

Extracellular Fatty Acid Synthase: A Possible Surrogate Biomarker of Insulin Resistance

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CONTEXT—Circulating fatty acid synthase (FASN) is a biomarker of metabolically demanding human diseases. The aim of this study was to determine whether circulating FASN could be a biomarker of overnutrition-induced metabolic stress and insulin resistance in common metabolic disorders.

RESEARCH DESIGN AND METHODS—Circulating FASN was evaluated in two cross-sectional studies in association with insulin sensitivity and in four longitudinal studies investigating the effect of diet- and surgery-induced weight loss, physical training, and adipose tissue expansion using peroxisome proliferator-activated receptor agonist rosiglitazone on circulating FASN.

RESULTS—Age- and BMI-adjusted FASN concentrations were significantly increased in association with obesity-induced insulin resistance in two independent cohorts. Both visceral and subcutaneous FASN expression and protein levels correlated inversely with extracellular circulating FASN ($P = -0.63$; $P < 0.0001$), suggesting that circulating FASN is linked to depletion of intracellular FASN. Improved insulin sensitivity induced by therapeutic strategies that decreased fat mass (diet induced, surgery induced, or physical training) all led to decreased FASN levels in blood (P values between 0.02 and 0.04). To discriminate whether this was an effect related to insulin sensitization, we also investigated the effects of rosiglitazone. Rosiglitazone did not lead to significant changes in circulating FASN concentration.

CONCLUSIONS—Our results suggest that circulating FASN is a biomarker of overnutrition-induced insulin resistance that could provide diagnostic and prognostic advantages by providing insights on the individualized metabolic stress. *Diabetes* 59: 1506–1511, 2010

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The main problem of obesity is not as much the degree of expansion of the adipose tissue as the increased susceptibility to cardiometabolic risk. As obesity becomes more prevalent, a fundamental diagnostic challenge is to identify among the obese population individuals whose obesity causes more metabolic stress and who, consequently, are more likely to develop type 2 diabetes and cardiovascular diseases. Identification of these individuals has enormous obvious therapeutic and economic implications. Whereas there is general agreement that epidemiologically obesity poses a cardiometabolic risk, it is also true that not all obese individuals develop cardiometabolic complications. Conversely, it is also true that some individuals develop metabolic complications inappropriately severe for their degree of obesity. This suggests that the fat mass per se may not be the best predictor and that a marker that could indicate the metabolic stress posed by a specific degree of obesity may be particularly useful. However, there are no good early predictor markers of the metabolic risk posed by obesity. Thus, new therapeutic targets and biomarkers useful for diagnostic and prevention purposes, ideally pathogenically linked, are urgently needed.

Accumulating evidence suggests that lipid metabolism is as important, if not more, to diabetes as carbohydrate metabolism. The anabolic effects of insulin are not limited to facilitating glucose uptake. In fact, insulin is the most lipogenic hormone and exerts important effects on protein metabolism. Insulin is an important regulator of fatty acid synthase (FASN), a key enzyme in de novo lipogenesis. Coordinately with acetyl CoA carboxylase (ACC), FASN determines the lipogenic flux from malonyl CoA into palmitate (1,2). Expression of the *FASN* gene is primarily regulated by hormonal and nutritional signals (3,4), and insulin particularly not only increases the rate of *FASN* gene transcription in murine cell lines and in primary human adipocytes but also increases human *FASN* gene expression and FASN enzymatic activity (5–7). Conversely, FASN is markedly inactivated under conditions of insulin resistance. Therefore, we hypothesized that a marker related to the de novo lipogenic pathway, and more specifically FASN, might provide some insights on the level of impairment of insulin sensitivity and metabolic stress.

