

*Current Perspective***Inter-organ Communication in the Regulation of Lipid Metabolism: Focusing on the Network Between the Liver, Intestine, and Heart**Masanori Ito<sup>1</sup> and Satomi Adachi-Akahane<sup>1,\*</sup><sup>1</sup>*Department of Physiology, School of Medicine, Faculty of Medicine, Toho University, 5-21-16 Omori-nishi, Ota-ku, Tokyo 143-8540, Japan**Received September 6, 2013; Accepted October 24, 2013*

**Abstract.** Recent studies have shown that lipid metabolism is regulated through the orchestration of multiple organs. Gut microbiota influences the metabolism of the liver through the production of fatty acids and phosphatidylcholine as well as the modulation of bile acid profile. Microbiota also affects the cardiovascular system through the production of metabolites from nutrients. MicroRNAs (miRNAs) are non-coding RNAs comprised of around 22 nucleotides in length. MiRNAs are released into blood flow from organs and interfere with the gene expression of target organs. MiRNAs are involved in the regulation of metabolic homeostasis including lipoprotein production and cardiovascular functions. Fatty acids are also circulating and distributed to each organ by fatty acid transporting proteins. Fatty acids can act as a ligand of G protein-coupled receptors, such as GPR41 and GPR43, and nuclear receptor PPAR $\alpha$ , which bear crucial roles in the regulation of energy expenditure. Therefore the inter-organ communication plays important roles in the systematic regulation of lipid metabolism. Studies on the inter-organ network system will contribute to the development of diagnostic and therapeutic strategies for metabolic diseases. This review discusses how lipid metabolism is regulated by the inter-organ communication, focusing on the network axis between the liver, intestine, and heart.

**Keywords:** inter-organ communication, lipid metabolism, liver, intestine, heart

**1. Introduction**

The molecular mechanism underlying the regulation of lipid metabolism has been extensively studied. Metabolic organs, such as the liver and adipose tissue, play critical roles at the center of the systemic regulation of lipid metabolism. The recent advances in modern ‘omics-technologies’ have revealed the involvement of microRNAs (miRNAs) and metabolites, in addition to hormones and cytokines, as key players in the inter-organ communication of lipid metabolism. This review discusses how lipid metabolism is regulated through the inter-organ communication, focusing on the network axis between the liver, intestine, and heart.

**2. Key-players of inter-organ communication****2.1. Fatty acids**

Fatty acids play important roles not only as sources for energy metabolism but also as components of membrane phospholipids. Fatty acids consist of an aliphatic chain attached to a carboxylic acid. Based on the number of carbon atoms in the hydrocarbon chain, fatty acids are classified as short-chain (less than six), medium-chain (six to twelve), and long-chain (more than twelve). They are further subdivided into saturated (no double bonds) or unsaturated (containing double bonds) fatty acids.

Long chain fatty acids, such as oleic acid, bind to nuclear transcription factor, PPAR $\alpha$ , as endogenous ligands. PPAR $\alpha$  is expressed in tissues that are responsible for fatty acid catabolism, such as the liver, intestine, heart, brown adipose tissue, muscle, and kidney. Fatty acid uptake and oxidation are stimulated by PPAR $\alpha$ -mediated gene expression.

Cellular uptake of fatty acids is mediated by fatty acid

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transport proteins (FATPs) (1). Six FATP genes have been identified in the genome of mammals. Various unique combinations of FATPs are expressed in a tissue-specific manner. FATP1 is expressed in adipose tissue, skeletal muscle, and heart. FATP2 is expressed in kidney and liver. FATP3 is expressed in lung, pancreas, and liver. FATP4 is ubiquitously expressed. FATP5 and FATP6 are expressed in the liver and heart, respectively. Studies using knockout/knockdown and transgenic mice revealed that FATPs are involved in the development of metabolic disease. For example, transgenic mice with cardiomyocyte-specific overexpression of FATP1 led to the accumulation of fatty acid in the heart, which caused hypertrophy associated with diastolic dysfunction and QT prolongation. On the other hand, liver-specific knockdown of FATP2/FATP5 resulted in the reduction of the uptake of fatty acid and the level of triglyceride in the liver, thus leading to an improvement of hepatosteatosis that was induced by a high-fat diet (1).

