	Oral	15, 30, 60 mg/kg	• Inhibits TLR4/TRAF6/TAK1/NF-κB activation in obese mice, • Promotes IRS1/PI3K/AKT expression against obesity	[13]
	Male C57BL/6J and ob/ob (B6/JGpt-*Lep*^{em1Cd25}/Gpt) mice	i.p. injection	20 mg/kg	• Initiates autophagy through the AMPK/ULK1 Path way activation • Boosts autophagy and lipase activity to promote lipolysis • Promotes Lipolysis via Interacting with the Glucocorticoid Receptor (GR)	[77]
	3T3-L1 cell lines	Cell treatment	0.05, 0.5, 5 μM	• inhibits adipocytes-specific genes (C/EBPα, leptin, aP2, and PPARγ), • Decreases angiogenic factors (VEGF-A and FGF-2) and MMPs (MMP-2 and MMP-9), • Enhances the mRNA expressions of angiogenic blockers (TSP-1, TIMP-1, and TIMP-2).	[19]
	3T3-L1 cell lines	Cell treatment	20, 50 μM	• Inhibits MCP-1, and TNF-α in adipocytes. • Promotes IL-10 expression to alleviate obesity-induced inflammation and insulin resistance.	[78]
	3T3-L1 cell lines	Cell treatment	10-40 μM	• Activates AMPK signaling pathway • Inhibits ERK/P38 and AKT	[79]

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				signaling pathways	
Diabetes	male ICR mice	Oral	30 mg/kg/day	• Down-regulation of PEPCK and G6Pase expression in the liver.	[80]
	Male Wistar rats (200–250 g)	oral	30, 100, 300 mg/kg BW	• The levels of InsR, IRS1, PI3Kp85, pAkt, and Glut4 in the skeletal muscle of diabetic rats may be enhanced by CK.	[81]
	MIN6 cell line	Cell treatment	2-32 μM	• CK significantly stimulates insulin release by up-regulating GLUT2 expression.	[37]
	male C57BL/KsJ db/db mice	oral	CK: Metformin 1:15	• Insulin and plasma glucose levels were raised when CK and MET were combined.	[82]
DN	HFD (high-fat diet)/STZ (streptozotocin)-induced DN mice model	intragastrically	10, 20, 40 mg/kg/day	• • suppress NADPH oxidase expression and block ROS-mediated NLRP3 inflammasome and NF-κB/p38 signaling pathway activation	[83]
DT	human tenocytes cell	Cell treatment	3, 10 μM	• Inhibits high glucose-induced apoptosis, inflammation, and oxidative stress • Normalize the MMP-9, MMP-13, TIMP-1, expressions. • Boosts PPARγ and antioxidant enzymes.	[84]
OP	Raw264.7 cells Balb/C female mice	Cell treatment, i.p. injection	10 μM 10 mg/kg	• Inhibits RANKL-induced osteoclast differentiation. • Inhibits ROS production by triggering NF-κB/ p65 and oxidative stress in Raw264.7 cells. • Inhibit bone resorption with macrophages generated from bone marrow.	[85]
	bone marrow mesenchymal	Cell treatment,	2.5-40 μM 10 μM	• Elevates Runx2 and β-catenin to promote osteogenic	[86]

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	stem cells male Sprague Dawley (SD) rats	i.p. injection		differentiation via the Wnt/ β-catenin pathway.	
OA	MC3T3E1 cell lines	Cell treatment	0.01-10 μM	• Increases ALP, Col-1, and Runx2 expression in preosteoblastic cells against osteoarthritis.	[31]
NAFLD	SD rats, HSC-T6 cells	i.p. injection Cell treatment	3mg/kg/day	• CK has anti-fibrotic and hepatoprotective effects	[87]
	HepG2 cells	Cell treatment	20 μM	• Suppresses of SREBP1c and activates PPAR-α	[33]
	HuH7 cells	Cell treatment	1 μM	• Inhibits lipid droplet and triglyceride accumulation via up-regulating AMPK/PPAR-α pathway.	[88]
HCC	HepG2 cells	Cell treatment	0, 5 and 10 μmol	• Enhances P21, and P27 expressions • Inhibits cyclin D1, cyclin-dependent kinase 4, and cell cycle progression to induce apoptosis	[89]

Abbreviations: DN=Diabetes nephropathy, DT=diabetic tendinopathy, OP=osteoporosis, OA=Osteoarthritis, NAFLD= Nonalcoholic fatty liver disease, HCC=Hepatocellular carcinoma.