In support of FASN being a potential marker for metabolic stress, there is evidence that FASN, an intracellular protein, can also be detected at increased levels in the extracellular space of human cancer cells (8–10). It has been suggested that when FASN is excessively accumulated beyond the metabolic needs of the cellular system,

- **Cross-sectional studies**
 - Association of circulating FASN with insulin sensitivity in 2 cohorts (n=135 and n=98)
 - Association with fatty acid synthase gene expression in 1 cohort (n=98).
- **Longitudinal studies**

Study of the changes in circulating FASN induced by:

 - Diet-induced weight loss. Cohort 1 (n=15).
 - Exercise improvement of insulin sensitivity. Cohort 2 (n=8).
 - Bariatric surgery-induced weight loss. Cohort 3 (n=10).
 - Rosiglitazone improvement of insulin sensitivity. Cohort 4 (n=16).

FIG. 1. Summary of the studies performed on circulating FASN levels.

FASN can be actively extruded from the metabolically stressed tumor cell (10). In fact, altered energy metabolism, and especially dysfunction of glucose/lipid metabolism, is an early hallmark that contributes to the pathophysiology of several human malignancies (3,11–13). Current evidence indicates that extracellular/circulating FASN occurs exclusively during invasive/metastatic cancer progression.

In support of our hypothesis, there is evidence that increased extracellular/circulating FASN levels are detectable in human diseases typically associated with insufficiently met increased energy demands (e.g., hypoxia or increased nutrient demand in rapidly growing carcinoma cells). Because AMP-activated protein kinase (AMPK) is essential to ensure intracellular energy homeostasis (14–17), we recently assessed whether activation of phosphorylation (activation) status of AMPK, known to spare de novo lipogenesis, may directly facilitate the release of FASN in response to cellular energy depletion. AMPK activation not only decreased de novo lipogenesis but also facilitated the release of extracellular FASN in human breast cancer cells (18). These results support the hypothesis that FASN release is an active and controlled process regulated and facilitated by AMPK activation in the context of energy depletion.

Obesity-induced insulin resistance is characterized by the relative inability to take up glucose, leading to intracellular glucopenia associated with increased intracellular lipid deposition in adipose tissue. Insulin resistance also impairs the process of de novo lipogenesis in adipose tissue. We hypothesized that in the context of insulin resistance, energy depletion caused by intracellular glucopenia, activation of AMPK, and impaired lipogenesis may be coupled with increased secretion of FASN, resulting in increased circulating FASN levels. As such, circulating FASN could become a surrogate bona fide marker of the degree of metabolic compromise caused by a certain degree of obesity.

RESEARCH DESIGN AND METHODS

To test the main hypothesis, we evaluated circulating FASN in different cohorts of subjects, summarized in Fig. 1. The cross-sectional association of circulating FASN with insulin sensitivity was studied in two independent cohorts and the relationship with adipose tissue FASN gene expression in one cohort. Furthermore, we evaluated the effects of diet-induced weight loss (cohort 1), exercise improvement of insulin sensitivity (cohort 2), bariatric

surgery-induced weight loss (cohort 3), and rosiglitazone-associated improvement of insulin sensitivity (cohort 4).

All study protocols have been approved by the ethics committee of the participant institutions. All participants gave written informed consent before taking part in the study.

Cross-sectional studies

Cohort 1. One hundred and thirty-five Caucasian men were consecutively recruited in an ongoing study dealing with nonclassical cardiovascular risk factors in northern Spain. Recruiting criteria and analysis of insulin sensitivity are described as supplemental material in an online appendix (<http://diabetes.diabetesjournals.org/cgi/content/full/db09-1756/DC1>).

Cohort 2. Paired samples of visceral and subcutaneous adipose tissue were obtained from 98 consecutively recruited Caucasian men ($n = 49$) and women ($n = 49$) who underwent open abdominal surgery for gastric banding, cholecystectomy, weight reduction surgery, abdominal injuries, or explorative laparotomy. After explantation, samples of visceral and subcutaneous adipose tissue were immediately frozen in liquid nitrogen. Human visceral and subcutaneous adipose tissue FASN gene expression was measured by quantitative real-time RT-PCR as previously described (19). Age ranged from 17 to 79 years and BMI from 21.7 to 61.5 kg/m². The study included 62 patients with impaired glucose tolerance or type 2 diabetes and 36 controls with normal glucose tolerance. All subjects had a stable weight, defined as the absence of fluctuations of >2% of body weight for at least 3 months before surgery. Patients with malignant diseases or any acute or chronic inflammatory disease, as determined by a leukocyte count of >7,000 Gpt/l, C-reactive protein levels of >50 mg/l, or clinical signs of infection, were excluded from the study.

