Fatty acid synthase gene expression in human adipose tissue: association with obesity and type 2 diabetes

J. Berndt · P. Kovacs · K. Ruschke · N. Klöting ·
M. Fasshauer · M. R. Schön · A. Körner · M. Stumvoll ·
M. Blüher

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Abstract

Aims/hypothesis Increased expression and activity of the lipogenic pathways in adipose tissue may contribute to the development of obesity. As a central enzyme in lipogenesis, the gene encoding fatty acid synthase (*FASN*) was identified as a candidate gene for determining body fat. In the present study we tested the hypothesis that increased *FASN* expression links metabolic alterations of excess energy intake, including hyperinsulinaemia, dyslipidaemia and altered adipokine profile to increased body fat mass.

Subjects and methods In paired samples of visceral and subcutaneous adipose tissue from 196 participants (lean or obese), we investigated whether *FASN* mRNA expression (assessed by PCR) in adipose tissue is increased in obesity and related to visceral fat accumulation, measures of insulin sensitivity (euglycaemic–hyperinsulinaemic clamp) and glucose metabolism.

Results *FASN* mRNA expression was increased by 1.7-fold in visceral vs subcutaneous fat. Visceral adipose tissue *FASN* expression was correlated with *FASN* protein levels, subcutaneous *FASN* expression, visceral fat area, fasting plasma insulin, serum concentrations of IL-6, leptin and retinol-binding protein 4 (RBP4), and inversely with measures of insulin sensitivity, independently of age, sex and BMI. Moreover, we found significant correlations between *FASN* expression and markers of renal function, including serum creatinine and urinary albumin excretion.

Conclusions/interpretation Increased *FASN* gene expression in adipose tissue is linked to visceral fat accumulation, impaired insulin sensitivity, increased circulating fasting insulin, IL-6, leptin and RBP4, suggesting an important role of lipogenic pathways in the causal relationship between consequences of excess energy intake and the development of obesity and type 2 diabetes.

J. Berndt · P. Kovacs · K. Ruschke · N. Klöting · M. Fasshauer ·
M. Stumvoll · M. Blüher (✉)
Department of Internal Medicine III, University of Leipzig,
Ph.-Rosenthal-Str. 27,
04103 Leipzig, Germany
e-mail: bluma@medizin.uni-leipzig.de

J. Berndt · P. Kovacs
Junior Research Group N06, Interdisciplinary Center of Clinical
Research Leipzig, Faculty of Medicine, University of Leipzig,
Leipzig, Germany

M. R. Schön
Department of Surgery II, University of Leipzig,
Leipzig, Germany

A. Körner
University Hospital for Children and Adolescents,
University of Leipzig,
Leipzig, Germany

Keywords Fatty acid synthase · Obesity · Type 2 diabetes

Abbreviations

CoA coenzyme A
CT computed tomography
FASN fatty acid synthase
RBP4 retinol-binding protein 4

Introduction

Fatty acid synthase (*FASN*) may play a role in the regulation of body weight and the development of obesity [1–4]. The body weight gain of normal subjects fed an excess energy intake (approximately 2.5 times energy expenditure, with the excess derived from carbohydrate)

cannot be explained by the increase in liver lipogenesis, suggesting that significant *de novo* lipogenesis occurs in other tissues, most likely adipose tissue [5]. However, although the key enzymes of fatty acid synthesis are present in human adipose tissue, their contribution to whole-body lipogenesis is considered to be lower than that of the liver [6, 7]. Feeding simple carbohydrates also substantially increases the activity of FASN [8], which is necessary for *de novo* synthesis of long-chain, saturated fatty acids from acetyl coenzyme A (CoA), malonyl CoA and NADPH [9]. Expression of the *FASN* gene and FASN activity are increased by insulin in cultured human adipocytes, suggesting that insulin sensitivity plays a role in their regulation [10]. Moreover, higher levels of *FASN* mRNA and FASN protein production were shown in insulin-resistant large adipocytes compared with small adipocytes in wild-type mice [11, 12].

