Regulatory Effects and Mechanisms of Chitosan and Its Derivatives on Animal Lipid Metabolism (Postprint)

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Abstract

Chitosan and its derivatives contain biologically active functional groups such as amino and hydroxyl groups, and exert regulatory effects on lipid metabolism when supplemented in animal diets. This review summarizes the metabolic kinetics of chitosan and its derivatives in animals and their modulatory effects on lipid metabolism. Additionally, it reviews the potential regulatory mechanisms from perspectives including lipid binding capacity, inhibition of pancreatic lipase, and modulation of adipocytokines, lipid metabolism-related enzymes and their gene expression, adenosine monophosphate-activated protein kinase, and lipid synthesis transcription factors. This provides a theoretical basis for further investigating the mechanisms by which chitosan and its derivatives influence animal lipid metabolism and for scientifically regulating lipid metabolism.

Full Text

Regulation and Mechanism of Chitosan and Its Derivatives on Lipid Metabolism in Animals

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Abstract

Chitosan and its derivatives contain bioactive functional groups such as amino and hydroxyl groups that exhibit lipid metabolism-regulating effects when added to animal diets. This review summarizes the metabolic kinetics of

chitosan and its derivatives in animals and their regulatory effects on lipid metabolism. The potential mechanisms are discussed from the perspectives of lipid binding capacity, pancreatic lipase inhibition, and modulation of adipocytokines, lipid metabolism-related enzymes and their gene expression, adenosine monophosphate-activated protein kinase (AMPK), and lipid synthesis transcription factors. This synthesis aims to provide a theoretical basis for further investigation into the mechanisms by which chitosan and its derivatives influence animal lipid metabolism and for the scientific regulation of lipid metabolism.

Keywords: chitosan; lipid metabolism; regulation mechanism

Chitosan is a polyglucosamine composed of -(1-4)-2-acetamido-D-glucose and -(1-4)-2-amino-D-glucose units, representing the second most abundant polymer in nature after cellulose. By inserting different functional groups into the chitosan molecule, various derivatives such as aminoethyl chitosan, carboxylated chitosan, and sulfated chitosan can be formed. The positively charged amino groups endow chitosan and its derivatives with unique, versatile functionalities. These compounds have attracted considerable attention for their anti-obesity and lipid-lowering effects, with numerous studies reporting their impacts on animal lipid metabolism. However, research findings show substantial variation, and studies on their underlying mechanisms remain limited. This review primarily synthesizes current knowledge on the metabolic kinetics of chitosan and its derivatives in animals and their effects on lipid metabolism and regulatory mechanisms, aiming to provide a reference for better utilization of these compounds to regulate animal lipid metabolism and improve animal health.

1.1 Absorption

Native chitosan possesses high molecular weight, resulting in low solubility and high viscosity characteristics. Consequently, early studies suggested that animal digestive tracts could not absorb chitosan and that no chitosan distribution occurred in vivo. Subsequent research has demonstrated, however, that mammalian cells can absorb chitosan and its derivatives and participate in intracellular metabolic processes, though their absorption rate and cellular activity depend heavily on molecular weight, solubility, and deacetylation rate. Chitosan and its derivatives with high water solubility and low molecular weight are more readily absorbed [1].

To enhance bioactivity, chitosan is often degraded into chitooligosaccharides, which have lower molecular weight and viscosity along with higher solubility. Studies using in vivo and in vitro models have shown that chitooligosaccharides are absorbed through small intestinal epithelial cells at higher rates than chitosan. Low molecular weight chitosan (13 and 22 kDa) can be transported across intestinal epithelial cells, whereas high molecular weight chitosan (230 kDa) cannot be absorbed [1].

