Adipose and Muscle Tissue Profile of CD36 Transcripts in Obese Subjects Highlights the Role of CD36 in Fatty Acid Homeostasis and Insulin Resistance

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OBJECTIVE

Fatty acid (FA) metabolism is tightly regulated across several tissues and impacts insulin sensitivity. CD36 facilitates cellular FA uptake, and *CD36* genetic variants associate with lipid abnormalities and susceptibility to metabolic syndrome. The objective of this study was to gain insight regarding the in vivo metabolic influence of muscle and adipose tissue CD36. For this, we determined the relationships between CD36 alternative transcripts, which can reflect tissue-specific CD36 regulation, and measures of FA metabolism and insulin resistance.

RESEARCH DESIGN AND METHODS

The relative abundance of alternative *CD36* transcripts in adipose tissue and skeletal muscle from 53 nondiabetic obese subjects was measured and related to insulin sensitivity and FA metabolism assessed by hyperinsulinemic–euglycemic clamps and isotopic tracers for glucose and FA.

RESULTS

Transcript 1C, one of two major transcripts in adipose tissue, that is restricted to adipocytes predicted systemic and tissue (adipose, liver, and muscle) insulin sensitivity, suggesting adipocyte CD36 protects against insulin resistance. Transcripts 1B and 1A, the major transcripts in skeletal muscle, correlated with FA disposal rate and triglyceride clearance, supporting importance of muscle CD36 in clearance of circulating FA. Additionally, the common *CD36* single nucleotide polymorphism rs1761667 selectively influenced *CD36* transcripts and exacerbated insulin resistance of glucose disposal by muscle.

CONCLUSIONS

Alternative *CD36* transcripts differentially influence tissue CD36 and consequently FA homeostasis and insulin sensitivity. Adipocyte CD36 appears to be metabolically protective, and its selective upregulation might have therapeutic potential in insulin resistance.

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The appropriate regulation of fatty acid (FA) metabolism is important for normal energy homeostasis, insulin sensitivity, and metabolic health. FA utilization is regulated across multiple tissues. Dietary-derived FAs taken up by adipose tissue are primarily converted to triglycerides (TGs) for storage in cytosolic lipid droplets.

PATHOPHYSIOLOGY/COMPLICATIONS

During periods of fasting and exercise, there is increased FA mobilization from adipocytes and FA delivery to tissues, particularly skeletal muscle, for uptake and oxidation (1). However, excessive plasma FA availability can result in FA fluxes that exceed the tissues' capability for FA utilization, leading to liver (2) and skeletal muscle insulin resistance (3). The scavenger receptor, CD36, plays an important role in facilitating cellular FA uptake and utilization (4,5). In mice, CD36 deletion impairs FA uptake and adaptive fuel flexibility by heart and skeletal muscle. In adipocytes, CD36 is important for FA uptake and FA release in response to lipolytic stimuli (6), influencing both energy storage and mobilization. Mutations in the *cd36* gene in the spontaneously hypertensive rat result in hyperlipidemia and insulin resistance (7).

In humans, complete CD36 deficiency (~6% of populations of Asian or African ancestry) results in reduced myocardial (8) and adipose tissue FA uptake (9) and in abnormalities of plasma lipid levels (10,11). Common single nucleotide polymorphisms (SNPs) in the *CD36* gene identified in African Americans and Caucasians influence levels of plasma lipids (11,12) and risk of metabolic syndrome (11) and diabetes (13). High levels of soluble CD36 in plasma (14) and increased monocyte CD36 expression (15) correlate with inflammation and insulin resistance. Increased hepatic CD36 expression is also observed in subjects with nonalcoholic fatty liver disease (16). The above observations indicate that both deficiency and high levels of CD36 can associate with negative metabolic effects in humans.

We hypothesized that the association of CD36 to metabolic disease might reflect its influence at the level of various organs, notably adipose and muscle tissues, which have high metabolic impact. The objective of this study was to gain insight into the relationship of muscle and adipose CD36 to serum FA homeostasis and organ insulin sensitivity in vivo. For this, we examined how tissue-specific alternative CD36 transcripts, which inform on local regulation of CD36 gene expression, relate to metabolic phenotypes of subjects who underwent a hyperinsulinemic–euglycemic clamp.