Recent studies have reported that inhibition of FASN in rodents induces profound weight loss and reduced food intake, suggesting that FASN may be involved in obesity through regulation of feeding behaviour and energy homeostasis [1, 2, 13, 14]. The FASN inhibitor C75 acts both centrally to reduce food intake and peripherally to increase fatty acid oxidation [1, 14], leading to reduced fat mass and resolution of fatty liver in mice [2] and chickens [15] with diet-induced obesity. Whether these effects also play a role in human obesity needs to be clarified, especially since adipose tissue lipogenic capacity and *FASN* expression have been shown to be lower in humans than in rodents [6]. In turkeys, *FASN* gene polymorphisms are associated with increased fat mass [16]. Furthermore, in genetic studies, a novel missense mutation in the human *FASN* gene resulting in a Val>Ile substitution at codon 1483 was shown to be associated with lower percentage body fat and BMI in Pima Indians [3] and in German children [17]. In humans, FASN protein is produced at high levels in liver, lung and intra-abdominal adipose tissue [18]. Taken together, these studies demonstrate that *FASN* is a candidate gene for the pathophysiology of human obesity and its related metabolic alterations.

We therefore tested the hypothesis that increased FASN activity and *FASN* expression is a mediator that links the metabolic consequences of caloric excess, including insulin resistance, dyslipidaemia and altered adipokine serum profile, to increased body fat mass. Thereby, our aim was to dissect the most significant correlates of altered *FASN* expression in obesity and type 2 diabetes. We studied *FASN* mRNA expression in adipose tissue using quantitative real-time RT-PCR in paired samples of visceral and subcutaneous adipose tissue from 196 volunteers who varied widely in terms of obesity, body fat distribution, insulin sensitivity and glucose tolerance.

Subjects and methods

Paired samples of visceral and subcutaneous adipose tissue were obtained from 196 men ($n=98$) and women ($n=98$) who underwent open abdominal surgery for gastric banding, cholecystectomy, appendectomy, weight reduction surgery, abdominal injuries or explorative laparotomy. The subjects' age ranged from 24 to 86 years and BMI ranged from 20.8 to 54.1 kg/m². Using an OGTT, we identified 67 individuals with either type 2 diabetes ($n=36$) or IGT ($n=31$). 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 leucocyte count of >7,000 Gpt/l, C-reactive protein levels of >50 mg/l or clinical signs of infection, were excluded from the study. After explantation, samples of visceral and subcutaneous adipose tissue were immediately frozen in liquid nitrogen. The study was approved by the ethics committee of the University of Leipzig. All participants gave written informed consent before taking part in the study.

Assays and measures of glucose metabolism Basal blood samples were taken after an overnight fast. Plasma insulin was measured with an enzyme immunometric assay for the IMMULITE automated analyser (Diagnostic Products, Los Angeles, CA). Plasma adiponectin and leptin levels were assessed by RIA (Linco Research, St Charles, MO). Serum IL-6 levels were measured using an ELISA (Quantikine IL-6; R&D Systems, Oxford, UK). RBP4 (retinol-binding protein 4) was measured as previously described [19]. Patients consumed a high-carbohydrate diet for the 3 days prior to the OGTT. Following an overnight fast, the OGTT was performed using 75 g of standardised glucose solution (Glucodex solution 75 g; Merieux, Montreal, QC, Canada). Venous blood samples were taken at 0, 60 and 120 min for measurement of plasma glucose concentrations. Insulin sensitivity was assessed using the euglycaemic–hyperinsulinaemic clamp method [20]. A cut-off value for insulin resistance was arbitrarily chosen from the euglycaemic–hyperinsulinaemic clamp results of more than 120 individuals with the same population background and underwent the clamp under the same conditions as previously reported [21].

Measures of body fat content BMI was calculated as weight (in kg) divided by the square of height (in m). Waist and hip circumferences were measured, and the WHR was calculated. Percentage body fat was measured by dual-energy X-ray absorptiometry (DEXA). In addition, abdominal visceral and subcutaneous fat areas were

calculated using computed tomography (CT) scans at the level of L4–L5.