5.1. Obesity

Obesity is a prevalent metabolic disease, defined by adipocyte hypertrophy, which results from an imbalance between energy expenditure and food intake. Obesity has been related to additional metabolic diseases such as insulin resistance, NAFLD, T2D, dyslipidemia, cardiovascular diseases, hypertension, and cancer [90]. Adipose tissue growth results in fat storage in preexisting adipocytes and the transformation of preadipocytes into mature adipocytes, a process called adipogenesis [91]. Under adipogenic conditions, more amount of free fatty acids will be produced by hypertrophic adipocytes [92]. It shows that obesity is linked to a lipid metabolism issue; there is an indication that 43.2% of obese people have hyperlipidemia [93]. An important function of adipose tissue is to regulate energy metabolism [94]. It's been proposed that one of the key strategies for treating obesity is to activate brown adipose tissue (BAT) and produce browning in white adipose tissue (WAT) [95].

CK might be the potential therapeutic agent against obesity via several signaling pathways (Figure 2). A study showed that C5BL/6J mice consumed a high-fat diet to induce obesity, whereas administration of CK (15, 30, 60 mg/kg) might successfully enhance the resistance to insulin and glucose tolerance, downregulate PPARγ expression, inhibit TLR4/TRAF6/TAK1/NF-κB stimulation in obese mice, and lower macrophage M1-type inflammatory cytokine levels in serum and adipose tissue in a dose-dependent manner. Furthermore, CK increased IRS1/PI3K/AKT expression which proved CK is an effective compound against obesity and early diabetes [96]. CK is a novel agonist of the glucocorticoid receptor (GR) used to treat obesity. In mice, CK was more effective than Orlistat

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in reducing blood lipids and weight [77]. CK treatment of 3T3-L1 adipocytes prevented lipid formation and the expression of genes particular to adipocytes (C/EBPα, leptin, aP2, and PPARγ), decreased angiogenic factors (VEGF-A and FGF-2) and MMPs (MMP-2 and MMP-9), whereas enhanced the mRNA expressions of angiogenic blockers (TSP-1, TIMP-1, and TIMP-2) [19]. CK had strong inhibitory effects on the rise of MCP-1 and TNF-α caused by the hypertrophic adipocyte supernatant. Additionally, it facilitated the expression of IL-10, prevented the induction of inflammatory macrophages, and enhanced the development of anti-inflammatory macrophages [78]. In early-stage adipogenesis, CK reduced the phosphorylation of protein kinase B (AKT), p38, and extracellular signal-regulated kinase [97]. Moreover, CK markedly elevated AMPK (AMP-activated protein kinase) and ACC (acetyl-CoA carboxylase) to suppress adipogenesis. In differentiated 3T3-L1 cells, the effect of CK on reducing PPAR-γ expression was restricted by AMPK pharmacological inhibition with dorsomorphin [79]. Therefore, by controlling lipid metabolism and energy metabolism, CK has the potential to be used as a treatment and preventive medicine for obesity.