Tissue-specific distribution of many mammalian genes is driven by alternate promoter usage (17) and in the case of the human *CD36* gene, several independently regulated promoters (18–20) yield six transcripts that encode the same protein. These transcripts can serve as indicators of tissue-specific gene expression and regulation in health and disease situations. We determined the relationships of alternative *CD36* transcripts in muscle and adipose tissues to FA metabolism and insulin resistance in a cohort of obese subjects. Our findings provide novel insight into the importance of CD36 in tissue FA partitioning and insulin sensitivity.

RESEARCH DESIGN AND METHODS

Study Subjects
This study used adipose and muscle samples collected from 53 subjects who underwent metabolic phenotyping (21,22). Subcutaneous adipose tissue was available from all subjects (38 women and 15 men; 10 African American, 40 Caucasian American, 2 American Indian, and 1 Asian) and skeletal muscle from 36 of the subjects (26 females and 10 men; 6 African American, 27 Caucasian, 2 American Indian, and 1 Asian). The exclusion criteria included history of consuming >20 g/day of alcohol, use of medications that affect glucose or lipid metabolism, or type 2 diabetes (confirmed by 2-h oral glucose tolerance test). All subjects provided written informed consent, and the study was approved by the Washington University Institutional Review Board.

Metabolic Characterization of Subjects
Study designs and methodology have been described (21,22), and the protocol followed is shown in Supplementary Fig. 1. Briefly, subjects were admitted to the clinical research unit at Washington University School of Medicine on the evening before the clamp procedure. Body fat mass (FM) and fat-free mass (FFM) were determined with dual-energy X-ray absorptiometry (Delphi-W densitometer, Hologic, Waltham, MA). Intra-abdominal and abdominal subcutaneous adipose tissue volumes were quantified by magnetic resonance imaging (Siemens, Iselin, NJ; ANALYZE 7.0 software, Mayo Foundation, Rochester, MN). Intrahepatic TG (IHTG) content

was measured using localized proton magnetic resonance spectroscopy (Siemens Magneton Vision Scanner, Siemens, Erlanger, Germany) as described (23). To determine hepatic, skeletal muscle, and adipose tissue insulin sensitivity [6,6-^{2}H$_2$]glucose, [2,2-^{2}H$_2$] palmitate, and 20% dextrose enriched with labeled glucose were infused (22,24). Blood samples were obtained before beginning the tracer infusion for background plasma glucose and palmitate tracer-to-tracee ratios (TTRs) then every 10 min during the final 30 min of the basal period and of the insulin clamp stage 1 (insulin infused at 20 mU/m^2 body surface area; 3–6 h) and stage 2 (50 mU/m^2 body surface area; 6–9 h) to determine glucose, free FA (FFA), and insulin concentrations and substrate kinetics.

Tissue samples were obtained from subcutaneous abdominal adipose tissue and from the quadriceps femoris muscle 60 min after starting the glucose tracer during the basal period (Supplementary Fig. 1). Tissues were immediately rinsed with ice-cold saline, frozen in liquid nitrogen, and stored at −80°C. For studying kinetics of very LDLs (VLDLs), after a 12-h overnight fast, subjects were given an intravenous bolus of [1,1,2,3,3-^{2}H$_5$] glycerol and a primed constant infusion of [5,5,5-^{2}H$_3$]leucine was started and maintained for 12 h (22). Blood samples were obtained before the start of tracer infusions to determine plasma concentrations of substrates, insulin, and background isotopic enrichments of glycerol and leucine in plasma, VLDL-TG, and VLDL apolipoprotein B (apoB) 100. Samples were then obtained at regular time points throughout to determine VLDL-TG and VLDL-apoB kinetics. Plasma glucose, palmitate and leucine TTRs, glycerol TTR in VLDL-TG, and leucine TTR in VLDL-apoB were determined with gas chromatography mass spectrometry.