Analysis of human *FASN* mRNA and *FASN* levels Human *FASN* gene expression was measured by quantitative real-time RT-PCR in a fluorescent temperature cycler using the TaqMan assay, and fluorescence was detected on an ABI PRISM 7000 sequence detector (Applied Biosystems, Darmstadt, Germany). Total RNA was isolated from paired subcutaneous and visceral adipose tissue samples using TRIzol (Life Technologies, Grand Island, NY), and 1 µg RNA was reverse transcribed with standard reagents (Life Technologies). From each RT-PCR sample, 2 µl was amplified in a 26-µl PCR using the Brilliant SYBR Green QPCR Core Reagent Kit from Stratagene (La Jolla, CA) according to the manufacturer's instructions. Samples were incubated in the ABI PRISM 7000 sequence detector for an initial denaturation at 95°C for 10 min, followed by 40 PCR cycles of 95°C for 15 s, 60°C for 1 min and 72°C for 1 min. The following primers were used: human *FASN* (accession no. NM 004104) 5'-CGCGTGGCCGGCTACTCCTAC-3' (sense) and 5'-CGGCTGCCACACGCTCCTCT-3' (anti-sense). SYBR Green I fluorescence emissions were monitored after each cycle. Human *FASN* mRNA expression was calculated relative to the expression of 18S rRNA,

determined by a premixed assay on demand for human 18S rRNA (PE Biosystems). Amplification of specific transcripts was confirmed by melting curve profiles (cooling the sample to 68°C and heating slowly to 95°C with measurement of fluorescence) at the end of each PCR. The specificity of the PCR was further verified by subjecting the amplification products to agarose gel electrophoresis. *FASN* protein expression (visceral and subcutaneous) was measured in a representative subgroup of lean ($n=10$), visceral ($n=10$), and subcutaneous obese ($n=10$) subjects by western blot analysis using a human *FASN*-specific primary antibody (H-300; Santa Cruz, Heidelberg, Germany). Three blots of the samples were scanned using a Molecular Dynamics Storm PhosphoImager and quantified with ImageQuant software.

Statistical analyses Data are shown as means±SEM unless stated otherwise. Before statistical analysis, non-normally distributed parameters were logarithmically transformed to approximate a normal distribution. Differences in expression between visceral and subcutaneous adipose tissue were assessed using the paired Student's *t* test, and differences between more than two groups were analysed by the Bonferroni–Holm test. Linear relationships were assessed by least-square regression analysis. Multivariate linear relation-