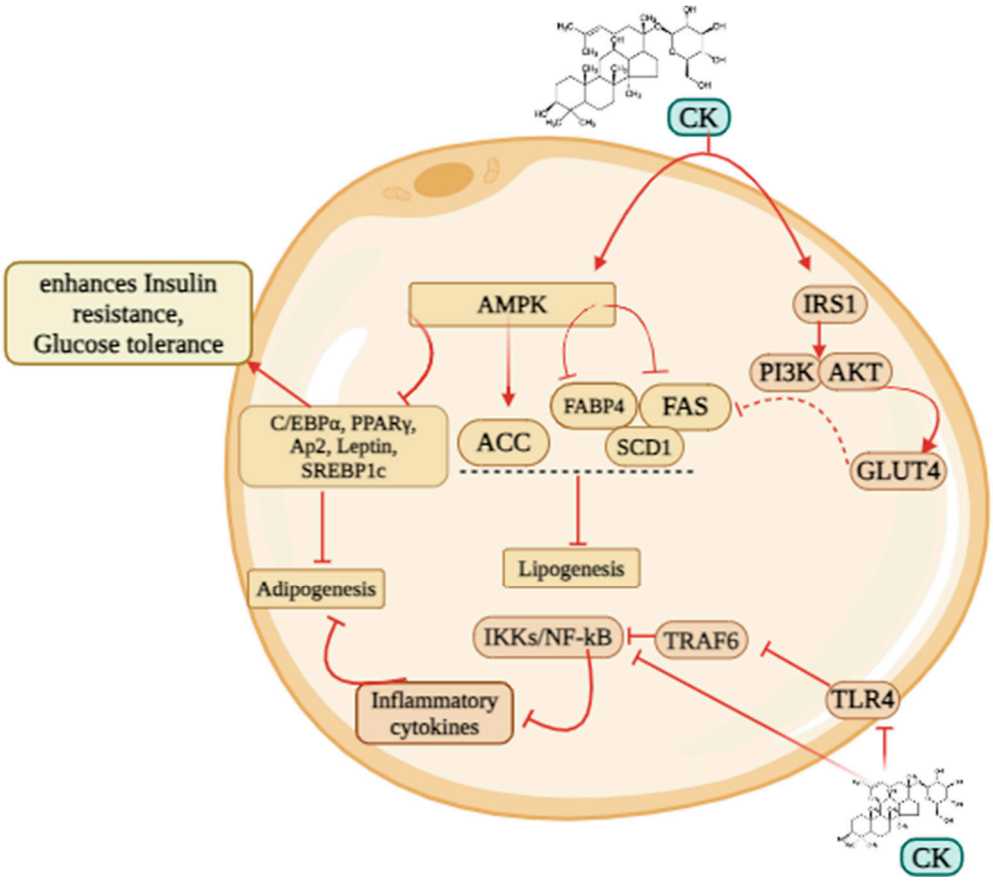

Figure 2. The pathway involves in obesity. CK inhibits adipogenesis via AMPK/PPARγ/CEBPα, and lipogenesis via AMPK/ACC/FAS pathway. Additionally, CK increases IRS1/PI3K/AKT expression against obesity. Furthermore, CK triggers TLR4/TRAF6/TAK1/NF-κB to minimize adipose tissue.

5.2. Diabetes and related complications

Diabetes mellitus (DM) is an emerging epidemic that can be linked to hereditary and environmental factors. Diabetes has complications that require treatment, including diabetic retinopathy, nephropathy, neuropathy, infertility, and cardiovascular disease. Type I diabetes, or T1D, and type II diabetes, or T2D, are the two primary subtypes of DM. An autoimmune condition called T1D kills beta cells in the pancreas and stops insulin from being released. On the other hand, T2D is characterized by high insulin levels and cell insulin resistance. According to epidemiological studies, there will be 629 million people with diabetes worldwide by 2045, up from a total of 425 million in 2017. Due to the potential harm that diabetes mellitus (DM) can do to an individual's

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quality of life, the condition needs to be controlled and managed as soon as possible [98]. To investigate the anti-diabetic activity, ICR mice were fed with CK (30mg/kg/day) for 4 weeks. Phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase), two glucose-producing genes, were down-regulated after CK treatment. The results showed that CK can reduce blood sugar levels and increase insulin sensitivity in type 2 diabetes caused by a high-fat diet and fasting. This is achieved by suppressing the expression of PEPCK and G6Pase in the liver [80]. Jiang et al studied CK (30, 100, 300 mg/kg/BW) on male Wistar rats to improve insulin sensitivity. They found that CK may improve insulin resistance and hyperglycemia in diabetic rates. Additionally, studies indicated that CK could increase the expression of Glut4, PI3Kp85, InsR, IRS1, and pAkt in the skeletal muscle of diabetic rats. These findings suggest that increased insulin sensitivity, which is directly linked to the PI3K/Akt signaling pathway, mediates the hypoglycemic action of CK [81]. Another study reveals that CK has strong stimulatory effects on insulin production in MIN6 cells by upregulating GLUT2 expression (Figure 3) [37].

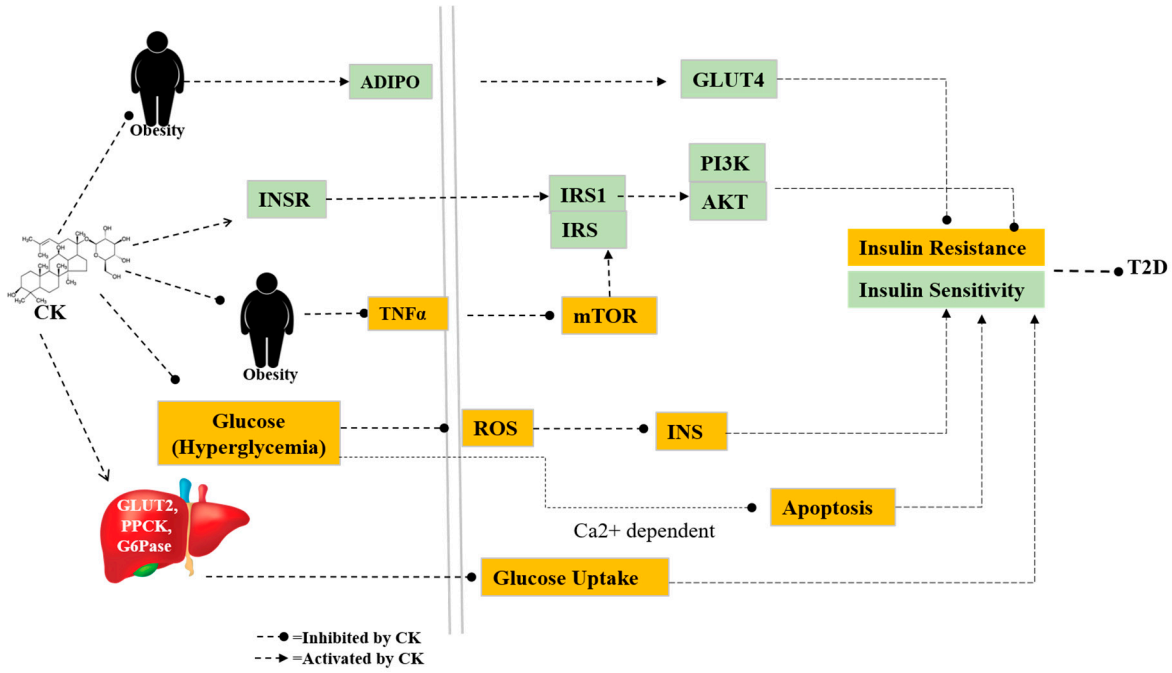

Figure 3. The pathway involves T2D. CK triggers insulin resistance and increases insulin sensitivity to inhibit T2D by regulating several genes.

