BACKGROUND: An emerging paradigm supports the notion that deregulation of fatty acid synthase (FASN)-catalyzed de novo FA biogenesis could play a central role in the pathogenesis of metabolic diseases sharing the hallmark of insulin-resistance.

CONTENT: We reviewed pharmacological and genetic alterations of FASN activity that have been shown to significantly influence energy expenditure rates, fat mass, insulin sensitivity, and cancer risk. This new paradigm proposes that insulin-resistant conditions such as obesity, type 2 diabetes, and cancer arise from a common FASN-driven "lipogenic state". An important question then is whether the development or the progression of insulin-related metabolic disorders can be prevented or reversed by the modulation of FASN status. If we accept the paradigm of FASN dysfunction as a previously unrecognized link between insulin resistance, type 2 diabetes, and cancer, the use of insulin sensitizers in parallel with forthcoming FASN inhibitors should be a valuable therapeutic approach that, in association with lifestyle interventions, would concurrently improve energy-flux status, ameliorate insulin sensitivity, and alleviate the risk of lipogenic carcinomas.

CONCLUSIONS: Although the picture is currently incomplete and researchers in the field have plenty of work ahead, the latest clinical and experimental evidence that we discuss illuminates a functional and drug-modifiable link that connects FASN-driven endogenous FA biosynthesis, insulin action, and glucose homeostasis in the natural history of insulin-resistant pathologies.

Fatty acids (FAs) for animal metabolism have 2 sources, exogenously derived (dietary) FAs and de novo endogenously synthesized FAs. The latter biosynthesis is catalyzed by FA synthase (FASN), which synthesizes long-chain FAs by using acetyl coenzyme A (CoA) as a primer, malonyl-CoA as a 2-carbon donor, and NADPH as reducing equivalent. The predominant product of FASN is a 16-carbon FA, palmitate, but FASN may also produce smaller amounts of myristate, laurate, and even shorter-chain FAs (1, 2). Based on the intracellular localization of FASN, 2 kinds of FASN proteins are classically recognized: cytosolic (FASN I) and mitochondrial FASN (FASN II). Whereas cytosolic FASN is primarily responsible for de novo FA biosynthesis, mitochondrial FASN provides the octanoyl precursor required for the essential lipoylation pathway (3). In addition, several studies have demonstrated that FASN can also occur in the extracellular space of human cancer cells (4–6).

There are 2 basic naturally occurring types of FASN architecture. The prototypical FASN (FASN I) is found in mammals and consists of a single gene that produces a polypeptide, which contains all of the reaction centers required to produce an FA (7). In prokaryotes and lower eukaryotes, such as yeast, bacteria, plants, and parasites, FA biosynthesis is accomplished by a series of monofunctional proteins in a dissociated type-II FASN system (FASN II). Importantly, an FASN II has been discovered and partially characterized in eukaryotic mitochondria (8–9). The possible role of mitochondrial type-II FASN in obesity and insulin resistance has not yet been explored.

Through selective pharmacologic and genetic manipulation of FASN we are beginning to accumulate evidence indicating that FASN-catalyzed de novo lipogenesis in the hypothalamus has an important impact on food intake and body weight homeostasis. This role has been the subject of recent excellent reviews (10–13). Here, we will focus on the role of FASN in periph-

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Nonstandard abbreviations: FA, fatty acids; FASN, FA synthase; CoA, coenzyme A; NAFLD, nonalcoholic fatty liver disease; AMPK, AMP-activated protein kinases; mTOR, mammalian target of rapamycin.
eral insulin resistance and in the pathogenesis of biologically aggressive subtypes of human malignancies exhibiting alterations in lipid metabolism.

**FASN STRUCTURE: A GIANT MOLECULAR-ASSEMBLY-LINE**

In contrast to FASN II, the type I FASN of fungi and animals are huge multifunctional polypeptides that integrate all the FA synthesis steps into large macromolecular assemblies. Mammalian FASN consists of 2 identical 270-kD polypeptide chains, each comprising all 7 required domains (i.e., beta-ketoacyl synthase, malonyl/acyetyltransferase, dehydrase, enoyl reductase, beta-ketoacyl reductase, acyl carrier protein, and thioesterase) that assemble into homodimers for enzymatic activity (14–21). First, a malonil group derived from malonyl-CoA is condensed with an acetyl group from acetyl-CoA. The resulting beta-ketoacyl derivative is then reduced in 3 consecutive steps: beta-ketoacyl reduction, beta-hydroxyacyl dehydration, and enoyl reduction, to the saturated acyl derivative, which then acts as a primer for further elongation and reduction cycles to yield ultimately a palmitoyl derivative. The latter is hydrolyzed by thioesterase to free palmitate. Early studies suggested that the FASN I enzymatic complex contained 7 separate enzymatic pockets as head-to-tail dimer with the beta-ketoacyl synthase and malonyl/acyetyltransferase domains of 1 monomer working together with the dehydrase, enoyl reductase, beta-ketoacyl reductase, acyl carrier protein, and thioesterase domains of the adjacent monomer (14–17). That is, 2 FASN monomers in the homodimeric form of the enzyme are arranged in a fully extended antiparallel orientation allowing functional interactions across the monomer interface. In 2005, Asturias et al. proposed an alternative head-to-head model for FASN organization, which predicts that the beta-ketoacyl synthase and malonyl/acyetyltransferase domains of both monomers lie closer to the center of the FASN dimer, where they can access the acyl carrier protein of either subunit (19). In 2006, Maier et al. reported the architecture of mammalian FASN from a 4.5-Å electron density map, in which most domains could be assigned, but no details were visible (20). Maier’s FASN map fundamentally agreed with the Asturias’ revised model and suggested that mammalian FASN is an intertwined dimer with a large dimerization interface running through the body of the molecule, perpendicular to the interface proposed in the classical scheme (20). Mammalian FASN therefore adopts an X-shape structure with a full set of active sites present in each of 2 semicircular “reaction chambers” on both sides of the molecule. These authors have recently determined mammalian FASN crystal structure at 3.2-Å resolution, which provides sufficient detail to understand both the folding and the connectivity of, at least, 5 catalytic domains (21). Although the flexibly tethered acyl carrier protein and thioesterase domains remain unresolved, the structure clearly reveals a complex architecture of alternating linkers and enzymatic domains. FASN architecture can now be segregated into 2 “arms”: a “selection/condensing arm” for addition of new building blocks, and a “modifying arm” for chemical processing of chain elongation intermediates [cartoon representations of this new FASN structure overview are elegantly presented in (21) and (22)]. Moreover, FASN structure exhibits an open and flexible design that is ideal for insertion or deletion of catalytic domains, particularly in the modifying arm. Surprisingly, Maier’s FASN structure has led to dentification of 2 additional nonenzymatic domains, a pseudo-ketoreductase and a peripheral pseudo-methyltransferase that is probably a molecular relic of an ancestral methyltransferase domain maintained in some related polyketide synthases. As a result of its modular architecture, which allows the variation of domain composition, mammalian FASN should be viewed as modular assembly line highly adaptable to new functions by achieving diverse product synthesis.

