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Liver stem/progenitor cells: their characteristics and regulatory mechanisms

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Liver stem cells give rise to both hepatocytes and bile duct epithelial cells also known as cholangiocytes. During liver development hepatoblasts emerge from the foregut endoderm and give rise to both cell types. Colony-forming cells are present in the liver primordium and clonally expanded cells differentiate into either hepatocytes or cholangiocytes depending on culture conditions, showing stem cell characteristics. The growth and differentiation of hepatoblasts are regulated by various extrinsic signals. For example, periportal mesenchymal cells provide a cue for bipotential hepatoblasts to become cholangiocytes, and mesothelial cells covering the parenchyma support the expansion of foetal hepatocytes by producing growth factors. The adult liver has an extraordinary capacity to regenerate, and after 70% hepatectomy the liver recovers its original mass by replication of the remaining hepatocytes without the activation of liver stem cells. However, in certain types of liver injury models, liver stem/progenitor-like cells, known as oval cells in rodents, proliferate around the portal vein, while the roles of such cells in liver regeneration remain a matter of debate. Clonogenic and bipotential cells are also present in the normal adult liver. In this minireview we describe recent studies on liver stem/progenitor cells by focusing on extracellular signals.

Keywords: cytokine/development/differentiation/hepatocyte/regeneration.

Abbreviations: 2-AAF, 2-acetylaminofluoren; AFP, alpha-fetoprotein; ALB, albumin; BMEL, bipotential mouse embryonic liver cell; CDE, choline-deficient, ethionine-supplemented; DDC, 3,5-diethoxycarbonyl-1,4-dihydro-collidine; Dlk, Delta-like protein 1; DPPIV, dipeptidyl peptidase IV; EpCAM, epithelial cell adhesion molecule; FGF, fibroblast growth factor; H-CFU-C, hepatic colony-forming unit in culture; MC, mesothelial cell; OSM, oncostatin M; PH, partial hepatectomy; STM, septum transversum mesenchyme; TNF, tumour necrosis factor; Wt1, Wilms' tumour 1.

The liver is a central organ for homeostasis owing to its numerous functions, including carbohydrate metabolism, glycogen storage, biosynthesis of various biochemical components including amino acids and nucleotides, lipid metabolism, urea synthesis, drug detoxification, production of plasma proteins and hormones, and destruction of erythrocytes. Because the liver is such an essential organ, liver diseases are often fatal. Liver insults such as hepatitis viruses, drugs, alcohol and genetic, metabolic and immune disorders can lead to steatosis, hepatitis, fibrosis, cirrhosis and cancer and liver disease is a major cause of death. The liver is also known as a unique organ that can regenerate, making it possible to transplant the liver from a living donor. However, the molecular mechanisms underlying organogenesis, maintenance, pathogenesis and regeneration of the liver are not well understood. As the liver is a large organ with a variety of functions, it has been used for many decades as a source to purify numerous enzymes for biochemical studies. By contrast, much progress has been made relatively recently in the characterization of each type of liver cell and analysis of their interactions. Those studies have been facilitated by new technologies such as genomics, mouse mutants and the development of various tools to isolate the cells of interest. In this review, we describe recent studies on liver stem/progenitor cells together with the environments that support their proliferation and differentiation during development and pathogenesis.

Liver architecture and liver stem cells

The liver is divided into lobules and each lobule consists of plates of hepatocytes lined by sinusoidal capillaries that radiate towards a central efferent vein (Fig. 1). Liver lobules are hexagonal and at each of six corners there is a portal triad of vessels consisting of a portal vein, hepatic artery and bile duct. Sinusoids are composed of liver-specific capillaries with fenestrated endothelial cells, hepatic stellate cells (Ito cells), liver-resident macrophages (Kupffer cells) and large granular lymphocytes (pit cells). The liver has a dual blood supply, namely, via the portal vein and the hepatic artery. The portal vein delivers the venous blood flowing from the intestines, pancreas and spleen. The hepatic artery supplies oxygen to the liver. The blood flows from a portal triad through a sinusoidal capillary to a central efferent vein. Hepatocytes are major parenchymal cells carrying out most of the metabolic functions and account for ~60% of the total liver cell population and 80% of the volume of the organ. Hepatocytes are highly polarized epithelial cells and

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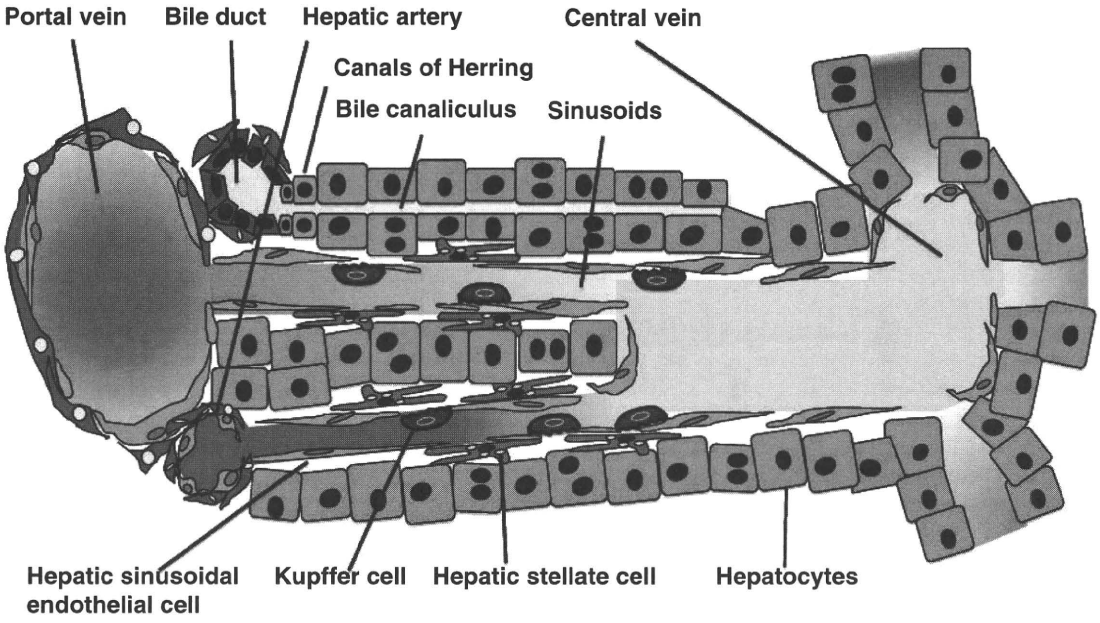

Fig. 1 Liver architecture. In the liver, blood flows from portal blood vessels through sinusoids to central efferent veins. Sinusoids are liver-specific capillaries with fenestrated endothelial cells, hepatic stellate cells (Ito cells) and blood cells such as liver-resident macrophages (Kupffer cells). Hepatocytes are highly polarized epithelial cells forming cords, and plates of hepatocytes are lined by sinusoidal capillaries that radiate towards a central efferent vein. Tight junctions formed between hepatocytes create a canaliculus that surrounds each hepatocyte. Bile salts produced in hepatocytes are excreted into canaliculi that are linked to bile ducts. The region that connects the bile canaliculus and the biliary tree is called 'canals of Hering'.

form cords. Their basolateral surfaces face fenestrated sinusoid endothelial cells, facilitating the transfer of materials between hepatocytes and blood flows. Tight junctions formed between hepatocytes create a canaliculus that surrounds each hepatocyte. Bile salts produced in hepatocytes are excreted into canaliculi that are linked to bile ducts at the portal triad. Bile ducts are formed by a specialized type of epithelial cell called a biliary epithelial cell or a cholangiocyte.

In general, stem cells are characterized by their ability to self-renew and differentiate to multiple lineages. As hepatocytes and cholangiocytes, the two types of liver epithelial cells, are derived from a common origin during organogenesis, those cells with the potential to proliferate and give rise to both types of liver epithelial cells are considered to be liver stem cells. Although there are many reports describing liver stem cells, the definitions of stem cells are rather vague in many of them. As it is not an easy task to distinguish stem cells from progenitors because of the difficulty of proving the unlimited self-renewal activity of stem cells in many situations, we use the term stem/progenitor cells to describe such cells in this review article.

The onset of liver development

Liver organogenesis begins at embryonic day (E) 8.5 in the mouse from the foregut endoderm. The ventral wall of the foregut endoderm faces the developing heart by approximately E8 and receives inductive signals for hepatic fate, such as fibroblast growth factor (FGF) from the heart (*1–3*) and bone morphogenetic protein from the septum transversum mesenchyme (STM) (*4*). Wnt2b is expressed in the lateral plate mesoderm adjacent to the endoderm destined to be the liver and is essential for the onset of liver development in zebrafish (*5*). By these signals, hepatoblasts emerge from the foregut endoderm and migrate as cords into the surrounding STM (*6*, *7*). Analysis of Flk1-deficient mouse embryos revealed that $Flk1^+$ endothelial cells are required for proliferation of hepatoblasts (*8*). Because hepatoblasts proliferate and give rise to both hepatocytes and cholangiocytes as described below, they are considered to be embryonic liver stem/progenitor cells.

Identification and characterization of hepatoblasts

As cell sorting using antibodies is a powerful means to isolate and characterize a specific cell type, efforts have been made to search for specific cell surface antigens on hepatoblasts (Fig. 2). Kubota and Reid (*9*) showed that the $RT1A1^-$ $OX18^{low}$ $ICAM\text{-}1^+$ fraction of E13 rat foetal liver contained hepatoblasts. Suzuki *et al.* (*10*) developed a single cell-based assay designated the hepatic colony-forming unit in culture (H-CFU-C) and showed that the $CD45^-$ $TER119^-$ $c\text{-}Kit^-$ $CD29^+$ $CD49f^+$ and $CD45^-$ $TER119^-$ $c\text{-}Kit^-$ $c\text{-}Met^+$ $CD49f^{+/low}$ fraction of E13.5 mouse liver contained hepatic progenitor/stem cells. They also showed that $CD45^-$ $TER119^-$ $c\text{-}Kit^-$ $c\text{-}Met^+$ $CD49f^{+/low}$ cells of E11.5 mouse liver had high H-CFU-C potential and that clonally expanding cells reconstituted the liver, pancreas and intestine *in vivo*. On the other hand, Minguet *et al.* (*11*) reported that $CD45^-$ $TER119^-$ $c\text{-}Kit^{low}$ cells in E11 mouse liver contained the earliest hepatic progenitors, also displaying features of

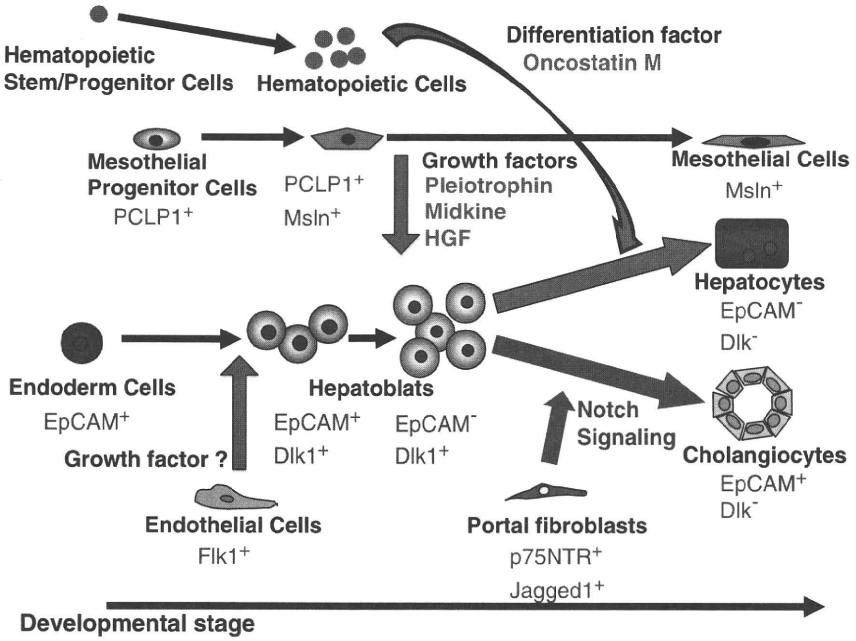

Fig. 2 Development of liver cells and expression of cell surface markers. The EpCAM$^+$DLK1$^+$ hepatoblasts emerge from EpCAM$^+$ foregut endoderm cells and form liver primordium. Then, the hepatoblasts dramatically reduce the expression of EpCAM. EpCAM is upregulated again in biliary epithelial cell precursor cells around the portal vein, where p75NTR$^+$ Jagged1$^+$ portal fibroblasts interacted with hepatoblasts. PCLP1$^+$ mesothelial progenitor cells produce growth factors for hepatoblasts to proliferate. OSM secreted by haematopoietic cells induces hepatocytic differentiation of hepatoblasts.

liver-repopulating stem cells. Delta-like protein 1 (Dlk1), also known as Pref-1, was strongly expressed in liver buds as early as E10.5 in mice. Dlk1$^+$ cells isolated from E14.5 livers expressed albumin (ALB) and formed colonies composed of the hepatocyte and cholangiocyte lineages in the presence of hepatocyte growth factor and epidermal growth factor, indicating that liver stem cell activity is present in this population (*12*). As in mouse foetal liver, Dlk1 is also expressed strongly in human foetal liver (*13*, *14*). Nierhoff *et al.* showed that murine foetal liver alpha-fetoprotein (AFP)$^+$/ALB$^+$ cells were positive for Dlk1 and E-cadherin and that purified E-cadherin$^+$ epithelial cells formed clusters in cell culture and differentiated along the hepatocytic lineage. Interestingly, AFP$^+$/E-cadherin$^+$ epithelial cells were Sca-1$^+$, but showed no expression of c-Kit. In order to examine their *in vivo* capacity, wild-type E12.5 mouse liver epithelial cells were transplanted into adult dipeptidyl peptidase IV (DPPIV) knockout mice, and DPPIV expression was used as a marker to discriminate the donor from recipient cells. This resulted in incorporation of the DPPIV$^+$ donor-derived cells into the hepatic parenchymal cords of the recipient liver, showing a repopulation and differentiation capacity of the E12.5 E-cadherin$^+$ cells (*15*).

Epithelial cell adhesion molecule (EpCAM) is expressed in HNF4α^+ hepatoblasts of liver buds as early as E9.5 in mice (Fig. 2). Colony-forming assays using sorted E11.5 liver cells revealed that the EpCAM$^+$ Dlk1$^+$ cell population contained *in vitro* colony-forming cells, indicating that liver stem cell activity is present in this population. EpCAM expression declined by E13.5 in mouse liver, while Dlk1 expression was sustained by E16.5 (*16*). In humans, Dan *et al.* (*17*) reported that multipotent progenitor cells derived from human foetal liver expressed EpCAM,and Schmelzer *et al.* (*18*) reported that pluripotent precursors of hepatoblasts expressed EpCAM and were located in ductal plates in human foetal liver. CD13 (aminopeptidase N) was detected on the cells of the Dlk1$^+$ hepatic stem/progenitor fraction. Colony formation assays revealed that hepatic stem/progenitor cells were enriched in the CD13$^+$ fraction, compared with the Dlk1$^+$ fraction, of non-haematopoietic cells in foetal liver (*19*).

Characteristics of foetal liver stem/progenitor cells

Dlk1$^+$ cells contain some clonogenic cells named hepatic progenitor proliferating on laminin that continuously proliferate on laminin-coated plates and differentiate to both hepatocytes and cholangiocytes depending on culture conditions, suggesting that they are liver stem cells (*20*). Bipotential cell lines, referred to as bipotential mouse embryonic liver cell (BMEL), were also obtained after a long latency in culture of foetal liver cells and they were shown to give rise to both hepatocytes and cholangiocytes in recipient mice, although the origin of BMEL was unknown (*21*). These cell lines are used to study the mechanisms of hepatocytic and/or cholangiocytic differentiation from liver stem cells.