1.2 Distribution

Xia et al. [2] used fluorescein-labeled chitosan to verify its distribution and metabolism in mice. The results showed that chitosan was primarily distributed in the liver, kidney, and muscle tissues, with the highest content in the liver 1 hour after oral administration, while kidney content became highest over time [2]. Wu et al. [3] conducted pharmacokinetic studies on chitosan microspheres and found that they were present in nearly all rat tissues, particularly the liver and kidney. The physicochemical properties of chitosan and its derivatives affect their in vivo distribution; lower molecular weight chitosan distributes more extensively in rat liver and kidney [1]. Additionally, when deacetylation degree decreases, chitosan accumulates more in mouse kidney than in liver and spleen [1].

1.3 Metabolism and Elimination

Lysozyme in animal plasma, liver, kidney, and urine can degrade chitosan. Rat liver and kidney play central roles in chitosan biodegradation, with degradation products in rat liver ranging from <1 kDa to 50 kDa for high molecular weight (>200 kDa) chitosan [4]. The kidney serves a critical function in chitosan excretion, with over 80% of chitosan eliminated via urine in rats [4]. Xia et al. [2] showed that after oral administration of chitosan in mice, it was excreted in its original form in feces at 2.5–5.0 hours and in urine at 6–12 hours, with partial degradation. Following intraperitoneal injection of chitosan (300 kDa), urinary chitosan molecular weights ranged from <10 kDa to 40 kDa [4], indicating that chitosan is primarily excreted through urine after hepatic and renal degradation.

2.1 Effects of Chitosan and Its Derivatives on Blood Lipid Content

Hyperlipidemia and hypercholesterolemia are risk factors for atherosclerosis and other cardiovascular dysfunctions. Most studies have demonstrated that chitosan and its derivatives can reduce blood lipid levels in animals, attracting attention for treating obesity and dyslipidemia. Research in animals (rodents, pigs, and poultry) and humans has shown that dietary supplementation with chitosan and its derivatives significantly decreases plasma triglycerides (TG), cholesterol (CHOL), low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C), and free fatty acid (FFA) levels while increasing plasma high-density lipoprotein cholesterol (HDL-C) content, thereby removing excess CHOL from tissues and transporting it to the liver for excretion [5-8].

The efficacy of chitosan and its derivatives in regulating blood lipids remains controversial. Although most trials show improvements in lipid profiles, some researchers question their clinical effectiveness. Keser et al. [9] found that chitosan only significantly reduced serum LDL-C levels in broilers without affecting other lipids. Wang et al. [10] reported that chitosan supplementation in pigs

only increased serum HDL-C without significantly affecting CHOL or TG. Human studies also showed that oral chitosan had no significant effect on plasma TG, HDL-C, LDL-C, or VLDL-C levels [11]. Conversely, Liu et al. [12] found that 7% chitosan supplementation in high-sucrose diets reduced plasma CHOL but increased plasma TG in rats. The regulatory effects of chitosan on blood lipids are influenced by multiple factors, including physicochemical properties and feeding duration, which may explain these inconsistent results. Trivedi et al. [11] recommended consuming chitosan approximately 15 minutes to 1 hour before meals to allow sufficient time for dissolution in gastric acid and effective binding of dietary fats. Jin et al. [13] reported that the lipid-lowering effects of chitosan correlate with its molecular weight and surface charge.

2.2 Effects of Chitosan and Its Derivatives on Lipid Accumulation

Reports on the effects of chitosan and its derivatives on lipid accumulation in animals are limited. High-fat diets readily induce lipid metabolism disorders, increase tissue lipoprotein lipase activity, reduce lipolysis rates in visceral and subcutaneous adipocytes, and promote TG accumulation in adipocytes. Chitosan and its derivatives can effectively inhibit intracellular lipid accumulation. Wu et al. [3] demonstrated that chitosan microspheres significantly improved TG content in rat adipocytes. Supplementing high-sucrose diets with 7% chitosan markedly increased lipolysis rates in rat epididymal fat pads and inhibited TG accumulation [12]. Keser et al. [9] also found that dietary chitosan reduced abdominal fat percentage in broilers. Furthermore, lipid accumulation accompanies adipocyte differentiation and hypertrophy. In vivo and in vitro data indicate that chitosan and its derivatives effectively inhibit adipocyte differentiation, hypertrophy, and adipose tissue weight, thereby reducing lipid accumulation [14-15].