Table 1 Anthropometric and metabolic characteristics of the study groups

	Lean		Predominantly visceraally obese		Predominantly subcutaneously obese	
	NGT	IGT/T2D	NGT	IGT/T2D	NGT	IGT/T2D
Men/women	28/25	5/8	9/12	10/11	29/26	17/16
Age (years)	48.8±2.37	39.0±3.2 ^a	61.2±2.6 ^b	61.4±3.7 ^b	57.8±2.2 ^b	53.6±3.2 ^{b,c}
BMI (kg/cm ²)	24.0±0.15	24.6±0.2	32.3±1.27 ^b	34.0±1.55 ^b	34.7±0.9 ^b	34.4±1.8 ^b
WHR	0.85±0.02	0.88±0.02	1.12±0.02 ^b	1.11±0.03 ^b	1.0±0.02 ^b	1.1±0.05 ^b
Body fat (%)	21.6±0.36	27±0.5 ^a	31.8±1.15 ^b	33.4±2.0 ^b	38.7±1.4 ^{b,c}	39.5±2.9 ^{b,c}
Visceral fat area (cm ²)	45.7±2.12	78±1.9 ^a	292.7±12.3 ^b	296.4±16.9 ^b	157.4±8.4 ^{b,c}	175.0±12.8 ^{a,b,c}
SC fat area (cm ²)	56.1±2.66	56±2.9	404±28.7 ^b	428±57 ^b	805.7±87.9 ^{b,c}	950.1±57.8 ^{a,b,c}
HbA _{1c} (%)	5.3±0.03	6.3±0.05 ^a	5.66±0.06 ^b	6.2±0.1 ^{a,b}	5.5±0.04	6.1±0.1 ^a
Fasting blood glucose (mmol/l)	5.3±0.05	7.8±0.2 ^a	5.3±0.10	6.2±0.2 ^{a,b}	5.3±0.08	6.4±0.2 ^{a,b}
Fasting plasma insulin (pmol/l)	30.1±1.77	125±24 ^a	125.3±16.8 ^b	262.3±23.9 ^{a,b}	112.0±9.0 ^b	282.0±46.4 ^{a,b}
2 h OGTT glucose (mmol/l)	5.9±0.1	13.5±2.9 ^a	6.3±0.20 ^b	12.0±1.1 ^a	6.0±0.1	11.6±1.0 ^a
Glucose infusion rate (mg kg ⁻¹ min ⁻¹)	96.0±1.23	59.0±5.9 ^a	48.0±6.6 ^b	26.3±4.9 ^{a,b}	65.2±4.1 ^{b,c}	49.3±9.9 ^{a,c}
NEFA (mmol/l)	0.3±0.02	0.6±0.01 ^a	0.7±0.06 ^b	0.8±0.08	0.5±0.04 ^{b,c}	0.6±0.1 ^c
Total cholesterol (mmol/l)	5.3±0.13	5.3±0.11	5.6±0.2 ^b	6.0±0.2 ^{a,b}	5.0±0.1	5.5±0.2 ^{a,c}
HDL-cholesterol (mmol/l)	1.6±0.08	1.1±0.09 ^a	1.3±0.05 ^b	1.41±0.08 ^{a,b}	1.4±0.05 ^b	1.5±0.1 ^b
LDL-cholesterol (mmol/l)	2.9±0.11	3.0±0.2	3.3±0.1 ^b	3.6±0.2 ^b	2.6±0.08 ^c	3.2±0.2 ^{a,c}
Leptin (pmol/l)						
Men	3.3±0.80	3.8±0.3	22.9±2.9 ^b	20.4±4.6 ^b	21.5±1.9 ^b	14.3±2.4 ^{a,b,c}
Women	8.4±1.0	9.2±0.5	32.3±3.7 ^b	38.6±6.0 ^b	28.0±2.2 ^b	32.3±3.2 ^b

Data are means±SEM

^a $p<0.05$ for IGT/T2D vs NGT within the same study group; ^b $p<0.05$ for visceral or subcutaneous obese vs lean; ^c $p<0.05$ for visceral obese vs subcutaneous obese

SC subcutaneous, T2D type 2 diabetes

Fig. 1 *FASN* mRNA expression in visceral and subcutaneous adipose tissue in lean, obese, NGT, IGT and type 2 diabetic subjects. *FASN* mRNA levels in the entire study population ($n=196$) (a), male ($n=98$) and female ($n=98$) participants (b), subgroups of lean ($n=53$), visceral obese (vis, $n=21$) and subcutaneous obese (SC, $n=55$) participants with NGT (c), subgroups of participants with NGT ($n=129$) or IGT or type 2 diabetes (IGT/T2D, $n=67$) (d). Visceral and subcutaneous fat areas were calculated using CT scans at the level of L4–L5. e *FASN* protein levels were measured in homogenates of visceral adipose tissue from lean ($n=10$), visceral ($n=10$), and subcutaneous obese ($n=10$) participants. For each group, a representative western blot and quantitation of the data from three independent experiments are shown. f Correlation between *FASN* mRNA expression and *FASN* protein levels in visceral adipose tissue ($R^2=0.5$, $p<0.001$). Values are means $\pm$ SEM. * $p<0.01$ for comparisons of fat depot-specific levels within the groups; # $p<0.01$ for differences between the obesity groups and lean subjects; + $p<0.05$ for differences between T2D and NGT

ships were assessed by a general linear model. Statistical software from the SAS Institute (Cary, NC) was used. A p value of <0.05 was considered to be statistically significant.