Despite extensive research on the antidiabetic effects of CK, Song et al studied CK on diabetic nephropathy (DN). DN mice model induced by HFD (high-fat diet) and STZ (streptozotocin) were administered CK intragastrically. The results demonstrated that CK dramatically reduced the growth of the glomerular mesangial matrix and considerably decreased the increased fasting blood glucose, serum creatinine, blood urea nitrogen, and 24-hour urine protein of the DN mice [83]. Additionally, It was observed that the expression of G6Pase and PEPCK in the liver and HepG2 was suppressed by CK. In the meantime, AMPK activation was markedly boosted upon CK administration, but FOXO1, HNF-4α, and PGC-1α expressions were significantly decreased [99]. It has been noted that diabetics with tendinopathy have a higher apoptotic tendency. Tendinopathy is a chronic illness that affects the tendons and causes a great deal of discomfort. It has a major negative influence on quality of life [100]. However, CK can effectively reduce the MMP system, inflammation, tenocyte apoptosis, and oxidative stress under hyperglycemia [84].

5.3. Osteoporosis

Osteoporosis (OP) represents an alarming clinical condition that typically manifests as rapid bone loss during menopause. It increases the possibility of a brittle fracture, which puts a great deal

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of strain on society. A growing number of people are experiencing OP as society ages. Hip fractures are expected to become more common worldwide by 2050 as population demographics change. OP occurs when the bone resorption (osteoclast) rate is greater than the bone formation (osteoblast) rate, leading to lowered bone density [101]. Osteoblasts are bone-decomposed cells that play a crucial role in maintaining bone homeostasis. During postmenopausal osteoporosis, the receptor activator for nuclear factor-κB ligand (RANKL) increases osteo-clastogenesis, which results in bone loss [102]. Nowadays, many medications, including calcitonin and bisphosphonates, are used to treat and prevent OP. On the other hand, documented accounts of the adverse effects of these medications in medical settings exist. As a result, to lower fracture rates and enhance patient quality of life, improved therapies for OP must be investigated [103].

Herbal supplementations have been extensively researched as potential sources for drug development because of their lesser toxicities. As an herbal product, CK showed an anti-osteoporotic effect by suppressing RANKL-induced osteoclast differentiation and ROS production by triggering NF-κB/P65 signaling pathway [85]. Additionally, CK elevated the Runx2 (master transcription factor) and β-catenin to promote osteogenic differentiation via the Wnt/ β-catenin pathway [86]. Furthermore, CK has a preventive effect on osteoarthritis via upregulating ALP, Col-1, and Runx2 in pre-osteoblastic MC3T3-E1 cell lines [31]. CK may reduce RANKL levels, boost the amount of osteoprotegerin (OPG) on AA-FLS in vitro, and prevent bone degradation in AA rats [104]. Furthermore, pretreatment with CK may prevent human CD4+ monocytes and murine RAW264.7 cells from proliferating into TRAP+ osteoclast-like cells in response to soluble RANKL (sRANKL) in a dose-dependent way. Moreover, CK inhibited the nuclear transcription factor of activated T cells (NFATc1) and RANK-associated NF-κB pathways in osteoclast progenitors. These suggest that GCK blocked osteoclastogenesis caused by RANKL through two different pathways [105]. Based on all of these data, CK appears to be a promising treatment for preventing dietary induction of OP (Figure 4).