In the past decade, a number of cell surface markers for foetal liver cells have been found and used to prospectively isolate and to localize them in the liver. While some studies used transplantation assays to

investigate the repopulation capacity, the ability to form a colony and differentiate to both lineages *in vitro* is a practical criterion to evaluate hepatoblasts in most of these studies. In the case of haematopoietic stem cells, a single purified stem cell can be shown to propagate and give rise to all kinds of haematopoietic cells for the long term in an irradiated recipient mouse, providing clear evidence for stemness *in vivo*, that is self-renewal ability and multi-lineage differentiation. By contrast, as liver repopulation assays require a large number of cells to be transplanted to demonstrate engraftment capacity, rigorous proof of stemness *in vivo* is difficult. Nonetheless, there is little doubt that hepatoblasts possess capacities of liver stem cells on the basis of numerous previous works as described above (*9–12*, *15–19*).

Differentiation of hepatoblasts to cholangiocytes

Bile ducts are formed only around the portal vein, suggesting that regionally specific signals induce cholangiocytes from hepatoblasts. Indeed, two signalling pathways, TGFβ/Activin and Notch, are specifically activated in hepatoblasts near the portal vein. TGFβ2 and TGFβ3 are predominantly expressed in the portal region (*22*), and the Onecut family of transcription factors, HNF6 (OC-1) and OC-2, promote expression of α2-macroglobulin and follistatin, inhibitors of the TGFβ/Activin pathway, in the parenchymal region (*23*). $Dlk1^+$ hepatoblasts express Notch2, whereas $p75NTR^+$ periportal fibroblasts express Jagged-1 (*24*). Forced expression of Notch intracellular domain in $Dlk1^+$ hepatoblasts resulted in differentiation to cholangiocytes (*25*). These results strongly suggest that cholangiocyte differentiation is induced by Notch signalling in the periportal region. Although differentiation of hepatoblasts to cholangiocytes by TGFβ and Notch signalling occurs in mid-gestation, surprisingly, hepatocytes turned to cholangiocytes and formed ectopic duct structures in the parenchyma by Notch activation after birth (*26*). These results indicate that not only hepatoblasts but also hepatocytes are competent to differentiate to cholangiocytes at least by the neonatal period (Fig. 2).

Immature cholangiocytes form a ductal plate, a single cell layer, around the portal vein. Tubular morphogenesis of bile ducts proceeds through the rearrangement of a single layer of the ductal plate. Recent studies on mice lacking Sox9, a transcription factor, or Notch 2 in the liver indicated the second wave of cholangiocyte differentiation adjacent to the initial single layer of the ductal plate, which was regulated by TGFβ and Notch pathways and involved in tubular morphogenesis. In a model proposed on the basis of those studies, after the initial induction of cholangiocytes near the portal vein, cholangiocyte differentiation and tubular morphogenesis progress in parallel (*22*, *26–30*). However, the precise mechanisms of bile duct morphogenesis have not been completely understood.

In addition, studies using mutant mice have implicated transcription factors including HES1, HNF6, HNF1β, Tbx3, FoxA2 and A3, FoxM1b, Hex and Sall4 in bile duct differentiation and/or morphogenesis (*31–38*) (Fig. 3). Although a network of these transcription factors and a link between transcription factors and Notch/TGFβ pathways are being uncovered (*39*), studies on gene expression and histology of mutant mice are insufficient to understand how these factors regulate complicated processes of tubulogenesis. As an alternative approach, *in vitro* culture systems allowing hepatoblasts to form bile duct structures are helpful to understand the lineage commitment of hepatoblasts and tubular morphogenesis (*22*, *25*, *40*, *41*).

Proliferation and differentiation of hepatocytes in foetal liver

At an early stage of hepatogenesis, endothelial cells contribute to the proliferation of hepatoblasts (*8*) and the vast majority of hepatoblasts become parenchymal hepatocytes at a later stage. The liver parenchyma is covered with the mesothelium consisting of the surface mesothelial cell (MC) layer, $ALCAM^+$ submesothelial cells and fibroblasts (*42*). At a later stage of hepatogenesis, MCs seem to contribute to the expansion of hepatoblasts (*43*). Foetal liver MCs are characterized by the expression of a sialomucin, PCLP1, and become adult liver MCs expressing mesothelin. Comparison of the gene expression profiles between foetal and adult MCs revealed that foetal $PCLP1^+$ MCs express various growth factors for hepatocytes such as Midkine and Pleiotrophin, and co-culture of $Dlk1^+$ foetal hepatocytes with $PCLP1^+$ foetal MCs in a transwell enhanced hepatocyte proliferation. Wilms' tumour 1 (Wt1) knockout mice were embryonic lethal, exhibiting impaired liver development. Cytokine production by Wt1 knockout MCs was reduced, while proliferation of $Dlk1^+$ cells from Wt1 knockout embryos was normal in a co-culture with wild-type MCs, indicating that defects in liver development of Wt1

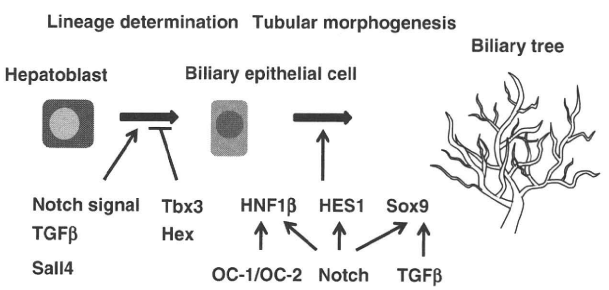

Fig. 3 Bile duct development. There are two steps in bile duct development. First, hepatoblasts are induced to differentiate to biliary epithelial cells around the portal vein. This step is promoted by TGFβ and Notch signals as well as a transcription factor, Sall4, whereas it is inhibited by two transcription factors, Tbx3 and Hex. Biliary epithelial cells then undergo tubular morphogenesis and form the biliary tree. Three transcription factors, HNF1β, HES-1 and Sox9, are involved in tubular morphogenesis. The Notch signal is upstream of all the three transcription factors, whereas OC-1 and OC-2, and the TGFβ signal are upstream of HNF1β and Sox9, respectively.

knockout mouse are due to MCs. MCs were also shown to delaminate and give rise to mesenchymal cells in the liver (*44*). These results indicate that the mesothelium is not only a protective sheet covering the liver parenchyma but also actively involved in liver organogenesis (Fig. 2).

Foetal liver is a major tissue for haematopoiesis, and hepatocytes acquire various metabolic functions at perinatal and postnatal stages. Mice lacking gp130, the common receptor subunit of the IL-6 family cytokines, develop liver with impaired functions, indicating that some of the IL-6 family cytokines are required for functional maturation of the liver (*45*). Oncostatin M (OSM), a member of the IL-6 family, strongly enhanced differentiation of foetal hepatocytes, while liver development is normal in OSM-deficient mice, suggesting that another member of the family may play a similar role. In the foetal liver, immigrating haematopoietic stem cells proliferate and produce numerous blood cells with the help of liver cells including hepatocytes and endothelial cells. Haematopoietic activity in foetal liver declines with hepatocyte differentiation (Fig. 2). As OSM is secreted from haematopoietic cells proliferating in the foetal liver and induces differentiation of hepatocytes, it is likely that OSM plays a role for coordination of liver development and haematopoiesis (*46*).

Adult liver stem/progenitor cells

Adult liver has a potential to regenerate under conditions of severe parenchymal loss, although hepatocytes and cholangiocytes are mitotically dormant under normal conditions. Hepatocytes themselves have a remarkable ability to self-replicate to restore liver mass (*47*) and are capable of at least 80 doublings by serial transplantation (*48*), allowing the liver to regenerate. Thus, the contribution of liver stem cells to regeneration after partial hepatectomy (PH) seems to be minimal if any. However, in liver injury that limits this pathway there is an accompanying expansion of a potential stem cell compartment in the periportal area, which is known as ductular reaction (*49–51*) (Fig. 4). These proliferating epithelial cells are often referred to as oval cells in rodents because of their oval nucleus (*52*). Upon activation of oval cells, they expand into liver parenchyma from the portal area, and selective damage of the periportal zone reduces oval cell proliferation, supporting the notion that oval cells are derived from the periportal region, in particular canals of Hering that connect the bile canaliculus and the biliary tree (*53*). In addition, an extrahepatic origin of oval cells such as bone marrow was also suggested (*54*); however, the exact origin of oval cells still remains to be established. While oval cells have been most extensively studied in rodents, similar cells have been found in various human liver diseases, such as chronic viral hepatitis, alcoholic liver disease, nonalcoholic fatty liver disease and fulminant hepatitis, and also implicated in tumourigenesis (*55*, *56*). Oval cells express both ALB and cytokeratin 19, which are hepatocytic and cholangiocytic markers, respectively, and are believed to differentiate to hepatocytic and biliary lineages, similar to hepatoblasts in the embryonic liver.

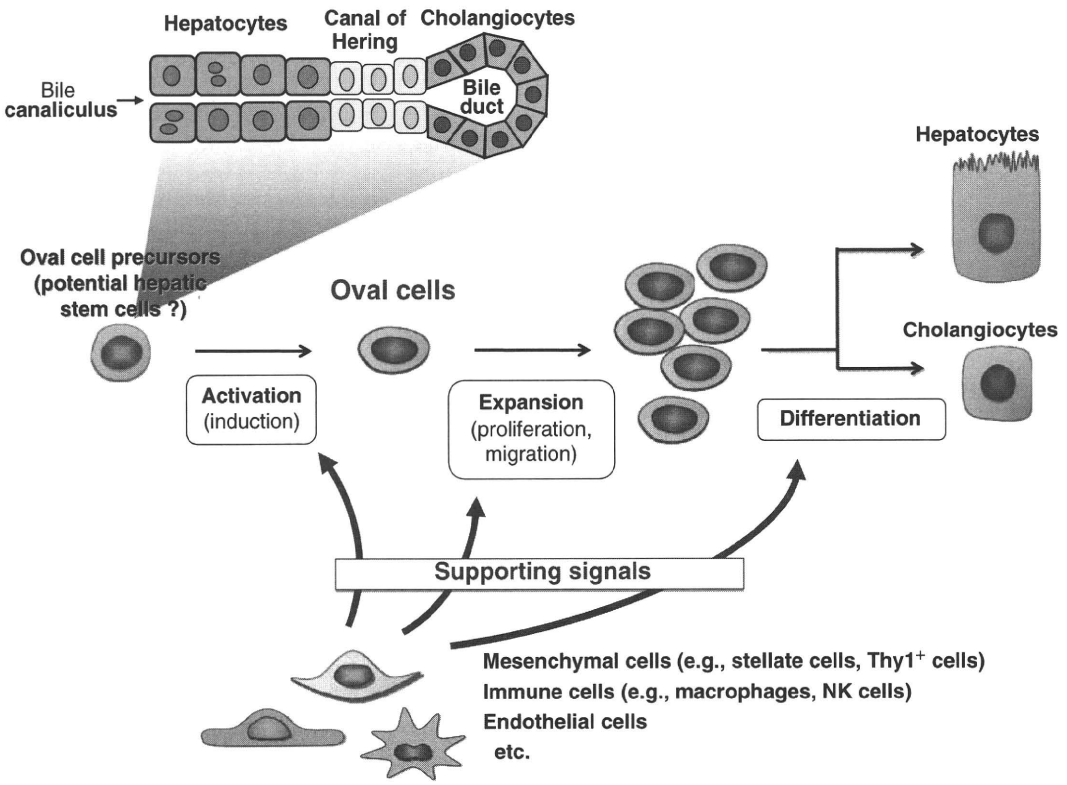

Fig. 4 Induction of oval cells. Oval cells and Thy1$^+$ cells are simultaneously induced in severe liver damage conditions. The origin of oval cells is still under debate. FGF7 is produced by Thy1$^+$ cells in response to liver damage conditions, and oval cells receive its signal.

Thus they are thought to be facultative stem/progenitor cells in the adult liver (Fig. 4).

The nature of oval cells as liver stem cells was debated in numerous reports of studies using various rodent models. The 2-acetylaminofluoren (2-AAF)/PH model, *i.e.* blocking hepatocyte proliferation by 2-AAF prior to PH, has been extensively used to characterize oval cells in rat (*57*, *58*). However, the same procedure does not induce oval cells in mice, and alternative protocols such as a choline-deficient, ethionine-supplemented (CDE) diet and 3,5-diethoxycarbonyl-1,4-dihydro-collidine (DDC) diet have been developed to induce oval cells in mice (*59–61*). Although the proliferating epithelial cells in the periportal region upon injury by various insults are collectively referred to as oval cells, it remains unclear whether or not the oval cells in different species by different protocols have common characteristics. A major problem in characterizing oval cells was the lack of appropriate cell surface markers to identify and isolate the oval cell compartment.

In the rat 2-AAF/PH model, Dabeva *et al.* reported that $EpCAM^+$ oval cells are bipotential adult hepatic progenitors (*62*, *63*). Suzuki *et al.* (*64*) reported that $CD133^+$ cells isolated from DDC-treated mouse liver could form large colonies in culture. These large colony-forming cells gave rise to both hepatocytes and cholangiocytes, while maintaining undifferentiated cells by self-renewing cell divisions. In order to isolate and characterize mouse oval cells, Okabe *et al.* (*65*) searched for cell surface molecules expressed on oval cells in mouse fed DDC diet. EpCAM was expressed in both mouse normal cholangiocytes and oval cells, and its related protein TROP2 was expressed exclusively in oval cells, establishing TROP2 as a novel marker to distinguish oval cells from normal cholangiocytes (*65*). Some of the $EpCAM^+$ cells isolated from injured liver proliferate to form colonies *in vitro*, and the clonally expanded cells differentiate into hepatocytes and cholangiocytes, suggesting that the oval cell fraction contains potential liver stem cells.

Interestingly, such cells with liver stem cell characteristics are also found in $EpCAM^+$ cells of the normal liver. Intriguingly, comparison of the colony formation of $EpCAM^+$ cells between normal and injured livers revealed little difference in the frequency of potential liver stem cell activity between them, strongly suggesting that most of the proliferating mouse oval cells represent transit-amplifying cells rather than stem cells. Bipotential clonal cell lines can be obtained from the healthy liver of adult mice and participate in liver regeneration in severe combined immune-deficient mice expressing urokinase-type plasminogen by the ALB promoter, where they differentiate in clusters of hepatocytes and occasionally bile ducts (*66*). Kamiya *et al.* (*67*) found progenitor cells in the $CD13^+CD49f^+CD133^+$ subpopulation of non-haematopoietic cells derived from postnatal livers. These results demonstrate the existence, in normal adult mouse liver, of a pool of clonogenic cells that are (or can become) bipotential.

As mentioned above, oval cells are induced in liver with severe or chronic damage. Chronic injury conditions in the liver are usually associated with inflammation, and the roles of lymphocytes and inflammatory responses in oval cell regulation have also been suggested (*68*, *69*). In accordance with this notion, several kinds of inflammatory cytokines, such as tumour necrosis factor (TNF)-α, lymphotoxin-β, interferon-γ and IL-6, have been shown to modulate oval cell response (*70*, *71*). Perhaps the best established inflammatory cytokine to be involved in oval cell response is a TNF family member ligand, TNF-like weak inducer of apoptosis (Tweak). Thus, transgenic mice overexpressing this cytokine in the liver exhibit periportal oval cell hyperplasia, while administration of a blocking anti-Tweak monoclonal antibody significantly reduced oval cell response in mice fed DDC diet (*72*). Furthermore, in mice lacking Fn14, the cognate receptor for Tweak, induction of oval cells was attenuated in both DDC diet and CDE diet models (*72*, *73*). These inflammatory cytokines are considered to function as part of the innate immune system sensing damage to the tissue and serve as the earliest signals for triggering the process of liver regeneration (Fig. 4).