Results

Visceral and subcutaneous *FASN* mRNA and *FASN* levels The anthropometric and metabolic characteristics of

the volunteers are summarised in Table 1. Analysis of 198 paired samples of visceral and subcutaneous adipose tissue revealed that *FASN* mRNA expression was ~ 1.7 -fold higher in visceral vs subcutaneous adipose tissue, both in men and women (Fig. 1a,b). There was no age-dependent effect on *FASN* gene expression (Table 2). To investigate whether these differences in expression can be explained by differences in body fat mass or fat distribution, we performed additional analyses in subgroups of lean ($\text{BMI}<25 \text{ kg/m}^2$) and obese ($\text{BMI}>30 \text{ kg/m}^2$) participants with normal

Table 2 Multivariate linear regression analyses showing the association of *FASN* mRNA expression in visceral subcutaneous fat with age and anthropometric and metabolic parameters for the entire study population ($n=198$)

	Visceral <i>FASN</i> mRNA β -Coefficient (p value)	Subcutaneous <i>FASN</i> mRNA β -Coefficient (p value)
Model 1		
Age (years)	0.13 (0.16)	0.02 (0.7)
Sex	-0.09 (0.31)	-0.022 (0.7)
Model 2		
Age (years)	-0.01 (0.88)	0.08 (0.39)
Sex	-0.09 (0.27)	-0.08 (0.4)
Body fat (%)	0.53 (<0.0001) ^a	0.29 (0.01) ^a
Model 3		
Age (years)	-0.017 (0.82)	0.076 (0.36)
Sex	-0.086 (0.25)	-0.088 (0.29)
Visceral fat area (cm ²)	0.47 (0.0001) ^a	0.2 (0.02) ^a
Model 4		
Age (years)	-0.06 (0.4)	0.006 (0.9)
Sex	-0.15 (0.075)	-0.1 (0.22)
Visceral fat area (cm ²)	0.35 (0.001)	0.1 (0.21)
GIR (mg kg ⁻¹ min ⁻¹)	-0.24 (0.02) ^a	-0.28 (0.002) ^a

^a Significant coefficients

GIR glucose infusion rate during the steady state of the euglycaemic-hyperinsulinaemic clamp

glucose tolerance. Based on CT scan measurement (L4–L5) of abdominal visceral and subcutaneous fat areas, obese subjects were further categorised as having predominantly visceral or subcutaneous obesity. Predominantly visceral obesity was defined as a ratio of visceral:subcutaneous fat area of >0.5, as previously described [22]. *FASN* mRNA levels were confirmed to be ~1.7-fold higher in visceral vs subcutaneous adipose tissue of all subgroups (Fig. 1c), and this increase was independent of differences in obesity and fat distribution. Visceral *FASN* mRNA expression was 2.1-fold higher and subcutaneous *FASN* mRNA expression was 2.4-fold higher in obese vs lean individuals (Fig. 1c). We went on to investigate whether impaired glucose metabolism in patients with either IGT type 2 diabetes is associated with altered *FASN* expression in different fat depots. Subgroup analyses demonstrated that IGT and type 2 diabetic patients were indistinguishable with regard to *FASN* mRNA measurements, and therefore these groups were studied together. Patients with IGT/type 2 diabetes had significantly higher *FASN* expression, both in visceral and subcutaneous fat, compared with individuals with NGT (Fig. 1d). *FASN* expression was ~1.4-fold higher in visceral vs subcutaneous fat in IGT/type 2 diabetic patients. In a representative subgroup of lean ($n=10$), predominantly

viscerally obese ($n=10$) and predominantly subcutaneously obese ($n=10$) participants, we measured visceral and subcutaneous *FASN* protein levels (Fig. 1e). In parallel with the relatively higher levels of visceral vs subcutaneous *FASN* mRNA expression, visceral *FASN* protein levels were significantly increased in all subgroups (Fig. 1e). There were significant correlations between *FASN* mRNA and protein levels in subcutaneous ($R^2=0.44$, $p<0.001$) and visceral fat depots ($R^2=0.5$, $p<0.001$; Fig. 1f). In addition, there was a significant correlation between visceral and subcutaneous *FASN* mRNA expression across the entire study cohort ($R^2=0.5$, $p<0.001$; Fig. 2a), as well as in each of the subgroups (data not shown).