Fermentation of dietary fiber by gut flora is a source of short chain fatty acids. These short chain fatty acids are ligands of G protein-coupled receptors such as GPR41 and GPR43. The knockout of these genes in mice revealed that short chain fatty acids induce the production of chemokines and cytokines during immune responses by activating GPR41 and GPR43 (2, 3). The disruption of GPR41 in mice also indicated that gut-derived short chain fatty acids raise energy expenditure and help to protect against obesity, presumably by stimulation of GPR41 that leads to the activation of sympathetic nervous system (4). These findings indicate that gut flora is involved in the regulation of the immune response and lipid metabolism through the production of short chain fatty acids.

## 2.2. miRNAs

MiRNAs are small RNAs of about 22 nucleotides in length. MiRNAs can be transferred from cell to cell by lipid-based carriers such as exosomes and lipoproteins. Exosomes contain miRNAs, non-coding RNAs, and mRNA. These RNAs are thought to function in intercellular communication (5). MiRNAs regulate the expression of gene products involved in lipid metabolism such as adipocyte differentiation, insulin signaling, and homeostasis of cholesterol. It is now well established that miRNAs play important roles in the post-transcriptional regulation of gene networks involved in lipid metabolism (5). Evolutionally conserved miRNAs, miR-33a and miR-33b, are positioned within the intron of the genes encoding transcription factors sterol regulatory element binding protein 2 (SREBP2) and SREBP1, respectively. The SREBP family of basic helix-loop-helix leucine zipper transcription factors is responsible for the regula-

tion of synthesis and uptake of cholesterol and fatty acids. Recent studies have demonstrated that miR-33a and miR-33b are co-transcribed with SREBP genes. MiR-33a and miR-33b repress the expression of gene products involved in fatty acid  $\beta$ -oxidation, cholesterol efflux (ABCA1, ATP-binding cassette subfamily A member 1), and the negative regulation of SREBPs, which results in the accumulation of cholesterol and triglycerides, thus enhancing the risk of atherosclerosis.

In the liver, miR-34a represses the expression of sirtuin 1 (SIRT1), a NAD<sup>+</sup>-dependent protein deacetylase (6). SIRT1 inhibits the expression of miR-34a through several mechanisms such as deacetylation of histone in the miR-34a-promoter region and farnesoid X receptor (FXR). The deacetylation of p53 also represses the expression of miR-34a, since acetylated p53 activates the transcription of miR-34a. FXR, when activated by deacetylation, induces the expression of small heterodimer partner (SHP). SHP binds to p53, and then p53 is released from miR-34a-promoter region. That is another mechanism of the repression of miR-34a by SIRT1. SIRT1 can affect other transcription factors such as PPAR $\alpha$ , liver X receptor (LXR), and SREBP. Patients with nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH) exhibit increased hepatic expression of miR-34a and low hepatic levels of SIRT1. In addition, miR-34a is induced in cardiac myocytes and involved in the regulation of cardiac function during aging. It causes dysfunction of contractility through downregulation of protein phosphatase-1 nuclear targeting subunit (PNUTS) that inhibits DNA damage responses, telomere attrition, and apoptosis in cardiac myocytes (7). Accordingly, the inhibition of miR-34a would be a novel therapeutic strategy against NAFLD, NASH, and cardiac dysfunction (6, 7).

The expression of miR-144 is stimulated by the activation of FXR. FXR, which is a nuclear receptor of bile acids, regulates genes such as bile acid export pump (BSEP) and CYP7A1. MiR-144 expressed in hepatocytes downregulates the expression level of ABCA1, thus leading to a reduction of cholesterol efflux for apoA-I to produce high density lipoprotein (HDL) (8). Since HDL exerts anti-atherosclerotic functions, miR-144 is an important risk factor for cardiovascular diseases.