**FASN and the Glucose-Insulin Axis in Liver and Adipose Lipogenic Tissues**

**LIVER FASN**

Nonalcoholic fatty liver disease (NAFLD) is associated with obesity, insulin resistance, and type 2 diabetes. Recent animal models have shown that modulating FASN in liver may be of relevance in understanding the pathophysiology of NAFLD (23). FASN catalyzes the last step in the FA biosynthetic pathway and is believed to be a determinant of the maximal capacity of a tissue, and liver in particular, to synthesize FAs by de novo lipogenesis. Deletion of the gene coding for FAS results in embryonic lethality (24). Liver-specific FASN-knockout in mice results in mutant mice that possess a similar phenotype to control animals when fed normal chow. Surprisingly, the lack of FASN did not protect against the development of fatty liver but rather exacerbated it under specific nutritional conditions (25). Indeed, when fed a low-fat/high-carbohydrate diet for 4 weeks, Liver-specific FASN-knockout mice developed hepatic steatosis due to a reduction in beta-oxidation, as evidenced by a 3-fold increase in hepatic malonyl-CoA concentrations and a significant decrease in blood ketone bodies (25). In fact, this mouse model led to the novel and interesting concept that “new fat” synthesized via FASN activity (mainly saturated palmitate) would specifically activate a pool of nuclear receptors (e.g., peroxisome proliferator-activated receptor alpha) and would in turn lead to enhanced beta-oxidation.

Until recently, the rate of hepatic de novo lipogenesis in humans (estimated by indirect calorimetry) was...
generally believed to be low (26). The availability of isotopic methods that measure the incorporation of $^{13}$C-[acetate or deuterated water into FAs has allowed more accurate determinations of hepatic de novo lipogenesis in humans. Donnelly and colleagues (27) have shown that the contribution of hepatic de novo lipogenesis to intrahepatic fat is $<5\%$ in healthy individuals but increases to $26\%$ in individuals with nonalcoholic fatty liver disease. It has also been shown that human de novo FA synthesis is stimulated by a eculoric low-fat/high-carbohydrate diet (28), and that lean as well as obese individuals fed a low-fat/high-carbohydrate diet have increased levels of hepatic de novo lipogenesis compared with individuals fed a high-fat/low-carbohydrate diet (29). In fact, because glucose is constantly required at a high rate by multiple tissues, mammals have evolved mechanisms to sense glucose levels and adapt the expression of genes to glucose availability and to adapt their metabolism to the nutritional environment (30). When abundant food supplies are accessible, nutrients are stored for subsequent use during periods of food shortage.

Very recent studies link hepatic lipogenesis to the consumption of specific nutrients. Ouyang and colleagues (31) investigated the dietary history of patients with evidence of biopsy-proven NAFLD without cirrhosis and controls matched for sex, age, and body mass index. Consumption of fructose in patients with NAFLD was nearly 2- to 3-fold higher than in controls (365 kcal vs 170 kcal; $P < 0.05$). In patients with NAFLD, hepatic mRNA expression of fructokinase, an important enzyme for fructose metabolism, and FASN were increased ($P = 0.04$ and $P = 0.02$, respectively) (31).

**ADIPOSE FASN**

The importance of the interrelationships between insulin action and FASN are exemplified by the findings in mice with a fat-specific insulin receptor knock-out. These mice have reduced adipose tissue mass, are protected against obesity, and have an extended life span. White adipose tissue of fat-specific insulin receptor knock-out mice is characterized by a polarization into 2 major populations of adipocytes, one small (<50 μm) and one large (100 μm), which differ with regard to basal triglyceride synthesis and lipolysis, as well as in the expression of FASN and other lipogenic genes (32). In fact, earlier studies found markedly blunted FA synthesis enzyme activities in large fat cells from old rats and suggested that this FA synthesis impairment, which was a primary defect in the insulin resistance of the large cells, was at least partly due to diminished cellular contents of FASN (33).