Studies of changing insulin action. The sample size and power calculations for the studies were based on our previous work (20), in which 12 subjects would enable the detection of 10% difference in insulin sensitivity index (S_i) with a 90% power at the 1% level.

Cohort 1: diet-induced weight loss. Fifteen Caucasian obese volunteers (12 female and three male, with an age range between 19 and 60 years) attending the Endocrinology Department at the University Clinic of Navarra were recruited. For more details, please see the online appendix.

Cohort 2. Eight healthy male subjects participated in a prospective randomized 12-week intensive-training intervention study. Inclusion criteria are shown in Appendix 1. In brief, each training session included 20 min of biking or running, 45 min of circle training, and 20 min of warming up and cooling down periods. All subjects completed a graded bicycle test to volitional exhaustion and had maximal oxygen uptake measured with an automated open-circuit gas-analysis system at baseline. The highest oxygen uptake per minute reached was defined as the maximal oxygen uptake (VO_{2max}), and subjects subsequently trained at their individual submaximal heart rate using heart rate monitors. At baseline and after 4, 8, and 12 weeks of training, blood samples were obtained in the fasting state.

Cohort 3. Ten women participated in a prospective weight loss study before and 12 months after gastric sleeve resection. The baseline BMI was 48.3 ± 4.2 kg/m², and the BMI 12 months after bariatric surgery was 37.2 ± 5.9 kg/m². Inclusion criteria can be found in the online appendix.

Cohort 4. We conducted a parallel-group randomized and controlled trial to evaluate the effects of rosiglitazone and physical exercise on endothelial function in patients with coronary artery disease and pre-diabetes over a 6-month period. Further information can be found in the online appendix.