Mesenchymal cells such as stellate cells have long been suggested to physically interact with oval cells and thus considered to induce some signals in them (*53*). Recent studies using several rat and mouse models have demonstrated that a population of mesenchymal cells expressing thymus cell antigen-1 (Thy-1; also known as CD90) resides in close proximity to and expands in parallel with oval cells (*74*) (H. Takase, T. Itoh and A. Miyajima, unpublished observation). Furthermore, these $Thy1^+$ cells were found to express FGF7, and its cognate receptor FGFR2b was detected in oval cells. FGF7 knockout mice showed a defect in oval cell response, while overexpression of FGF7 *in vivo* in normal mouse liver led to induction and proliferation of cells with markers of oval cells in the periportal area. Together, these results strongly suggest that FGF7 plays a key role in adult liver stem/progenitor cell response as well as that the $Thy1^+$ cells may serve as the niche for oval cells by providing this cytokine (Takase, H., Itoh, T. and Miyajima A., unpublished observations). As a signal related to oval cell response, several recent studies have implicated the canonical Wnt/β-catenin pathway in oval cell regulation (*75–78*) (Fig. 4). The Wnt/β-catenin pathway is well known to play important roles in stem cell regulation including self-renewal in various other organs and tissues, and also in carcinogenesis including liver tumours. In both rat and mouse models, expression of some Wnt ligands in damaged liver and concomitant activation of the β-catenin pathway in oval cells were observed. In conditional knockout mice lacking β-catenin in both hepatocytes and cholangiocytes, DDC diet-induced oval cell response in the liver was significantly reduced, although not completely abrogated. While several factors have been shown to be involved in oval cell response, the precise modes of their actions and their relationship are currently unclear and should be determined.

Concluding remarks

Traditionally, research on liver biology mostly relied on relatively crude cell separation methods based on cell density and centrifugation. In the last decade, identification of specific cell surface markers for each of the liver cell types, production of corresponding monoclonal antibodies and cell sorting techniques have together revolutionized the field and enabled us to perform much more detailed characterization of liver cells, particularly non-parenchymal cells including the stem/progenitor cells. It has also become possible to analyse the modes of interaction among different types of these cells *in vivo* by means of combinatorial use of specific markers/antibodies as well as *in vitro* with co-culture systems using the isolated viable cell populations. Elucidation of the molecular basis for the signals that regulate development, proliferation and differentiation of liver stem/progenitor cells should not only advance our understanding of the basic pathophysiology of the liver but also help to establish better protocols to generate mature hepatocytes and other liver cells *in vitro* for cell-based therapy, transplantation and drug discovery.

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Conflict of interest

None declared.

References

1. Douarin, N.M. (1975) An experimental analysis of liver development. *Med. Biol.* **53**, 427–455
2. Gualdi, R., Bossard, P., Zheng, M., Hamada, Y., Coleman, J.R., and Zaret, K.S. (1996) Hepatic specification of the gut endoderm *in vitro*: cell signaling and transcriptional control. *Genes Dev.* **10**, 1670–1682
3. Jung, J., Zheng, M., Goldfarb, M., and Zaret, K.S. (1999) Initiation of mammalian liver development from endoderm by fibroblast growth factors. *Science* **284**, 1998–2003
4. Rossi, J.M., Dunn, N.R., Hogan, B.L., and Zaret, K.S. (2001) Distinct mesodermal signals, including BMPs from the septum transversum mesenchyme, are required in combination for hepatogenesis from the endoderm. *Genes Dev.* **15**, 1998–2009
5. Ober, E.A., Verkade, H., Field, H.A., and Stainier, D.Y. (2006) Mesodermal Wnt2b signalling positively regulates liver specification. *Nature* **442**, 688–691
6. Sosa-Pineda, B., Wigle, J.T., and Oliver, G. (2000) Hepatocyte migration during liver development requires Prox1. *Nat. Genet.* **25**, 254–255
7. Zaret, K. (1998) Early liver differentiation: genetic potentiation and multilevel growth control. *Curr. Opin. Genet. Dev.* **8**, 526–531
8. Matsumoto, K., Yoshitomi, H., Rossant, J., and Zaret, K.S. (2001) Liver organogenesis promoted by endothelial cells prior to vascular function. *Science* **294**, 559–563
9. Kubota, H. and Reid, L.M. (2000) Clonogenic hepatoblasts, common precursors for hepatocytic and biliary lineages, are lacking classical major histocompatibility complex class I antigen. *Proc. Natl. Acad. Sci. USA* **97**, 12132–12137
10. Suzuki, A., Zheng, Y., Kondo, R., Kusakabe, M., Takada, Y., Fukao, K., Nakauchi, H., and Taniguchi, H. (2000) Flow-cytometric separation and enrichment of hepatic progenitor cells in the developing mouse liver. *Hepatology* **32**, 1230–1239
11. Minguet, S., Cortegano, I., Gonzalo, P., Martinez-Marin, J.A., de Andres, B., Salas, C., Melero, D., Gaspar, M.L., and Marcos, M.A. (2003) A population of c-Kit(low)(CD45/TER119)- hepatic cell progenitors of 11-day postcoitus mouse embryo liver reconstitutes cell-depleted liver organoids. *J. Clin. Invest.* **112**, 1152–1163
12. Tanimizu, N., Nishikawa, M., Saito, H., Tsujimura, T., and Miyajima, A. (2003) Isolation of hepatoblasts based on the expression of Dlk/Pref-1. *J. Cell. Sci.* **116**, 1775–1786
13. Floridon, C., Jensen, C.H., Thorsen, P., Nielsen, O., Sunde, L., Westergaard, J.G., Thomsen, S.G., and Teisner, B. (2000) Does fetal antigen 1 (FA1) identify cells with regenerative, endocrine and neuroendocrine potentials? A study of FA1 in embryonic, fetal, and placental tissue and in maternal circulation. *Differentiation* **66**, 49–59
14. Yanai, H., Nakamura, K., Hijioka, S., Kamei, A., Ikari, T., Ishikawa, Y., Shinozaki, E., Mizunuma, N., Hatake, K., and Miyajima, A. (2010) Dlk-1, a cell surface antigen on foetal hepatic stem/progenitor cells, is expressed in hepatocellular, colon, pancreas and breast carcinomas at a high frequency. *J. Biochem.* **148**, 85–92
15. Nierhoff, D., Ogawa, A., Oertel, M., Chen, Y.Q., and Shafritz, D.A. (2005) Purification and characterization of mouse fetal liver epithelial cells with high in vivo repopulation capacity. *Hepatology* **42**, 130–139
16. Tanaka, M., Okabe, M., Suzuki, K., Kamiya, Y., Tsukahara, Y., Saito, S., and Miyajima, A. (2009) Mouse hepatoblasts at distinct developmental stages are characterized by expression of EpCAM and DLK1: drastic change of EpCAM expression during liver development. *Mech. Dev.* **126**, 665–676
17. Dan, Y.Y., Riehle, K.J., Lazaro, C., Teoh, N., Haque, J., Campbell, J.S., and Fausto, N. (2006) Isolation of multipotent progenitor cells from human fetal liver capable of differentiating into liver and mesenchymal lineages. *Proc. Natl. Acad. Sci. USA* **103**, 9912–9917
18. Schmelzer, E., Zhang, L., Bruce, A., Wauthier, E., Ludlow, J., Yao, H.L., Moss, N., Melhem, A., McClelland, R., Turner, W., Kulik, M., Sherwood, S., Tallheden, T., Cheng, N., Furth, M.E., and Reid, L.M. (2007) Human hepatic stem cells from fetal and postnatal donors. *J. Exp. Med.* **204**, 1973–1987
19. Kakinuma, S., Ohta, H., Kamiya, A., Yamazaki, Y., Oikawa, T., Okada, K., and Nakauchi, H. (2009) Analyses of cell surface molecules on hepatic stem/progenitor cells in mouse fetal liver. *J. Hepatol.* **51**, 127–138
20. Tanimizu, N., Saito, H., Mostov, K., and Miyajima, A. (2004) Long-term culture of hepatic progenitors derived from mouse Dlk+ hepatoblasts. *J. Cell. Sci.* **117**, 6425–6434
21. Strick-Marchand, H., Morosan, S., Charneau, P., Kremsdorf, D., and Weiss, M.C. (2004) Bipotential mouse embryonic liver stem cell lines contribute to liver regeneration and differentiate as bile ducts and hepatocytes. *Proc. Natl. Acad. Sci. USA* **101**, 8360–8365
22. Antoniou, A., Raynaud, P., Cordi, S., Zong, Y., Tronche, F., Stanger, B.Z., Jacquemin, P., Pierreux, C.E., Clotman, F., and Lemaigre, F.P. (2009) Intrahepatic bile

ducts develop according to a new mode of tubulogenesis regulated by the transcription factor SOX9. *Gastroenterology* 136, 2325–2333
23. Clotman, F., Jacquemin, P., Plumb-Rudewiez, N., Pierreux, C.E., Van der Smissen, P., Dietz, H.C., Courtoy, P.J., Rousseau, G.G., and Lemaigre, F.P. (2005) Control of liver cell fate decision by a gradient of TGF beta signaling modulated by Onecut transcription factors. *Genes Dev.* 19, 1849–1854
24. Suzuki, K., Tanaka, M., Watanabe, N., Saito, S., Nonaka, H., and Miyajima, A. (2008) p75 Neurotrophin receptor is a marker for precursors of stellate cells and portal fibroblasts in mouse fetal liver. *Gastroenterology* 135, 270–281 e273
25. Tanimizu, N. and Miyajima, A. (2004) Notch signaling controls hepatoblast differentiation by altering the expression of liver-enriched transcription factors. *J. Cell. Sci.* 117, 3165–3174
26. Zong, Y., Panikkar, A., Xu, J., Antoniou, A., Raynaud, P., Lemaigre, F., and Stanger, B.Z. (2009) Notch signaling controls liver development by regulating biliary differentiation. *Development* 136, 1727–1739
27. Geisler, F., Nagl, F., Mazur, P.K., Lee, M., Zimber-Strobl, U., Strobl, L.J., Radtke, F., Schmid, R.M., and Siveke, J.T. (2008) Liver-specific inactivation of Notch2, but not Notch1, compromises intrahepatic bile duct development in mice. *Hepatology* 48, 607–616
28. Lozier, J., McCright, B., and Gridley, T. (2008) Notch signaling regulates bile duct morphogenesis in mice. *PLoS One* 3, e1851
29. Sparks, E.E., Huppert, K.A., Brown, M.A., Washington, M.K., and Huppert, S.S. (2010) Notch signaling regulates formation of the three-dimensional architecture of intrahepatic bile ducts in mice. *Hepatology* 51, 1391–1400
30. Tchorz, J.S., Kinter, J., Muller, M., Tornillo, L., Heim, M.H., and Bettler, B. (2009) Notch2 signaling promotes biliary epithelial cell fate specification and tubulogenesis during bile duct development in mice. *Hepatology* 50, 871–879
31. Clotman, F., Lannoy, V.J., Reber, M., Cereghini, S., Cassiman, D., Jacquemin, P., Roskams, T., Rousseau, G.G., and Lemaigre, F.P. (2002) The onecut transcription factor HNF6 is required for normal development of the biliary tract. *Development* 129, 1819–1828
32. Coffinier, C., Gresh, L., Fiette, L., Tronche, F., Schutz, G., Babinet, C., Pontoglio, M., Yaniv, M., and Barra, J. (2002) Bile system morphogenesis defects and liver dysfunction upon targeted deletion of HNF1beta. *Development* 129, 1829–1838
33. Hunter, M.P., Wilson, C.M., Jiang, X., Cong, R., Vasavada, H., Kaestner, K.H., and Bogue, C.W. (2007) The homeobox gene Hhex is essential for proper hepatoblast differentiation and bile duct morphogenesis. *Dev. Biol.* 308, 355–367
34. Kodama, Y., Hijikata, M., Kageyama, R., Shimotohno, K., and Chiba, T. (2004) The role of notch signaling in the development of intrahepatic bile ducts. *Gastroenterology* 127, 1775–1786
35. Krupczak-Hollis, K., Wang, X., Kalinichenko, V.V., Gusarova, G.A., Wang, I.C., Dennewitz, M.B., Yoder, H.M., Kiyokawa, H., Kaestner, K.H., and Costa, R.H. (2004) The mouse Forkhead Box m1 transcription factor is essential for hepatoblast mitosis and development of intrahepatic bile ducts and vessels during liver morphogenesis. *Dev. Biol.* 276, 74–88
36. Li, Z., White, P., Tuteja, G., Rubins, N., Sackett, S., and Kaestner, K.H. (2009) Foxa1 and Foxa2 regulate bile duct development in mice. *J. Clin. Invest.* 119, 1537–1545
37. Oikawa, T., Kamiya, A., Kakinuma, S., Zeniya, M., Nishinakamura, R., Tajiri, H., and Nakauchi, H. (2009) Sall4 regulates cell fate decision in fetal hepatic stem/progenitor cells. *Gastroenterology* 136, 1000–1011
38. Suzuki, A., Sekiya, S., Buscher, D., Izpisua Belmonte, J.C., and Taniguchi, H. (2008) Tbx3 controls the fate of hepatic progenitor cells in liver development by suppressing p19ARF expression. *Development* 135, 1589–1595
39. Raynaud, P., Carpentier, R., Antoniou, A., and Lemaigre, F.P. (2011) Biliary differentiation and bile duct morphogenesis in development and disease. *Int. J. Biochem. Cell Biol.* 43, 245–256
40. Tanimizu, N., Miyajima, A., and Mostov, K.E. (2007) Liver progenitor cells develop cholangiocyte-type epithelial polarity in three-dimensional culture. *Mol. Biol. Cell* 18, 1472–1479
41. Tanimizu, N., Miyajima, A., and Mostov, K.E. (2009) Liver progenitor cells fold up a cell monolayer into a double-layered structure during tubular morphogenesis. *Mol. Biol. Cell* 20, 2486–2494
42. Asahina, K., Tsai, S.Y., Li, P., Ishii, M., Maxson, R.E. Jr, Sucov, H.M., and Tsukamoto, H. (2009) Mesenchymal origin of hepatic stellate cells, submesothelial cells, and perivascular mesenchymal cells during mouse liver development. *Hepatology* 49, 998–1011
43. Onitsuka, I., Tanaka, M., and Miyajima, A. (2010) Characterization and functional analyses of hepatic mesothelial cells in mouse liver development. *Gastroenterology* 138, 1525–1535, 1535 e1521–1526
44. Ijpenberg, A., Perez-Pomares, J.M., Guadix, J.A., Carmona, R., Portillo-Sanchez, V., Macias, D., Hohenstein, P., Miles, C.M., Hastie, N.D., and Munoz-Chapuli, R. (2007) Wt1 and retinoic acid signaling are essential for stellate cell development and liver morphogenesis. *Dev. Biol.* 312, 157–170
45. Kamiya, A., Kinoshita, T., Ito, Y., Matsui, T., Morikawa, Y., Senba, E., Nakashima, K., Taga, T., Yoshida, K., Kishimoto, T., and Miyajima, A. (1999) Fetal liver development requires a paracrine action of oncostatin M through the gp130 signal transducer. *EMBO J.* 18, 2127–2136
46. Kinoshita, T., Sekiguchi, T., Xu, M.J., Ito, Y., Kamiya, A., Tsuji, K., Nakahata, T., and Miyajima, A. (1999) Hepatic differentiation induced by oncostatin M attenuates fetal liver hematopoiesis. *Proc. Natl. Acad. Sci. USA* 96, 7265–7270
47. Michalopoulos, G.K. (2010) Liver regeneration after partial hepatectomy: critical analysis of mechanistic dilemmas. *Am. J. Pathol.* 176, 2–13
48. Overturf, K., al-Dhalimy, M., Ou, C.N., Finegold, M., and Grompe, M. (1997) Serial transplantation reveals the stem-cell-like regenerative potential of adult mouse hepatocytes. *Am. J. Pathol.* 151, 1273–1280
49. Alison, M.R., Golding, M.H., and Sarraf, C.E. (1996) Pluripotential liver stem cells: facultative stem cells located in the biliary tree. *Cell Prolif.* 29, 373–402
50. Roskams, T.A., Theise, N.D., Balabaud, C., Bhagat, G., Bhathal, P.S., Bioulac-Sage, P., Brunt, E.M., Crawford, J.M., Crosby, H.A., Desmet, V., Finegold, M.J., Geller, S.A., Gouw, A.S., Hytiroglou, P., Knisely, A.S., Kojiro, M., Lefkowitch, J.H., Nakanuma, Y., Olynyk, J.K., Park, Y.N., Portmann, B., Saxena, R., Scheuer, P.J., Strain, A.J., Thung, S.N., Wanless, I.R., and West, A.B. (2004) Nomenclature of the finer branches of the biliary tree: canals, ductules, and ductular reactions in human livers. *Hepatology* 39, 1739–1745
51. Theise, N.D., Saxena, R., Portmann, B.C., Thung, S.N., Yee, H., Chiriboga, L., Kumar, A., and Crawford, J.M.