Correlation of *FASN* mRNA expression with parameters of obesity, glucose metabolism, and insulin sensitivity In the entire cohort ($n=198$), univariate regression analysis revealed significant positive correlations between visceral and subcutaneous *FASN* mRNA expression and BMI, percentage body fat, visceral and subcutaneous fat area, WHR, fasting plasma insulin, HbA_{1c}, NEFA, leptin, IL-6 and RBP4. There was an inverse correlation between visceral *FASN* mRNA expression and glucose uptake during the steady state of the euglycaemic-hyperinsulinaemic clamp. The correlation between visceral *FASN* mRNA expression and BMI ($R^2=0.24$, $p<0.0001$), visceral fat area ($R^2=0.22$, $p<0.001$; Fig. 2b), glucose infusion rate during the steady state of an euglycaemic-hyperinsulinaemic clamp ($R^2=0.2$, $p<0.01$; Fig. 2c), fasting plasma insulin concentrations ($R^2=0.13$, $p=0.03$; Fig. 2d), IL-6 ($R^2=0.2$, $p<0.0001$), and RBP4 ($R^2=0.2$, $p<0.01$; Fig. 3a) remained significant even upon adjusting for age, sex and percentage body fat. Interestingly, there were no significant correlations between subcutaneous *FASN* gene expression and anthropometric and metabolic parameters beyond those with age, sex and percentage body fat. Multivariate linear regression analysis revealed percentage body fat, visceral fat area and glucose infusion rate during clamp as significant predictors of visceral *FASN* mRNA expression, whereas only glucose infusion rate was a significant predictor of subcutaneous *FASN* mRNA expression (Table 2).

Of the adipokines, IL-6 was found to be the best predictor of visceral *FASN* mRNA expression (Table 3). Further analysis of other serum markers of obesity and/or insulin resistance showed that leptin and RBP4 serum concentrations are significant predictors of visceral *FASN* mRNA expression, and these associations were independent of age, sex and percentage body fat (Table 3).

Higher visceral *FASN* mRNA expression is related to increased markers of renal dysfunction Increased *FASN* expression was reported in experimental chronic renal failure in animal models [23]. We therefore investigated

Fig. 2 Correlations between visceral (vis) *FASN* mRNA expression and **a** subcutaneous (SC) *FASN* mRNA expression ($R^2=0.5$, $p<0.001$), **b** intraabdominal fat area (IAFA) ($R^2=0.22$, $p<0.001$), **c** glucose uptake during the steady state of an euglycaemic–hyperinsulinaemic clamp and ($R^2=0.2$, $p<0.01$) **d** fasting plasma insulin concentration ($R^2=0.13$, $p=0.03$). Data are log transformed to achieve normal distribution

the relationships of *FASN* mRNA expression in obese subjects and patients with IGT/type 2 diabetes with parameters of renal function. We found a significant correlation between visceral *FASN* expression and serum creatinine concentrations ($R^2=0.26$, $p=0.005$; Fig. 3b), independent of age, sex and percentage body fat. However, adjusting for serum creatinine in multivariate regression analyses did not change significant relationships between *FASN* expression and percentage body fat, visceral fat area, glucose infusion rate during the steady state of the euglycaemic–hyperinsulinaemic clamp, fasting plasma in-

sulin and IL-6 concentrations. Moreover, in IGT/type 2 diabetic patients, urinary albumin excretion was correlated with visceral *FASN* mRNA expression ($R^2=0.15$, $p=0.03$), even after adjusting for age, sex and percentage body fat.