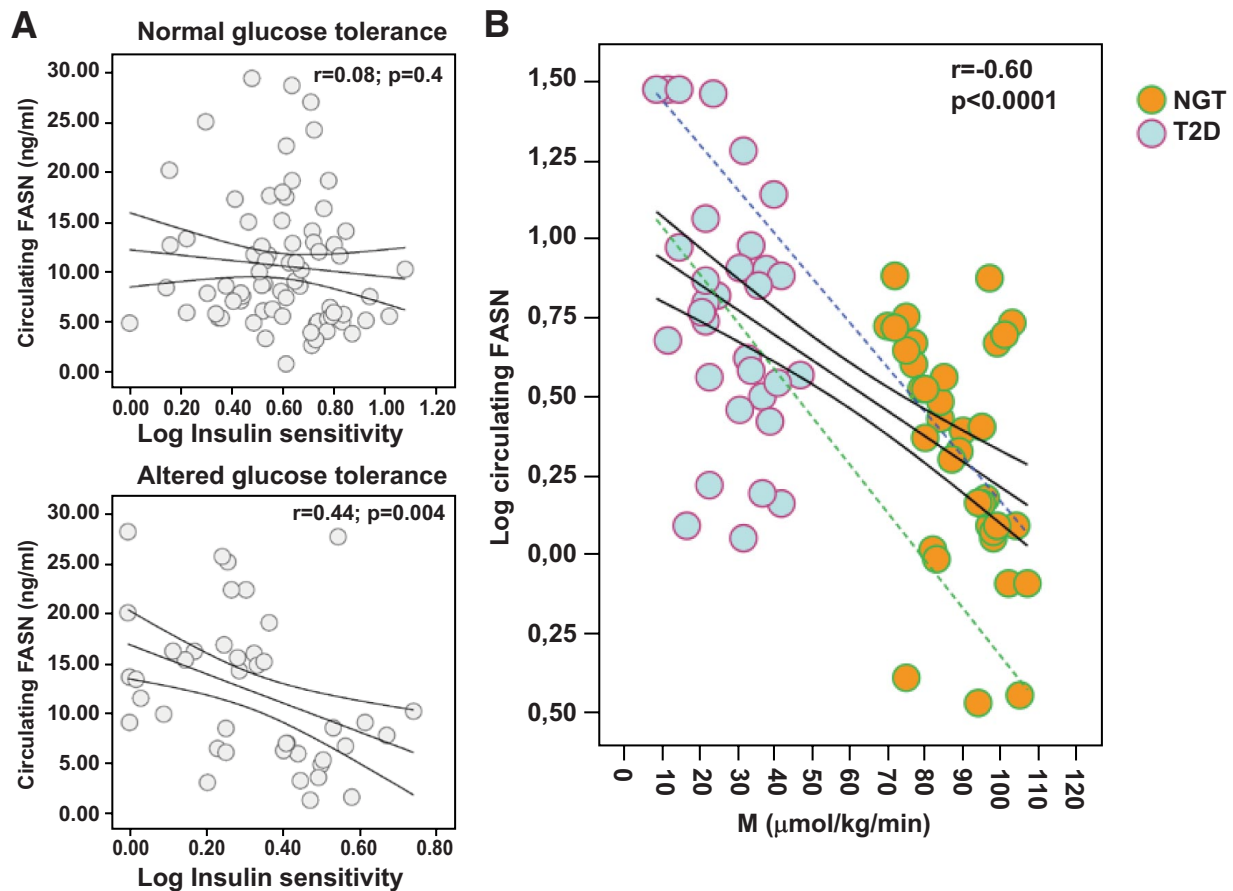


FIG. 2. A: Linear association between circulating FASN concentration and insulin sensitivity according to glucose tolerance status (*upper panel*: normal glucose tolerance; *lower panel*: altered glucose tolerance). Although the absolute value of circulating FASN is reported, the statistical analysis was performed using its log-transformed value. **B:** Linear association between log circulating FASN concentration and insulin sensitivity in all subjects (solid line with 95% CI) and according to glucose tolerance status (dotted lines). The association was also significant in each subgroup separately ($r = -0.43$, $P = 0.01$, in subjects with normal glucose tolerance [NGT] and $r = -0.39$, $P = 0.02$, in subjects with type 2 diabetes).

Analytical methods. FASN concentrations, centralized in a single laboratory, were measured in serum without additives by a sandwich enzyme immunoassay (FAS-Detect ELISA; FASgen, Baltimore, MD) according to the manufacturer's instructions. The within- and between-run coefficients of variation were $<10\%$, and the detection limit was 3.22 ng/ml. FASN concentrations were also measured by Western blot (see below) in 10 subjects (five insulin resistant and five insulin sensitive). Circulating FASN was also measured by Western blot in 10 subjects (five insulin resistant and five insulin sensitive). The remaining analytical methods and statistical analyses are described in the online appendix.

RESULTS

Serum FASN concentration is inversely associated with insulin sensitivity. Cross-sectional studies revealed that age- and BMI-adjusted serum FASN concentrations were inversely associated with insulin sensitivity in two independent cohorts of subjects. In the first cohort, the association was significant in all subjects as a whole ($r = -0.20$; $P = 0.02$), especially in subjects with altered glucose tolerance ($r = -0.44$; $P = 0.004$) (Fig. 2A). In the second cohort, the association was highly significant ($r = -0.60$; $P < 0.0001$) (Fig. 2B). As hypothesized, the highest levels of FASN concentrations in serum were observed in obese subjects with altered glucose tolerance (online appendix Table 1). Also in support of our hypothesis, FASN gene expression in visceral and subcutaneous adipose tissue correlated negatively with extracellular FASN in the obese glucose-intolerant group of subjects ($r = -0.44$, $P < 0.01$, and $r = -0.31$, $P < 0.05$, respectively). As