Calculations
Metabolic and isotopic steady states were achieved during the last 30 min of the basal period and stages 1 and 2 of the clamp procedure, and the Steele equation for steady-state conditions was used to calculate substrate kinetics (25). Glucose rate of disappearance (Rd) from plasma was assumed to equal the glucose rate of appearance (Ra) during basal conditions; during the clamp

procedure, glucose Rd was assumed to equal the sum of endogenous glucose Ra and the rate of infused glucose. Hepatic insulin sensitivity index (HISI) was determined by calculating the reciprocal of the hepatic insulin resistance index (product of basal endogenous glucose production rate, in μmol·kg FFM^{-1}·min^{-1} and fasting plasma insulin in mU/L) (26). Skeletal muscle insulin sensitivity was assessed as the relative increase in glucose Rd during insulin infusion (24). The suppression of palmitate Ra during stage 1 of the clamp procedure was used as a measure of adipose tissue insulin sensitivity. Total FFA Ra was calculated by dividing palmitate Ra by the proportional contribution of palmitate to total plasma FFA concentration. FFA Rd was expressed in μmol/kg FFM per min, which indicates the amount of FFA released into the circulation in relation to the amount of lean tissues that use FFA (i.e., index of FFA availability).

Hepatic secretion rates of VLDL-TG and VLDL-apoB were calculated by multiplying plasma VLDL-TG or VLDL-apoB concentration by VLDL-TG or VLDL-apoB fractional turnover rate, determined by fitting the TTR data to a compartmental model. Plasma VLDL-TG clearance rate was calculated as VLDL-TG Rd divided by VLDL-TG concentration (22).

RNA Isolation and Quantitative RT-PCR

Total RNA was extracted from the tissue biopsies and from normal human dermal microvascular endothelial cells (HMVECs; Lonza) using TRIzol (Invitrogen) and quantified (NanoDrop ND1000). For tissue distribution, total RNA from various human tissues was purchased from Stratagene for liver (catalog number 540017), heart (540011), skeletal muscle (540029), and pancreas (540023); Agilent for tongue (540149), and Clontech for adipose tissue (636558). Synthesis of cDNA (SuperScript III, Invitrogen) using random hexamer primers was followed by quantitative RT-PCR (qRT-PCR) in triplicate (SYBR Green PCR Master Mix on the ABI 7500 RT PCR System, Applied Biosystems) as described (22). Relative expression of CD36, peroxisome proliferator–activated receptor (PPAR)-γ and CEB/Pα was normalized to the human acidic ribosomal phosphoprotein P0 (RPLP0). Human *CD36* alternative transcript assays were detected using

forward primers unique to the first exons (untranslated 5′sequence), and reverse primers were positioned at exon 2, which is common to all the transcripts (18,19). The primers for qRT-PCR are shown in Supplementary Table 1. The average reaction efficiency (slope = 10[−1/slope]−1) for each primer pair was 96 ± 0.05%.

Human THP-1 monocytes (ATCC, Rockville, MD) were cultured in RPMI 1640 (ATTC 30–2001) with 10% heat-inactivated FBS, 100 μg/mL penicillin/streptomycin and 0.05 mmol/L 2-mercaptoethanol. Cells (4×10^5 cells/well) were treated (18–24 h) with phorbol myristic acid (50 ng/mL) to drive differentiation into macrophages and switched to fresh medium for 24 h prior to RNA isolation. Simpson–Golabi–Behmel syndrome (SGBS) human preadipocytes (27) were differentiated in serum-free Dulbecco's modified Eagle's medium with 2 μmol/L rosiglitazone, 250 nmol/L dexamethasone, 0.5 mmol/L 3-isobutyl-1-methylxanthine, 1 μmol/L cortisol, 0.2 nmol/L triiodothyronine, and 10 nmol/L insulin. RNA was isolated 14 days later for exon qRT-PCR. Adipose tissue CD36 protein levels were determined as previously described (22).

Statistical Analysis

Distribution normality for transcripts and metabolic variables was assessed using the Shapiro–Wilk test. Correlations were calculated using Spearman rank-order coefficients. The data were adjusted for the effects of age, sex, BMI, and visceral adipose tissue (VAT) on insulin sensitivity. Neither of these variables significantly impacted the observations, and the data presented are unadjusted correlation coefficients with corresponding P values. Differences between rs1761667 genotypes were determined by Student t tests with significance at $P < 0.05$ (two-tailed).

RESULTS

Tissue Distribution of Alternative CD36 Transcripts in Humans

The human *CD36* gene exhibits six alternative promoters yielding transcripts 1A–1F, with distinctive tissue distribution as shown in Fig. 1*A* and *B*. The most ubiquitous transcript is 1B, which is nearly exclusive in liver, monocytes, and macrophages. In adipose tissue and skeletal muscle, 1B transcripts are expressed several fold higher relative to

1C and 1A. Transcripts 1D, 1E, and 1F are orders of magnitude less abundant (Supplementary Fig. 2), except for exon 1D in microvascular endothelial cells (HMVEC) (Fig. 1*D*). We focused our analysis on the predominant transcripts in adipose and muscle tissues 1A–1C.