Differentiation and function of white adipocyte are regulated by miR-378 (6). Mitogen-activated protein kinase 1 (MAPK1) is a target gene of miR-378. MAPK1 phosphorylates PPAR $\gamma$  to inhibit its transcriptional activity. Since PPAR $\gamma$  serves as a master transcriptional regulator of adipogenesis, miR-378 promotes adipogenesis by inhibiting the expression of MAPK1 and activation of PPAR $\gamma$ . MiR-378 is also expressed in cardiac myocytes, and its level is down-regulated during

hypertrophic development of heart failure (9). In the heart, miR-378 targets growth factor receptor-bound protein 2 (GRB2), insulin-like growth factor 1 receptor (IGF1R), kinase suppressor of ras 1 (KSR1), as well as MAPK1. MiR-378 serves as an endogenous negative regulator of cardiac hypertrophy through the repression of the RAS signaling pathway (9). More information on the miRNA profile associated with cardiac disease has been emerging (10). As summarized in the above section, circulating miRNAs will be useful biomarkers of various diseases, including cardiovascular disease, diabetes, and metabolic disorders. Therefore miRNAs are promising targets for the development of novel strategies for therapeutics of cardiac and metabolic diseases.

### 2.3. Microbiota

Gut microbiota contributes to human health from birth to old age through the production of metabolites such as short chain fatty acids, bile acids, and vitamins (11). As reviewed by Purchiaroni et al., gut microbiota supports energy metabolism, immune system, and trophic functions in the host (12). The composition of flora in the gut can be influenced by a patients' lifestyle (diet, age, illness, medication, etc.). Alternation of microbiota causes various kinds of diseases. As described in the next sections, gut bacteria have emerged as critical regulators in metabolic disorders such as fatty liver and cardiovascular diseases.

## 3. Examples of inter-organ communication

### 3.1. Intestine and liver

Bile acids are derived from cholesterol. Bile acids play crucial roles in the emulsification and the absorption of dietary fat. Therefore, the entero-hepatic circulation of bile acids is important for the absorption of fat. The bile acid is also important as a natural ligand of the nuclear receptor FXR and the G protein-coupled receptor TGR5. The autocrine and paracrine systems regulate the bile acid metabolism in hepatocytes and enterocytes. For instance, fibroblast growth factor 15/19 (FGF15/19), secreted from the intestine by the stimulation of bile acids through activation of FXR, represses the expression of apical sodium-dependent bile acid transporter (ASBT) in the intestine and CYP7A1 that is the rate-limiting enzyme of bile acid synthesis in the liver (13). We found that disruption of the steroidogenic acute regulatory protein (StAR)-related lipid transfer domain containing 10 (*Stard10*) gene results in an increase in the biliary secretion of bile acids from the liver and the impairment of enterohepatic cycling of bile acids by downregulation of ASBT, thus preventing the accumulation of fat in the liver. STARD10 turned out to be

involved in the regulation of ASBT through the regulation of specific peroxisome proliferator-activated receptor  $\alpha$  (PPAR $\alpha$ )-target genes (14). ASBT inhibitor 264W94 augmented the stimulation of TGR5 by bile acids and thus led to enhanced glucagon-like peptide-1 (GLP-1) secretion in the intestine and lowered the plasma glucose level. Such a beneficial effect of ASBT inhibitor may offer a new therapeutic strategy for type 2 diabetes (15).

Dietary habits and nutrient intake are correlated with NAFLD (16). NAFLD progresses to the more serious disease NASH. NASH may progress to cirrhosis with subsequent liver failure and increases the risk for hepatocellular carcinoma. Since fructose and the fructose metabolite glyceraldehyde act as endogenous toxins and induce hepatic inflammation, a fructose-enriched diet may cause NASH (17). Gut microbiota affects the host's lipid composition in the serum, with the most notable effects on triglyceride. The activity of lipoprotein lipase (LPL) is increased by gut microbiota, as gut microbiota suppresses an inhibitor of LPL, angiopoietin-like protein 4 / fasting-induced adipose factor (Angptl4 / Fiaf). Thus gut microbiota increases the clearance of triglyceride via LPL. Although the underlying mechanism has not been clear, conventional mice have higher levels of phosphatidylcholine (16:0/18:1) in both serum and the liver compared with germ-free mice (18). A recent study showed that phosphatidylcholine (16:0/18:1) is a physiological agonist of PPAR $\alpha$  (19). Then, an increase in phosphatidylcholine (16:0/18:1) and stimulation of the PPAR $\alpha$ -mediated activation of LPL may be another explanation for the enhancement of triglyceride clearance by microbiota (18).