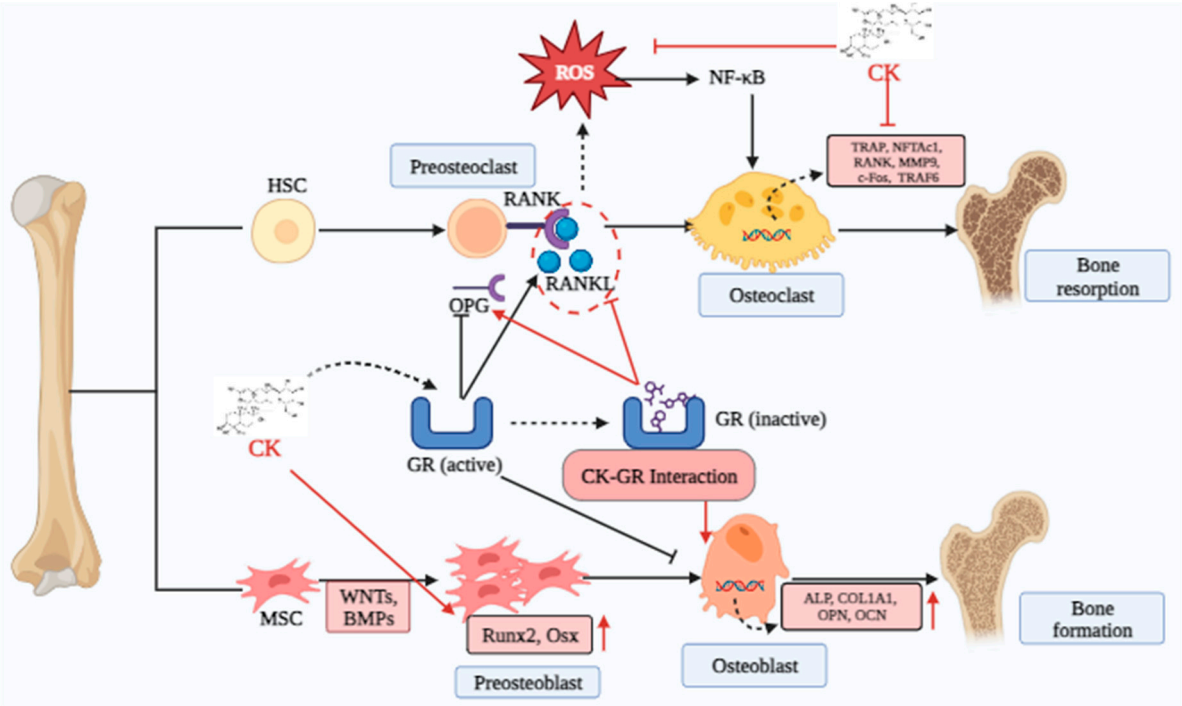

Figure 4. CK is effective in inhibiting osteoclast by interacting with glucocorticoid receptors and this interaction triggers RANKL which attaches to RANK receptor to produce osteoclast. CK also inhibits ROS and TRAP, NFTAC1, MMPs, C-FOX, and TRAF6 to minimize bone resorption. On the other hand, CK increases the expression of preosteoblastic (Runx2, Osx) and Osteoblastic genes (ALP, COL1A1, OPN, OCN) that increase bone formation.

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5.4. Non-alcoholic Fatty liver disease (NAFLD)

NAFLD also known as hepatic steatosis or the formation of triglyceride in the liver which is not induced by consuming alcohol [106]. NAFLD is a broad term for a range of pathologies that include nonalcoholic fatty liver (NAFL), which is the first stage of NAFLD, nonalcoholic steatohepatitis (NASH), which is defined by the beginning of inflammation brought on by lipotoxicity, and severe NASH symptoms that include fibrosis. According to reports, the overall incidence of NAFLD might reach 15% to 18% among Asian nations and 30% in Western nations. Obesity is closely linked to this fatty liver disease. The prevalence of NAFLD is predicted to increase globally in light of the present obesity pandemic. [87]. A growing amount of clinical data indicates that NAFLD is a major risk factor for the emergence of liver cirrhosis, liver fibrosis, and liver cancer [107]. However, it is still unclear how NAFLD develops and how it leads to fibrosis and chronic liver disease. The "two-hit" theory first came forward by Day et al. in 1998. First, there is an initial metabolic change that results in insulin resistance, hyperglycemia, and hepatocyte triglyceride formation, which causes hepatic steatosis. The second hit causes the injury to worsen and progress, leading to cirrhosis, inflammation, fibrosis, and steatohepatitis [108]. On the other hand, the "multiple parallel hits" theory postulates that several parallel factors, including adipokines and cytokines secreted abnormally, dysfunctional mitochondria, stress to the endoplasmic reticulum, gut endogenous endotoxin, metabolism of lipids, lipotoxicity, oxidative stress, and genetic susceptibility cause NAFLD [109]. Still, the FDA has not approved any particular medications for NAFLD. Medicines such as atorvastatin calcium tablets and fenofibrate are commonly used to manage blood lipid levels [110]. However, using lipid-lowering medications can lead to certain negative side effects [111]. Therefore, finding novel medications that treat NAFLD with great efficacy and few adverse effects is urgent.