Transcript 1C is not present in human macrophages or endothelial cells (Fig. 1*C* and *D*), thus in adipose tissue, 1C expression would provide an in vivo assessment of adipocyte *CD36*. In contrast, 1B expression reflects adipocytes and resident macrophages in addition to a minor contribution from endothelial cells. As shown in Fig. 1*C* and *E*, differentiation of monocytes to macrophages and of preadipocytes to adipocytes associated with general increases in all transcripts expressed by each cell type.

Adipose Tissue Transcript 1C Strongly Associates With Insulin Sensitivity

To determine metabolic relevance of tissue CD36, levels of total *CD36* mRNA and alternative transcripts 1A–1C were determined in adipose ($n = 53$) and skeletal muscle ($n = 36$) biopsies from obese individuals who underwent body composition measurements and hyperinsulinemic–euglycemic clamps (Table 1). In adipose tissue, transcripts 1B and 1C both correlated with total *CD36* mRNA and protein. These correlations were stronger with 1C despite its lower abundance as compared with 1B (Table 2), supporting contribution of both transcripts to *CD36* mRNA and protein abundance.

Total adipose tissue *CD36* mRNA inversely correlated with IHTG content (Table 2), and modest positive correlations were observed with the HISI and with insulin suppression of lipolysis (palmitate Ra), an index of adipose tissue insulin sensitivity.