Gut microbiota also affects the bile acid profile of host tissues. Gut microbiota promotes de-conjugation of bile acids and produce secondary bile acid. The bile acid profiles were compared using a germ-free and conventional rats. Tauro-muricholic acid, which is the primary bile acid specific to rodents, was abundant in germ-free rats. Since tauro-muricholic acid has minimal effect on the activation of FXR, the expression of FXR-activated genes was reduced in the germ free rats (20). Obesity is associated with alterations in the composition of the microbiota that contributes to metabolic liver disease, including NASH (21). Deoxycholic acid has been reported to be increased by obesity associated alterations of gut microbiota. Deoxycholic acid induces the secretion of various inflammatory and tumor-promoting factors in the liver, thus facilitating the development of hepatocellular carcinoma by generation of reactive oxygen species (22).

Short chain fatty acid, produced by microbiota in the gut, activates GPR43 to suppress insulin signaling via the G(i/o)bc-PLC-PKC-PTEN signaling pathway in white

adipose tissue, which leads to a reduction of the insulin-mediated fat accumulation (23). On the other hand, in the liver and muscles, the insulin sensitivity and energy expenditure are enhanced by the activation of GPR43 (23). Therefore short chain fatty acid inhibits the accumulation of lipids in adipose tissue and promotes the metabolism of unincorporated lipids and glucose in other tissues through the activation of GPR43.

### 3.2. Liver and heart

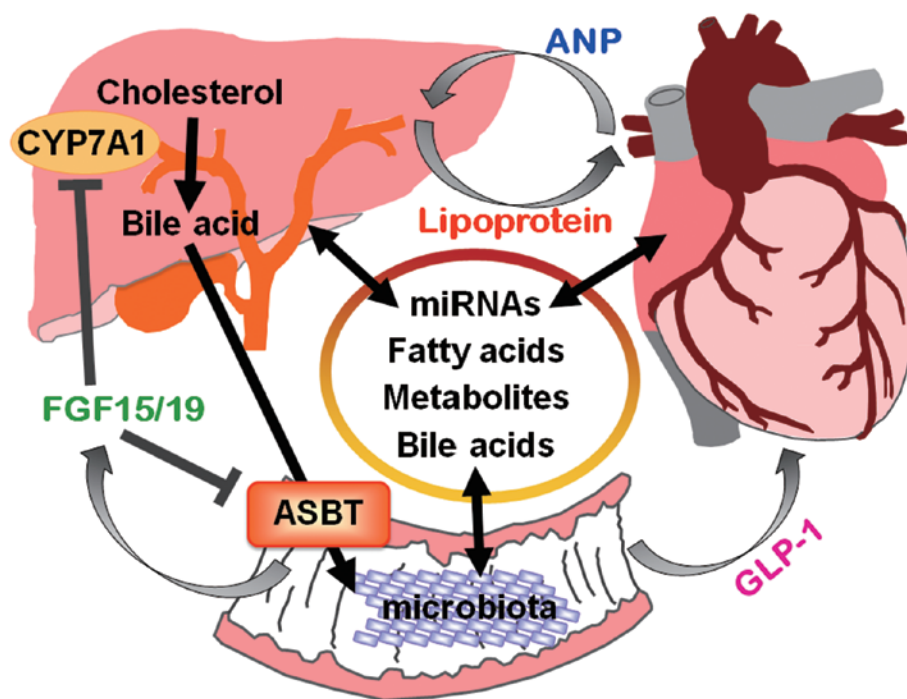
Atherosclerosis is the most common form of coronary artery disease. Inflammation is the initial cause of atherosclerosis. Roles of lipoprotein profile in atherosclerosis were investigated using many genetically modified mouse models (24). HDL-cholesterol (HDL-C) has anti-atherogenic properties (25 – 27). The elevation of HDL-C level has been the subject of cardiovascular medicine. Phospholipids are involved in lipoprotein metabolism by reducing intestinal absorption of cholesterol, enhancing biliary cholesterol excretion, and modulating transcriptional factors such as SREBP. Dietary phospholipids have the effects of increasing HDL-C and apoA-I levels and reducing cholesterol and triglyceride levels (28). Since NAFLD is associated with cardiovascular risk factors such as the chronic inflammatory state, hyperlipidemia, and oxidative stress, NAFLD