Discussion

FASN is the central enzyme in de novo lipogenesis, catalysing the conversion of malonyl CoA into palmitate

Fig. 3 Correlation of *FASN* mRNA expression with **a** RBP4 ($R^2=0.2$, $p<0.01$) and **b** creatinine serum concentrations ($R^2=0.26$, $p=0.005$). Data are log transformed to achieve normal distribution

Table 3 Multivariate linear regression analysis of adipokines and parameters of dyslipidaemia as predictors of visceral *FASN* mRNA expression adjusted for age, sex and percentage body fat ($n=196$)

Parameter	Visceral <i>FASN</i> mRNA		Subcutaneous <i>FASN</i> mRNA	
	β -Coefficient	<i>p</i> value	β -Coefficient	<i>p</i> value
IL-6	0.36	<0.001	0.3	0.005
Leptin	0.28	0.008	0.24	0.009
RBP4	0.26	0.005	0.1	0.09
NEFA	0.14	0.06	0.09	0.1
Triacylglycerol	0.02	0.7	0.01	0.7
Adiponectin	−0.01	0.8	−0.1	0.08

[8, 9]. In a recent study it was demonstrated that insulin, both alone and in combination with dexamethasone, increased human *FASN* gene expression and concomitantly elevated FASN activity [24]. Although the contribution of human adipose tissue to whole-body lipogenesis is considered to be low and less than that of liver [6, 7], adipose tissue remains an important site of endogenous fatty acid synthesis [25]. Expression of the *FASN* gene is primarily regulated by hormonal and nutritional signals [26]. FASN activity measurements and *FASN* mRNA levels are frequently used as markers of de novo lipogenesis, and changes in FASN activity are primarily due to alterations in the transcription rate of the *FASN* gene [10, 24, 27]. It was demonstrated in both murine cell lines and primary human adipocytes that insulin and angiotensin II increase the rate of *FASN* gene transcription [10, 27]. In the present study we demonstrate that both visceral and subcutaneous *FASN* mRNA expression are closely correlated with FASN protein levels in both depots.

The important role of *FASN* as a candidate gene for the development of obesity was demonstrated in interventional [1, 2, 14] and genetic studies [3, 16, 17]. Inhibition of FASN in rodents causes reduced food intake and profound weight loss [1, 2]. It is noteworthy that adipose tissue lipogenic capacity and *FASN* expression and FASN production are lower in humans vs rodents [6], and therefore it still needs to be clarified whether the results of rodent studies are applicable for the human regulation of adipose lipogenesis. However, a missense substitution in the *FASN* gene was found to be associated with percentage body fat in non-diabetic Pima Indians [3].

Consequences of excess energy intake include hyperinsulinaemia, elevated triacylglycerol and NEFA serum concentrations, increased or decreased circulating adipokines and others. These outcomes could potentially lead to higher *FASN* expression and FASN activity, resulting in increased lipogenesis. Thus, we hypothesised that *FASN* expression plays a role in the causal relationship between

the metabolic consequences of excess energy intake and increased fat mass. We therefore investigated in vivo whether increased *FASN* gene expression in human adipose tissue contributes to the development of obesity. We also aimed to determine which metabolic abnormalities are significantly related to *FASN* mRNA expression in adipose tissue. *FASN* gene expression was studied in adipose tissue using quantitative real-time RT-PCR in paired samples of visceral and subcutaneous adipose tissue from 196 volunteers who varied widely in terms of obesity, body fat distribution and metabolic parameters. We used correlational analysis to dissect whether and to what extent *FASN* mRNA expression is explained by the variability in anthropometric and metabolic parameters. *FASN* mRNA expression was significantly higher in visceral vs subcutaneous adipose tissue, independent of BMI or body fat distribution. This finding is in accordance with high *FASN* expression in intra-abdominal adipose tissue [18]. Although there was a correlation between visceral and subcutaneous *FASN* mRNA expression, the fact that visceral *FASN* expression was in general higher than subcutaneous expression suggests that FASN predominantly mediates the adverse effects of obesity and excess energy intake in visceral fat. *FASN* gene expression is significantly higher in obese vs lean individuals, supporting our previous results that *FASN* expression and FASN levels correlate with adipocyte size [11, 12]. In contrast to our results, Diraion et al. [4] found that *FASN* mRNA expression was decreased in the subcutaneous adipose tissue of obese vs lean individuals [4]. Differences in nutritional status, metabolic parameters and the size of the study population (the previous study [4] was of much smaller size) might explain these divergent findings.