Chitosan and its derivatives can also stimulate fatty acid oxidation in rats, reduce FFA levels, and prevent lipid accumulation in non-adipose tissues [15].

2.3 Influence of Physicochemical Properties on Lipid Metabolism

Chitosan and its derivatives constitute a large family of compounds with diverse physical and chemical properties, and their capacity to bind fats and regulate lipid metabolism closely relates to these properties. Molecular weight is the primary factor affecting their physicochemical characteristics. Due to better solubility and absorbability, researchers favor low molecular weight chitosan and its derivatives for lipid-lowering properties [1], as they exhibit superior CHOL clearance and greater inhibition of lipid accumulation and adipocyte differentiation compared to high molecular weight chitosan. Recent research indicates that lipid-binding capacity correlates with specific molecular weight ranges. Jin et al. [13] showed that in an in vitro digestive tract model, chitosan

with a molecular weight of 3 kDa exhibited the strongest fat and CHOL binding capacity, with binding ability decreasing at molecular weights above or below 3 kDa. However, contradictory findings exist; Yao et al. [16] reported that in rats fed high-fat diets, only high molecular weight chitosan reduced plasma CHOL and increased fecal CHOL, while low molecular weight chitosan had no significant effect.

The deacetylation process generates positively charged amino groups in chitosan. Higher deacetylation degrees provide more free amino groups and positive charges, enhancing the ability to bind lipids and bile acids through electrostatic interactions. Additionally, particle size affects lipid-lowering efficacy. With fixed molecular weight, smaller particles have larger total surface areas, and fine-particle chitosan effectively reduces plasma and liver lipid levels in rats [5]. In vitro experiments show that powdered chitosan has greater oil absorption capacity than flake chitosan [2].

3.1 Lipid Binding Mechanism

The lipid binding mechanism was the earliest proposed mechanism for chitosan-mediated lipid metabolism regulation. In the digestive tract, chitosan forms chitosan-lipid complexes that interfere with dietary fat digestion and absorption, promoting fecal excretion of dietary fats.

Trautwein et al. [17] confirmed that chitosan supplementation in high-fat diets significantly increased fecal fat excretion and reduced apparent fat digestibility by 50% in rats. Guha et al. [18] suggested that chitosan can bind approximately four times its own weight in lipids in vitro. Using an in vitro digestive tract model, Jin et al. [13] concluded that 1 g of chitosan sample could absorb 2–8 g of peanut oil or 50–65 mg of CHOL.

The lipid binding mechanism may involve four aspects. First is electrostatic attraction: the positive charges on chitosan amino groups facilitate binding to anionic substances such as fatty acids and bile acids, interrupting intestinal lipid absorption and promoting bile acid excretion. To compensate for fecal losses, hepatic CHOL is accelerated into bile acid conversion, manifesting as lipid- and CHOL-lowering effects. Therefore, under equivalent molecular weight conditions, higher deacetylation degrees provide more positive charges and stronger lipid-binding capacity. However, electrostatic attraction cannot explain increased neutral lipids like CHOL in feces. Second is encapsulation: chitosan dissolves in the stomach to form viscous emulsions, and as polysaccharide chains polymerize, the viscous chitosan encapsulates fat droplets, forming protective films. When the complex reaches the small intestine, chitosan precipitates with encapsulated fats, preventing lipase from digesting lipids, which are then excreted in feces [2]. Third is embedding: fat molecules can embed within chitosan' s long chains, with larger molecular weights and longer chains trapping more fat molecules [17]. Thus, lipid-binding capacity increases with molecular weight. Fourth is adsorption: at equivalent molecular weight, pow-

dered chitosan has smaller particle size, larger surface area, and more open pore structures than flake chitosan, facilitating adsorption [2]. However, chitosan dissolves under gastric acidic conditions, potentially weakening this adsorption function in vivo.