Interestingly, the 1B and 1C adipose CD36 transcripts displayed strikingly different relationships to measures of insulin sensitivity. There were no associations with transcript 1B despite its abundance in adipose tissue. On the other hand, strong correlations were observed with transcript 1C (Table 2 and Supplementary Fig. 3). Transcript 1C negatively correlated with IHTG content ($P < 0.001$) and positively with liver ($P < 0.001$), adipose tissue ($P < 0.001$), and skeletal muscle ($P < 0.0001$) insulin sensitivity. Expression of transcript 1A did not relate to insulin sensitivity systemically or at the
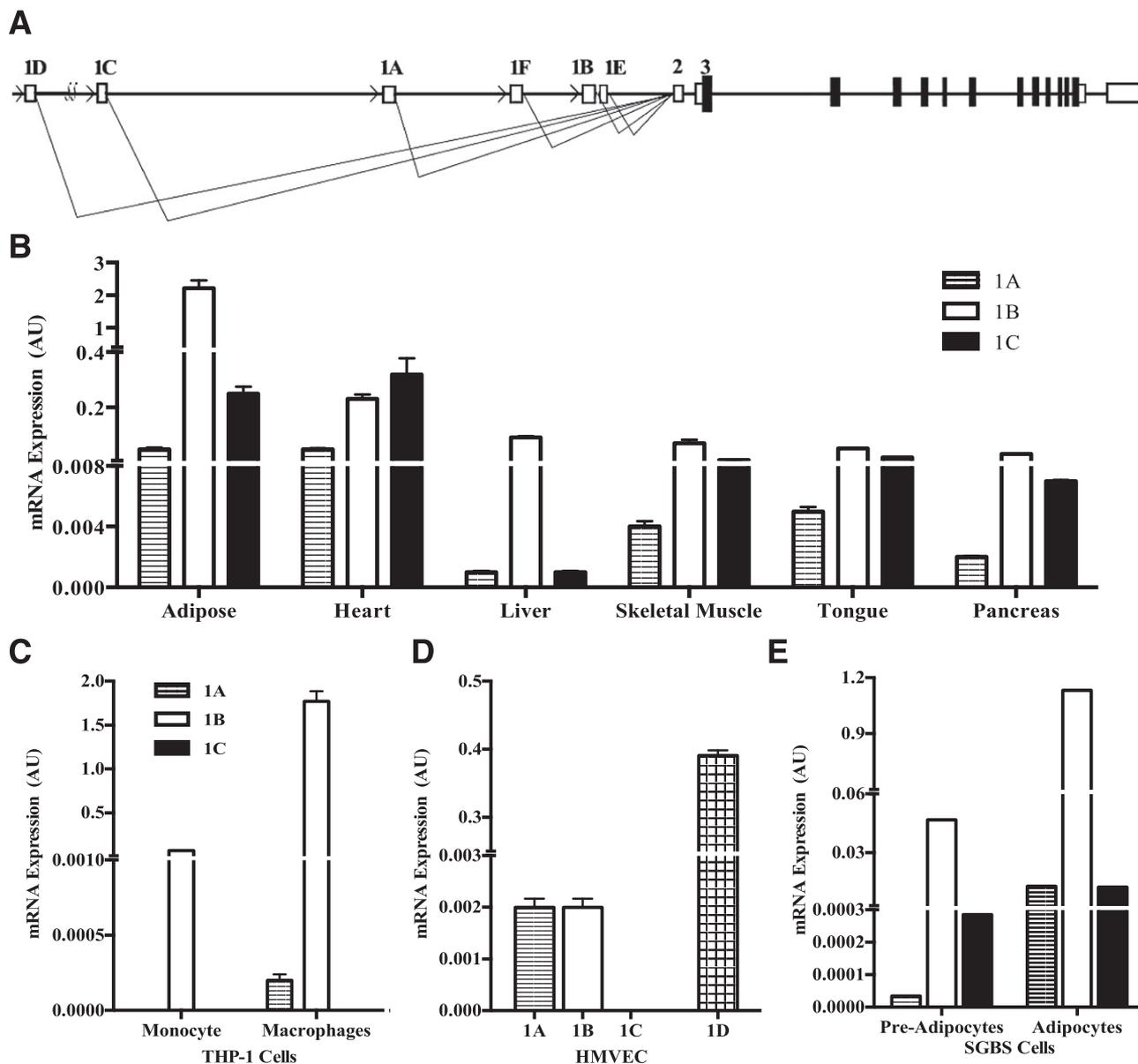
Figure 1—Distribution of alternative CD36 first exon transcripts in human tissues and cells. *A*: Diagram of the *CD36* gene illustrating position of the untranslated (empty boxes) *CD36* first exon regions (1A–1F) relative to coding exons 3–14 (filled boxes). The bent lines illustrate splicing between the alternative first exons and exon 2. *B*: Relative expression of the major alternative transcripts 1A, 1B, and 1C in commercially available human tissue (adipose tissue, heart, liver, skeletal muscle, tongue, and pancreas). *C*: Human THP-1 monocytes and macrophages where 1B is the predominant transcript. *D*: HMVECs showing predominance of transcript 1D. *E*: Human preadipocytes and adipocytes (SGBS cell line) showing higher abundance of 1B relative to the 1A and 1C transcripts. Expression (arbitrary units) of the transcripts normalized to the human *RPLPO* gene is shown, and data are representative of duplicate determinations from at least two experiments. Details for differentiation of THP-1 and SGBS cells are in RESEARCH DESIGN AND METHODS. AU, arbitrary units.

organ level. These findings suggest that adipocyte-specific transcript 1C is a sensitive marker of multiorgan (adipose, muscle, and liver) insulin sensitivity.

Adipose Transcript 1C Inversely Correlates With Visceral Fat Tissue

Our study cohort did not include lean subjects and had a relatively narrow BMI range (Table 1), which should minimize the confounding effects of BMI on metabolic parameters. However,

equally obese subjects differ in the amount of VAT (21,22). Consistent with the strong relationship of this depot to insulin resistance, VAT volume inversely correlated ($P = 0.009$) to adipose tissue transcript 1C. Total adipose tissue CD36 mRNA showed a modest relationship to the percentage of FM, reflecting associations by both 1C and 1B transcripts (Table 2). Overall, all relationships observed between total and 1C adipose CD36 transcripts and measures

of insulin sensitivity were not affected when the data were adjusted for BMI or VAT. This suggested that the correlations obtained were not driven by adiposity in this cohort.

