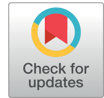

METABOLISM

Glutamine Regulates Skeletal Muscle Immunometabolism in Type 2 Diabetes

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Diabetes 2022;71:624–636 | https://doi.org/10.2337/db20-0814

Dysregulation of skeletal muscle metabolism influences whole-body insulin sensitivity and glucose homeostasis. We hypothesized that type 2 diabetes–associated alterations in the plasma metabolome directly contribute to skeletal muscle immunometabolism and the subsequent development of insulin resistance. To this end, we analyzed the plasma and skeletal muscle metabolite profile and identified glutamine as a key amino acid that correlates inversely with BMI and insulin resistance index (HOMA-IR) in men with normal glucose tolerance or type 2 diabetes. Using an in vitro model of human myotubes and an in vivo model of diet-induced obesity and insulin resistance in male mice, we provide evidence that glutamine levels directly influence the inflammatory response of skeletal muscle and regulate the expression of the adaptor protein GRB10, an inhibitor of insulin signaling. Moreover, we demonstrate that a systemic increase in glutamine levels in a mouse model of obesity improves insulin sensitivity and restores glucose homeostasis. We conclude that glutamine supplementation may represent a potential therapeutic strategy to prevent or delay the onset of insulin resistance in obesity by reducing inflammatory markers and promoting skeletal muscle insulin sensitivity.

Type 2 diabetes is a chronic disease characterized by insulin resistance and disturbed glucose homeostasis that currently affects >400 million people worldwide. Obesity is a major risk factor for developing type 2 diabetes (1,2). Skeletal muscle accounts for the majority of insulin-stimulated whole-body glucose disposal (3). Therefore, dysregulation of skeletal muscle metabolism profoundly influences whole-body insulin sensitivity and glucose homeostasis (3). While the molecular links between obesity and type 2 diabetes remain incompletely understood, chronic inflammation is strongly correlated with the development of insulin resistance (4). Nevertheless, the role of inflammation in modulating skeletal muscle metabolism and whole-body insulin sensitivity remains to be fully appreciated.

Obesity increases systemic and local inflammatory processes, with an infiltration of immune cells, such as macrophages and T cells, into the peripheral tissues controlling whole-body glucose homeostasis, including skeletal muscle (5). This dynamic cross talk between immune and metabolic processes is referred to as immunometabolism (6,7). Alterations of metabolic pathways in immune or metabolic cells can drive inflammatory responses that lead to the production and release of cytokines, thereby exerting autocrine, paracrine, and/or endocrine effects on glucose and energy homeostasis. In the context of overnutrition and obesity, an overload of nutrients and metabolites directly contributes to the low-grade inflammatory state associated with these conditions (8). For example, several lipid species, such as saturated fatty acids, ceramides or lipid-derived eicosanoids, activate immune cells and induce inflammation in myocytes, thereby leading to insulin resistance

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Received 10 September 2020 and accepted 23 December 2021

This article contains supplementary material online at https://doi.org/10.2337/figshare.17433230.

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(9,10). Furthermore, in obese insulin-resistant adipose tissue, alterations in amino acid levels, in particular reduced glutamine concentrations, are linked to increased glycolysis and inflammation in adipocytes, resulting in dysregulated immunometabolism (11). Thus, altered levels of intercellular metabolites, including lipids and amino acids, may influence peripheral insulin sensitivity in obesity and type 2 diabetes.

Recent advances in metabolomic techniques have revealed that elevated lactate and branched chain amino acids (BCAAs), as well as an altered lipid composition, constitute a plasma metabolomic signature of type 2 diabetes (12). Changes in metabolite levels, such as BCAAs, reflect both dietary intake and disturbed catabolism in various organs (13). However, few studies have characterized the metabolomic signature of both peripheral tissues and plasma in individuals with normal glucose tolerance (NGT) or type 2 diabetes. Alterations in plasma metabolite levels in type 2 diabetes may affect the function of peripheral tissues controlling glucose and energy homeostasis through the modulation of the extracellular environment. Both an excess or an insufficient amount of metabolites can affect the cellular metabolic state, which may in turn affect transcriptional activity via intermediate metabolites that constitute substrates or cosubstrates for enzymes modifying chromatin and/or the activity of transcriptional regulators (14). Through a comparative analysis of the plasma and skeletal muscle metabolomic signature of well-phenotyped subjects, specific metabolites involved in the pathophysiological mechanism underlying the development of type 2 diabetes can be identified.

We hypothesized that type 2 diabetes–associated alterations in the plasma metabolome not only are biomarkers of the disease, but also directly contribute to an altered immunometabolic state in skeletal muscle and subsequent development of insulin resistance. In this study, we identified glutamine as a key amino acid that is inversely correlated with BMI and HOMA of insulin resistance (HOMA-IR) index in men with NGT or type 2 diabetes. Using an in vitro model of human myotubes and an in vivo model of diet-induced obesity and insulin resistance in male mice, we provide evidence that extracellular glutamine levels can directly influence the inflammatory response of skeletal muscle and regulate the expression of the adaptor protein GRB10. Moreover, we demonstrate that a systemic increase in glutamine levels improves peripheral insulin sensitivity and restores glucose homeostasis.

RESEARCH DESIGN AND METHODS

Participants

The study was approved by the regional ethics committee of Stockholm, Sweden, and conducted according to the Declaration of Helsinki. Informed written consent was obtained from each participant. Male volunteers with type 2 diabetes or NGT were matched for age and BMI (exclusion criteria and study design are presented in Supplementary Fig. 1). Clinical characteristics of the participants are presented in Table 1. The groups are subsets of a larger study designed to characterize the plasma and skeletal muscle metabolome in men with NGT or type 2 diabetes (15). Plasma and vastus lateralis skeletal muscle biopsies were collected after an overnight fast. Daily oral glucose-lowering medications were taken after the collection of skeletal muscle and plasma samples to reduce direct drug-related effects. However, longer effects of the medication on the metabolome may persist.