The contribution of human adipose tissue to whole-body lipogenesis is considered to be low and less than that of liver (34, 35). However, adipose tissue remains an important site of endogenous FA synthesis (36). Studies in humans fed with a high carbohydrate diet demonstrated that total body fat synthesis significantly exceeded hepatic de novo lipogenesis, suggesting that adipose tissue may be the major site for FA synthesis, with the adipose tissue accounting for up to 40% of whole-body lipogenesis under these conditions (37, 38). The fatty acid synthase (FASN) gene is over-transcribed in the adipose tissue of genetically obese rats (39), whereas it has recently been demonstrated that the human FASN gene and de novo lipogenesis are coordinately regulated in human adipose tissue (40). Moreover, FASN gene expression in adipose tissue appears to be linked to visceral fat accumulation (41). Considering that pharmacological inhibition of FASN activity blocks adipocytic differentiation and leads to the reduction of adipocyte number (42), 2 parameters that determine adipose tissue mass, it is reasonable to suggest that the induction of FASN-catalyzed adipocyte lipogenesis may contribute to obesity.

Both visceral and subcutaneous FASN mRNA expressions were recently demonstrated to be closely correlated with FASN protein levels in both depots, impaired insulin sensitivity and adipokine profile (increased proinflammatory cytokines) (41). However, several previous studies have addressed the expression of FASN in obese vs lean individuals with opposite results. Other authors found decreased lipogenic capacity of subcutaneous adipose tissue and decreased FASN expression in obese fasted individuals with a large, long-term excess in body mass (43–45). Similar observations were reported in ob/ob mice with established obesity (46, 47). This scenario could be different in recent-onset, dynamic obesity. In fact, during the period of dynamic obesity with rapidly expanding fat stores, Zucker rats have a large increase in adipose tissue lipogenic capacity (39). Although the possibility that the expression of lipogenic genes is also increased in adipose tissue of humans with dynamic obesity remains to be elucidated (41), the decreased expression of lipogenic genes could be a late and adaptive process aimed at limiting or preventing a further development of fat mass.

The decrease of FASN gene expression in adipose tissue contrasts with the enhanced hepatic lipogenesis, and the ultimate mechanism behind this discrepancy is still unclear. Insulin stimulates the transcription of lipogenic genes in rat hepatocytes and adipocytes, and also in human adipocytes (48, 49). It is possible that the difference in insulin concentration between portal and peripheral plasma plays a role in the in vivo observed difference between liver and adipose tissue lipogenic capacity in obese patients in some studies. The raised leptin levels of obese individuals could also play...
a role. There are data supporting a suppressive action of leptin on the transcription of FASN (50). Tumor necrosis factor α, whose expression and secretion by adipocytes is increased in obesity (51), could also explain the decrease in FASN mRNA levels, because it reduces the expression of several genes including FASN, in adipocytes (52).

Several studies have assessed the effect of carbohydrate overfeeding on glucose-induced whole body de novo lipogenesis, but only 4 have evaluated simultaneously adipose tissue FASN expression in lean and overweight humans. In one of these studies, whole-body de novo lipogenesis after overfeeding was lower ($P < 0.001$) and glycogen synthesis was higher ($P < 0.001$) in overweight than in normal-weight individuals. Carbohydrate overfeeding for 4 days led to a significant stimulation of FASN mRNA in both lean and overweight individuals: adipose tissue FASN mRNA increased by a mean of 1.8-fold, and the percentage stimulation was comparable in lean vs overweight individuals after carbohydrate overfeeding (45). The authors concluded that stimulation of adipose lipogenic enzymes was not higher in overweight individuals. Carbohydrate overfeeding did not stimulate whole-body net de novo lipogenesis nor expression of lipogenic enzymes in adipose tissue to a larger extent in overweight than lean individuals (45). In another study, 12 lean and 7 obese volunteers were given 2 eucaloric diets (10% vs 30% fat; 75% vs 55% carbohydrate; sugar/starch 60:40) each for 2 weeks by a random-order cross-over design (53). FASN mRNA in abdominal and gluteal adipose tissues was compared to hepatic de novo lipogenesis measured in serum by isotopic and nonisotopic methods. The low-fat high-sugar diet induced a 4-fold increase in maximum hepatic de novo lipogenesis ($P < 0.001$) but only a 1.3-fold increase in adipose tissue FASN mRNA ($P = 0.029$) and no change in cytokine mRNA (tumor necrosis factor α and IL-6). There was a borderline significant positive correlation between changes in FASN mRNA and hepatic de novo lipogenesis ($P = 0.039$). Compared to lean individuals, obese individuals had lower levels of FASN mRNA and higher levels of cytokine mRNA ($P < 0.001$) (53). In 2 other studies, no significant change in FASN mRNA expression was found after carbohydrate overfeeding in fasting conditions (54, 55).

**FASN IN THE PATHOGENESIS OF HUMAN CANCER**

The paradox of a common phenotypic behavior of cancer cells (i.e., unrelenting growth with invasion of surrounding normal tissues) considering their major genotypic diversity strongly suggests that a limited set of phenotypic mannerisms should exist in virtually all aggressive human malignancies. One of them has been recognized since the 1920s, with Otto Warburg’s observation that human cancer cells avidly consume glucose and produce lactic acid under aerobic conditions. High levels of carbon flux through aerobic glycolysis have become a hallmark of the transformed phenotype, because they may provide cancer cells a growth advantage in the tumor microenvironment (56). Remarkably, tumor cells catabolize glucose at a rate that exceeds bioenergetic need by shifting from oxidative to glycolytic metabolism. In this regard, exacerbated glycolysis is not only an important source of energy for tumors but it further provides important precursors for FA synthesis through the pentose phosphate pathway. In this scenario, tumor cells could redirect the excess glycolytic end product pyruvate toward de novo FA synthesis, which is necessary to maintain a constant supply of lipids and lipid precursors to fuel membrane production and lipid-based posttranslational modification of proteins in a highly proliferating cell population (Fig. 1).