(1999) The canals of Hering and hepatic stem cells in humans. *Hepatology* **30**, 1425–1433
52. Farber, E. (1956) Similarities in the sequence of early histological changes induced in the liver of the rat by ethionine, 2-acetylamino-fluorene, and 3′-methyl-4-dimethylaminoazobenzene. *Cancer Res.* **16**, 142–148
53. Paku, S., Schnur, J., Nagy, P., and Thorgeirsson, S.S. (2001) Origin and structural evolution of the early proliferating oval cells in rat liver. *Am. J. Pathol.* **158**, 1313–1323
54. Petersen, B.E., Bowen, W.C., Patrene, K.D., Mars, W.M., Sullivan, A.K., Murase, N., Boggs, S.S., Greenberger, J.S., and Goff, J.P. (1999) Bone marrow as a potential source of hepatic oval cells. *Science* **284**, 1168–1170
55. Fausto, N. (2004) Liver regeneration and repair: hepatocytes, progenitor cells, and stem cells. *Hepatology* **39**, 1477–1487
56. Lee, J.S., Heo, J., Libbrecht, L., Chu, I.S., Kaposi-Novak, P., Calvisi, D.F., Mikaelyan, A., Roberts, L.R., Demetris, A.J., Sun, Z., Nevens, F., Roskams, T., and Thorgeirsson, S.S. (2006) A novel prognostic subtype of human hepatocellular carcinoma derived from hepatic progenitor cells. *Nat. Med.* **12**, 410–416
57. Evarts, R.P., Nagy, P., Marsden, E., and Thorgeirsson, S.S. (1987) A precursor-product relationship exists between oval cells and hepatocytes in rat liver. *Carcinogenesis* **8**, 1737–1740
58. Laishes, B.A. and Rolfe, P.B. (1981) Search for endogenous liver colony-forming units in F344 rats given a two-thirds hepatectomy during short-term feeding of 2-acetylaminofluorene. *Cancer Res.* **41**, 1731–1741
59. Akhurst, B., Croager, E.J., Farley-Roche, C.A., Ong, J.K., Dumble, M.L., Knight, B., and Yeoh, G.C. (2001) A modified choline-deficient, ethionine-supplemented diet protocol effectively induces oval cells in mouse liver. *Hepatology* **34**, 519–522
60. Preisegger, K.H., Factor, V.M., Fuchsbichler, A., Stumptner, C., Denk, H., and Thorgeirsson, S.S. (1999) Atypical ductular proliferation and its inhibition by transforming growth factor beta1 in the 3,5-diethoxycarbonyl-1,4-dihydrocollidine mouse model for chronic alcoholic liver disease. *Lab. Invest.* **79**, 103–109
61. Wang, X., Foster, M., Al-Dhalimy, M., Lagasse, E., Finegold, M., and Grompe, M. (2003) The origin and liver repopulating capacity of murine oval cells. *Proc. Natl. Acad. Sci. USA* **100 (Suppl. 1)**, 11881–11888
62. Yovchev, M.I., Grozdanov, P.N., Joseph, B., Gupta, S., and Dabeva, M.D. (2007) Novel hepatic progenitor cell surface markers in the adult rat liver. *Hepatology* **45**, 139–149
63. Yovchev, M.I., Grozdanov, P.N., Zhou, H., Racherla, H., Guha, C., and Dabeva, M.D. (2008) Identification of adult hepatic progenitor cells capable of repopulating injured rat liver. *Hepatology* **47**, 636–647
64. Suzuki, A., Sekiya, S., Onishi, M., Oshima, N., Kiyonari, H., Nakauchi, H., and Taniguchi, H. (2008) Flow cytometric isolation and clonal identification of self-renewing bipotent hepatic progenitor cells in adult mouse liver. *Hepatology* **48**, 1964–1978
65. Okabe, M., Tsukahara, Y., Tanaka, M., Suzuki, K., Saito, S., Kamiya, Y., Tsujimura, T., Nakamura, K., and Miyajima, A. (2009) Potential hepatic stem cells reside in EpCAM+ cells of normal and injured mouse liver. *Development* **136**, 1951–1960
66. Fougere-Deschatrette, C., Imaizumi-Scherrer, T., Strick-Marchand, H., Morosan, S., Charneau, P., Kremsdorf, D., Faust, D.M., and Weiss, M.C. (2006) Plasticity of hepatic cell differentiation: bipotential adult mouse liver clonal cell lines competent to differentiate *in vitro* and *in vivo*. *Stem Cells* **24**, 2098–2109
67. Kamiya, A., Kakinuma, S., Yamazaki, Y., and Nakauchi, H. (2009) Enrichment and clonal culture of progenitor cells during mouse postnatal liver development in mice. *Gastroenterology* **137**, 1114–1126, 1126 e1111–1114
68. Knight, B., Akhurst, B., Matthews, V.B., Ruddell, R.G., Ramm, G.A., Abraham, L.J., Olynyk, J.K., and Yeoh, G.C. (2007) Attenuated liver progenitor (oval) cell and fibrogenic responses to the choline deficient, ethionine supplemented diet in the BALB/c inbred strain of mice. *J. Hepatol.* **46**, 134–141
69. Strick-Marchand, H., Masse, G.X., Weiss, M.C., and Di Santo, J.P. (2008) Lymphocytes support oval cell-dependent liver regeneration. *J. Immunol.* **181**, 2764–2771
70. Akhurst, B., Matthews, V., Husk, K., Smyth, M.J., Abraham, L.J., and Yeoh, G.C. (2005) Differential lymphotoxin-beta and interferon gamma signaling during mouse liver regeneration induced by chronic and acute injury. *Hepatology* **41**, 327–335
71. Knight, B., Yeoh, G.C., Husk, K.L., Ly, T., Abraham, L.J., Yu, C., Rhim, J.A., and Fausto, N. (2000) Impaired preneoplastic changes and liver tumor formation in tumor necrosis factor receptor type 1 knockout mice. *J. Exp. Med.* **192**, 1809–1818
72. Jakubowski, A., Ambrose, C., Parr, M., Lincecum, J.M., Wang, M.Z., Zheng, T.S., Browning, B., Michaelson, J.S., Baetscher, M., Wang, B., Bissell, D.M., and Burkly, L.C. (2005) TWEAK induces liver progenitor cell proliferation. *J. Clin. Invest.* **115**, 2330–2340
73. Tirnitz-Parker, J.E., Viebahn, C.S., Jakubowski, A., Klopcic, B.R., Olynyk, J.K., Yeoh, G.C., and Knight, B. (2010) Tumor necrosis factor-like weak inducer of apoptosis is a mitogen for liver progenitor cells. *Hepatology* **52**, 291–302
74. Yovchev, M.I., Zhang, J., Neufeld, D.S., Grozdanov, P.N., and Dabeva, M.D. (2009) Thymus cell antigen-1-expressing cells in the oval cell compartment. *Hepatology* **50**, 601–611
75. Apte, U., Thompson, M.D., Cui, S., Liu, B., Cieply, B., and Monga, S.P. (2008) Wnt/beta-catenin signaling mediates oval cell response in rodents. *Hepatology* **47**, 288–295
76. Hu, M., Kurobe, M., Jeong, Y.J., Fuerer, C., Ghole, S., Nusse, R., and Sylvester, K.G. (2007) Wnt/beta-catenin signaling in murine hepatic transit amplifying progenitor cells. *Gastroenterology* **133**, 1579–1591
77. Itoh, T., Kamiya, Y., Okabe, M., Tanaka, M., and Miyajima, A. (2009) Inducible expression of Wnt genes during adult hepatic stem/progenitor cell response. *FEBS Lett.* **583**, 777–781
78. Yang, W., Yan, H.X., Chen, L., Liu, Q., He, Y.Q., Yu, L.X., Zhang, S.H., Huang, D.D., Tang, L., Kong, X.N., Chen, C., Liu, S.Q., Wu, M.C., and Wang, H.Y. (2008) Wnt/beta-catenin signaling contributes to activation of normal and tumorigenic liver progenitor cells. *Cancer Res.* **68**, 4287–4295

Antiobesity Effect of Eicosapentaenoic Acid in High-Fat/High-Sucrose Diet–Induced Obesity

Importance of Hepatic Lipogenesis

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OBJECTIVE—Given the pleiotropic effect of eicosapentaenoic acid (EPA), it is interesting to know whether EPA is capable of improving obesity. Here we examined the anti-obesity effect of EPA in mice with two distinct models of obesity.

RESEARCH DESIGN AND METHODS—Male C57BL/6J mice were fed a high-fat/high-sucrose diet (25.0% [w/w] fat, 32.5% [w/w] sucrose) (HF/HS group) or a high-fat diet (38.1% [w/w] fat, 8.5% [w/w] sucrose) (HF group) for 4–20 weeks. A total of 5% EPA was administered by partially substituting EPA for fat in the HF/HS + EPA and HF + EPA groups.

RESULTS—Both the HF/HS and HF groups similarly developed obesity. EPA treatment strongly suppresses body weight gain and obesity-related hyperglycemia and hyperinsulinemia in HF/HS-fed mice (HF/HS + EPA group), where hepatic triglyceride content and lipogenic enzymes are increased. There is no appreciable effect of EPA on body weight in HF-fed mice (HF + EPA group) without enhanced expression of hepatic lipogenic enzymes. Moreover, EPA is capable of reducing hepatic triglyceride secretion and changing VLDL fatty acid composition in the HF/HS group. By indirect calorimetry analysis, we also found that EPA is capable of increasing energy consumption in the HF/HS + EPA group.

CONCLUSIONS—This study is the first demonstration that the anti-obesity effect of EPA in HF/HS-induced obesity is associated with the suppression of hepatic lipogenesis and steatosis. Because the metabolic syndrome is often associated with hepatic lipogenesis and steatosis, the data suggest that EPA is suited for treatment of the metabolic syndrome. ***Diabetes* 59:2495–2504, 2010**

The metabolic syndrome has been defined as a cluster of visceral fat obesity, impaired glucose metabolism, atherogenic dyslipidemia (high plasma triglyceride and low HDL cholesterol), and hypertension (1). There is considerable evidence that visceral fat obesity is a key etiological factor in the metabolic syndrome (2). Enhanced hepatic lipogenesis and hepatic steatosis also appear to play an important role in the pathogenesis of the metabolic syndrome (3). Indeed, nonalcoholic fatty liver disease may constitute the common features of the metabolic syndrome.

Numerous epidemiological studies and clinical trials have revealed that fish oil and n-3 polyunsaturated fatty acids (PUFAs) reduce the risk of coronary heart disease (4). Eicosapentaenoic acid (EPA), one of the major n-3 PUFAs contained in fish oil, has a variety of pharmacological effects such as lipid-lowering (5), anti-platelet (6), anti-inflammatory (7), and anti-atherogenic effects (8,9). Recently, the Japan EPA Lipid Intervention Study (JELIS), a large-scale prospective randomized clinical trial, demonstrated that EPA delays the onset of cardiovascular events via cholesterol-independent mechanisms (10,11), but the molecular mechanisms remain to be elucidated. In a recent sub-analysis of the JELIS, EPA had a great risk reduction of coronary artery events of 53% in patients with high triglycerides and low HDL cholesterol (11), suggesting that EPA may be effective to reduce the incidence of atherosclerosis in the metabolic syndrome. These findings are supported by our recent observations that EPA administration results in decreases in remnant-like particle-triglyceride, small dense LDL, and C-reactive protein and an increase in adiponectin in patients with the metabolic syndrome (12,13).

Given the pleiotropic effect of EPA, it is interesting to know whether highly purified EPA is capable of improving obesity. There is currently a controversy as to the anti-obesity effect of EPA; it has been effective (13,14), has been ineffective (15), or has even increased visceral fat accumulation (16). On the other hand, it is noteworthy that EPA suppresses hepatic lipogenesis and steatosis by reducing mRNA and active protein of sterol regulatory element binding protein-1c (SREBP-1c) (17–19). We, therefore, examined the impact of hepatic lipogenesis on the anti-obesity effect of highly purified EPA.

Here, we demonstrate that EPA strongly suppresses body weight gain and obesity-related hyperglycemia and hyperinsulinemia in high-fat (HF)/high-sucrose (HS)-induced obese mice with enhanced hepatic lipogenesis but not in HF-induced obese mice without enhanced hepatic lipogenesis. This study is the first demonstration that the anti-obesity effect of EPA is related to the suppression of hepatic lipogenesis. Given that the metabolic syndrome is often associated with hepatic lipogenesis and steatosis, the data of this study suggest that EPA is suited for the treatment of the metabolic syndrome.

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RESEARCH DESIGN AND METHODS

Highly purified EPA ethyl ester (purity >98%) was obtained from Nippon Suisan Kaisha (Tokyo, Japan). Ethyl palmitate (purity >95%) was purchased from Wako (Tokyo). Nine-week-old male C57BL/6J mice were obtained from CLEA Japan (Tokyo) and acclimated for 1 week before the experiment. Mice were housed under controlled temperature and lighting (0730–1930 light, 1930–0730 dark cycle) with free access to water and a fish meal-free diet (fish meal-free F1: 4.4% fat; Funabashi Farm, Funabashi, Japan). All experiments were carried out in accordance with the guidelines for the use and care of laboratory animals of Mochida Pharmaceutical (numbers 1523, 1524, 2354, and 2389).

Diets. The outline of experiments and composition of diets are in supplementary Tables 1 and 2, respectively (available in an online appendix at http://diabetes.diabetesjournals.org/cgi/content/full/db09-1554/DC1).

Plasma analysis. Blood samples were collected via the retro-orbital sinus of nonfasted mice under light anesthesia every 2 weeks. Plasma glucose, total cholesterol, triglycerides, free fatty acid, and insulin were determined by commercially available kits. Plasma concentrations of leptin and adiponectin were measured with the respective enzyme-linked immunosorbent assay kits (leptin: Morinaga, Yokohama, Japan; adiponectin: R&D Systems, Minneapolis, MN).

Hepatic triglyceride content. Liver lipids were extracted by the method of Folch et al. (20). Hepatic triglyceride content was measured with a reagent from Wako.

Gene expression analysis. Expression levels of some genes were determined by quantitative real-time PCR using primers and probes shown in supplementary Table 3. Expression of other genes was determined using TaqMan gene expression assays (Applied Biosystems, Foster City, CA). The assay IDs of TaqMan (R) gene expression assays are shown in supplementary Table 4. 18s rRNA was measured using TaqMan (R) rRNA control reagents (Applied Biosystems) as a control.

Enzymatic activities. Acyl-CoA oxidase, fatty acid synthase (FAS), and hydroxyacyl-CoA dehydrogenase activities were measured spectrophotometrically (21–23).

Cytoplasmic and nuclear protein extracts and Western blot analysis. Cytoplasmic and nuclear proteins were extracted as described (24,25) with slight modifications. The samples were equally pooled from all the mice of each group (n = 7–10), and then 30 μg protein per lane was separated by SDS-PAGE. Western blot analysis was performed using anti–SREBP-1 antibody (H160; Santa Cruz Biotechnology, Santa Cruz, CA). We used anti–β-actin (Cell Signaling Technology, Beverly, MA) and anti-TATA binding protein (TBP) (Abcam, Cambridge, U.K.) antibodies as cytoplasmic and nuclear controls, respectively. The blots were visualized with the ECL Western blotting analysis system (GE Healthcare, Buckinghamshire, U.K.).

Indirect calorimetry analysis. The volumes of consumed O_2 (VO_2) and produced CO_2 (VCO_2) were measured by indirect calorimetry using an Oxymax system (Columbus Instruments, Columbus, OH). The O_2 and CO_2 contents were recorded every 10 min from 1130 to 0900. Mice had unrestricted access to food and water. The respiratory exchange ratio (RER) was calculated by VCO_2/VO_2.