Table 1—Clinical characteristics of the study participants

	Lean		Overweight		Effect, *P*	
Clinical parameter	NGT	T2D	NGT	T2D	Overweight	T2D
N of participants	7	6	7	13	—	—
Age, years	57.3 ± 9.5	65.3 ± 2.7	61.4 ± 10.2	63.6 ± 6.1	0.721	0.097
BMI, kg/m^2	24.1 ± 0.58	24.6 ± 0.36	27.2 ± 1.65	27.6 ± 1.2	<0.001***	0.321
W/H ratio	0.91 ± 0.04	0.98 ± 0.06	0.94 ± 0.05	0.99 ± 0.06	0.296	0.007**
FPG, mmol/L	5.27 ± 0.4	8.42 ± 2.28	5.36 ± 0.32	8.77 ± 1.76	0.679	<0.001***
Serum insulin, pmol/L	40.1 ± 15.4	44.2 ± 17	64.8 ± 17.8	85.9 ± 37.5	0.002**	0.193
HbA_{1c}, mmol/mol	34 ± 5.32	51 ± 6.13	38.1 ± 2.54	51.9 ± 6.42	0.241	<0.001***
HOMA-IR	1.61 ± 0.5	3.03 ± 1.48	2.27 ± 0.73	5.51 ± 2.92	0.033*	0.002**
Serum C-peptide, nmol/L	0.59 ± 0.12	0.69 ± 0.15	0.72 ± 0.131	0.99 ± 0.40	0.035*	0.057
Plasma TGs, mmol/L	0.89 ± 0.33	1.19 ± 0.44	1.21 ± 0.71	1.47 ± 0.78	0.212	0.240
Plasma cholesterol, mmol/L	4.94 ± 0.53	4.35 ± 0.85	5.56 ± 0.67	4.65 ± 0.76	0.099	0.005**
Plasma HDL, mmol/L	1.24 ± 0.13	1.42 ± 0.21	1.36 ± 0.26	1.21 ± 0.312	0.523	0.869
Plasma LDL, mmol/L	3.3 ± 0.54	2.43 ± 0.76	3.67 ± 0.44	2.77 ± 0.83	0.174	0.001**

Data are presented as mean ± SEM. Overweight was defined as BMI ≥25 kg/m^2. Effect of BMI (overweight) and type 2 diabetes (T2D) was measured by two-way ANOVA. No interactions were significant for the parameters shown. FPG, fasting plasma glucose; TG, triglyceride; W/H, waist to hip. *$P < 0.05$. **$P < 0.01$. ***$P < 0.001$.

Untargeted metabolomic analysis was performed by Metabolon, Inc. (Durham, NC). Microarray analysis was performed on total RNA and hybridized to Affymetrix GeneChip Human Transcriptome Array 2.0 or mouse Clariom D (Thermo Fisher Scientific, MA).

Animal Experiments

C57BL/6J male mice were purchased from Charles River Laboratories (Sulzfeld, Germany). Experimental procedures were approved by the Stockholm North Animal Ethical Committee (Stockholm, Sweden). At 6–8 weeks of age, mice were fed either a standard chow diet (4% kcal from fat) (R34; Lantmännen) or a 60% fat diet (Research Diet, New Brunswick, NJ). Three separate experiments were performed with mice fed a high-fat diet (HFD) for either 17 (experiments 1 and 2) or 15 weeks (experiment 3). Prior to euthanasia, mice received daily intraperitoneal injection of glutamine (1 g/kg body wt) or PBS (20 mL/kg body wt) for 14 days. To assess body composition (experiment 1), total lean and fat masses were determined in conscious mice using the EchoMRI-100 system (Echo Medical Systems). At day 15, blood glucose was measured after 4 h of fasting (One Touch Ultra 2 Glucose Meter; LifeScan), and animals were then euthanized under general anesthesia. Plasma insulin levels were quantified using the Ultra-Sensitive Mouse Insulin ELISA Kit (Crystal Chem). To assess insulin tolerance (experiment 2), mice were fasted for 4 h, and 0.75 IU/kg body wt of insulin was injected intravenously, and the rate of glucose disappearance between 0 and 15 min was measured as described (16). To assess glucose tolerance (experiment 3), mice were fasted for 4 h, and glycemia was monitored during 150 min after intraperitoneal injection of 1.5 g/kg body wt of glucose.

Cell Culture Experiments

Primary cells were isolated from vastus lateralis skeletal muscle biopsies derived from healthy volunteers as described (17). For the high/low glutamine experiment, DMEM glutamine-free medium was used and supplemented to 0.5 or 10 mmol/L final concentration (glutamine; Life Technologies) during the 8-day differentiation period or after differentiation for 48 h. BPTES, a glutaminase 1 inhibitor, was purchased from Sigma-Aldrich (SML0601) and added to the cell medium at a concentration of 10 μmol/L for 24 h. For the silencing experiments, differentiated cells were transfected with 10 nmol/L of either Ambion Silencer Select Negative Control #2 (cat. no. 4390847) or an siRNA against *GRB10* (Ambion Silencer Select siRNA s6125; Life Technologies). Lipopolysaccharide (LPS) was purchased from Sigma-Aldrich (L2630) and added to the cell medium at a concentration of 100 ng/mL for 24 h.

Insulin Signaling and Western Blot Analysis

Extensor digitorum longus (EDL) (experiment 1) and soleus (experiment 3) muscles were incubated with Krebs-Henseleit buffer under continuous gassing (95% oxygen/5% carbon dioxide) at 30°C in the absence (basal) or presence of a submaximal concentration (0.36 nmol/L) of insulin (Actrapid; Novo Nordisk) for 20 min. Western blot analysis was performed as described (18). The primary antibodies used were anti-GRB10 (C-11) (Santa Cruz Biotechnology), anti–phosphorylated AS160 Thr^{642} (cat. no. 8881; Cell Signaling Technology), anti–phosphorylated AKT Thr^{308} (cat. no. 4056; Cell Signaling Technology), and anti–phosphorylated AKT Ser^{473} (cat. no. 9270; Cell Signaling Technology).

Statistical Analysis

Analyses were performed using either R 3.5.2 or GraphPad Prism 8.1 software (GraphPad Software, Inc.). The statistical tests used are indicated in the figure legends.

Data and Resource Availability

The data sets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

RESULTS

Plasma Glutamine Levels Are Associated With BMI and HOMA-IR

We performed a metabolomic analysis of plasma and vastus lateralis skeletal muscle biopsies from a cohort of 14 men with NGT and 19 men with type 2 diabetes, separated into lean and overweight subgroups based on BMI. Anthropometric and clinical parameters are shown in Table 1. Plasma metabolites were correlated with BMI, HOMA-IR, and waist-to-hip ratio (Fig. 1*A*–*B* and Supplementary Fig. 1*B*–*C*). Of these, monosaccharides, including glucose and mannose, were the metabolites most strongly associated positively with the clinical parameters (Fig. 1*A*–*B*). The amino acids glutamine and glutamate were also correlated with BMI and HOMA-IR index (glutamine: $r = -0.42$; $P = 0.015$ for both BMI and HOMA-IR) (glutamate: $r = 0.54$; $P = 0.0001$ and $r = 0.62$; $P = 0.001$, respectively, for BMI and HOMA-IR), with glutamine and glutamate showing an inverse correlation (Fig. 1*A*–*B*). The BCAA isoleucine, which has previously been identified as biomarker of type 2 diabetes (19), increased in parallel with HOMA-IR and BMI. In skeletal muscle, the BCAAs isoleucine and leucine displayed a positive association with BMI and HOMA-IR, while glutamine was negatively associated with HOMA-IR ($r = -0.38$; $P = 0.03$) (Fig. 1*C*–*D*). When comparing the plasma metabolome between men with NGT or type 2 diabetes, we found a trend for decreased glutamine in type 2 diabetes, while the glutamate level was significantly increased (Supplementary Fig. 1*D*–*K*). To further dissect the relationship between plasma glutamine/glutamate levels and glucose metabolism independently of adiposity, the participants were stratified based on BMI, revealing that glutamine and glutamate levels were selectively altered in overweight men with type 2 diabetes (Fig. 1*E*–*F*). This