Various researches have shown a significant potency of CK against NAFLD (Figure 5). Chen et al. demonstrated that CK is beneficial for treating NAFLD through hepato-protective and anti-fibrotic effects [87]. Another study depicted that, CK activates AMPK and increases ACC and mononyl CoA levels in the AMPK signaling pathway to stimulate fatty acid oxidation. CK can suppress triglyceride accumulation in the liver by inhibiting lipogenic markers such as SREBP1c, SDC1, and FAS and enhancing lipolytic markers including PPAR- α, and CD36 [33]. AMPK also inhibits the formation of free fatty acids by reducing TG hydrolysis via direct phosphorylation and inactivation of hormone-sensitive lipase. Additionally, the activation of AMPK is related to elevated expression of PPAR- α, and subsequent decrease of SREBP1c and PPAR-γ activity in adipocytes and hepatocytes. Moreover, CK might be directly mediating its beneficial impacts by activating AMPK [88]. To minimize the toxicity of CK, Yue et al [112] develop natural nano-CK that acts as an mTOR inhibitor to change lipid metabolism. In steatosis hepatocytes, nano-CK can alleviate lipotoxicity and restore lipid homeostasis by encouraging lipid export and blocking DNL and lipid absorption, all of which create a feedback loop regulated by mTOR. Furthermore, CK has a definite hepatoprotective impact on sodium valproate-induced hepatotoxicity, as shown by Zhou et al [113]. These beneficial effects were mediated by reducing oxidative stress through the suppression of lipid peroxidation and the upregulation of the protective antioxidant system; controlling the peroxisome pathway through the downregulation of soluble epoxide hydrolase, and controlling iron homeostasis through the upregulation of hepcidin. In the case of hepatocellular carcinoma, CK caused a G0/G1 phase arrest, blocked cell cycle progression, and induced apoptosis through the upregulation of p21Cip1 and p27Kip1, and downregulation of cyclin D1 and cyclin-dependent kinase 4 in HepG2 cells. This was accomplished by the mitochondrial system via a modulation of the ratio of Bcl-2 to Bax [89].

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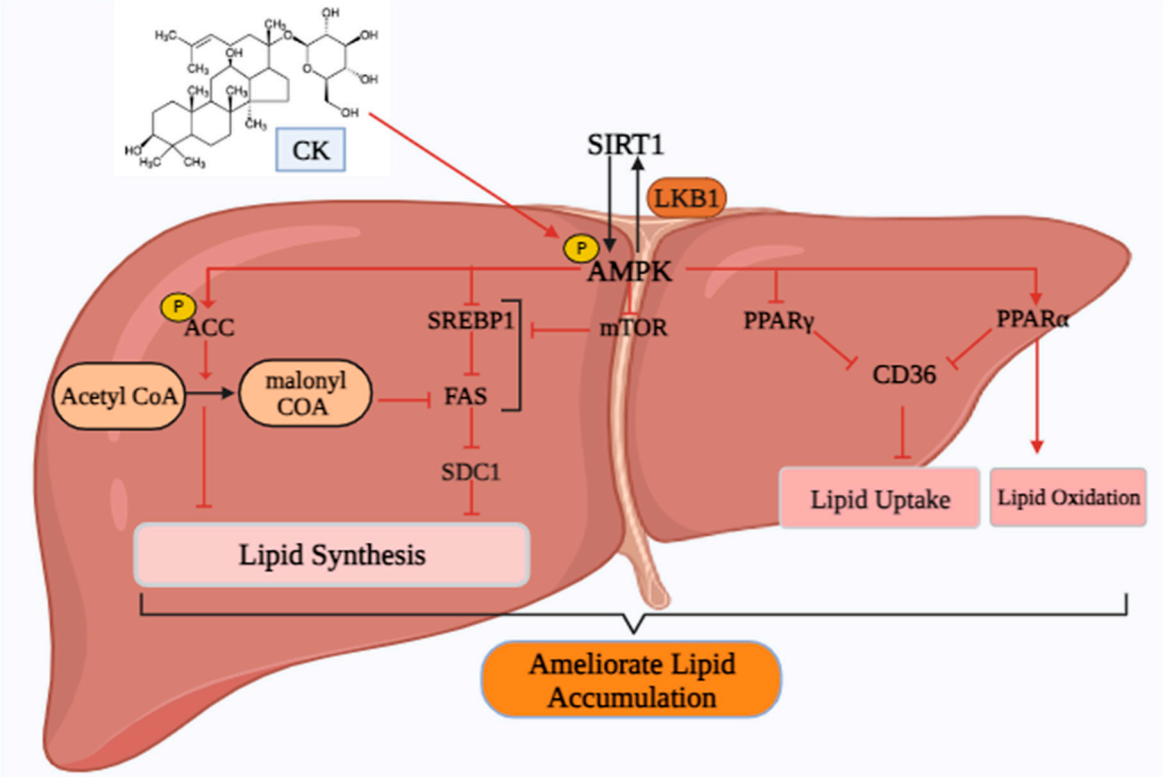

Figure 5. Activity of CK on NAFLD pathway. CK increases the levels of the AMPK/SIRT1 pathway that increases ACC and inhibits the SREBP1/FAS/SDC1 pathway to minimize lipid synthesis. CK also PPARα to produce lipid oxidation.

Thus, as a monotherapy, in conjunction with other medications, or nanoformulation of CK may prove to be a promising therapeutic for alleviating hepatic problems associated with other metabolic diseases or diminishing fatty liver.