FASN concentration normally remains at very low levels in noncancerous cells. Although FASN activity is not known to be regulated by allosteric effectors or covalent modification, the expression of FASN is highly dependent on nutritional conditions in lipogenic tissues. As described above, FASN-catalyzed endogenous FA biosynthesis in liver and adipose tissue is stimulated by a high-carbohydrate diet, whereas it is suppressed by the presence of small amounts of FAs in the diet and by fasting. FASN is also highly expressed in hormone-sensitive cells. During the menstrual cycle, the expression of FASN in the human endometrium is closely linked to the expression of the proliferation antigen Ki-67, estrogen receptor, and progesterone receptor, which suggest a functional connection between FASN and the estradiol ($E_2$)/estrogen receptor–dependent signaling in the normal control of endometrial cell proliferation (57). In normal mammary glands, the stimulation of FASN expression and activity at lactation is considered to be due to stimulatory effects of cortisol, prolactin, and insulin, facilitated by decreased production of progestins. Although the ultimate mechanisms responsible for cancer-associated FASN overexpression are not completely understood, 4 main mechanisms have been demonstrated: enhanced transcription of the FASN gene, gain of FASN gene copies, enhanced translation of FASN mRNA, and increased stabilization of FASN protein (Fig. 2). These modes of regulation of FASN, all of which are not mutually exclusive and may concurrently occur in tumor cells, efficiently prevent it from responding to physiological regulators, thus resulting in constitutive tumor-associated FASN overexpression and hyperactivity (Fig. 3). Importantly, the classic consideration of FASN-driven endogenous FA biogenesis as a minor anabolic-energy–storage pathway is no
longer valid in the cancer setting because the FASN-driven lipogenic phenotype, by conferring powerful growth and survival advantages upon extracellular biophysical and/or metabolic stresses, necessarily appears to accompany the pathogenesis and natural history of most human cancers (58, 59).

FASN, a Drug-Treatment Target to Disrupt the Pandemic Linkage Between Insulin Resistance, Type 2 Diabetes, and Cancer

Despite the recognized contribution of obesity to the increased incidence and/or death from various types of human malignancies, relatively little information exists on the ultimate molecular mechanisms by which obesity contributes to tumor formation and invasive/metastatic progression. The strongest empirical evidence supports the view that adiposity-related increases in insulin (e.g., chronic hyperinsulinemia, type 2 diabetes) and steroid hormones (e.g., E2, testosterone, progesterone) are the big actors connecting obesity and cancer (60). According to this “classical” view, the relationship of overweight/obesity to cancer is associated with adipose tissue as an active and metabolic “organ” that acts through endocrine, autocrine, and paracrine processes to promote cancer growth. Therefore, both the genesis and the progression of cancer may be

Fig. 1. Glucose metabolism and FA biosynthesis pathways.

About 25 enzymes are involved in the metabolism of glucose to fatty acids (FAs). The figure shows the key elements of the main synthetic pathways and their connections. Following cellular uptake by glucose transporters, glucose is phosphorylated by hexokinases (HK) to glucose-6-phosphate. Most of glucose-6-phosphate enters the glycolytic pathway to generate pyruvate and ATP. Pyruvate is converted to acetyl-CoA, which enters the citric acid cycle in the mitochondria. Depending on the oxygen availability citrate can be fully oxidized to generate ATP via oxidative phosphorylation, or it can be transported to the cytoplasm where it is converted back to acetyl-CoA (the requisite building block for FA synthase) by ATP citrate lyase (ACLY). Under anaerobic conditions pyruvate can also be used as electron acceptor, resulting in the lactate dehydrogenase (LDH)-catalyzed production of lactate, which is secreted from the cell. A portion of the acetyl-CoA is carboxylated to malonyl-CoA by acetyl-CoA carboxylase α (ACACA), the primary rate-limiting enzyme and site of pathway regulation. FASN, the main biosynthetic enzyme, performs the condensation of acetyl-CoA and malonyl-CoA to produce the 16-carbon saturated fatty acid palmitate and other saturated long-chain FAs, a process that is dependent on NADPH as reducing equivalents. NADPH (which is essential for FA synthesis) is provided in a reaction catalyzed by malic enzyme (not shown) or can be acquired via the pentose phosphate pathway. Saturated long-chain FAs can be further modified by elongases or desaturases to form more complex FAs, which are used for the synthesis of various cellular lipids such as phospholipids, triglycerides and cholesterol esters or for acylation of proteins.
Fig. 2. Molecular mechanisms regulating FASN expression in cancer cells. (A), Enhanced transcription of FASN gene. Growth Factors (GF) and GF Receptors (GFR) have emerged as major contributors, at the transcriptional level, to FASN overexpression in tumor cells. Steroid hormones (SH) including estradiol (E2), progestins (P), and androgens (A) also have an important role regulating FASN gene expression and FASN biosynthesis as part of the SH-driven Continuation...
caused by different obesity-related biological factors acting through various mechanisms, including changes in the synthesis and bioavailability of sex hormones, insulin resistance, release of growth factors and/or proinflammatory cytokines, and abnormal energetic disposal and expenditure. FASN-catalyzed endogenous FA biosynthesis, however, has been consistently forgotten as a feasible molecular “bridge” actively involved in the obesity-cancer pandemic linkage. Interestingly, recent experimental and epidemiological evidence could be exploited as proof-of-concept to definitely establish a causal role of FASN dysfunction in insulin-related metabolic disorders, including cancer.