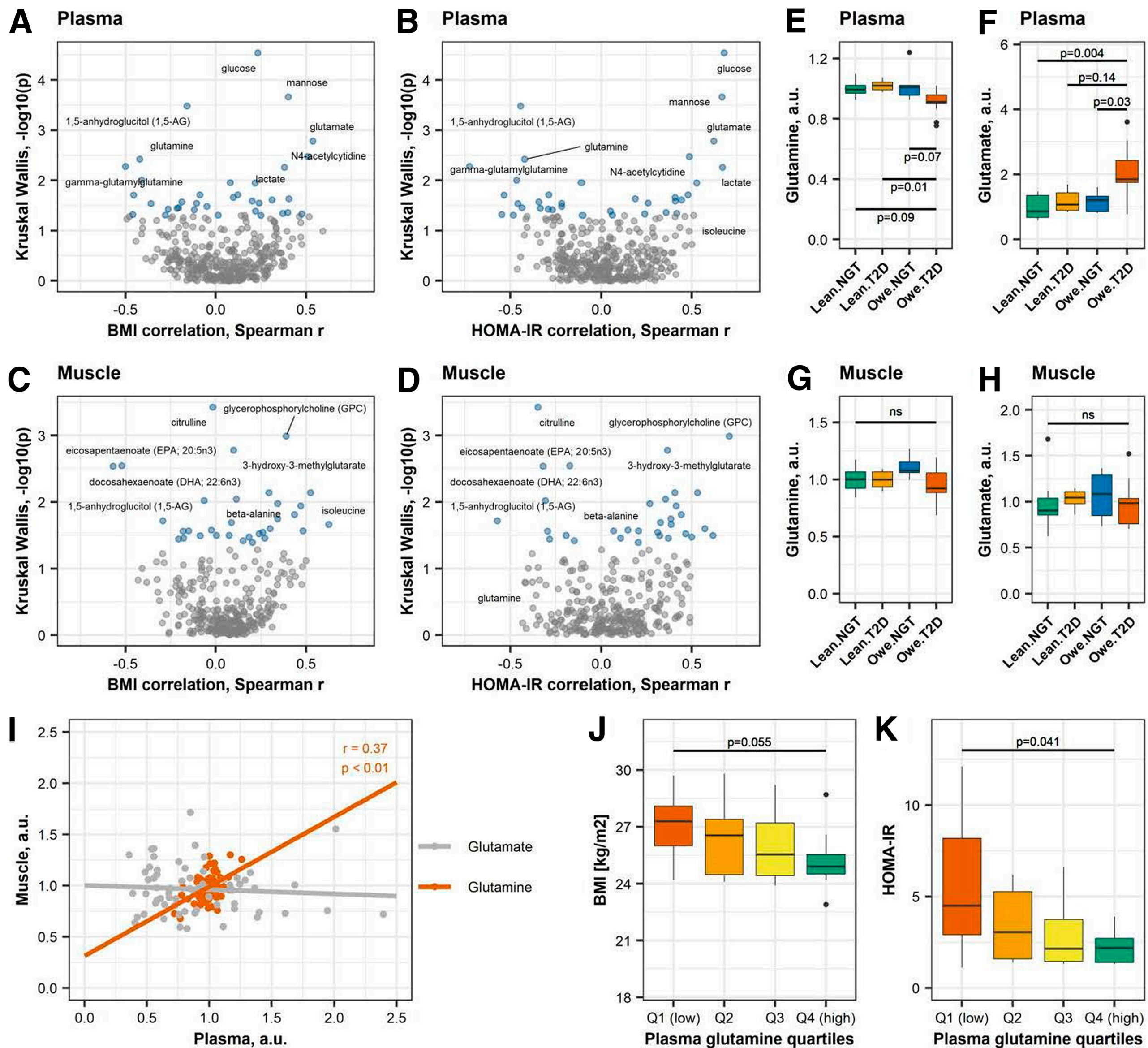

Figure 1—Plasma glutamine levels are associated with BMI and HOMA-IR. *A–D*: Volcano plots of metabolites in plasma and skeletal muscle correlating with BMI and HOMA-IR. Spearman correlation and Kruskal-Wallis comparison across groups. Blue color indicates significance at $P < 0.05$. *E–F*: Plasma glutamine and glutamate levels. Kruskal-Wallis test with Dunn multiple comparison test. *G–H*: Skeletal muscle glutamine and glutamate levels. Kruskal-Wallis test. *I*: Spearman correlation of glutamine and glutamate in plasma and skeletal muscle. *J*: BMI of individuals ranked based on plasma glutamine quartiles (Qs). Ordinary one-way ANOVA with uncorrected Fisher least significant difference comparison. *K*: HOMA-IR of individuals ranked based on plasma glutamine Qs. Kruskal-Wallis test with uncorrected Dunn test. *n* = 9, 8, 8, and 7 per Q, respectively. a.u., arbitrary unit; ns, not significant; Owe, overweight; T2D, type 2 diabetes.

relationship was not recapitulated in lean participants with type 2 diabetes, suggesting that modulation of plasma glutamine/glutamate levels was not a marker of glucose dysregulation or overweight per se, but rather a reflection of the differences in combined adiposity and insulin resistance between the groups.

In skeletal muscle, glutamine and glutamate content was not statistically altered in overweight men or men with type 2 diabetes (Fig. 1*G–H*). No differences in the gene expression of the enzymes responsible for glutamine synthesis (encoded by *GLUL*) or hydrolysis (encoded by *GLS*) (Supplementary Fig. 2*B–C*) were noted. Nevertheless, skeletal muscle glutamine, but not glutamate, was correlated with its respective plasma concentration (Fig. 1*I*), suggesting a direct link between plasma and intramuscular glutamine concentration. Plasma and skeletal muscle glutamine levels had similar associations with indices of glucose metabolism, including fasting insulin and glucose, C-peptide, and HOMA-IR (Supplementary Fig. 2*A*). Accordingly, participants with the highest plasma glutamine level (quartile 4) displayed lower BMI and HOMA-IR, while lower plasma glutamine level (Q3–Q1) was progressively

associated with increased BMI and HOMA-IR (Fig. 1*J*–*K*). Based on these associations, we hypothesized that high plasma glutamine levels may be protective with respect to obesity-induced insulin resistance.