6. Mechanism of TP^{53} regulating pathway in metabolic diseases

Numerous studies have been conducted on the tumor suppressor activity of TP^{53}, also known as p^{53}, which has long been considered the "guardian of the genome" [114]. As a transcription factor, p^{53} primarily transactivates gene expression in response to genotoxic or oncogenic stress, hence exerting its tumor-suppressive properties. P^{53} initiates programmed cell death (apoptosis) in response to severe or protracted DNA damage, mainly by activating genes that encode pro-apoptotic proteins. P53 has been implicated in aging, innate and adaptive immunity, development, reproduction, and neuronal degeneration, among other physiological processes. P^{53} has been implicated in innate and adaptive immunity, development, reproduction, and neuronal degeneration, aging, among other physiological processes [115]. Furthermore, the link between p^{53} and metabolic disorders has emerged as an additional area of study for p^{53} researchers.

In 2009, Minamino and his colleagues provided the first evidence that p53 plays a role in the development of obesityT2D by demonstrating diet-induced IR in A^{y} transgenic mice [116]. This research demonstrated that p53 activity was inhibited by siRNA knock-down in cells or TP53 gene knock-out in mice, which reduced proinflammatory cytokines expression in the adipose tissue and alleviated senescence, ultimately averting the development of IR. Diabetes is often caused by three main factors: 1) abnormalities in glucose homeostasis; 2) functional deficits in pancreatic insulin secretion; and 3) the emergence of insulin resistance. Based on existing in vitro and in vivo data, p53 appears to be able to significantly affect each of these three pathways. The pro-inflammatory cytokines TNF-α and IFN-γ have been demonstrated to work in concert to boost apoptosis, p53 activation, and ROS generation in a pancreatic beta cell line and insulin-producing islet cells [117]. Lastly, it was discovered that ROS were also implicated in FFA-mediated beta cell death and that

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NAPDH oxidase 2 (NOX2) was the downstream regulator of both ROS production and p53 activation [118]. Inflammatory cytokines, ROS, and free fatty acids can all function upstream of p53 to cause p53-mediated death in pancreatic beta cells. All things considered, these scientists discovered that reduction of pancreatic beta cell activity lowers insulin output, leading to hyperglycemia and diabetes. Studies conducted in vitro and in vivo showed that elevated release of FFAs caused ROS-induced DNA damage and p53 upregulation in adipose tissue. While p53 inhibition reduced inflammation, p53 activation increased the expression of proinflammatory adipokines through the NF-κB signaling pathway, which in turn led to adipose tissue inflammation, insulin resistance, and diabetes. Obese human patients and mouse models have both shown these obesity-related alterations in p53 expression. It is believed that dysfunctional adipose tissue-related chronic inflammation fosters an environment that is conducive to tumor growth and progression [119].

Fatty liver, also known as hepatic steatosis, constitutes one of the most prevalent side effects of type 2 diabetes and obesity [120]. Yahagi et al. demonstrated that independent of the presence of obesity, p53 activation is critical to the pathophysiology of fatty liver disease [121]. Comparably, Derdak et al. showed that p53 activation increases hepatic steatosis and insulin resistance in both non-alcoholic fatty liver disease (NAFLD) and alcoholic liver disease (ALD); interestingly, the same group discovered that p53 activity can be inhibited to improve the symptoms of both ALD and NAFLD [122] [123]. These investigations also provided potential mechanistic explanations, proposing that PTEN and miRNA34a are induced by p53, respectively, and that this is the cause of ALD and NAFLD formation. Another group used p53-knockout mice to confirm that p53 plays a role in the development of NAFLD, possibly via its downstream target p66shc [124]. Notably, p53 expression and the degree of steatosis are positively associated in human liver tissues, indicating that these symptoms shown in models of mice are physiologically relevant [125].

After reviewing the research, it is summarized that ginsenosides CK and TP53 gene plays a vital role in regulating metabolic diseases. However, the role of CK against the TP53 regulating pathway in metabolic diseases is unclear. We already reported that CK showed strong activity to regulate TP53 in the case of obesity and OP [126]. Thus, to better support the clinical use of CK, additional research is required to enhance the pertinent mechanisms of CK.

7. Synthesis of CK analogues and their pharmacological activity

Numerous biological actions of CK have been reported in the above sections. However, its use in medicine is limited by the poor bioavailability of CK after oral administration, which is thought to be a limiting factor. Researchers are concerned with increasing the intestinal absorption of CK by modifying the structure. In the early stages of treating asthma, CK was found to exhibit strong action against IgE. In 2019, Ren et al [127] reported couples of CK analogues were synthesized via straight forwarded methods. The produced compounds were assessed on the anti-IgE activities utilizing an in vivo airway hyper responsiveness experiment and an ovalbumin-induced asthmatic mouse model. They found that compounds T1, T2, T3, T8, and T12 (the analogues of CK) showed either superior or comparable anti-asthmatic effects compared to CK. Furthermore, Huang et al. [128] reported six derivatives of CK, among them structures 1 and 2 were highly potent to activate the LXRα (Liver X Receptor α) expression and showed lower toxicity than CK. They also demonstrated that structures 1, 2, and 4 enhanced the expression of ABCA1 (ATP-binding cassette transporter) mRNA levels. It has been documented that CK preferentially accumulates in the liver where it converts to fatty acid esters. Because the ester of CK was not eliminated by bile acid as CK was, it remained in the liver for a longer period. CK-octyl ester showed moderate detoxification and showed anti-liver cancer activity in murine-H22 cells and in vivo [129]. In addition, the novel ester prodrugs of CK (CK-butyl, and CK-octyl) have shown higher bioavailability due to their highly lipophilic properties than the CK. Even, the findings indicated that the permeability co-efficient of CK was lower than the esters [130]. These findings establish a groundwork for further alterations of CK and apply the new structures to metabolic diseases.