Glycerol release from white adipose tissue. Glycerol release from the epididymal fat ex vivo was measured according to the method of Schweiger et al. (26) with slight modifications.

Hepatic triglyceride secretion rate. A dose of 500 mg/kg tyloxapol (also known as Triton WR-1339; Sigma-Aldrich, St. Louis, MO) in saline was injected intravenously. Mice were fasted overnight before the injection. Immediately before and 1 h after the injection, blood samples were collected and plasma triglyceride concentrations were determined as described above. The hepatic triglyceride secretion rate was calculated as described by Steiner et al. (27).

Fatty acid composition of VLDL. Blood samples were collected via the inferior vena cava of mice 4 h after the tyloxapol injection. Plasma was isolated, and VLDL fraction was obtained by ultracentrifugation, according to the method of Werner et al. (28). Fatty acid composition was analyzed by gas chromatography.

Statistical analysis. Data are presented as means ± SE. ANOVA using a split-plot model was used for the line plots of indirect calorimetry analysis (Fig. 6*C* and *E*). Other data were assessed by a t test. $P < 0.05$ was considered a significant difference.

RESULTS

Effect of EPA on HF/HS- and HF-induced obesity. Both HF/HS and HF groups gained weight with increases in the mesenteric, epididymal, retroperitoneal, and subcutaneous white adipose tissue (WAT) weights relative to the control group after a 20-week feeding (experiments 1 and 2) (Fig. 1*A–D*). There were no appreciable differences between HF/HS and HF groups. The HF/HS-induced increases in body weight and WAT weights were markedly suppressed by treatment with EPA (HF/HS + EPA group). By contrast, EPA treatment did not affect an HF-induced increase in body weight and WAT weights (HF + EPA group). In the HF/HS group, obesity-induced WAT inflammation was evident, as revealed by elevated expression of mRNAs for F4/80, monocyte chemoattractant protein-1 (MCP-1), and tumor necrosis factor-α. EPA treatment markedly reduced F4/80 and MCP-1 mRNA expression in WAT (Fig. 1*E*). In WAT from the HF group, expression of F4/80, MCP-1, and tumor necrosis factor-α mRNAs was elevated, which was not affected by EPA (Fig. 1*F*). In this study, caloric intake in the HF/HS group was roughly equivalent to that in the HF/HS + EPA group (Table 1).

Effect of EPA on HF/HS- and HF-induced metabolic abnormalities. In experiment 1, the HF/HS group developed obvious hyperinsulinemia, hyperglycemia, and hepatic steatosis, which were significantly improved by EPA (HF/HS + EPA group) (Fig. 2*A*, *C*, and *E*). The liver weights after a 20-week feeding were 1.33 ± 0.03, 3.10 ± 0.30, and 1.42 ± 0.06 g in the control, HF/HS, and HF/HS + EPA groups, respectively (n = 10 for the control and HF/HS groups, n = 7 for the HF/HS + EPA group; $P < 0.01$ for the control group versus HF/HS group and the HF/HS group versus HF/HS + EPA group). Similarly, the HF group developed hyperglycemia and hyperinsulinemia; however, EPA had a marginal impact on HF-induced hyperglycemia and hyperinsulinemia (Fig. 2*B* and *D*). Although EPA treatment ameliorated hepatic steatosis in the HF group (Fig. 2*F*), liver weight was not decreased by EPA (data not shown). Plasma leptin concentrations were markedly elevated in the HF/HS group relative to the control group and were significantly reduced by EPA ($P < 0.01$) (Table 1). Although HF/HS did not affect plasma adiponectin concentration, EPA treatment resulted in an ~1.5-fold elevation of plasma adiponectin (Table 1), which is consistent with our previous report (13).

Effect of EPA on gene expression in the HF/HS group. Enhanced expression of genes related to β-oxidation (29), uncoupling proteins (UCPs) (30), and glucose oxidation (31) are known to suppress obesity. However, they were not reduced or rather elevated in the liver and WAT from the HF/HS group relative to the control group (Fig. 3*A* and *E*). Moreover, in the skeletal muscle, except UCP-3, gene expression was not increased by EPA treatment (HF/HS + EPA group) relative to the HF/HS group (Fig. 3*C*). These observations suggest that enhancement of β-oxidation, UCPs, and glucose oxidation does not play a major role in the anti-obesity effect of EPA.

Enhanced lipolysis may promote the degradation of triglycerides accumulated in WAT, thereby leading to the suppression of obesity. Expression of mRNAs for hormone-sensitive lipase and adipose triglyceride lipase in WAT from the HF/HS group did not differ significantly from that in the control group. On the other hand, expression of mRNAs for the β3-adrenergic receptor (β3-AR), triglyceride hydrolase (TGH)-1, and TGH-2 in the HF/HS group was significantly lower than in the control group ($P < 0.01$), which was reversed in the HF/HS + EPA group (Fig. 3*E*).

It is also possible that enhanced lipogenesis promotes energy accumulation through triglyceride synthesis from diet-derived carbohydrates and fatty acids, thereby stimulating obesity and WAT accumulation. In WAT, there was

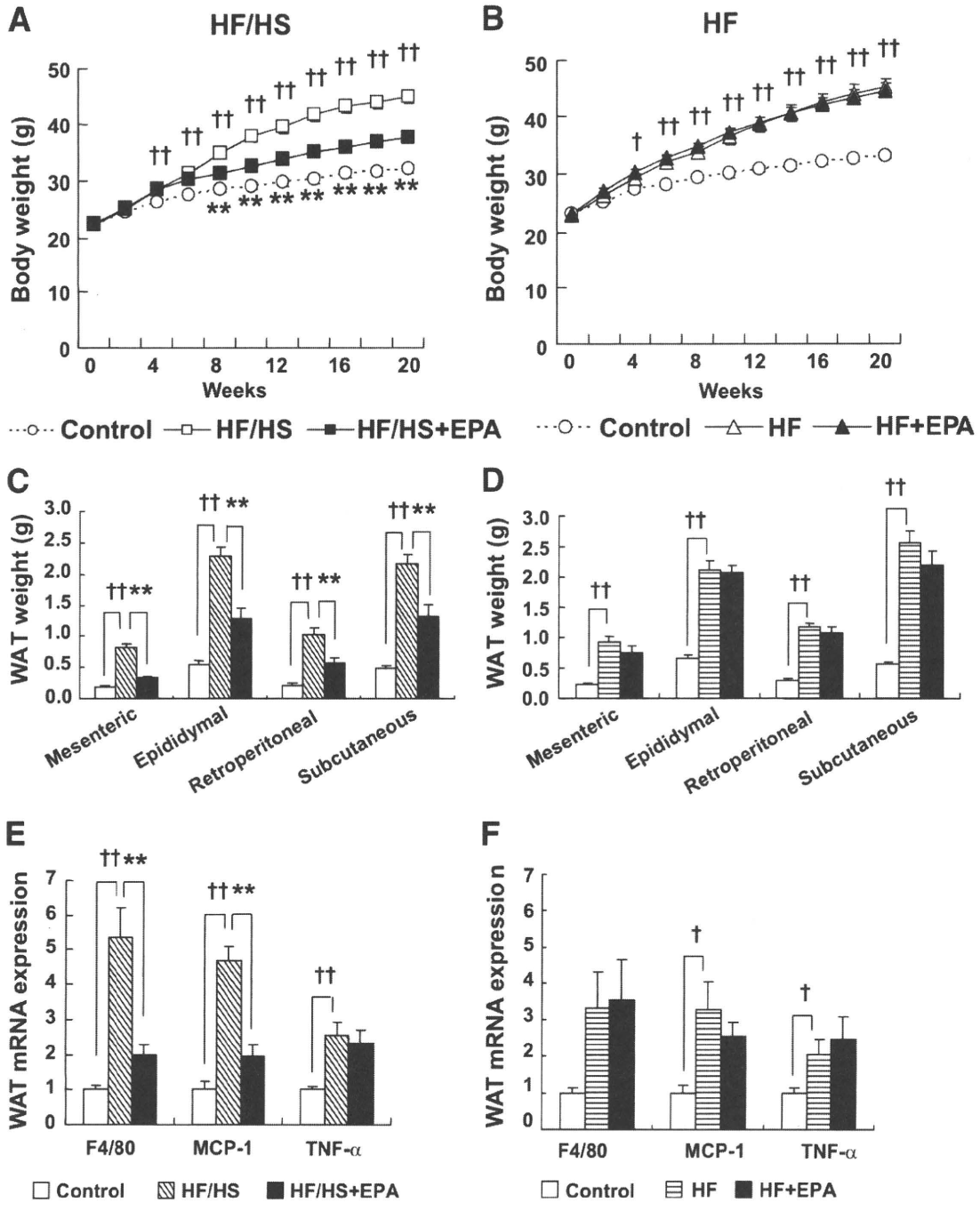

FIG. 1. Effect of EPA on HF/HS- and HF-induced obesity. *A* and *B*: Body weight change. *C* and *D*: WAT weights. *E* and *F*: Proinflammatory gene expression in the epididymal WAT. n = 7–10. †$P < 0.05$; ††$P < 0.01$ vs. control group. **$P < 0.01$ vs. HF (/HS) group.

no appreciable difference in acetyl-CoA carboxylase (ACC)-1 and FAS mRNA expression between the HF/HS and control groups. Expression of ACC-1 and FAS mRNAs was not increased in the HF/HS + EPA group relative to the HF/HS group. Stearoyl-CoA desaturase (SCD)-1 mRNA expression in the HF/HS group was elevated relative to the control group, which was unaffected by EPA treatment (Fig. 3*E*). In the liver, ACC-1, FAS, and SCD-1 mRNA expression in the HF/HS group was elevated relative to the control group, which was reduced by EPA treatment (HF/HS + EPA group) (Fig. 3*A*). In this study, hepatic SREBP-1 protein, a master regulator of lipogenic enzymes, was also increased in the HF/HS group, which was reduced by EPA treatment (Fig. 3*F*).

Effect of EPA on enzymatic activity in the HF/HS group. In this study, elevated enzymatic activities of hepatic FAS and acyl-CoA oxidase in the HF/HS group were reduced by EPA treatment (Fig. 3*B*). Elevated activities of hydroxyacyl-CoA dehydrogenase in the skeletal muscle from the HF/HS group was also reduced by EPA treatment (Fig. 3*D*).

Effect of EPA on gene expression and enzymatic activity in the HF group. We next examined mRNA levels and activities of lipogenic enzymes in the HF group, where EPA did not improve obesity and WAT accumulation (Fig. 1*B* and *D*). Notably, expression of mRNA for lipogenic enzymes and FAS activity in the liver were not elevated in the HF group relative to the control group (Fig.

TABLE 1
Food intake and plasma parameters in experiment 1 and 2 at 18 weeks of feeding

	Experiment 1			Experiment 2		
	Control	HF/HS	HF/HS + EPA	Control	HF	HF + EPA
Food intake (kcal/day/mouse)	8.56	11.15	10.58	9.84	12.72	13.96
Leptin (ng/ml)	4.60 ± 0.82	44.60 ± 5.43††	15.46 ± 2.74**	6.46 ± 0.67	47.02 ± 5.39††	36.24 ± 5.55
Adiponectin (μg/ml)	10.05 ± 0.59	10.78 ± 0.51	15.00 ± 1.45*	10.85 ± 0.92	14.51 ± 0.76††	15.33 ± 1.03
Total cholesterol (mg/dl)	96.8 ± 5.8	236.4 ± 9.6††	104.6 ± 5.5**	101.6 ± 3.7	193.6 ± 7.3††	109.2 ± 7.2**
Triglyceride (mg/dl)	157.2 ± 9.2	105.6 ± 8.1††	80.6 ± 3.7*	143.6 ± 11.6	113.2 ± 12.4	88.0 ± 4.6
Free fatty acid (mEq/l)	0.695 ± 0.060	0.771 ± 0.053	0.593 ± 0.038*	0.670 ± 0.022	0.681 ± 0.059	0.663 ± 0.025

Data are means ± SE (n = 7–10), except for food intake. The results of the food intake are presented as means of two cages. ††$P < 0.01$ vs. the control group; *$P < 0.05$; **$P < 0.01$ vs. the HF (/HS) group.

4*A* and *B*). Moreover, the hepatic nuclear SREBP-1 protein was reduced by a HF diet (Fig. 4*E*), suggesting that HF diet induces obesity without enhancement of hepatic lipogenesis. Although EPA reduced FAS activity, mRNA of lipogenic enzymes was not affected by EPA (Fig. 4*A* and *B*). Treatment with EPA did not affect the activities of β-oxi-

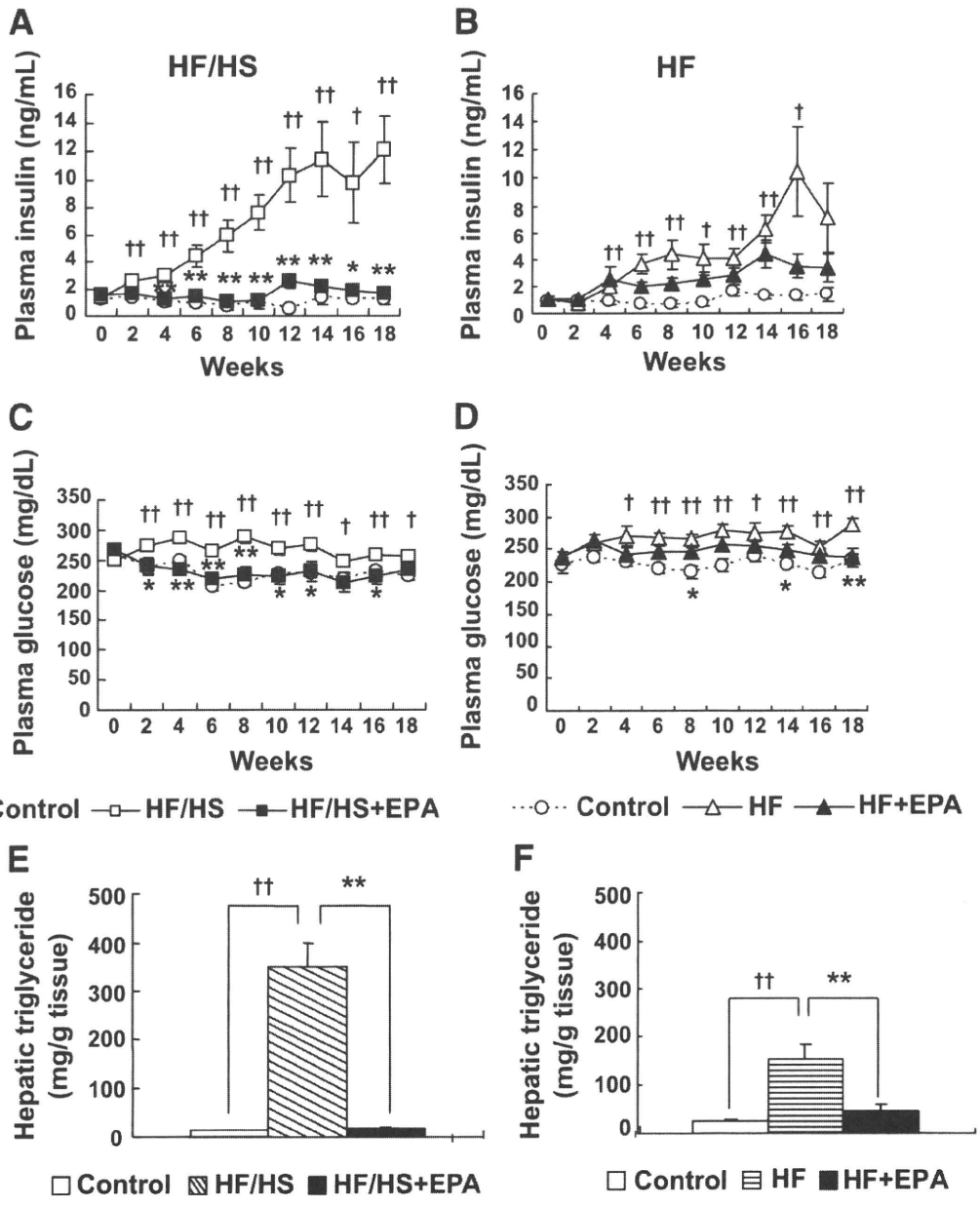

FIG. 2. Effect of EPA on HF/HS- and HF-induced metabolic abnormalities. *A* and *B*: Plasma insulin. *C* and *D*: Plasma glucose. *E* and *F*: Hepatic triglyceride content. †$P < 0.05$; ††$P < 0.01$ vs. control group. *$P < 0.05$; **$P < 0.01$ vs. HF (/HS) group.