**ALTERATION OF FASN ACTIVITY: GENETIC AND PHARMACOLOGICAL EVIDENCE**

A good proof-of-concept strategy to characterize FASN-driven endogenous FA biogenesis as a molecular link connecting worldwide obesity and cancer epidemics might consider a novel missense substitution in the FASN gene, which has recently been suggested to exert a protective effect against obesity in Pima Indians, one of the most obese populations in the world also having the highest reported prevalence of type 2 diabetes, and in German children (61, 62). This Val1483Ile substitution (missense mutation) at codon 1483 of the FASN gene, which is positioned within the peak of linkage to percentage of body fat on chromosome 17q25, appears to associate with lower percentage body fat and body mass index, with enhanced substrate oxidation rate, and with a protective effect in the development of obesity in white boys but not in girls. The change of Val for Ile in the 1483 position of the amino acid sequence is located within the interdomain region of the FASN protein that is close to 2 active centers of the FAS dimer. For this reason, this change in the structure of the FASN protein may alter the configuration of the catalytically active enzyme and thus, putatively, alter its biological activity. We recently evaluated adipose tissue FASN activity according to this FASN gene polymorphism and the association of this FASN gene variant with adiposity and insulin sensitivity in white adults. Preliminary analyses strongly suggest that individuals with a Val1483Ile polymorphism in the FASN gene could be protected from metabolic disorders associated with obesity. Thus, FASN activity significantly decreases in adipose tissue samples from carriers of the FASN Val1483Ile variant, and those individuals with decreased FASN activity linked to this polymorphism had increased insulin sensitivity (Fernandez-Real JM, Menendez JA, unpublished data). Considering that overweight and obesity associate with an increased risk of cancer development, and that a high level of FASN-catalyzed de novo FA biogenesis positively correlates with aggressive behaviors and poorer prognoses in various human carcinomas, future studies could examine whether FASN gene alterations leading to reduced FASN biosynthetic activity not only protect against obesity but further differentially modify the incidence and/or death from cancer in lean, overweight, and obese individuals.

**INSULIN RESISTANCE, CANCER, AND FASN: LESSONS FROM METFORMIN**

Hyperinsulinemia occurring in newly diagnosed breast cancer patients is strongly associated with obesity, a well-recognized adverse prognostic factor in breast cancer disease. Accordingly, studies of nondiabetic

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**Fig. 2. Continued.**

Cellular response leading to proliferation in hormonally responsive tumors. The effects of GF/GFR on FASN are complex and involve activation and/or cross-talk between multiple signal transduction pathways including the phosphatidylinositol-3 kinase (PI3K)/protein kinase B (AKT) and the mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK1/2) signaling cascades. The regulatory effects of SH on FASN gene expression, downstream of the SH Receptors (SHR) estrogen receptor (ER), progesterone receptor (PR), and androgen receptor (AR) also involve aberrant activation of the PI3K/AKT and MAPK ERK1/2 signal transduction pathways. These ultimately stimulate FASN expression through modulation of the expression and/or nuclear maturation of the transcription factor sterol regulatory element-binding protein (SREBP)-1c, which binds to and activates sterol regulatory elements in the promoter region of the target gene FASN. Cross-talk between GF/GFR and SH/SHR converging on PI3K-AKT and MEK/ERK cascades amplify responses of FASN expression in hormone-responsive cancer cells. (B). Gain of FASN gene copies. 17q22–17q24, the region to which the human FASN gene has been mapped, is prone to high-level amplification in tumor cells and, accordingly, gain of FASN gene copy number has recently been shown in prostate cancer tissues. It remains to be elucidated whether FASN gene amplification similarly occurs in other tumor types. (C). Enhanced translation of FASN mRNA. The 5′- and 3′-untranslated regions of FASN mRNA are involved in selective translational induction that is mediated by mammalian target of rapamycin (mTOR) and its downstream effector p70S6K1. (d) Increased stabilization of FASN protein. FASN protein has been found to interact with USP2a, a preproteasomal ubiquitin-specific protease. USP2a removes ubiquitin (which serves as a tag for regulated proteasomal destruction) from FASN, thus stabilizing the enzyme. It should be noted that these four pathways regulating FASN may operate concurrently in cancer cells.
Fig. 3. Molecular control of endogenous FASN levels in normal and cancer cells: similarities and differences. A similarity between FASN-catalyzed endogenous FA biogenesis in lipogenic tissues (A), hormone-sensitive tissues (B), and tumor cells (C) is that control of endogenous FASN levels preferentially occurs through modulation of the expression and/or maturation status of the transcription factor sterol regulatory element binding protein-1c (SREBP-1c), a critical intermediate of the pro- and antilipogenic actions of nutrients and hormones that stimulates FASN gene transcription when interacting with a complex SREBP-binding site at the endogenous FASN gene promoter. It is obvious, however, that the upstream mechanisms controlling FASN expression in cancer cells must be different, because tumor-associated FASN is insensitive to nutritional signals. Note that the upstream cascade depicted in panels (A–C) all interact with the downstream cascade depicted in (D), upstream lipogenic transduction. In lipogenic tissues such as hepatocytes and adipocytes (A), FASN gene expression is stimulated by a high carbohydrate diet, whereas it is inhibited by dietary FAs and fasting. This nutritional regulation of FASN expression in lipogenic cells is partially mediated by hormones which, through a modification of either the expression levels and/or the maturation status of SREBP-1c (see below) via PI-3 K /Akt and/or Ras/Raf/MEK1/MEK2 /ERK1/ERK2 MAPK transduction cascades, ultimately stimulate (insulin, triiodothyronine, glucocorticoids) or inhibit (leptin, glucagon, cyclic AMP) FASN-dependent lipogenesis. In hormone-sensitive tissues such as fetal lung, cycling endometrium, and lactating breast (B), steroid hormones (SH) including estradiol (E2), progestins (P), androgens (A), and prolactin (PRO) drive FASN gene expression and the FASN biosynthetic pathway downstream of their respective SH Receptors (SHR) by converging, at least in part, on the PI3K/AKT and MAPK ERK1/2 signal transduction pathways. In tumor cells (C), SREBP-1c expression and/or maturation will be Continued on page 433
women with early-stage breast cancer have shown that women with the highest fasting insulin levels had 3 times the risk of recurrence and death compared with women with the lowest insulin levels (63). On the other hand, recent clinical studies have revealed that treatment with the biguanide metformin, a first-line treatment for patients with type 2 diabetes, is significantly associated with reduced cancer risk (64, 65). In a study of more than 10 000 diabetic patients being treated with metformin or other sulfonylureas, those who were treated with sulfonylureas had an increased risk of cancer-related mortality compared to those patients on metformin. In a second study with a smaller cohort, it was observed that patients treated with metformin had a lower incidence of cancer compared to patients on other treatments. Importantly, this protective effect appeared to improve with higher doses of metformin. These 2 independent studies have led to the investigation of the use of metformin as an antineoplastic agent. Although the mechanism by which insulin and insulin sensitizers such as metformin influence breast cancer growth is the subject of intense research, it seems that insulin may signal, at least in part, through its own receptor to activate a cascade of proliferative and anti-apoptotic events. But, how might metformin treatment reduce cancer risk? Metformin is classically considered to function as an insulin sensitization agent that promotes reduced circulating insulin and glucose concentrations in hyperglycemic and hyper-insulinemic patients. Interestingly, metformin has many other beneficial effects, including the lack of additional weight gain or moderate weight loss when compared to other sulfonylureas, which work through the activation of the AMP-activated protein kinases (AMPK), very sensitive indicators of cellular energy status (66). The ability of metformin to activate AMPK has been suggested to constitute a direct (i.e., insulin-independent) mechanism of metformin against cancer cells, because it lastly promotes the inhibition of the AMPK downstream effector, mammalian target of rapamycin (mTOR) (67).