Glutamine Administration Improves Glucose Homeostasis in HFD-Fed Mice

To assess whether increased glutamine levels could reverse or ameliorate the development of obesity-induced metabolic disturbances, three independent groups of C57BL/6J mice were fed an HFD for 15–17 weeks (experiments 1–3). During the last 14 days of the diet, the mice were injected daily intraperitoneally with either PBS or 1 g/kg glutamine. Chow-fed mice injected with PBS were used as controls for the effects of the HFD (Fig. 2*A*). Seventeen weeks of HFD led to the development of obesity, with increased body weight, fat mass percentage, and fasting glycemia (Fig. 2*B*–*D*). Glutamine administration did not alter body weight or fat mass percentage in the HFD-fed mice (Fig. 2*B*–*C*). Despite this, 14-day glutamine administration reversed the fasting hyperglycemia in HFD-fed mice ($P < 0.05$) (Fig. 2*D*). This reduction of hyperglycemia was accompanied by a reduction in the fasting insulin level and HOMA-IR as compared with PBS-treated HFD-fed mice (Fig. 2*E*–*F*). In a separate experiment (experiment 3) of mice fed an HFD for 15 weeks, glucose tolerance was improved in glutamine-treated versus PBS-treated mice (Fig. 2*G*–*H*). Furthermore, insulin sensitivity (experiment 2) was improved, as indicated by the increased plasma glucose disappearance rate after an intravenous insulin injection (Fig. 2*I*–*J*). Given that skeletal muscle is a major insulin-sensitive organ, we assessed insulin signaling in isolated glycolytic EDL and oxidative soleus muscles. Submaximal insulin-stimulated (0.36 nmol/L) phosphorylation of AKT at Ser^{473} was decreased in EDL muscle of HFD-fed mice as compared with control chow-fed mice, and glutamine administration tended to lessen this HFD-induced insulin signaling impairment (Fig. 2*K*). Phosphorylation of AKT at Thr^{308} showed a similar pattern (Fig. 2*L*). As a proxy for AKT activity, we determined phosphorylation of AS160, a direct AKT substrate that plays a role in GLUT4 translocation (20). Insulin-stimulated phosphorylation of AS160 was increased in EDL muscle of glutamine-treated HFD-fed mice as compared with chow-fed or PBS-treated HFD-fed mice (P = 0.02 and P = 0.006, respectively) (Fig. 2*M*), indicating that glutamine treatment increased EDL muscle AKT activity. Conversely, glutamine did not affect insulin-stimulated AKT or AS160 phosphorylation in soleus muscle (Supplementary Fig. 3*A*–*D*), suggesting a fiber-type difference in the response to glutamine treatment.

Glutamine Suppresses Gene Transcripts Annotated to Inflammation-Related Pathways

To further decipher the molecular mechanisms by which glutamine administration affects the skeletal muscle profile, we performed a transcriptomic analysis on mouse quadriceps skeletal muscle. Microarray analysis showed that HFD altered the skeletal muscle transcriptome in PBS- or glutamine-treated mice as compared with chow-fed mice, with 1,300 and 1,290 genes altered, respectively, between treatments (false discovery rate <0.2) (Fig. 3*A*–*B*). Fifty-one genes were differentially regulated between PBS- and glutamine-treated HFD-fed mouse skeletal muscle, with most of the transcripts decreased in response to glutamine (Fig. 3*C*–*D*). Moreover, genes altered by the glutamine treatment (false discovery rate <0.2; HFD plus glutamine versus HFD plus PBS) displayed a negative correlation with their fold change in response to an HFD (HFD plus PBS versus chow plus PBS), suggesting that glutamine treatment reversed the HFD-induced upregulation of gene expression (Fig. 3*F*). Interestingly, a pathway enrichment analysis revealed that glutamine treatment decreased mRNA expression of genes annotated to pathways linked to the regulation of inflammation and extracellular matrix organization as compared with PBS-treated HFD-fed and chow-fed conditions (Fig. 3*E*). Accordingly, gene expression analysis by quantitative PCR showed that expression of the inflammatory chemokine *Ccl2* was decreased in mice receiving glutamine treatment as compared with PBS-treated HFD-fed mice, and *Tnf* (tumor necrosis factor α) and *Cxcl1* followed a similar pattern. Gene-encoding macrophage surface markers such as *Cd68*, *Adgre1* (F4/80) , and *Itgax* (CD11c) were decreased in glutamine-treated HFD-fed mice and were not significantly different from those in chow-fed mice (Fig. 3*G*). Collectively, our results provide evidence to suggest that the beneficial effects of glutamine on skeletal muscle metabolism may be conferred by downregulating inflammation and immune cell infiltration.

Skeletal Muscle Expression of the Adaptor Protein GRB10 Is Associated With Plasma Glutamine Levels

We next compared genes altered by glutamine administration in HFD-fed mice ($P < 0.05$) with the genes altered in skeletal muscle of humans with high or low plasma glutamine levels ($P < 0.05$) and identified a small subset of genes responding similarly in both species (Fig. 4*A* and Supplementary Table 1). Among this subset, we focused on *GRB10*, a gene encoding an adaptor protein that interacts with the insulin receptor, for its role in the development of insulin resistance in adipocytes and myotubes (21). Human *GRB10* expression was lower in skeletal muscle of individuals with higher plasma glutamine levels and inversely correlated with plasma glutamine levels (Fig. 4*B*–*C*). The same pattern was noted in mouse models, where *Grb10* gene expression was increased in quadriceps of PBS-treated HFD-fed mice and was decreased after glutamine administration (Fig. 4*D*). Grb10 protein content followed a similar profile (Fig. 4*E*). *Grb10* expression was also significantly correlated with HOMA-IR in mouse models, with a similar relationship observed in humans (Fig. 4*F*). Gene silencing of *GRB10* in cultured