The results of the present study also demonstrate that adipose *FASN* gene expression is higher in patients with type 2 diabetes or IGT vs individuals with NGT. Independently of age, sex and body fat mass, increased *FASN* expression was shown to be primarily related to impaired insulin sensitivity, whereas no correlation was found between *FASN* expression and acute or chronic hyperglycaemia, which goes beyond the relationships with BMI or percentage body fat. Since *FASN* gene expression is increased by insulin stimulation [10], elevated plasma insulin concentrations in insulin-resistant states could represent a potential mechanism for the increased *FASN* expression in the adipose tissue of obese and insulin-resistant subjects. As previously shown in mice with an adipose tissue-specific deletion of the insulin receptor, impaired insulin sensitivity in fat does not lead to decreased FASN protein production in large adipocytes [11].

Our study design also allowed us to investigate the relationships between *FASN* mRNA expression and serum concentrations of different adipokines and circulating

markers of insulin resistance. In multivariate linear regression analyses we found that circulating levels of IL-6 predict visceral *FASN* expression, independently of age, sex and percentage body fat. Visceral *FASN* gene expression was also found to be related to serum concentrations of RBP4 and leptin. Leptin could directly induce *FASN* mRNA expression in adipose tissue, since adipocyte-selective reduction of the leptin receptor in mice has been observed to decrease *FASN* mRNA expression [28]. However, experimentally increased plasma leptin concentrations in rats resulted in a decrease of *FASN* mRNA levels in fat [29]. Notably, in chickens, leptin was shown to increase *FASN* gene expression in the liver [30]. Therefore, we cannot exclude the possibility that increased serum concentrations of leptin in obese subjects contribute to increased *FASN* mRNA expression in fat. Circulating levels of RBP4 were identified as another significant predictor of *FASN* mRNA expression. Since RBP4 is closely associated with obesity and insulin resistance [19, 31], further studies are required to elucidate a potential direct role of RBP4 in stimulating *FASN* gene expression. Multivariate regression analysis also identified a significant association of visceral *FASN* expression and creatinine serum concentrations. Moreover, in patients with type 2 diabetes/IGT, we found a significant correlation between microalbuminuria and adipose tissue *FASN* mRNA expression. These results confirm previous studies in rodents, showing that *FASN* gene expression is upregulated in experimental chronic renal failure [23, 32]. However, patients with renal dysfunction were excluded from our study. Notably, adjusting for either serum creatinine or microalbuminuria had no effect on significant relationships between *FASN* expression, percentage body fat, visceral fat area and parameters of insulin resistance. Therefore, *FASN* expression appears to be independently related to renal function, but elucidation of the exact mechanisms will require more sophisticated methods and study designs.

In conclusion, although it is not possible to establish a true causability chain, our data are compatible with the notion that increased *FASN* expression in adipose tissue links excess energy intake and accumulation of body fat. Thereby, adipose *FASN* mRNA expression is significantly related to obesity, predominantly visceral fat accumulation, impaired insulin sensitivity and circulating adipokines. Moreover, in addition to chronic hyperinsulinaemia, elevated leptin and RBP4 serum concentrations in obesity might contribute to increased *FASN* expression. Thus, inhibition of *FASN* specifically in adipose tissue may be a promising therapeutic approach in the prevention and treatment of obesity and its complications.

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