Importantly, a recent study evaluating the effects of metformin on the growth of human cancer cells in mice provided with either a control diet or a high-en-

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**Fig. 3. Continued.**

constitutively driven by the aberrant hyperactivation of PI3K/AKT and/or MAPK ERK1/2 pathways in response to a variety of oncogenic changes including overproduction of GF (e.g., EGF, IGF-1, heregulin), ligand-dependent or -independent hyperactivation of GFRs (e.g., EGFR, ERBB2) and/or gain or loss of function of components of the signaling cascade (e.g., loss of PTEN function). SH can also stimulate FASN gene expression in hormone-dependent tumor cells by upregulating the expression and/or nuclear maturation of SREBP-1c, a process that also appears to be driven by the activation of PI3K/AKT and MAPK ERK1/2 signaling cascades occurring in response to the specific binding of SHs such as A, P, and E2 to their counterpart receptors (AR, PR, and ER, respectively). It should be noted that cross-talk between GF, GFR, SH, and SHR further ensures robust responses of FASN gene in tumor cells. This model does not exclude that fundamental differences in the ability of tumor-associated FASN gene to respond to nutritional signals may also synergistically interact with oncogenic signals to further maintain and/or enhance a lipogenic phenotype in tumor cells in spite of, for instance, high levels of circulating dietary FAs. Downstream lipogenic transduction. The defining feature of the SREBP pathway is the proteolytic release of a membrane-bound transcription factor SREBP (D). Proteolytic cleavage frees it to move through the cytoplasm to the nucleus. Once in the nucleus, SREBP can bind the specific DNA sequence (the SREBP binding site) that is found the control region of the FASN gene promoter. This binding to SREBP-B5 leads to the increased transcription of the FASN gene. The approximately 120 kDa SREBP precursor transcriptionally inactive protein is anchored in the membrane of the endoplasmic reticulum (ER) and nuclear envelope by virtue of two membrane-spanning helices in the middle of the protein. Both the amino-terminal transcription factor domain and the COOH-terminal regulatory domain face the cytoplasm. The 2 membrane-spanning helices are separated by a loop that lies in the lumen of the ER. Two separate, site-specific proteolytic cleavages are necessary for release of the transcriptionally active amino-terminal domain. These cleavages are carried out by 2 distinct proteases, called site-1 protease (S1P) and site-2 protease (S2P). The regulated release of transcriptionally active SREBPs also requires SREBP cleavage activating protein (SCAP), which forms a complex with SREBP. When cellular demand for endogenous FAs rises, the SREBP:SCAP complex exits the ER and travels to the Golgi apparatus to encounter active S1P, which cleaves SREBP at site-1, cutting it into 2 halves that remain bound in the membrane. The newly generated amino-terminal half of SREBP is cleaved by S2P at the site-2 lying within its membrane-spanning helix. This releases the cytoplasmic portion of SREBP, which then travels to the nucleus, where it activates transcription of FASN gene. Supporting this model, inhibitors of PI3K and MAPK have been found to downregulate SREBP-1c and decrease FASN gene transcription, ultimately reducing endogenous lipogenesis. FASN overexpression by oncogenic stimuli can also be abrogated by deletion of the major SREBP binding site from the FASN promoter. Furthermore, active AKT can stimulate the synthesis and nuclear accumulation of activated SREBP-1c which, in turn, up-regulates FASN gene transcription. SH can also upregulate the proteolytic activation of SREBP-1c, thus directly enhancing the expression of one of its primary lipogenic target genes (i.e., FASN).
ergy diet (which leads to weight gain and systemic insulin resistance with hyperinsulinemia) demonstrated that metformin significantly attenuated the effect of diet on tumor growth (tumors of mice on the high energy diet were nearly twice the volume of those of mice on the control diet (68). However, metformin failed to modulate mice tumor growth on the control diet. Mechanistically, metformin attenuated the increased insulin–receptor activation associated with the high-energy diet and also led to increased phosphorylation of AMPK, 2 actions which would be expected to decrease neoplastic proliferation.