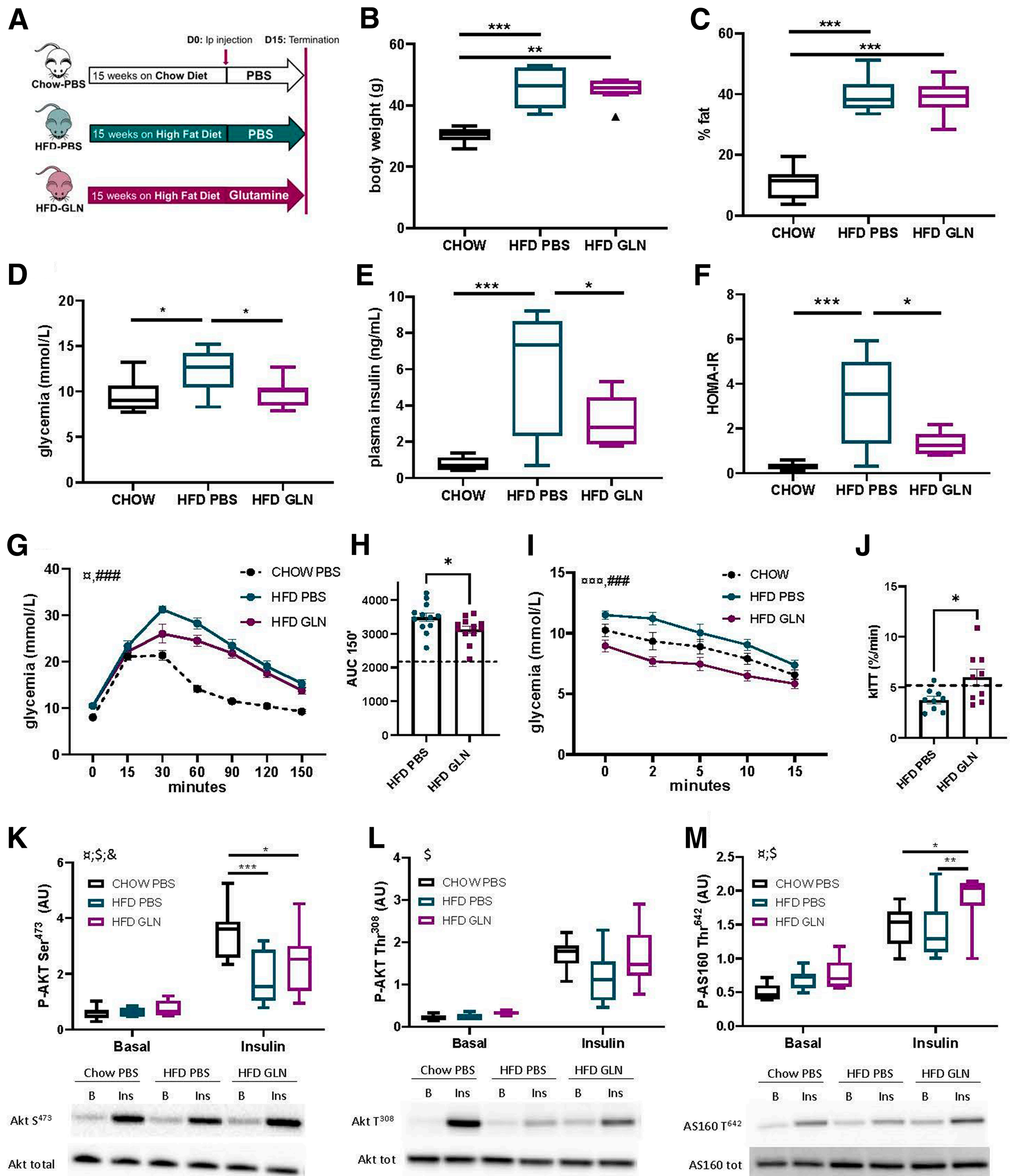

Figure 2—Glutamine (GLN) administration improves glucose homeostasis in HFD-fed mice. *A*: Experimental plan. *B*: Final body weight (day 15) expressed in grams. *C*: Total body fat percentage measured by magnetic resonance imaging. *D–F*: Glycemia (*D*), plasma insulin level (*E*), and calculated HOMA-IR (*F*) after 4-h fasting (n = 6, 8, and 8, respectively). Statistical difference was determined using one-way ANOVA or Kruskal-Wallis. *G–H*: Glucose tolerance test (*G*) and 150′ area under the curve (AUC) (*H*) (n = 8, 12, and 12, respectively). *I–J*: Insulin tolerance test (*I*) and glucose disappearance rate (KITT) (*J*) (n = 8, 9, and 9, respectively). For both tests, statistical differences were determined using two-way ANOVA and unpaired t test between the HFD groups. ¤, group effect; #, time effect. Dashed lines represent the chow-fed PBS-treated group response. *K–M*: Akt phosphorylation (P-AKT) (*K–L*) and AS160 phosphorylation (P-AS160) (*M*) in lysates of EDL skeletal muscle incubated in the absence or presence of a submaximal dose of insulin (0.36 nmol/L) for 20 min. Data are mean ± SEM in panels *G–I*. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Statistical effect was measured using two-way ANOVA in panels *K–M*. $, insulin effect; &, interaction effect; ¤, group effect; AU, arbitrary unit.