expected, these results were especially robust in the extreme of the spectrum corresponding with obese subjects who had developed type 2 diabetes ($r = -0.63$; $P < 0.0001$) (Fig. 3A). The relevance of these findings was confirmed with the protein levels in serum (Fig. 3B) and adipose tissue (not shown). This indicates that under metabolic stress and in association with insulin resistance, intracellular levels of FASN may decrease by the combined effects of decreased biosynthesis coupled with increased secretion of FASN protein, leading to increased plasma levels.

Studies of changing insulin action. From our results indicating that insulin resistance, a state where de novo lipogenesis and FASN biosynthesis is severely compromised, is associated with increased FASN levels in plasma, we hypothesized that strategies aiming at improving insulin sensitivity systemically should reverse this effect. To address this, we evaluated four different strategies known to improve insulin sensitivity but with a different impact on lipid biosynthesis: 1) lifestyle changes (diet-induced weight loss and physical training-induced improvement of insulin sensitivity), 2) surgery-induced weight loss, and 3) pharmacological intervention. An alternative AMPK-independent strategy was to promote safe fat accumulation and lipogenesis mediated by rosiglitazone in vivo (cohort 4), which we predicted would not result in decreased FASN secretion.

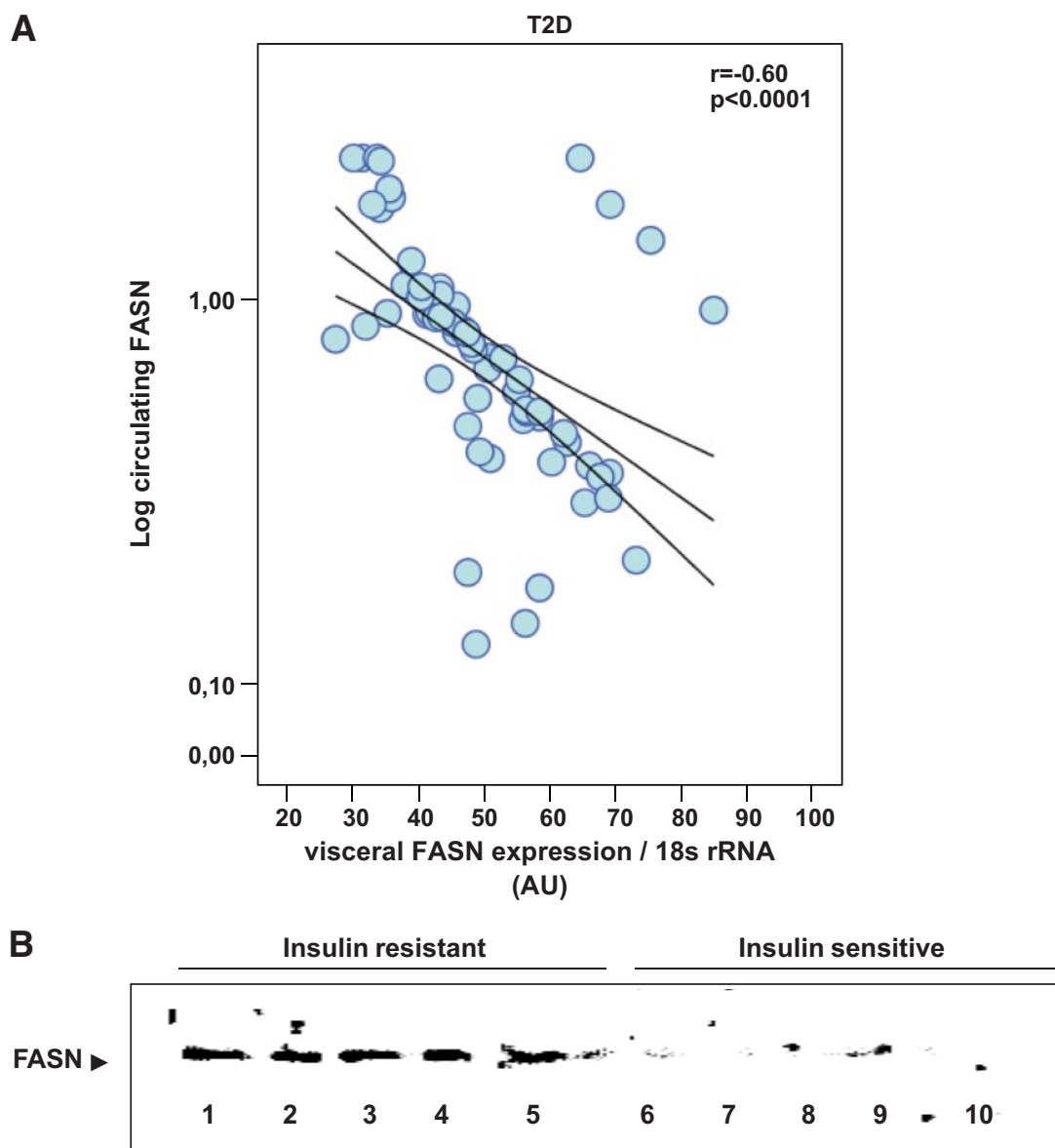


FIG. 3. A: Linear association between log circulating FASN concentration and FASN gene expression in visceral adipose tissue in subjects with type 2 diabetes. **B:** Western blot showing the fragment recognized by the antibody used in the enzyme-linked immunosorbent assay in insulin-resistant (lanes 1–5) and insulin-sensitive (lanes 6–10) subjects. Previous experience indicates that FASN is an ordered cellular response