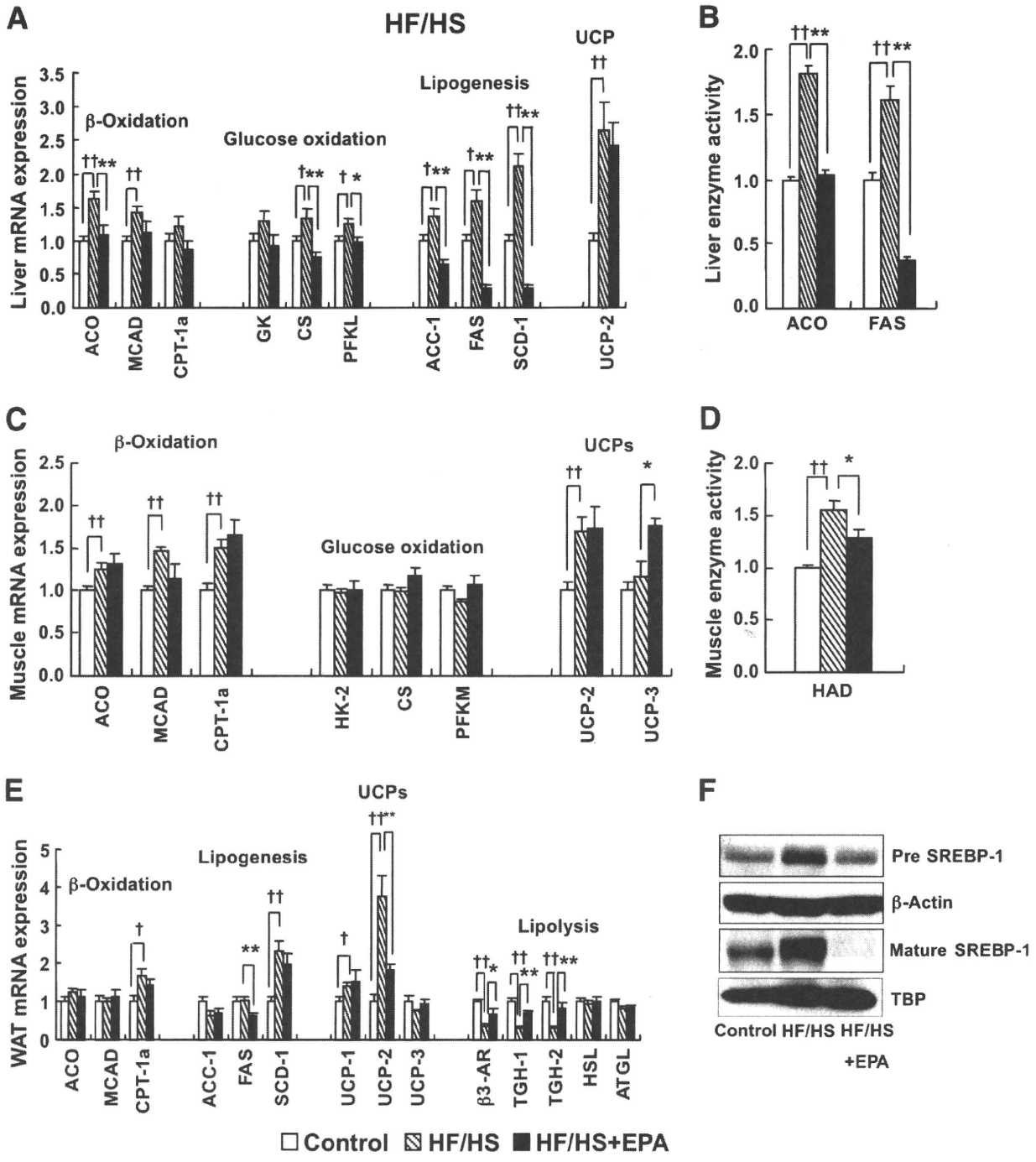

FIG. 3. Effect of EPA on energy metabolism–related genes in the HF/HS groups. *A* and *B*: Liver. *C* and *D*: Skeletal muscle. *E*: Epididymal WAT. *F*: Hepatic SREBP-1 protein in the HF/HS group. n = 7–10. †P < 0.05; ††P < 0.01 vs. control group. *P < 0.05; **P < 0.01 vs. HF/HS group. ACO, acyl-CoA oxidase; ATGL, adipose triglyceride lipase; CPT-1a, carnitine palmitoyltransferase-1a; CS, citrate synthase; GK, glucokinase; HAD, hydroxyacyl-CoA dehydrogenase; HK-2, hexokinase-2; HSL, hormone-sensitive lipase; MCAD, acetyl-CoA dehydrogenase, medium chain; PFKL, phosphofructokinase, liver; PFKM, phosphofructokinase, muscle, B-type.

dation enzymes in the liver and muscle or the mRNA expression of lipogenesis or lipolysis-related proteins in WAT from the HF group (Fig. 4*B–D*). Expression of mRNAs for β3-AR and TGH-1 in WAT from the HF group was lower than in the control group. TGH-2 mRNA expression tended to be low relative to the control group, but the difference was not statistically significant (Fig. 4*D*).

Effect of EPA on triglyceride secretion and VLDL fatty acid composition in the HF/HS group. In the liver, triglycerides are synthesized via lipogenesis and secreted as VLDL, which in turn delivered to peripheral tissues such as WAT. Evidence has suggested that some species of fatty acids affect triglyceride accumulation in adipocytes (32). We therefore examined hepatic triglyceride secretion and VLDL fatty acid composition in the HF/HS group (experiment 3). We found the enhanced secretion of triglycerides in the HF/HS group relative to the control group (Fig. 5*A*). Analysis of VLDL fatty acid composition revealed that palmitic (C16:0), palmitoleic (C16:1 n-7), and oleic acids (C18:1 n-9) are increased and stearic acid (C18:0) is

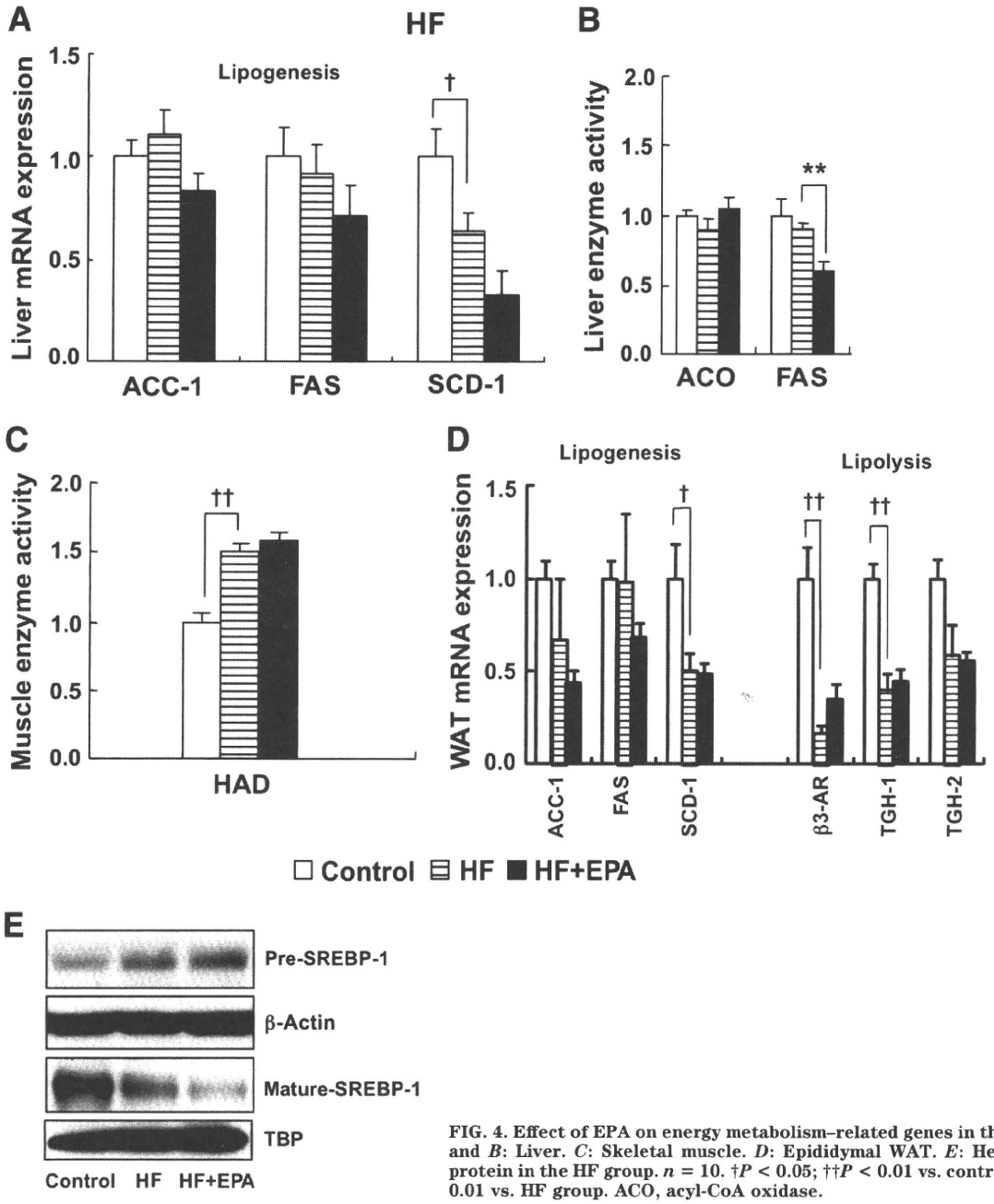

FIG. 4. Effect of EPA on energy metabolism–related genes in the HF groups. *A* and *B*: Liver. *C*: Skeletal muscle. *D*: Epididymal WAT. *E*: Hepatic SREBP-1 protein in the HF group. $n = 10$. †$P < 0.05$; ††$P < 0.01$ vs. control group. **$P <$ 0.01 vs. HF group. ACO, acyl-CoA oxidase.

decreased in the HF/HS group relative to the control group (Fig. 5*B*). In this study, EPA (C20:5 n-3) was markedly increased in VLDL obtained from the HF/HS + EPA group relative to the HF/HS and control groups. Increased hepatic triglyceride secretion and the aberrant VLDL fatty acid composition in the HF/HS group were all reversed by EPA treatment (Fig. 5*A* and *B*). Linoleic acid (C18:2 n-6) was markedly decreased in both HF/HS and HF/HS + EPA groups relative to the control group.

Effect of EPA on WAT lipolysis in the HF/HS group. Because reduced expression of β3-AR, TGH-1, and TGH-2 mRNAs in WAT from the HF/HS group was restored by EPA treatment (Fig. 3*E*), we examined the effect of EPA on WAT lipolysis in the HF/HS group (experiment 4). There was no significant difference in glycerol release ex vivo between HF/HS and control groups (Fig. 6*A*). Treatment with isoproterenol, a β-AR agonist, stimulated glycerol release in the control group, which was markedly reduced in the HF/HS group. Interestingly, glycerol release with or without isoproterenol stimulation was higher in the HF/HS + EPA group than in the HF/HS group (Fig. 6*A*).

Effect of EPA on energy consumption in the HF/HS group. There was a marked difference in energy accumulation between HF/HS and HF/HS + EPA groups, despite similar caloric intake (Fig. 1 and Table 1). However, except UCP-3 in the skeletal muscle, gene expression of β-oxidation enzymes and UCPs were unchanged by EPA treatment (Fig. 3*A*, *C*, and *E*). Furthermore, the activities of β-oxidation–related enzymes were reduced by EPA (Fig. 3*B* and *D*). We, therefore, examined the effect of EPA on O_2 consumption and RER using indirect calorimetry (experiment 4). HF/HS feeding increased O_2 consumption and significantly reduced RER 6 weeks after the experiment (Fig. 6*C–F*). In this study, EPA treatment significantly increased the HF/HS-induced O_2 consumption and inhibited the HF/HS-induced decrease in RER throughout

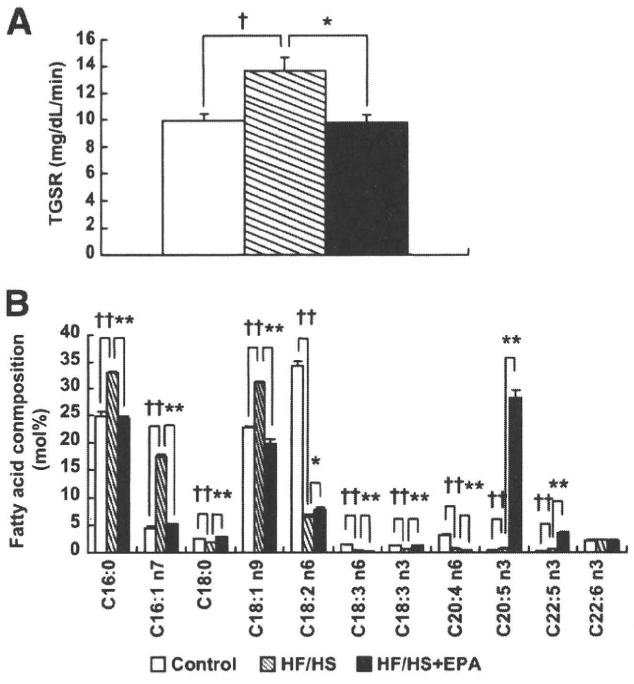

FIG. 5. Effect of EPA on triglyceride secretion rate (TGSR) and VLDL fatty acid composition in the HF/HS group. *A*: TGSR. *B*: VLDL fatty acid composition. $n = 6$. †$P < 0.05$; ††$P < 0.01$ vs. control group. *$P < 0.05$; **$P < 0.01$ vs. HF/HS group.

the experimental period ($P < 0.01$; 130 data points per mouse) (Fig. 6*C* and *E*). The mean values of RER for the light and dark cycles in the HF/HS + EPA group were significantly higher than those of the HF/HS group ($P < 0.01$) (Fig. 6*F*), whereas there was no significant difference in mean values of VO_2 between HF/HS and HF/HS + EPA groups (Fig. 6*D*). EPA also significantly increased O_2 consumption 2 weeks after the experiment ($P < 0.01$) (data not shown).

After 8 weeks of feeding, expression levels of mRNA of UCP-1 in brown adipose tissue (BAT) were unchanged by the HF/HS and HF/HS + EPA groups (Fig. 6*B*). There was no significant difference in UCP-3 mRNA expression in the skeletal muscle among control, HF/HS, and HF/HS + EPA groups (data not shown). EPA also suppressed body weight gain (control group, 28.47 ± 0.64 g; HF/HS group, 35.73 ± 0.99 g; HF/HS + EPA group, 30.78 ± 0.59 g) and epididymal WAT accumulation (control group, 0.36 ± 0.04 g; HF/HS group, 1.51 ± 0.14 g; HF/HS + EPA group, 0.80 ± 0.07 g) (experiment 4). There was no appreciable difference in caloric intake among control, HF/HS, and HF/HS + EPA groups (control group, 14.48 ± 2.03 kcal/day/mouse; HF/HS group, 13.76 ± 0.24 kcal/day/mouse; HF/HS + EPA group, 12.36 ± 0.67 kcal/day/mouse).