These above-mentioned epidemiological and experimental results are consistent with prior hypothesis-generating epidemiological studies that suggest metformin may reduce cancer risk and improve cancer prognosis, and further contribute to the rationale for evaluation of the FASN-related antineoplastic activity of metformin in hyperinsulinemic cancer patients. Considering that activation of AMPK is well known to inhibit the expression of gluconeogenic genes and promote the expression of enzymes required for FA oxidation, suppression of the lipogenic phenotype might explain, at least in part, the protective effects of metformin on cancer risk and progression. Activated AMPK chronically decreases the expression of the transcriptional regulator SREBP-1c (sterol regulatory element binding protein 1c), thus suppression of the transcriptional expression of FASN- and AMPK-related inhibition of mTOR may synergistically function to further suppress mTOR-regulated FASN expression at the translational level (69). Goodwin and colleagues recently completed a prospective phase II clinical trial of 32 nondiabetic breast cancer patients that showed that metformin reduced fasting insulin level by 22%, and this group is now planning a multicenter phase III randomized, double-blind, placebo-controlled trial of metformin in early-stage breast cancer patients (70). In addition to investigating the effects of metformin-based interventions on cancer outcomes, it would be of interest to address biological questions such as the impact of baseline insulin and FASN levels on therapeutic benefit, differential effects according to p53 and/or HER2 status, and the molecular mechanisms by which metformin-based interventions work.

**FASN as a Master Controller of Insulin-Sensitivity Status: From Diabetes to Cancer and Back?**

FASN-catalyzed de novo FA biogenesis could play a central role in the pathogenesis of metabolic diseases sharing an insulin-resistance “hallmark”. On one hand, FASN-catalyzed endogenous FA biogenesis appears to be necessary to integrate a number of signaling pathways that regulate lipid metabolism, proliferation, and survival in human epithelial cells. On the other hand, FASN lipogenic activity might coordinate the expression and/or activation status of genes and/or proteins closely related to the natural history of insulin-related metabolic diseases. This unexpected ability of FASN activity to modulate the expression status of key genes involved in the glucose–insulin axis is strongly supported when considering the figures from gene-expression profiling conducted to globally study the cellular processes affected by FASN blockade and the associations between FASN gene expression and serum glucose in vivo in humans (see supplemental Fig. 1 available at www.clinchem.org with the online version of this paper). Selected FASN target genes involved in the insulin-glucose metabolic axis are shown in Table 1.

A global assessment on the ultimate role of FASN expression and/or activity in humans is largely precluded because immunohistochemical and/or mRNA studies should be performed in tissue biopsies from individuals. In this regard, the quantitative determination of FASN molecules in blood might be considered a noninvasive and objective method to easily and rapidly identify FASN-related metabolic altered states of insulin sensitivity in human subjects. Several studies have demonstrated that FASN, a cytoplasmic protein, can also occur in the extracellular space of human cancer cells and in the blood of cancer patients (3–5). The current dogma in the field is that circulating FASN can be detected solely in the serum of cancer patients and can be used as a diagnostic marker. Accordingly, circulating FASN concentrations have been found to increase in parallel with different clinical stages compared with those measured in healthy individuals, and these findings have supported the perception that the extracellular form of FASN should be considered a tumor marker that can enable assessment of cancer virulence, because its upregulation is more pronounced in the late stages of tumors (3–5). That is, the excess intracellular FASN, which increases during progression of human cells toward malignancy, is actively excluded from the tumor cell in a stage-related manner. In the above-mentioned studies, no clinical or anthropometrical characteristics of the healthy volunteers were reported other than age and sex. We recently envisioned that, because energy metabolism, and especially dysfunction of glucose/lipid metabolism, is an early and nearly universal hallmark in most human malignancies, increased concentrations of extracellular/circulating FASN might also occur in other metabolic disorders in which insulin resistance is prominent, such as obesity, type 2 diabetes, or altered glucose tolerance. Intriguingly, we observed a strong relationship between higher concentrations of circulating FASN and the most pronounced insulin resistance in the absence of any evidence of concurrent neoplasias, and improvement in insulin action following weight loss led...
to decreased concentration of circulating FASN. In our hands, these unprecedented clinical findings were further supported by the following experimental evidence: administration of insulin sensitizers downregulated FASN release from cultured human adipose tissue explants, and triggering of insulin resistance following treatment of isolated adipocytes with inflammatory stimuli upregulated the amount of extracellular FASN.