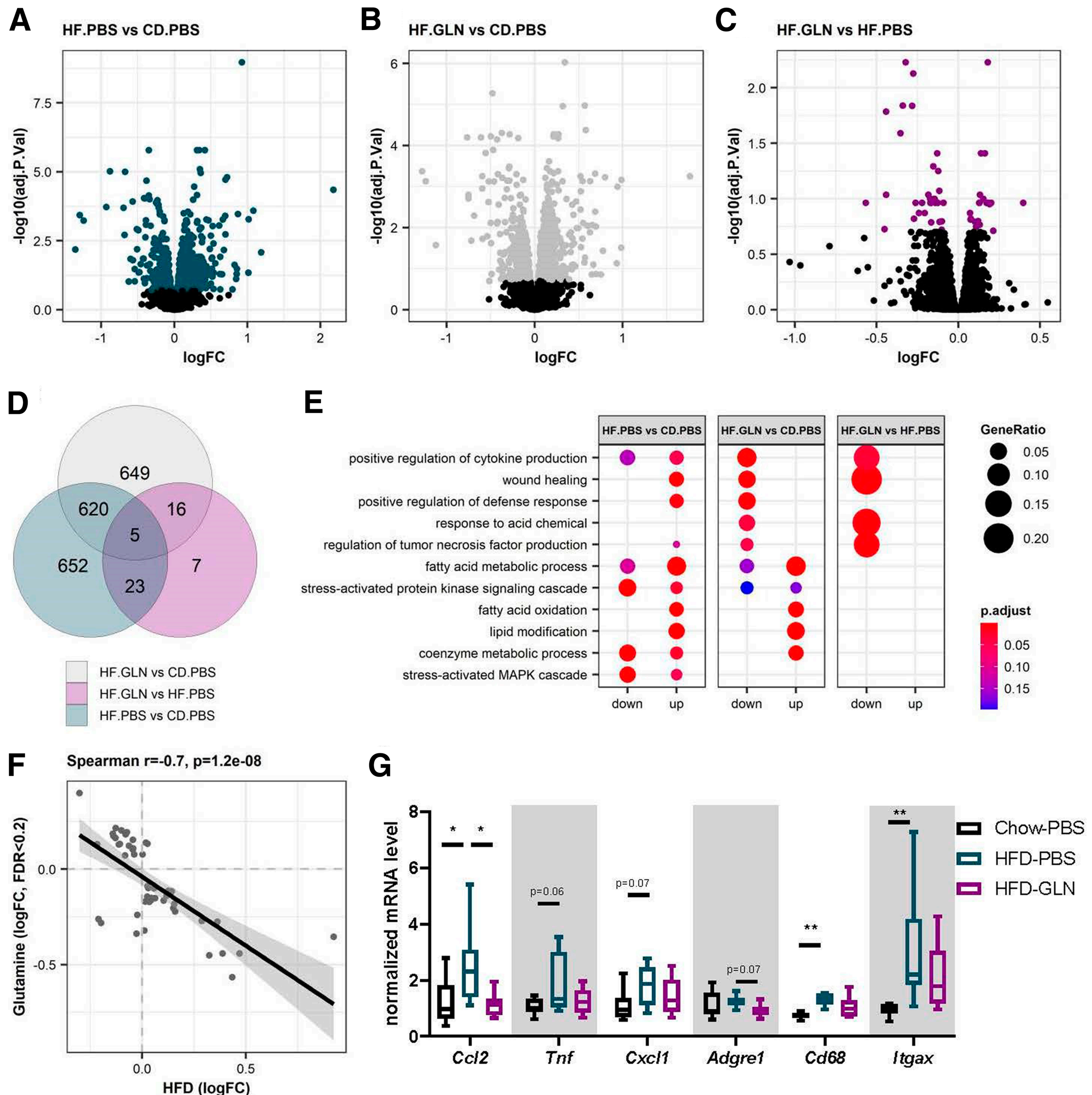

Figure 3—Glutamine (GLN) suppresses gene transcripts annotated to inflammation-related pathways. *A–C*: Changes in gene expression between HFD (HF)–fed PBS-treated and chow diet (CD)–fed PBS-treated (*A*), HFD-fed GLN-treated and CD-fed PBS-treated (*B*), and HFD-fed GLN-treated and HFD-fed PBS-treated (*C*) conditions are represented in volcano plots. Colored dots represent genes significantly altered between conditions (false discovery rate [FDR] <0.2). *D*: Significantly regulated genes (FDR <0.2) overlapped in a Venn diagram. *E*: Gene ontology analysis performed based on genes with FDR <0.2 showing downregulated and upregulated pathways between conditions. *F*: Spearman correlation between the logarithm of fold change (logFC) of the genes altered between HFD-fed GLN-treated and HFD-fed PBS-treated conditions GLN *y*-axis) and logFC between HFD-fed PBS-treated and CD-fed PBS-treated conditions (HFD *x*-axis). *G*: Gene expression analysis of cytokine and immune cell markers in quadriceps muscle as measured by quantitative PCR. Data are mean ± SEM. $^*P < 0.05$, $^{**}P < 0.01$. Statistical effect was measured using one-way ANOVA or Kruskal-Wallis for panel *G*. $n = 8$ mice per group. MAPK, mitogen-activated protein kinase.

human skeletal muscle cells increased insulin-stimulated glucose uptake, as well as insulin-stimulated AKT phosphorylation (21), suggesting that the reduction in GRB10 protein may have partly accounted for the enhanced insulin signaling in glutamine-treated HFD-fed mice. Because the gene ontology analysis revealed that the glutamine effect was primarily related to pathways controlling inflammation, we treated skeletal muscle cells with LPS for 24 h to determine whether there is an association between inflammation and *GRB10* expression. As