of a living cell that actively extrudes excess intracellular FASN molecules from the cell (reference 10). The extracellular FASN molecules are present in various immunoreactive forms and show different expression patterns. In serum of breast cancer patients, the FASN is a small molecule similar to the form in breast cancer cell lysate. We here substantiate that a 150 kDa fragment is preferentially detected in serum from subjects with insulin resistance.

The results showed that stabilization at low body weight using a diet-induced weight loss strategy (characteristics of the subjects shown in online appendix Table 2) led to significantly decreased circulating FASN concentration in those with altered glucose tolerance (i.e., initially elevated FASN plasma levels) and remained essentially unchanged in subjects with normal glucose tolerance (Fig. 4A) in whom circulating FASN levels initially were not affected. Exercise also reduced significantly circulating FASN concentrations in young men without any comorbidities (Fig. 4B). Similarly, surgery-induced weight loss by gastric sleeve resection also led to a significant lowering of serum FASN concentration (Fig. 4C). These experimental paradigms clearly indicate that under conditions where there is an improvement in insulin sensitivity associated with increased bioenergetic efficiency or

decreased intracellular FASN, there is a decrease in FASN levels in serum.

As an alternative, we used rosiglitazone in vivo (cohort 4), an AMPK-independent insulin sensitizer that binds and activates nuclear receptor peroxisome proliferator-activated receptor γ and can be predominantly considered anabolic, activating lipogenesis and promoting fat deposition. Under these conditions, FASN accumulated in adipose tissue, but this was not associated with a parallel increase in FASN in plasma (12.2 ± 3 ng/ml vs. 11.4 ± 4 ng/ml; $P = 0.5$) in vivo.