Nevertheless, Gang et al. [19] found that dietary chitosan did not affect fecal fat excretion in mice and therefore did not reduce fat absorption. Pokhis et al. [20] suggested that chitosan-lipid complexes are partially excreted and partially utilized by colonic bacteria for energy, so fecal fat detection does not significantly increase, indicating chitosan lacks substantial fat-binding capacity. Stoll et al. [21] demonstrated that chitosan limits body weight in humans consuming both high-fat and high-carbohydrate diets, suggesting lipid-lowering mechanisms beyond simple lipid binding.

3.2 Inhibition of Pancreatic Lipase Activity

Pancreatic lipase is the key enzyme degrading dietary TG, converting it into one monoglyceride and two fatty acids. Chitosan and its derivatives are considered competitive inhibitors of pancreatic lipase, reducing intestinal lipid and CHOL absorption [22]. Jin et al. [13] showed that chitosan of various molecular weights (1–9 kDa) could inhibit pancreatic lipase activity to some degree in an in vitro digestive tract model. However, the specific mechanisms underlying this inhibition remain unclear, with limited reports requiring further investigation.

3.3 Regulation of Adipocytokine Secretion

Adipocytes are primary energy storage sites that also play crucial endocrine roles in energy balance. Adipocytokines regulate energy metabolism, inflammation, and insulin sensitivity through local and systemic actions. Chitosan and its derivatives modulate adipocytokine secretion by inhibiting adipocyte differentiation and hypertrophy and reducing adipose tissue weight, thereby suppressing lipogenesis [12,23].

Interleukin-6 (IL-6) exhibits both pro- and anti-inflammatory effects while acting as an inhibitor of lipid accumulation and an inducer of lipolysis and oxidation. In cultured 3T3-L1 adipocytes, chitooligosaccharides downregulate adipogenesis-related genes including peroxisome proliferator-activated receptor (PPAR) and CCAAT/enhancer-binding proteins by increasing IL-6 promoter transcriptional activity and upregulating IL-6 gene expression, thereby reducing adipocyte differentiation and lipid accumulation [23].

Energy balance is controlled by a complex neuroendocrine system involving peripheral signals derived from adipocytes. Leptin (LEP) is an adipocyte-secreted hormone proportional to white adipose mass and serves as a key regulator of energy balance, playing an epigenetic regulatory role in adipocyte differentiation. LEP informs the brain about whole-body energy storage status, enabling adipose tissue to drive central control of energy balance. LEP regulates appetite

and energy expenditure in rodents, reducing body fat content. Researchers have found that chitosan and its derivatives can weaken animal appetite and feed intake by increasing plasma LEP levels, accompanied by increased LEP content and gene expression [7]. Given LEP' s critical role in appetite suppression, a potential link between chitosan and appetite control has been proposed [7]. Bahar et al. [23] discovered that during adipocyte differentiation, chitooligosaccharides inhibit LEP gene promoter demethylation, suppressing adipocyte maturation and lipogenesis. Additionally, LEP can activate AMPK to stimulate fatty acid oxidation and prevent lipid accumulation in non-adipose tissues. Chiu et al. [15] also found that chitosan reverses high-fat diet-induced inhibition of hepatic AMPK activity in rats by inducing LEP secretion, thereby stimulating fatty acid oxidation.

Furthermore, reducing tumor necrosis factor- (TNF-) can regulate lipid metabolism and enhance hepatic lipid synthesis in rats, while adipocyte hypertrophy increases plasma TNF- levels. Adiponectin inhibits lipogenesis. Chitosan and its derivatives promote adiponectin secretion and reduce TNF- levels in rat adipocytes [14].