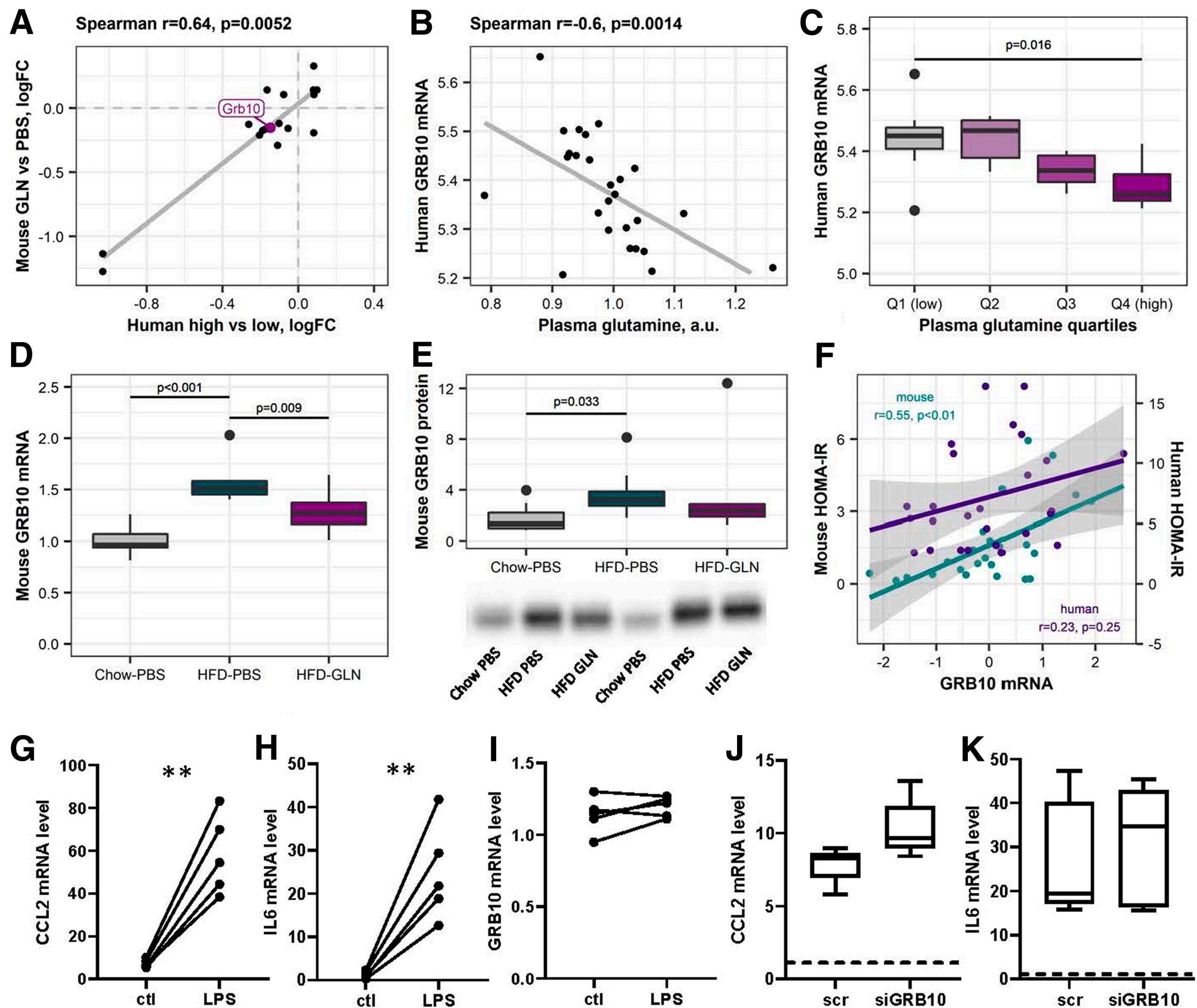

Figure 4—Skeletal muscle expression of the adaptor protein GRB10 is associated with plasma glutamine (GLN) levels. *A*: Correlation between the subset of genes altered between both skeletal muscle of participants with high glutamine (quartile 4 [Q4]) versus low GLN (Q1) and skeletal muscle of GLN-treated versus PBS-treated HFD-fed mice ($P < 0.05$). *B*: Correlation between plasma GLN level and human skeletal muscle expression of *GRB10* ($n = 27$). *C*: *GRB10* expression level measured by gene array in human skeletal muscle per Q of plasma GLN concentration. *D–E*: mRNA (*D*) and protein level (*E*) of GRB10 in quadriceps of mice; Kruskal-Wallis test with Dunn multiple comparison test ($n = 8$). *F*: Spearman correlation between *GRB10* gene expression and HOMA-IR in mice and humans. *G–I*: *CCL2* (*G*), *IL-6* (*H*), and *GRB10* (*I*) mRNA levels in skeletal muscle cells after 24-h treatment with 100 ng/mL LPS. *J–K*: *CCL2* (*J*) and *IL-6* (*K*) mRNA levels in response to LPS after transfection with scramble siRNA (scr) or siRNA targeting *GRB10* (siGRB10). The dashed line represents the expression in scr control (ctl) condition. Paired Student *t* test. $n = 5$. $^{**}P < 0.01$. a.u., arbitrary unit; logFC, logarithm of fold change.

expected, LPS induced a strong increase in interleukin-6 (*IL-6*) and *CCL2* expression; however, *GRB10* mRNA was unchanged (Fig. 4*G–I*). Furthermore, *GRB10* silencing (Supplementary Fig. 3*E*) did not affect the LPS-induced upregulation of *IL-6* or *CCL2* expression (Fig. 4*J–K*), suggesting these transcriptional events are regulated in an independent manner in response to glutamine.

Glutamine Metabolism by Glutaminase Regulates *IL-6* and *GRB10* Transcription in Myotubes

To decipher the molecular mechanism by which glutamine regulates the skeletal muscle transcriptional profile and exerts a beneficial effect on metabolism, we differentiated primary human skeletal muscle cells during 8 days in presence of 0.5 (low) or 10 mmol/L (high) glutamine (Fig. 5*A*). Gene expression and secretion of the inflammatory cytokines *IL-6* and *CCL2* were decreased in myotubes exposed to high glutamine (Fig. 5*B–C* and Supplementary Fig. 3*F–G*), indicating that glutamine directly affects the expression of inflammatory cytokines in skeletal muscle. In myotubes, glutamine can be used as a substrate for several cellular pathways, including the hexosamine pathway, the TCA cycle, and the glutathione system. Modulation of these metabolic pathways can ultimately regulate