leads to a high risk of cardiovascular disease (29). FXR and TGR5 can be therapeutic targets of NAFLD and type 2 diabetes, as the activation of TGR5 increases GLP-1, the insulin secretion and sensitivity, and thus up-regulates the energy expenditure. The activation of FXR reduces lipogenesis and enhances the consumption of bile acid (30).

With respect to the heart to liver axis, atrial natriuretic peptide (ANP) affects liver function. It has been reported that ANP protects hepatocytes from the ischemia–reperfusion injury, presumably by repressing Kupffer cell activation during inflammatory process, although the underlying mechanism of the effect remains to be clarified (31).

### 3.3. Intestine and heart

L-Carnitine is an abundant nutrient included in red meat and contains a trimethylamine structure. The trimethylamine structure is derived from the metabolite of phosphatidylcholine. Intestinal microbiota produces trimethylamine-*N*-oxide (TMAO) from dietary phosphatidylcholine or L-carnitine. TMAO alters cholesterol and sterol metabolism in macrophages, liver, and intestine and therefore potentially induces the development of atherosclerosis (32). Therefore antibiotics that abolish the TMAO-producing microbes or inhibit



**Fig. 1.** Inter-organ communication between the liver, intestine, and heart in the systemic regulation of lipid metabolism through miRNAs, fatty acids, bile acids, microbiota, as well as hormones. ANP, atrial natriuretic peptide; ASBT, apical sodium dependent bile acid transporter; CYP7A1, cytochrome P450, family 7, subfamily A, polypeptide 1; FGF15/19, Fibroblast growth factor 15/19; GLP-1, glucagon-like peptide-1; miRNA, microRNA.



specific microbial metabolic pathways should be useful for reducing the risk of cardiovascular events (33, 34).

Intestinal microbiota converts cyanidin-3-*O*- $\beta$ -glucoside (Cy-3-G) into protocatechuic acid (PCA). PCA exerts antiatherosclerotic effects through the up-regulation of ABCA1 and ABCG1. PCA also reverses cholesterol transport by suppressing the expression of miR-10b in macrophages (35). These findings suggest that gut microbiota is a novel target for the prevention and treatment of cardiovascular diseases.

GLP-1, secreted from the intestine, activates the GLP-1 receptor of atrial cardiac myocytes to stimulate the secretion of ANP. ANP induces cGMP-mediated smooth muscle relaxation and natriuresis, thus leading to a reduction of blood pressure (36). Such a gut–heart axis defines a novel strategy for the treatment of high blood pressure

#### 4. Summary

In this review, we discussed the important roles of inter-organ communications in the regulation of lipid metabolism, focusing on the network axis between the liver, intestine, and heart. As summarized in Fig. 1, miRNA and fatty acids, in addition to hormones, play important roles in the inter-organ communication in the regulation of lipid metabolism. Microbiota can influence the metabolism of the liver and also affect the cardiovascular system through the production of short chain fatty acids and secondary bile acids. Therefore the communication between organs would be critical for understanding the pathophysiology of lipid metabolism and for developing novel therapeutic strategy for diseases. However, mechanisms in regulating lipid metabolism through orchestration of the inter-organ communication remain to be elucidated.

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