DISCUSSION

To the best of our knowledge, we have described the following for the first time: 1) A relationship between

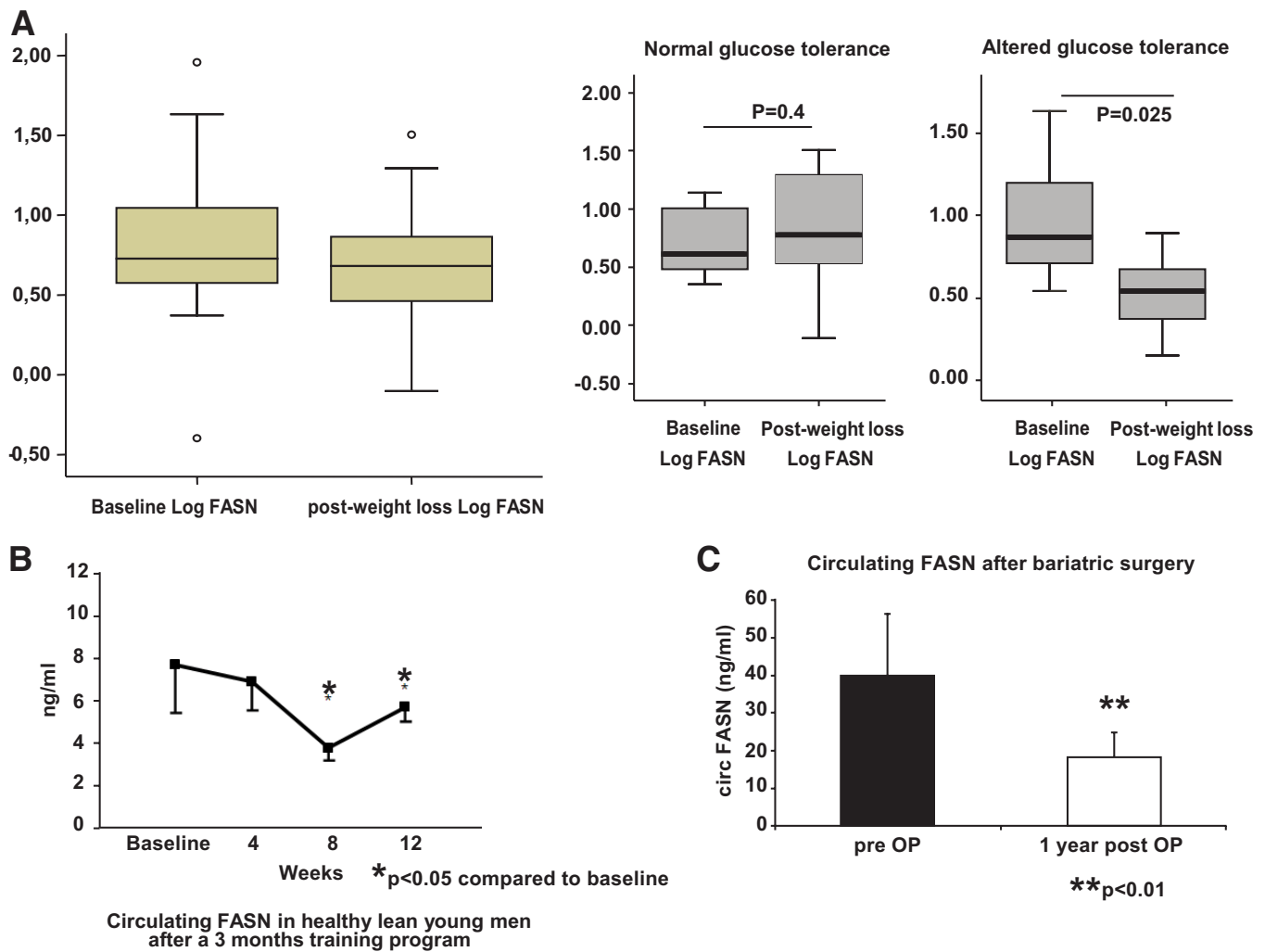


FIG. 4. A: Change in log circulating FASN concentration after weight loss in all subjects (left panel) and according to glucose tolerance status (right panels). B: Change in circulating FASN in healthy lean young men after 3-month physical training-induced improvement of insulin action. *P = 0.05 compared with baseline. C: Change in circulating FASN after surgery-induced weight loss. **P = 0.01.