DISCUSSION

This study demonstrates that hepatic steatosis is more severe in the HF/HS group than in the HF group. Hyperinsulinemia also develops more rapidly in the HF/HS group than in the HF group, although both HF/HS and HF groups similarly develop obesity and WAT accumulation. In this study, we found that EPA ameliorates HF/HS-induced obesity, WAT inflammation, fatty liver, hyperinsulinemia, and hyperglycemia. By contrast, there is no effect of EPA on obesity in the HF group. These observations indicate the differential effect of EPA on metabolic parameters between HF/HS and HF groups. Expression and activities of hepatic lipogenic enzymes are increased in the HF/HS group, which are abolished by EPA treatment. By contrast, expression of hepatic lipogenic enzymes is not increased in the HF group, where EPA is ineffective against visceral fat accumulation and obesity. It seems that the lower expression of SREBP-1 in the HF/HS + EPA group results in the reduction of hepatic lipogenic enzymes. These observations, taken together, suggest that suppression of enhanced hepatic lipogenesis contributes to the anti-obesity effect of EPA. It is noteworthy that mice with liver-specific disruption of SCD-1 are resistant to high-carbohydrate diet–induced obesity but are sensitive to HF diet–induced obesity (33). In this study, we confirmed that EPA markedly reduces hepatic lipogenic enzymes including SCD-1. The phenotypic effect of liver-specific deficiency of SCD-1 is similar to that observed in this study, thereby supporting the concept that EPA exerts the anti-obesity effect at least in part through the suppression of hepatic lipogenesis.

Deficit of lipogenic enzymes or administration of lipogenic enzyme inhibitors has been reported to inhibit obesity. For instance, administration of FAS inhibitors lowers body weight by reducing food intake (34). Global deficiency of ACC-2 (35) or SCD-1 (36) or administration of antisense oligonucleotide against SCD-1 (37) also shows resistance to obesity through enhanced expression of β-oxidation enzymes or UCPs. On the other hand, neither anorectic effect nor upregulation of β-oxidation enzymes or UCPs has been reported with EPA. Then, how does reduced hepatic lipogenesis inhibit obesity or triglyceride accumulation in WAT? Here, we demonstrated that EPA markedly suppresses the HF/HS-induced hepatic triglyceride secretion and palmitic, palmitoleic, and oleic acids in VLDL. In this study, despite the enhanced hepatic lipogenesis and triglyceride secretion, plasma triglyceride concentrations are reduced in the HF/HS group relative to the control group. This may be because of HF/HS-induced activation of lipoprotein lipase in WAT, as suggested elsewhere (38). Previous studies with mice lacking VLDL receptor or apolipoprotein E demonstrated that VLDL metabolism is closely related to obesity (39,40). There are also several previous reports showing the relationship between triglycerides delivered from the liver and fat accumulation in the adipose tissue. For instance, obese subjects have exhibited enhanced VLDL-triglyceride secretion from the liver (41). Moreover, adenoviral overexpression of diacylglycerol-acyl transferase-1, which catalyzes the final step of hepatic triglyceride synthesis, has resulted in enhanced VLDL-triglyceride secretion and thus obesity (42). On the other hand, there is a report that triglyceride accumulation in WAT is enhanced by a certain species of fatty acids such as oleic and palmitic acids, among which, oleic acid tends to induce triglyceride accumulation in 3T3-L1 adipocytes (32). Moreover, plasma content of palmitoleic acid is positively correlated with obesity in humans (43,44). In this study, we showed that hepatic expression of SCD-1 is enhanced in the HF/HS group and is markedly suppressed by EPA. SCD-1 is known to catalyze the conversion from stearic and palmitic acids to oleic and palmitoleic acids, respectively. Increased oleic and palmitoleic acids in VLDL and hepatic gene expression of SCD-1 in the HF/HS group are both suppressed by EPA. It is, therefore, conceivable that increases in the quantity (i.e., secretion rate) and quality (i.e., fatty acid composition) of triglycerides delivered from the liver contribute to

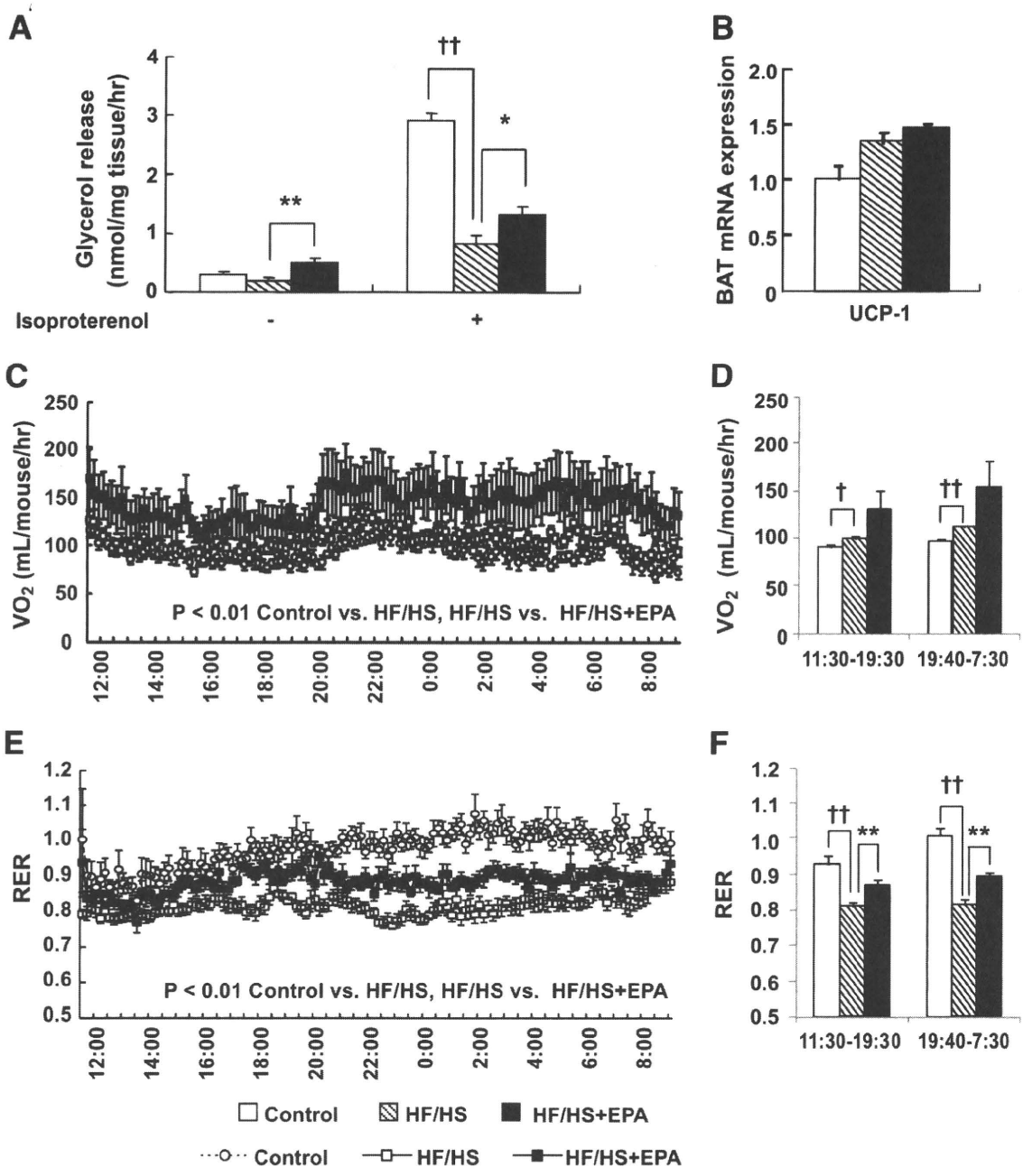

FIG. 6. Effect of EPA on WAT lipolysis and energy consumption in the HS/HF group. *A*: Glycerol release from epididymal WAT with or without 10 μmol/l isoproterenol. *B*: UCP-1 mRNA expression in BAT. Indirect calorimetry analysis of VO_2 (*C* and *D*) and RER (*E* and *F*). $n = 9$. ††$P < 0.01$ vs. control group. *$P < 0.05$; **$P < 0.01$ vs. HF/HS group.

triglyceride accumulation and thus obesity in the HF/HS group. Collectively, we speculate that the anti-obesity effect of EPA is due at least in part to its impact on the quantity and quality of triglycerides through the suppression of hepatic lipogenesis.

Lipogenesis is an efficient means to transform the energy accumulated in the body but not to consume energy. Because EPA results in marked reduction of body weight and fat accumulation in the HF/HS group, it is obligatory that EPA consumes energy. Here, we demonstrated that EPA enhances energy consumption and reverses the decreased RER in the HF/HS group. In this regard, Rustan et al. (45) reported that treatment with docosahexaenoic acid + EPA increases RER, but they did not show increased energy consumption. These observations suggest that energy accumulated not as triglycerides is consumed inside and/or outside the liver. Enhanced energy consumption accompanied by enhanced expression of β-oxidation enzymes and UCPs was reported in SCD-1 knockout mice (36) and mice treated with antisense oligonucleotides of SCD-1 (37). In this study, although EPA strongly suppresses hepatic SCD-1 mRNA expression, it does not enhance β-oxidation enzymes and UCPs. There are appreciable changes in gene expression, which suggests the EPA-induced increase in energy consumption. Expression of β-oxidation and glucose oxidation genes in the liver, skeletal muscle, and WAT is not increased by EPA treatment. Moreover, activities of acyl-CoA oxidase and hydroxyacyl-CoA dehydrogenase in the liver and skeletal muscle, respectively, were reduced by EPA, suggesting a minor contribution of

β-oxidation to enhanced energy consumption. This discussion is also supported by increased RER by EPA.

Expression of mRNAs for UCPs except UCP-3 in the skeletal muscle is not also increased when treated with EPA. Although UCP-3 mRNA expression in the skeletal muscle is only slightly increased by EPA (experiment 1), EPA fails to enhance expression of UCP-3 mRNA in the skeletal muscle as well as UCP-1 mRNA in BAT in experiment 4, suggesting a minor contribution of UCPs to EPA-induced energy consumption. In this regard, our preliminary data show no significant increase in O_2 consumption in the HF + EPA group relative to the HF group (data not shown). These observations, taken together, suggest that the suppressed hepatic lipogenesis is related to enhanced energy consumption in the HF/HS + EPA group. The molecular mechanisms by which EPA enhances energy consumption remain to be elucidated. We also found that EPA only partly restores the otherwise reduced WAT lipolysis in the HF/HS group. Similar to the HF/HS group, β3-AR, TGH-1, and TGH-2 mRNA expression is reduced in the HF group, but EPA fails in the restoration of the reduced gene expression. These observations suggest that EPA restoration of lipolysis is the consequence of the anti-obesity effect of EPA.

The mechanism underlying the anti-obesity effect of EPA has not been extensively studied, and the anti-obesity effect of EPA reported so far is somewhat controversial. Indeed, there is a report showing that EPA does not prevent visceral fat accumulation in rats with HF/HS-induced obesity, where EPA was administered for a much shorter period of time (15). On the other hand, Oh-i et al. (16) reported that EPA promotes body weight gain in rats fed an HF diet through the reduction of brain leptin transport or leptin resistance. The authors used diet without carbohydrate such as sucrose and fructose, when EPA may not exert the anti-obesity effect. In this study, the fatty acid composition was different between the HF/HS and HF diets used. Although the difference might modify the phenotype of our models, this would not invalidate our conclusion on the association between the anti-obesity effect of EPA and suppression of hepatic lipogenesis. In analyzing the anti-obesity effect of EPA, we should be careful of the doses of EPA administered, period of administration, species of animals used, etc., in addition to the proportion of sucrose versus fat in the diet.

Given that hepatic lipogenesis in the HF group was roughly comparable to that in control group, it is likely that hepatic lipogenesis is less associated with HF-induced obesity. This may be related to no appreciable anti-obesity effect of EPA in the HF group, even with a downregulation of hepatic lipogenic genes. In this regard, Kuda et al. (46) reported that treatment with fish oil tends to prevent HF-induced body weight gain, although not with statistical significance. This may be because of the potential difference in the anti-obesity effect between EPA used in this study and fish oil or the doses of administration. On the other hand, EPA reduced hepatic triglyceride in both the HF/HS and HF groups. Rustan et al. (47) reported that EPA inhibits incorporation of other fatty acids into triglyceride, which may explain the EPA-induced reduction of hepatic triglyceride accumulation in the HF group.

In conclusion, this study is the first demonstration that EPA prevents visceral fat accumulation and obesity, possibly through the suppression of hepatic lipogenesis and enhancement of energy consumption (Fig. 7). Because the metabolic syndrome is often associated with enhanced lipogenesis and steatosis or nonalcoholic fatty liver disease, this study suggests that EPA may be effective to improve visceral fat accumulation and hepatic steatosis in patients with the metabolic syndrome. Our data also suggest that EPA is suited for the treatment of the metabolic syndrome.

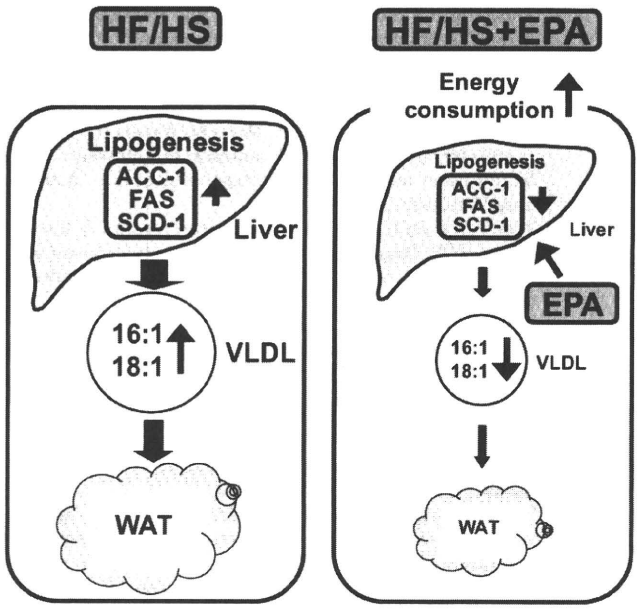

FIG. 7. Possible mechanism underlying the anti-obesity effect of EPA. EPA prevents WAT accumulation in HF/HS-induced obese mice possibly through the suppression of hepatic lipogenesis and enhancement of energy consumption.

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A.S. researched data, wrote the manuscript, and contributed to discussion. H.K. and Y.O. wrote the manuscript, contributed to discussion, and reviewed/edited the manuscript. T.N., M.O., M.N., K.M., M.I., and T.S. contributed to discussion and reviewed/edited the manuscript.