8. Discussion and future perspective

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According to reviewed databases involving numerous studies, we summarized that CK is mostly associated with metabolic disorders, such as obesity, NAFLD, OP, diabetes mellitus, and its complications. This systemic review highlights the beneficial effects of CK against the four major metabolic diseases and the related pathways. It demonstrates the variety of ways in which CK can contribute to metabolic diseases such as enhancing IR, inhibiting glucose uptake, boosting glucose tolerance and insulin sensitivity, inhibiting bone resorption, increasing bone formation, triggering lipid synthesis, lipid uptake, boosting lipid oxidation, and blocking the inflammatory cytokines. In this study, we demonstrated that CK 1) enhances AMPK and inhibits adipogenic genes (C/EBPα, PPARγ, Ap2, leptin, and SREBP1c), lipogenic genes (FAS, FABP4, and SCD1). CK also inhibits the IKKs/ NF-kB pathway to trigger obesity mediated-inflammation. 2) Increases the expression of Glut4, PI3Kp85, InsR, IRS1, and pAkt to block the IR and increases GLUT2/PPCK/G6Phase pathway to reduce gluconeogenesis, inhibits ROS/INS and apoptosis to increase insulin sensitivity. 3) Inactivate GR that inhibits osteoclast differentiations, bone resorption and increases osteoblastic differentiation. Osteoclast production is inhibited and osteoblast development is promoted by the stimulation of the activity of genes associated with osteogenesis, such as Runx2, osterix and the suppression of the expression of genes related to osteoclasts, such as C-Fos and NFAC1. Meanwhile, CK is a novel agonist of the GR to treat obesity and OP. Furthermore, 4) ameliorates lipid accumulation by activating the AMPK/SIRT1 pathway in thev case of NAFLD. Research on CK in the future may focus on atherosclerosis, cardiovascular disease, fatty liver, hyperlipidemia, and other conditions.

Although numerous studies have established the toxicity of CK, our review revealed that the toxicity of CK is mostly dependent on the dosage and timing of administrations and the sex of subjects also affects the hepatotoxicity. Generally, doses of CK used to treat metabolic disorders in mice and rats are less than 100 mg/kg. However, doses up to 120 mg/kg in mice and rats can cause hepatotoxicity and nephrotoxicity [41]. Beagle dogs were administered 4, 12, and 36 mg/kg oral dosees for 26 weeks and did not exhibit any visible toxicity in the 4-12 mg/kg groups. 36mg/kg groups exhibited reduced body weight, enhancing plasma enzymes, and nephrotoxicity [46]. Studies showed that the dose of CK administrations did not exceed 100 mg/ml body weight ranging from for treating metabolic diseases. Additionally, in the cell lines treatments, the range of administration was 0.1-64 μg/ml showed more than 80% cell viability. Although, several cells showed cell cytotoxicity in different concentrations. Thus, it can be suggested that it is difficult for CK to cause bio-toxicity at normal doses. The development of bio-toxicity is more closely linked to an increase in CK dose than it is to an extension in administration time. As a result, the development and application of CK depend significantly on the management of doses administered.

It is well recognized that the potential for the treatment of CK is usually limited because of poor water solubility, and bioavailability and membrane permeability [131]. When it comes to medications that are poorly soluble in water, co-crystals can increase their bioavailability without altering their pharmacological action [132]. It is recommended that co-crystals of CK undergo research as an antimetabolite to increase oral bioavailability. Another recommendation is to change the chemical structure of CK or modify its dosage forms such that it dissolves better in intestinal fluids and has a higher oral bioavailability. Research on anti-metabolic disorders and other CK-related pharmacological effects should focus on comparing and examining the safety and pharmacological effects of injectable and oral CK metabolites in vivo. In addition, researchers are interested more in CK analogues due to their less cytotoxicity, more bioavailability, better membrane permeability, and higher efficacy compared to CK in various diseases. CK analogues could be drug candidate because of their physiochemical properties and pharmacological action.

To sum up, providing conceptual knowledge for the clinical use of CK as a potential anti-metabolic disease agent, the current study offers a thorough review and summary of the pharmacokinetics studies, toxicity, and physical and chemical characteristics of CK.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

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