Muscle CD36 Transcripts Associate With FA and VLDL Kinetics but Not With Insulin Sensitivity

Total adipose tissue *CD36* mRNA negatively correlated with secretion rate of VLDL-TG and VLDL-apoB. Transcripts 1A,

Table 1—Characteristics of the study subjects

N (male/female)	53 (15/38)
Age, years	41 ± 2
BMI, kg/m^2	35.9 ± 0.6
Glucose, mg/dL	94 ± 0.93
Insulin, μIU/mL	14.5 ± 1.2
IHTG, % liver volume	13 ± 2
TGs, mg/dL	136 ± 9
Total cholesterol, mg/dL	170 ± 4
HDL cholesterol, mg/dL	45 ± 2
LDL cholesterol, mg/dL	100 ± 3

Values are mean ± SEM unless otherwise noted.

1B, and 1C all negatively related to apoB-100 production rate, but only 1C showed a negative correlation with VLDL-TG production rate.

In skeletal muscle, there were no significant relationships between total or individual CD36 transcripts and measures of skeletal muscle or systemic insulin sensitivity (data not shown). However, muscle transcripts 1A and 1B and total *CD36* mRNA correlated with FFA Rd, and a weaker correlation was observed with VLDL-TG clearance (Table 2).

Overall, the data presented in Table 2 showed a differential relationship between adipose and muscle *CD36* transcripts as related to metabolic phenotypes. Adipose tissue transcripts positively correlated with multiple organ insulin sensitivity and inversely with hepatic secretion of VLDL while muscle transcripts positively associated with uptake of FFA and VLDL clearance.

Differential Metabolic Relationships of CD36 Transcripts Might Reflect Transcript-Specific Regulation

The individual transcripts (1A–1C) did not correlate across adipose and muscle tissues (data not shown), suggesting differential, tissue-specific regulation. A search of the reported literature and available databases identified PPARγ binding sites within 1 kb of the transcription start site of 1B and 1C (Supplementary Table 2). Several conserved C/EBP consensus sites were identified in the 1C promoter and both C/EBPα and C/EBPβ function as important coregulators with PPARγ (28,29). Thus it is possible that transcript 1C constitutes a major PPARγ-C/EBP target in adipose tissue. Consistent with this, *CD36* transcripts in adipose tissue samples correlated with expression of *CEBPA* and *PPARG* (*n* = 36) (Supplementary Fig. 4), with the strongest relationships observed with the 1C transcript (Supplementary Fig. 4*A* and *D*).

Common CD36 Promoter SNP Influences CD36 Transcripts and Exacerbates Muscle Insulin Resistance

SNPs in the *CD36* gene associate with metabolic phenotypes related to complications of obesity, as reviewed recently (30). A haplotype including *CD36* SNP rs1761667 (A/G), a common (global frequency of the G allele is 0.58) promoter variant that localizes upstream of exons 1A and 1B, has been reported to influence serum FFA and the susceptibility to coronary artery disease in type 2 diabetic subjects (31). In addition, the G allele of this SNP associated with increased monocyte and platelet CD36 protein (12,32). We tested whether the rs1761667 genotype has differential effects on *CD36* transcripts. Our findings suggest that the G allele for this SNP modifies CD36 expression in muscle (Fig. 2*A*–*C*) in a transcript-specific manner. When we grouped subjects based on IHTG content, which is a predictor of insulin resistance (Supplementary Table 3), the rs1761667 G/G genotype significantly exacerbated impairment of insulin-stimulated muscle glucose uptake in the more insulin-resistant, high-IHTG subgroup (Fig. 2*D*).

CONCLUSIONS

CD36 has been documented to facilitate myocardial and adipose tissue FA uptake in humans, and common variants in the gene were recently associated with blood lipids and metabolic syndrome risk (10,30). However, little is known regarding the in vivo role of CD36 in FA metabolism and insulin sensitivity in humans. The findings from this study support the physiological influence of CD36 on FA homeostasis and insulin responsiveness and provide insight into the association between the *CD36* gene and metabolic disease. The relationships we identified suggest an underappreciated role of adipocyte CD36 in regulating systemic metabolism. The negative relationships uncovered between adipose tissue CD36 transcript 1C and hepatic TG accumulation, VLDL output, and insulin resistance of liver and muscle suggest a protective metabolic role of adipocyte CD36, but the exact nature of this role remains to be determined. It is possible that it reflects CD36-mediated FA partitioning in adipose tissue away from other organs. In addition, adipocyte CD36 might influence metabolism of different tissues through modulating the secretion of adipokines. Adiponectin levels are reduced in the serum and adipose tissue of the *cd36*-null mouse (33), and genetic studies identified associations between *CD36* SNPs and plasma adiponectin in humans (34).