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REFERENCES

1. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, Gordon DJ, Krauss RM, Savage PJ, Smith SC Jr, Spertus JA, Costa F. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement Circulation 2005;112:2735–2752
2. Matsuzawa Y, Funahashi T, Nakamura T. Molecular mechanism of metabolic syndrome X: contribution of adipocytokines adipocyte-derived bioactive substances. Ann N Y Acad Sci 1999;892:146–154
3. Marchesini G, Brizi M, Bianchi G, Tomassetti S, Bugianesi E, Lenzi M, McCullough AJ, Natale S, Forlani G, Melchionda N. Nonalcoholic fatty liver disease: a feature of the metabolic syndrome. Diabetes 2001;50:1844–1850
4. Kris-Etherton PM, Harris WS, Appel LJ, American Heart Association. Nutrition Committee. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. Circulation 2002;106:2747–2757
5. Ando M, Sanaka T, Nihei H. Eicosapentanoic acid reduces plasma levels of remnant lipoproteins and prevents in vivo peroxidation of LDL in dialysis patients. J Am Soc Nephrol 1999;10:2177–2184
6. Tamura Y, Hirai A, Terano T, Takenaga M, Saitoh H, Tahara K, Yoshida S. Clinical and epidemiological studies of eicosapentaenoic acid (EPA) in Japan. Prog Lipid Res 1986;25:461–466
7. Arita M, Yoshida M, Hong S, Tjonahen E, Glickman JN, Petasis NA,

Blumberg RS, Serhan CN. Resolvin E1, an endogenous lipid mediator derived from omega-3 eicosapentaenoic acid, protects against 2,4,6-trinitrobenzene sulfonic acid-induced colitis. Proc Natl Acad Sci U S A 2005;102:7671–7676
8. Yamada H, Yoshida M, Nakano Y, Suganami T, Satoh N, Mita T, Azuma K, Itoh M, Yamamoto Y, Kamei Y, Horie M, Watada H, Ogawa Y. In vivo and in vitro inhibition of monocyte adhesion to endothelial cells and endothelial adhesion molecules by eicosapentaenoic acid. Arterioscler Thromb Vasc Biol 2008;28:2173–2179
9. Mita T, Watada H, Ogihara T, Nomiyama T, Ogawa O, Kinoshita J, Shimizu T, Hirose T, Tanaka Y, Kawamori R. Eicosapentaenoic acid reduces the progression of carotid intima-media thickness in patients with type 2 diabetes. Atherosclerosis 2007;191:162–167
10. Yokoyama M, Origasa H, Matsuzaki M, Matsuzawa Y, Saito Y, Ishikawa Y, Oikawa S, Sasaki J, Hishida H, Itakura H, Kita T, Kitabatake A, Nakaya N, Sakata T, Shimada K, Shirato K, Japan EPA lipid intervention Study (JELIS) Investigators. Effects of eicosapentaenoic acid on major coronary events in hypercholesterolaemic patients (JELIS): a randomised open-label, blinded endpoint analysis. Lancet 2007;369:1090–1098
11. Saito Y, Yokoyama M, Origasa H, Matsuzaki M, Matsuzawa Y, Ishikawa Y, Oikawa S, Sasaki J, Hishida H, Itakura H, Kita T, Kitabatake A, Nakaya N, Sakata T, Shimada K, Shirato K, JELIS Investigators, Japan. Effects of EPA on coronary artery disease in hypercholesterolemic patients with multiple risk factors: sub-analysis of primary prevention cases from the Japan EPA Lipid Intervention Study (JELIS). Atherosclerosis 2008;200:135–140
12. Satoh N, Shimatsu A, Kotani K, Sakane N, Yamada K, Suganami T, Kuzuya H, Ogawa Y. Purified eicosapentaenoic acid reduces small dense LDL, remnant lipoprotein particles, and C-reactive protein in metabolic syndrome. Diabetes Care 2007;30:144–146
13. Itoh M, Suganami T, Satoh N, Tanimoto-Koyama K, Yuan X, Tanaka M, Kawano H, Yano T, Aoe S, Takeya M, Shimatsu A, Kuzuya H, Kamei Y, Ogawa Y. Increased adiponectin secretion by highly purified eicosapentaenoic acid in rodent models of obesity and human obese subjects. Arterioscler Thromb Vasc Biol 2007;27:1918–1925
14. Pérez-Matute P, Pérez-Echarri N, Martínez JA, Marti A, Moreno-Aliaga MJ. Eicosapentaenoic acid actions on adiposity and insulin resistance in control and high-fat-fed rats: role of apoptosis, adiponectin and tumour necrosis factor-α. Br J Nutr 2007;97:389–398
15. Raclot T, Groscolas R, Langin D, Ferré P. Site-specific regulation of gene expression by n-3 polyunsaturated fatty acids in rat white adipose tissues. J Lipid Res 1997;38:1963–1972
16. Oh-I S, Shimizu H, Sato T, Uehara Y, Okada S, Mori M. Molecular mechanisms associated with leptin resistance: n-3 polyunsaturated fatty acids induce alterations in the tight junction of the brain. Cell Metab 2005;1:331–341
17. Yahagi N, Shimano H, Hasty AH, Amemiya-Kudo M, Okazaki H, Tamura Y, Iizuka Y, Shionoiri F, Ohashi K, Osuga J, Harada K, Gotoda T, Nagai R, Ishibashi S, Yamada N. A crucial role of sterol regulatory element-binding protein-1 in the regulation of lipogenic gene expression by polyunsaturated fatty acids. J Biol Chem 1999;274:35840–35844
18. Sekiya M, Yahagi N, Matsuzaka T, Najima Y, Nakakuki M, Nagai R, Ishibashi S, Osuga J, Yamada N, Shimano H. Polyunsaturated fatty acids ameliorate hepatic steatosis in obese mice by SREBP-1 suppression. Hepatology 2003;38:1529–1539
19. Kajikawa S, Harada T, Kawashima A, Imada K, Mizuguchi K. Highly purified eicosapentaenoic acid prevents the progression of hepatic steatosis by repressing monounsaturated fatty acid synthesis in high-fat/high-sucrose diet-fed mice. Prostaglandins Leukot Essent Fatty Acids 2009;80:229–238
20. Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 1957;226:497–509
21. Molero JC, Waring SG, Cooper A, Turner N, Laybutt R, Cooney GJ, James DE. Casitas b-lineage lymphoma-deficient mice are protected against high-fat diet-induced obesity and insulin resistance. Diabetes 2006;55:708–715
22. Nepokroeff CM, Lakshmanan MR, Porter JW. Fatty-acid synthase from rat liver. Methods Enzymol 1975;35:37–44
23. Osumi T, Hashimoto T. Acyl-CoA oxidase of rat liver: a new enzyme for fatty acid oxidation. Biochem Biophys Res Commun 1978;83:479–485
24. Hannah VC, Ou J, Luong A, Goldstein JL, Brown MS. Unsaturated fatty acids down-regulate SREBP isoforms 1a and 1c by two mechanisms in HEK-293 cells. J Biol Chem 2001;276:4365–4372
25. Inoue N, Shimano H, Nakakuki M, Matsuzaka T, Nakagawa Y, Yamamoto T, Sato R, Takahashi A, Sone H, Yahagi N, Suzuki H, Toyoshima H, Yamada N. Lipid synthetic transcription factor SREBP-1a activates p21WAF1/CIP1, a universal cyclin-dependent kinase inhibitor. Mol Cell Biol 2005;25:8938–8947
26. Schweiger M, Schreiber R, Haemmerle G, Lass A, Fledelius C, Jacobsen P, Tornqvist H, Zechner R, Zimmermann R. Adipose triglyceride lipase and hormone-sensitive lipase are the major enzymes in adipose tissue triacylglycerol catabolism. J Biol Chem 2006;281:40236–40241
27. Steiner G, Haynes FJ, Yoshino G, Vranic M. Hyperinsulinemia and in vivo very-low-density lipoprotein-triglyceride kinetics. Am J Physiol 1984;246: E187–E192
28. Werner A, Havinga R, Bos T, Bloks VW, Kuipers F, Verkade HJ. Essential fatty acid deficiency in mice is associated with hepatic steatosis and secretion of large VLDL particles. Am J Physiol Gastrointest Liver Physiol 2005;288:G1150–G1158
29. Tanaka T, Yamamoto J, Iwasaki S, Asaba H, Hamura H, Ikeda Y, Watanabe M, Magoori K, Ioka RX, Tachibana K, Watanabe Y, Uchiyama Y, Sumi K, Iguchi H, Ito S, Doi T, Hamakubo T, Naito M, Auwerx J, Yanagisawa M, Kodama T, Sakai J. Activation of peroxisome proliferator-activated receptor delta induces fatty acid beta-oxidation in skeletal muscle and attenuates metabolic syndrome. Proc Natl Acad Sci U S A 2003;100:15924–15929
30. Son C, Hosoda K, Ishihara K, Bevilacqua L, Masuzaki H, Fushiki T, Harper ME, Nakao K. Reduction of diet-induced obesity in transgenic mice overexpressing uncoupling protein 3 in skeletal muscle. Diabetologia 2004;47:47–54
31. Wu C, Kang JE, Peng LJ, Li H, Khan SA, Hillard CJ, Okar DA, Lange AJ. Enhancing hepatic glycolysis reduces obesity: differential effects on lipogenesis depend on site of glycolytic modulation. Cell Metab 2005;2:131–140
32. Madsen L, Petersen RK, Kristiansen K. Regulation of adipocyte differentiation and function by polyunsaturated fatty acids. Biochim Biophys Acta 2005;1740:266–286
33. Miyazaki M, Flowers MT, Sampath H, Chu K, Otzelberger C, Liu X, Ntambi JM. Hepatic stearoyl-CoA desaturase-1 deficiency protects mice from carbohydrate-induced adiposity and hepatic steatosis. Cell Metab 2007;6:484–496
34. Loftus TM, Jaworsky DE, Frehywot GL, Townsend CA, Ronnett GV, Lane MD, Kuhajda FP. Reduced food intake and body weight in mice treated with fatty acid synthase inhibitors. Science 2000;288:2379–2381
35. Abu-Elheiga L, Oh W, Kordari P, Wakil SJ. Acetyl-CoA carboxylase 2 mutant mice are protected against obesity and diabetes induced by high-fat/high-carbohydrate diets. Proc Natl Acad Sci U S A 2003;100:10207–10212
36. Ntambi JM, Miyazaki M, Stoehr JP, Lan H, Kendziorski CM, Yandell BS, Song Y, Cohen P, Friedman JM, Attie AD. Loss of stearoyl-CoA desaturase-1 function protects mice against adiposity. Proc Natl Acad Sci U S A 2002;99:11482–11486
37. Jiang G, Li Z, Liu F, Ellsworth K, Dallas-Yang Q, Wu M, Ronan J, Esau C, Murphy C, Szalkowski D, Bergeron R, Doebber T, Zhang BB. Prevention of obesity in mice by antisense oligonucleotide inhibitors of stearoyl-CoA desaturase-1. J Clin Invest 2005;115:1030–1038
38. Surwit RS, Feinglos MN, Rodin J, Sutherland A, Petro AE, Opara EC, Kuhn CM, Rebuffé-Scrive M. Differential effects of fat and sucrose on the development of obesity and diabetes in C57BL/6J and A/J mice. Metabolism 1995;44:645–651
39. Goudriaan JR, Tacken PJ, Dahlmans VE, Gijbels MJ, van Dijk KW, Havekes LM, Jong MC. Protection from obesity in mice lacking the VLDL receptor. Arterioscler Thromb Vasc Biol 2001;21:1488–1493
40. Gao J, Katagiri H, Ishigaki Y, Yamada T, Ogihara T, Imai J, Uno K, Hasegawa Y, Kanzaki M, Yamamoto TT, Ishibashi S, Oka Y. Involvement of apolipoprotein E in excess fat accumulation and insulin resistance. Diabetes 2007;56:24–33
41. Mittendorfer B, Patterson BW, Klein S. Effect of sex and obesity on basal VLDL-triacylglycerol kinetics. Am J Clin Nutr 2003;77:573–579
42. Yamazaki T, Sasaki E, Kakinuma C, Yano T, Miura S, Ezaki O. Increased very low density lipoprotein secretion and gonadal fat mass in mice overexpressing liver DGAT1. J Biol Chem 2005;280:21506–21514
43. Okada T, Furuhashi N, Kuromori Y, Miyashita M, Iwata F, Harada K. Plasma palmitoleic acid content and obesity in children. Am J Clin Nutr 2005;82:747–750
44. Paillard F, Catheline D, Duff FL, Bouriel M, Deugnier Y, Pouchard M, Daubert JC, Legrand P. Plasma palmitoleic acid, a product of stearoyl-coA desaturase activity, is an independent marker of triglyceridemia and abdominal adiposity. Nutr Metab Cardiovasc Dis 2008;18:436–440
45. Rustan AC, Hustvedt BE, Drevon CA. Dietary supplementation of very long-chain n-3 fatty acids decreases whole body lipid utilization in the rat. J Lipid Res 1993;34:1299–1309
46. Kuda O, Jelenik T, Jilkova Z, Flachs P, Rossmeisl M, Hensler M, Kazdova L, Ogston N, Baranowski M, Gorski J, Janovska P, Kus V, Polak J, Mohamed-Ali V, Burcelin R, Cinti S, Bryhn M, Kopecky J. n-3 Fatty acids and rosiglitazone improve insulin sensitivity through additive stimulatory effects on muscle glycogen synthesis in mice fed a high-fat diet. Diabetologia 2009;52:941–951
47. Rustan AC, Nossen JO, Christiansen EN, Drevon CA. Eicosapentaenoic acid reduces hepatic synthesis and secretion of triacylglycerol by decreasing the activity of acyl-coenzyme A:1,2-diacylglycerol acyltransferase. J Lipid Res 1988;29:1417–1426

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Review

Adipose tissue macrophages: their role in adipose tissue remodeling

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ABSTRACT

The adipose tissue secretes a large number of bioactive substances, adipocytokines, which may be involved in a variety of physiologic and pathologic processes. Unbalanced production of pro- and anti-inflammatory adipocytokines seen in visceral fat obesity contributes critically to the development of the metabolic syndrome. Evidence has accumulated indicating that obesity is associated with a state of chronic, low-grade inflammation, suggesting that inflammation may be a potential mechanism, whereby obesity leads to insulin resistance. Indeed, obese adipose tissue is characterized by adipocyte hypertrophy, followed by increased angiogenesis, immune cell infiltration, extracellular matrix overproduction, and thus, increased production of proinflammatory adipocytokines during the progression of chronic inflammation. The dynamic change found in the adipose tissue can be referred to as "adipose tissue remodeling," in which stromal cells change dramatically in number and cell type during the course of obesity. Among stromal cells, infiltration of macrophages in the adipose tissue precedes the development of insulin resistance in animal models, suggesting that they are crucial for obesity-related adipose tissue inflammation. We have demonstrated that a paracrine loop involving saturated fatty acids and TNF-α derived from adipocytes and macrophages, respectively, aggravates obesity-induced adipose tissue inflammation. Notably, saturated fatty acids, which are released from hypertrophied adipocytes via the macrophage-induced lipolysis, serve as a naturally occurring ligand for TLR4 complex, thereby activating macrophages. Understanding the molecular mechanism underlying adipose tissue remodeling may lead to the identification of novel, therapeutic strategies to prevent or treat obesity-induced adipose tissue inflammation. *J. Leukoc. Biol.* 88: 33–39; 2010.

Abbreviations: ATF3=activating transcription factor 3, CLS=crown-like structure, DAMP=damage-associated molecular pattern, EPA=eicosapentaenoic acid, ER=endoplasmic reticulum, HMGB1=high-mobility group box-1, MKP-1=MAPK phosphatase-1, PAMP=pathogen-associated molecular pattern , PPARγ/δ=peroxisome proliferator-activated receptor γ/δ, PRR=pattern-recognition receptor, S100A8/A9=S100 calcium-binding protein A8/A9, SVF=stromal vascular fraction

Introduction

The metabolic syndrome is a constellation of visceral fat obesity, impaired glucose metabolism, atherogenic dyslipidemia, and blood pressure elevation, which all increase independently the risk of atherosclerotic diseases, such as ischemic heart disease and cerebral stroke [1–5]. The molecular basis for the clustering of such independent risks of atherosclerosis has not been fully elucidated, and visceral fat obesity is considered most important [1–5]. Evidence has accumulated indicating that obesity is associated with a state of chronic, low-grade inflammation, suggesting that inflammation may be a potential mechanism, whereby obesity leads to insulin resistance [1–4].

Adipose tissue secretes a large number of adipocytokines such as leptin, MCP-1, and adiponectin, which may be involved in a variety of physiologic and pathologic processes [1–3, 5, 6]. Unbalanced production of pro- and anti-inflammatory adipocytokines seen in visceral fat obesity critically contributes to the development of many aspects of the metabolic syndrome [1–5]. There is considerable evidence that obese adipose tissue is markedly infiltrated by macrophages; they may participate in the inflammatory pathways that are activated in the adipose tissue [7–9]. Notably, macrophage infiltration and inflammation-related gene expression in the adipose tissue precede the development of insulin resistance in animal models [7, 8], suggesting that infiltrated macrophages are an important source of inflammation in the adipose tissue. This review summarizes the role of macrophages in adipose tissue inflammation.

ADIPOSE TISSUE REMODELING

In addition to lipid-laden, mature adipocytes, the adipose tissue is composed of various cell types; the remaining SVF includes preadipocytes, endothelial cells, fibroblasts, and immune cells [10]. In contrast to "acute inflammation," which resolves by an active termination program [11], "chronic inflammation" is characterized by sustained interaction between

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