3.4.1 Regulation of Lipid Metabolism-Related Enzyme Activity and Gene Expression

Chitosan and its derivatives participate in animal lipid metabolism by regulating the activity and gene expression of lipid metabolism-related enzymes. Research shows they modulate lipid metabolism by inhibiting lipid synthesis and transport-related enzyme activities and gene expression while upregulating lipid oxidation and catabolism-related genes. Using digital gene expression profiling and gene ontology analysis, Choi et al. [22] found that chitooligosaccharide supplementation in high-fat diets altered the expression of 965 genes in rats, with 640 upregulated and 325 downregulated. Enriched genes were primarily involved in lipid metabolism, cellular ketone metabolism, organic acid metabolism, steroid metabolism, and fatty acid -oxidation. Chitooligosaccharides mainly affected signaling pathways including PPAR signaling, fatty acid metabolism, unsaturated fatty acid biosynthesis, lysosomes, bile secretion, bile acid synthesis, and steroid hormone synthesis [22]. Rahman et al. [14] investigated chitooligosaccharide effects on 3T3-L1 adipocyte differentiation, revealing differential changes in numerous protein spots, with 50 proteins showing significant changes (6 upregulated).

3.4.2 Regulation of AMPK and Lipid Synthesis Transcription Factors

AMPK, a crucial sensor of cellular energy homeostasis, plays a key role in regulating lipid metabolism in liver and adipose tissue. AMPK inhibits liver X receptor (LXR) activity, thereby suppressing hepatic TG accumulation and fatty acid synthase activation. Chitosan supplementation significantly acti-

vates AMPK phosphorylation, attenuating high-fat diet-induced expression of LXR , PPAR , and sterol-regulatory element binding protein 1c (SREBP1c) in rat liver, epididymal, and perirenal adipose tissues [15]. Recent studies show that chitooligosaccharides activate AMPK through calcium-sensing receptor-phospholipase C-inositol trisphosphate (IP3) receptor channels [24]. Moreover, chitosan and its derivatives induce LEP and IL-6 secretion, reversing high-fat diet-induced AMPK inhibition in rat liver, epididymal, and perirenal adipose tissues [14-15].

LXRs and PPARs are ligand-activated transcription factors known as nuclear receptors that play important roles in fatty acid and CHOL metabolism, emerging as key metabolic regulators. LXR triggers downstream fatty acid synthase gene expression by inducing SREBP1c, increasing fatty acid synthesis. The PPAR family includes PPAR , PPAR , and PPAR , where PPAR controls adipocyte differentiation and lipid storage while PPAR reduces hyperlipidemia. For energy balance, PPAR induces fatty acid -oxidation when hepatic fatty acid levels are elevated. Chitosan and its derivatives inhibit LXR and PPAR gene expression while promoting PPAR expression in rat white adipose tissue [22].

Hepatic CHOL conversion to bile acids represents a crucial pathway for CHOL elimination. Cholesterol 7 -hydroxylase (CYP7A1) is the rate-limiting enzyme in bile acid synthesis. High-fat diets supplemented with chitosan activate PPAR , which forms a complex with retinoid X receptor and binds to CYP7A1 regulatory elements, activating hepatic CYP7A1 expression [15]. 3-Hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) and sterol-regulatory element binding protein-2 (SREBP-2) are key enzymes in CHOL synthesis, and high-fat/high-CHOL diet-induced LXR activation upregulates their expression. Dietary chitosan inhibits LXR protein expression in high-fat diet-fed rats, downregulating HMGR and SREBP-2 protein expression [25]. Additionally, ATP binding cassette transporter A1 (ABCA1), a hepatic transporter protein, promotes CHOL transfer to apolipoprotein A (apoA) to form HDL-C. Dietary chitosan promotes ABCA1 gene expression, alleviating hepatic CHOL accumulation in high-fat diet-fed rats [25].