An important concept that is illustrated by this study is the metabolic relevance of alternative transcripts, which

Table 2—CD36 transcripts in adipose tissue correlate positively with measures of insulin sensitivity while transcripts in skeletal correlate with FFA Rd and VLDL clearance

	Total	1A	1B	1C
Adipose tissue				
IHTG, %	−0.39*	0.08	−0.24	−0.49**
HISI	0.45*	−0.02	0.27	0.47**
Insulin stimulation of glucose Rd, %	0.27	0.23	0.22	0.57***
Insulin suppression of palmitate Ra,† %	0.52*	0.22	0.30	0.56**
VLDL-TG production rate, μmol/min	−0.45*	−0.14	−0.20	−0.40*
VLDL-apoB100 production rate,† nmol/min	−0.44*	−0.49*	−0.55**	−0.49*
CD36 total mRNA	—	0.27	0.58**	0.68***
CD36 protein,‡	0.75***	0.32	0.46*	0.58**
VAT, cm^3	−0.09	−0.39*	−0.10	−0.47*
FM, %	0.43*	0.05	0.47*	0.36*
Skeletal muscle				
VLDL-TG clearance, mL/min	0.35*	0.38*	0.40*	−0.16
FFA Rd, μmol/kg FFM/min	0.58**	0.45*	0.54**	−0.32

Shown are Spearman *r* correlations and corresponding *P* values. Protein expression level in arbitrary units. *$P < 0.05$. **$P < 0.001$. ***$P < 0.0001$. †*n* = 36. ‡*n* = 26 for skeletal muscle.
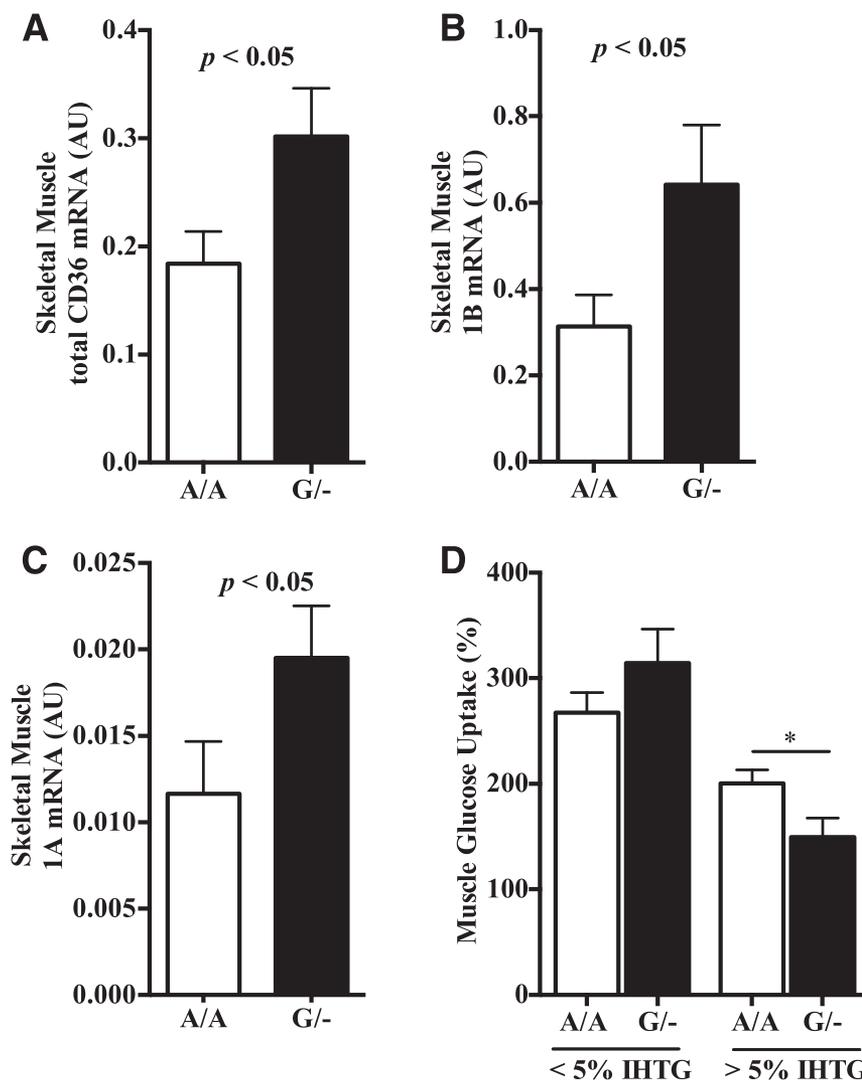
Figure 2—CD36 common promoter SNP rs1761667 influences skeletal muscle CD36 expression and muscle glucose uptake. Expression is compared between genotype groups: (*A*) total skeletal muscle *CD36* mRNA, (*B*) skeletal muscle 1B, and (*C*) skeletal muscle 1A. Sample size $n = 12$ A/A and 15 G/−. *D*: Insulin-stimulated muscle glucose uptake. Rs1761667 G/− genotype further impairs insulin-stimulated glucose uptake in subjects with high IHTGs. Subjects were subdivided into those having lower or higher than 5% IHTG. Rs1761667 genotype distribution in the subgroups: IHTG <5%, $n = 8$ A/A, and $n = 17$ G/−. For IHTG >5%, $n = 11$ A/A and $n = 17$ G/−; see Supplementary Table 2 for subject characteristics in the subgroups. AU, arbitrary units. *$P = 0.01$.