### Table 1. Selected FASN target genes involved in the insulin-glucose metabolic axis.

<table>
<thead>
<tr>
<th>Gene</th>
<th>x-Fold change</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CAV1</strong> (caveolin 1, caveolae protein, 22kDa)</td>
<td>+6.6</td>
<td>Effective insulin signaling in the adipocyte may be strictly dependent on location of at least 2 insulin-responsive elements to caveolae (insulin receptor and the glucose transporter GLUT4), as well as on a direct functional interaction between caveolin-1 and the insulin receptor</td>
</tr>
<tr>
<td><strong>ITGB1</strong> (integrin, alpha 1 (fibronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12)) and <strong>ITGA6</strong> (integrin, alpha 6)</td>
<td>+6.0 and 7.0</td>
<td>Members of the integrin family of receptors that regulate cell migration through interactions with insulin growth factors (IGFs) and IGF binding proteins (IGFBPs)</td>
</tr>
<tr>
<td><strong>TNC</strong> (tenascin C)</td>
<td>+7.3</td>
<td>TNC participates in the ability of IGFBPs to modify IGF actions dependent on the amount that is associated to the extracellular matrix</td>
</tr>
<tr>
<td><strong>SPP1</strong> (secreted phosphoprotein 1)</td>
<td>+7.6</td>
<td>A bone-derived factor playing a key role in the recently emerged role of the skeleton as an endocrine organ with effects on body weight control and glucose homeostasis</td>
</tr>
<tr>
<td><strong>IGFBP-3</strong> (insulin-like growth factor binding protein-3)</td>
<td>+7.9</td>
<td>IGFBP-3, the major circulating carrier protein for IGFs, modulates IGF actions and also possesses intrinsic activities. In vitro and in vivo findings suggest that IGFBP-3 has potent insulin-antagonizing capability, supporting its role in cytokine-induced insulin resistance and other mechanisms involved in the development of type-2 diabetes</td>
</tr>
<tr>
<td><strong>THBD</strong> (thrombomodulin)</td>
<td>+8.0</td>
<td>THBD has been shown to play a role in the association between insulin resistance with accelerated atherosclerosis, especially coronary heart disease. Plasma-soluble THBD appears to reflect endothelial damage in the state of insulin resistance in patients with type-2 diabetes</td>
</tr>
<tr>
<td><strong>THBS1</strong> (thrombospondin 1)</td>
<td>+8.9</td>
<td>An antiangiogenic factor and regulator of transforming growth factor beta activity, obesity, adipose inflammation, and insulin resistance; THBS1 is a true adipokine that is highly expressed in obese, insulin-resistant individuals, and is highly correlated with adipose inflammation</td>
</tr>
<tr>
<td><strong>TGFB1</strong> (transforming growth factor, beta 1)</td>
<td>+9.3</td>
<td>TGFB1 is a potent growth inhibitor of normal breast epithelia that is able to indirectly mediate its growth-inhibitory effects by inducing the secretion of IGFBP3, which sequesters and prevents IGFs from binding and transducing mitogenic signals through the IGF receptors</td>
</tr>
<tr>
<td><strong>IGF2</strong> (insulin-like growth factor 2 (somatomedin A))</td>
<td>+9.9</td>
<td>This peptide produces marked insulin-like effects in various targets tissues</td>
</tr>
<tr>
<td><strong>IGFBP-1</strong> (insulin-like growth factor binding protein-1)</td>
<td>+10.0</td>
<td>IGFBP-1 is negatively regulated by insulin. Thus, elevated insulin and body fat are associated with decreased IGFBP-1 levels cross-sectionally, further indicating that IGFBP-1 levels may be altered through change in insulin over time and are a potential serum marker of insulin resistance</td>
</tr>
<tr>
<td><strong>GPC3</strong> (glypican 3)</td>
<td>+11.2</td>
<td>GPC3 is a cell-surface heparan-sulfate proteoglycan that acts as a growth suppressor by sequestering or downregulating IGF2. GPC2 may play roles in glucose transport through its direct interaction with GLUT4</td>
</tr>
<tr>
<td><strong>SPARC</strong> (secreted protein, acidic, cysteine-rich (osteonectin))</td>
<td>+11.3</td>
<td>Elevated adipocyte tissue expression of SPARC (secreted protein acidic and rich in cysteine), which markedly occurs in different animal models of obesity, is a newly identified autocrine/paracrine factor that could affect key functions in adipose tissue physiology and pathology</td>
</tr>
</tbody>
</table>

* Genes modified by >7.0-fold following 6-h treatment with the FASN blocker cerulenin compared to untreated control cells. The fold-change values represent the mean from 2 replicates.
ing FASN inhibitors should represent a valuable therapeutic approach that, in association with lifestyle interventions, would concurrently improve energy flux status, ameliorate insulin sensitivity, and alleviate the risk of lipogenic carcinomas (Supplemental Fig. 2b). Auspiciously, we are beginning to accumulate experimental and clinical evidence supporting the notion that insulin reduction might be exploited as a therapeutic modality in some human tumors, especially breast carcinomas. Trials of metformin in patients with type-2 diabetes as well as in patients with breast cancer receiving metformin as adjuvant treatment, primarily based on its ability to lower circulating insulin levels, might offer a good opportunity to directly identify the clinical relevance of disrupting the insulin-FASN axis in the prevention and/or treatment of type 2 diabetes and cancer.

FUTURE PERSPECTIVES

If insulin-resistant states such as obesity, type 2 diabetes, and cancer might arise from a common FASN-driven lipogenic state, then interventions aimed to modulated FASN expression and/or activity should significantly influence energy expenditure rates, fat mass, insulin sensitivity, and cancer risk susceptibility (see Supplemental Fig. 2a available at www.clinchem.org with the online version of this paper). A role for lipogenic enzymes in glucose homeostasis is further supported by the fact that mutant mice for the lipogenic enzyme acetyl-CoA carboxylase α are protected against obesity and diabetes induced by high-energy diets. Further studies are required to definitely elucidate whether the development and/or the progression of insulin-related metabolic disorders can be prevented or reversed by the modulation of FASN status. Accepting the paradigm of FASN dysfunctions as a previously unrecognized molecular bridge that functionally links insulin resistance, type 2 diabetes, and cancer, the use of insulin sensitizers along with forthcoming FASN inhibitors should represent a valuable therapeutic approach that, in association with lifestyle interventions, would concurrently improve energy flux status, ameliorate insulin sensitivity, and alleviate the risk of lipogenic carcinomas (Supplemental Fig. 2b). Auspiciously, we are beginning to accumulate experimental and clinical evidence supporting the notion that insulin reduction might be exploited as a therapeutic modality in some human tumors, especially breast carcinomas. Trials of metformin in patients with type-2 diabetes as well as in patients with breast cancer receiving metformin as adjuvant treatment, primarily based on its ability to lower circulating insulin levels, might offer a good opportunity to directly identify the clinical relevance of disrupting the insulin-FASN axis in the prevention and/or treatment of type 2 diabetes and cancer.

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