Chitosan reduces expression of downstream fatty acid and TG synthesis-related genes including fatty acid synthase, acetyl-CoA carboxylase, fatty acid desaturase, ATP-citrate lyase, and diacylglycerol acyltransferase, while upregulating lipid oxidation and catabolism-related genes such as acyl-CoA oxidase and carnitine palmitoyltransferase, by inhibiting LXR and SREBP1c expression and activating PPAR [15,25]. In obese patients, hepatic steatosis relates not only to lipid synthesis and catabolism but also to lipid flux. Fatty acids are transported into hepatocytes via fatty acid transport proteins (FATP), while fatty acid-binding proteins (FABP) bind hydrophobic fatty acids for diffusion into the cytosol, where they are esterified into TG and accumulate in the liver. High-fat diets supplemented with chitosan effectively inhibit hepatic FATP and FABP expression in rats, with nuclear receptors playing key regulatory roles [15].

Collectively, current research indicates that chitosan and its derivatives regulate

animal lipid metabolism by activating AMPK to modulate key transcription factors including nuclear receptors and SREBPs and their downstream genes involved in lipid metabolism (synthesis, transport, catabolism, oxidation). The regulatory mechanism is illustrated in Figure 1 [Figure 1: see original paper].

Figure 1. Molecular regulation mechanism of chitosan and its derivatives. AMPK: adenosine monophosphate-activated protein kinase; LXR : liver X receptor ; PPAR : peroxisome proliferator-activated receptor ; SREBP1c: sterol-regulatory element binding protein 1c; PPAR : peroxisome proliferator-activated receptor ; ABCA1: ATP binding cassette transporter A1; HMGR: 3-hydroxy-3-methylglutaryl coenzyme A reductase; FAS: fatty acid synthase; ACC: acetyl-CoA carboxylase; FADS: fatty acid desaturase; Acly: ATP-citrate lyase; DGAT: diacylglycerol acyltransferase; FATP: fatty acid transport protein; FABP: fatty acid-binding proteins; AOX1: alcohol oxidase 1; CPT: carnitine palmitoyltransferase; CYP7A1: cholesterol 7 -hydroxylase; TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol.

However, Egan et al. [7] found that chitosan supplementation in conventional diets upregulated PPAR expression in pig hypothalamus while downregulating orexigenic gene expression in small intestine and hypothalamus, suggesting that dietary chitosan exerts anti-obesity effects and reduces body fat by altering appetite and feeding behavior to influence satiety signals. Chia et al. [26] reported that subcutaneous chitosan injection in mice fed conventional diets significantly activated PPAR in brain and stomach, downregulating apolipoprotein B (apoB) and ghrelin gene expression. apoB combines with microsomal transport protein to incorporate lipids into apoB, forming VLDL particles, and downregulation of apoB expression helps reduce TG and CHOL levels. Furthermore, Xu et al. [27] found that chitosan increased hepatic LDL-C receptor mRNA levels in rats, eliminating CHOL-rich lipoprotein particles. High-fat diets supplemented with chitosan inhibited hepatic acyl coenzyme A: cholesterol acyltransferase (ACAT) gene expression [5]. ACAT is responsible for intestinal CHOL absorption and VLDL assembly; high ACAT activity accelerates hepatic VLDL release, elevating blood CHOL levels.

4 Summary

In summary, chitosan and its derivatives effectively reduce blood lipid levels and lipid deposition in animals through mechanisms primarily involving lipid binding, pancreatic lipase inhibition, and regulation of adipocytokines, lipid metabolism-related enzyme activities and gene expression, AMPK, and lipid synthesis transcription factors. However, current research has focused mainly on humans and rats, with limited studies in other animal species. Controversies persist regarding their effects on lipid metabolism and underlying regulatory mechanisms, necessitating further investigation to provide theoretical foundations for scientifically regulating animal lipid metabolism using chitosan and its derivatives.

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