are common for mammalian genes (17). For CD36, alternative promoter usage confers tissue and cell-specific CD36 distribution and differential sensitivity to regulation. From a clinical perspective, aberrant use of a particular CD36 promoter could be a factor in metabolic abnormalities and might be relevant to disease mechanisms. Determining the CD36 tissue transcript profile in the context of obesity could provide useful clues related to the metabolic status of various tissues and to the potential mechanisms underlying obesity-associated complications such as metabolic syndrome, diabetes, and cardiovascular disease. CD36 downregulation has been proposed as an effective target in

hyperlipidemia, insulin resistance, and inflammation (35,36). As more knowledge becomes available related to the regulation of individual CD36 transcripts, this might allow for the design of therapies that target select CD36 transcripts and specific tissues.

Our data show that adipocyte CD36, which is uniquely represented by 1C, predicts insulin sensitivity while there is lack of association with adipose 1B. This difference might reflect the independent regulation of the two adipose transcripts. While both the 1B and 1C promoters contain PPARγ sites, unlike 1C, 1B lacks proximal (within 1 kb) C/EBP motifs, perhaps accounting for the stronger relation of 1C to adipose

tissue *PPARG* and *CEBPA* mRNA expression. In addition, 1B has several conserved response elements for factors that impair insulin sensitivity (glucocorticoids [37], interferon-γ [38]) or are linked to inflammation (NFKβ, CK2, SpI-1/PU.1) (39).

In muscle, the positive correlations observed between the major CD36 transcripts and the FFA rate of disposal and VLDL-TG clearance support the important quantitative role of muscle CD36 in serum lipid utilization in humans. The lack of association between muscle CD36 transcripts and insulin sensitivity that we report in this study does not rule out involvement of muscle CD36 in the etiology of insulin resistance. Our data

focused on the contribution of transcriptional regulation to tissue CD36 levels and as a result do not consider the impact of posttranscriptional regulation. Previous studies have documented alterations in intracellular trafficking and posttranslational processing of CD36 in the diabetic muscle (5,39,40). The data suggest that transcriptional regulation might play a more important role in determining CD36 function in adipose tissue as compared with muscle.

Our study demonstrates that the effects of a disease-associated CD36 SNP might be tissue and transcript specific, and this should be considered when evaluating the functional impact of particular SNPs and their clinical utility as potential biomarkers of disease susceptibility. *CD36* SNP rs1761667 (A/G) worsened insulin resistance of muscle glucose disposal, and this effect could be consequent to its impact on the CD36 transcript profile, which influences how tissue CD36 is regulated in response to metabolic challenges. The finding that the effect of rs1761667 was only significant in the insulin-resistant subgroup of our cohort would be consistent with earlier findings showing an association between the rs1761667 SNP with coronary disease only in diabetic subjects (31).

In summary, these novel findings shed light on the physiological role of human CD36 and its genetic variants and highlight the link between CD36 and insulin resistance. The findings will be useful for designing therapeutic approaches that target tissue-specific CD36 transcripts in order to improve FA homeostasis and insulin resistance.

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