severe insulin resistance and elevated levels of circulating FASN. 2) Circulating FASN was inversely associated with adipose tissue FASN expression. This supports the hypothesis that circulating extracellular FASN levels increase in parallel with the metabolic stress of the cells, as indicated by their decreased amounts of intracellular FASN levels. 3) Decreased concentration of circulating FASN is a good correlate of improvement in insulin action and metabolic control, as evidenced during subtle lifestyle interventions or surgery-induced weight loss. And 4) FASN plasma levels work as a marker of insulin sensitivity only in the context of metabolic stress, given that rosiglitazone, which promotes lipid biosynthesis and storage, did not lead to changes in circulating FASN.

We cannot exclude the possibility that the liver could also contribute to circulating FASN. In fact, weight loss patients typically improve in metabolic function quickly after massive weight loss, but liver disease caused by obesity is slower to reverse.

Our results indicate that states of overnutrition-induced insulin resistance and decreased de novo lipogenesis are associated with increased FASN in serum. Situations that improve insulin sensitivity either through decreasing substrate availability or optimizing fat deposition are associated with normalization of FASN in serum. In this respect, FASN

levels in plasma of obese individuals could be considered a marker of metabolic failure in the context of overnutrition-induced insulin resistance and metabolic stress.

The fact that FASN can be secreted actively from the cell in response to AMPK pharmacological activation (18) suggest that this could be a mechanism of physiological relevance in the context of nutritional deprivation. It is known that AMPK activation decreases lipogenesis de novo through its well-known effect of phosphorylating and inactivating ACC. Our results support the hypothesis that secretion of FASN could be a regulated process aiming to eliminate unnecessary FASN activity under conditions where lipogenesis is spared. It could be speculated that since FASN is a fundamental determinant of malonyl CoA levels, it would make biological sense to have a coupled mechanism associated with activation of AMPK-induced inactivation of ACC that prevented the collapse of malonyl CoA and its subsequent effects promoting uncontrolled fatty acid oxidation. In our opinion, in situations known to induce energy depletion and AMPK activation (fasting, weight loss, and physical exercise), the increased release and turnover of FASN may provide a mechanism to extrude this enzyme when is no longer needed under conditions of depleted energy stores.

Another fundamental question is whether extracellular FASN displays any signaling capacity. In our opinion, this is quite unlikely because the ~150 kDa NH₂-terminal fragment would likely not have FASN activity because several essential domains would be missing: ACP, TE, KR, and ER and probably the second half of the DH domain (21). The fragment likely is still capable of dimerization through the KS domains (E. Brignole, personal communication). Furthermore, the fragment cannot carry substrates (acyl or malonyl) because the ACP is absent. The fragment probably resembles a 142 kDa fragment that results from limited proteolysis of FASN with kallikrein (22) and splits the DH domain in half. Interestingly, plasma kallikrein is increased in diabetic patients (23).

Earlier studies demonstrated that cultured cancer cells can excrete immunoreactive FASN into the extracellular space in a time-dependent and hormone-independent manner (8–10). The current view is that circulating FASN can be detected solely in the serum of cancer patients. Here, we demonstrate that increased levels of extracellular/circulating FASN are also a common feature associated with hypernutrition-induced metabolic disturbances. Furthermore, our data suggest that measurements of FASN may have the added value of providing a measure of metabolic failure associated with a certain degree of obesity. We can envisage that FASN markers could have a better predictive value about metabolic stress and complications than BMI alone.

In summary, here we provide data indicating that FASN can be actively secreted under condition of overnutrition-induced metabolic stress and that FASN per se may be not only a diagnostic marker but also prognostic of obesity-associated metabolic complications. Thus, we hypothesize that FASN markers could be used to identify and prioritise obese patients for aggressive therapeutic intervention. We believe our data are interesting enough to warrant large-scale studies.

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No potential conflicts of interest relevant to this article were reported.

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