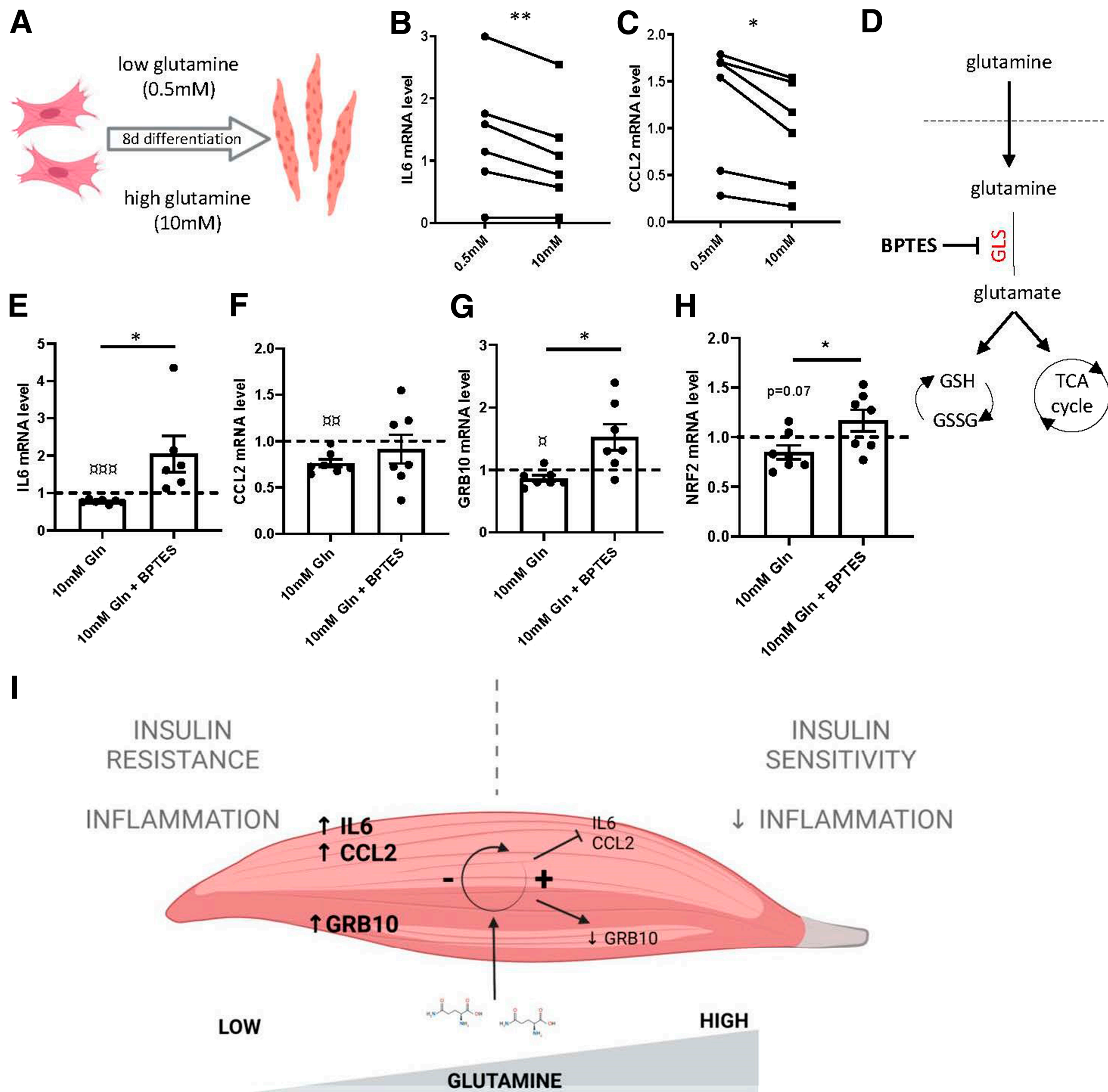

Figure 5—Glutamine (GLN) metabolism by glutaminase (GLS) regulates IL-6 and GRB10 transcription in myotubes. *A–C*: Skeletal muscle cells were differentiated during 8 days in myotubes in low (0.5 mmol/L) or high (10 mmol/L) concentration of glutamine (*A*), and *IL-6* (*B*) and *CCL2* (*C*) mRNA levels were measured ($n = 6$). *D*: BPTES is an inhibitor of GLS1 that converts GLN to glutamate. *E–H*: BPTES was added for 24 h, and *IL-6* (*E*), *CCL2* (*F*), *GRB10* (*G*), and *NRF2* (*H*) mRNA levels were measured ($n = 7$). Statistical effect was measured using paired Student *t* test or Wilcoxon test. *$P < 0.05$, **$P < 0.01$ for difference between BPTES treated and untreated. ¤$P < 0.05$, ¤¤$P < 0.01$, ¤¤¤$P < 0.001$ for difference between high and low GLN conditions (indicated by dashed line). *I*: Graphical abstract.

transcription factor activity (22). To identify potential pathways by which glutamine modulates gene expression, we incubated myotubes with an inhibitor of glutaminase 1 activity, BPTES. BPTES inhibits the conversion of glutamine to glutamate, which is the precursor for glutaminolysis and glutathione synthesis (Fig. 5*D*). A 24-h BPTES exposure increased the expression of *IL-6*, but not *CCL2* (Fig. 5*E–F*). *GRB10* expression was decreased in response to high glutamine exposure ($P = 0.03$) and upregulated after BPTES treatment (Fig. 5*G*). Presumably, increased glutamine integration into glutathione could affect the cellular oxidative stress status and therefore the regulation of inflammatory cytokines. Therefore, we measured the expression of the antioxidant enzyme glutathione peroxidase 1 (*GPX1*) and of the transcription factor nuclear factor erythroid 2–related factor 2 (*NRF2*), which is

activated in response to oxidative stress and regulates the expression of cytoprotective genes. *NRF2* mRNA levels showed a trend toward decreasing in response to high glutamine (P = 0.07) and increased after BPTES treatment, while *GPX1* showed an opposite pattern (Fig. 5*H* and Supplementary Fig. 3*H*). Similarly, in differentiated myotubes exposed to a high glutamine medium for 48 h, we found decreased CCL2 expression, a trend toward reduced GRB10 expression, and unchanged IL-6 expression (Supplementary Fig. 3*I–L*). Collectively, our results suggest that modulation of extracellular glutamine levels regulates *GRB10* expression and inflammation in myotubes. Moreover, the effect of glutamine on transcriptional activity is partly mediated through glutamine metabolism by glutaminase and its role as a substrate in intermediary metabolism.

DISCUSSION

We provide evidence that plasma glutamine levels are associated with insulin resistance and BMI in men. Moreover, glutamine administration directly improved HFD-induced impairments in glucose homeostasis in a mouse model. Our data suggest that the beneficial effect of glutamine treatment is mediated through modulation of the skeletal muscle immunometabolic profile, leading to decreased inflammation and improved insulin sensitivity (Fig. 5*I*). Comparative analysis of the skeletal muscle transcriptomic profile in humans and mouse models identified that GRB10, an adaptor protein involved in insulin signaling, is associated with glutamine levels and insulin sensitivity. These glutamine-dependent changes in inflammatory cytokines and GRB10 expression require glutamine metabolism by glutaminase, the first enzyme in glutaminolysis.

Analysis of paired samples of plasma and vastus lateralis skeletal muscle from men with either NGT or type 2 diabetes allowed us to compare circulating and skeletal muscle metabolomic signatures of the same individual. With this comparative analysis, we previously identified changes in plasma amino acids (15) and now revealed that low glutamine levels and high glutamate levels are associated with BMI and HOMA-IR. This is consistent with evidence that a high glutamine-to-glutamate ratio was associated with reduced risk of incident type 2 diabetes in a large-scale population cohort (23) and insulin sensitivity in obese patients (11). Of note, plasma glutamine levels were unaltered in the lean men with type 2 diabetes, possibly indicating that glutamine levels are implicated in the development of peripheral insulin resistance, whereas impaired glucose homeostasis in lean individuals with type 2 diabetes is often associated with defective insulin secretion (24,25). We found that plasma glutamine, but not glutamate, correlated with skeletal muscle levels, in line with previous reports showing that skeletal muscle is the major contributor to circulating glutamine levels (26). Thus, glutamine availability may be of importance in the pathogenesis of obesity-related type 2 diabetes.

Glutamine supplementation is beneficial for diseases associated with a hypercatabolic state, such as critical illness or sepsis (27). Glutamine administration is also beneficial during high-intensity exercise, where oxidative stress and transient inflammation occur, through increased levels of the antioxidant glutathione and an inhibition of the proinflammatory nuclear factor-κB pathway (28). In metabolic diseases, oral glutamine supplementation induces weight loss and improves glucose metabolism in rodent models of HFD-induced obesity (23,29,30) and in obese humans (31,32). However, glutamine is primarily metabolized by the intestine when administrated orally (27), and part of its beneficial effect may be mediated through changes in the microbiome (33) and/or incretin release. In contrast, parenteral glutamine administration increases circulatory glutamine and glutamine availability for peripheral organs, such as skeletal muscle (27,34). In the current study, 2-week glutamine treatment in HFD-fed mice improved glucose tolerance and insulin sensitivity, concomitant with enhanced EDL muscle insulin signaling and increased AKT activity. The insulin-sensitizing effect of glutamine appears to be lower in oxidative soleus muscle, which may be due to an intrinsic fiber-type difference in insulin signaling or metabolism (35). Other organs, such as adipose tissue, could also contribute to the glutamine-associated improvements in whole-body glucose homeostasis. Conversely, glutamine administration did not affect body weight or body fat composition, suggesting that the improvements in insulin sensitivity are independent of adipose tissue loss. Using a shorter HFD exposure, we showed that a similar 2-week glutamine treatment protocol resulted in an attenuation of fat mass gain as compared with the HFD-fed control group, with reductions in adipocyte size, decreased immune cell infiltration, and improved glucose metabolism (11). In the current study, the 2-week glutamine treatment was initiated after a longer period (15 weeks) of HFD in severely obese mice. Therefore, we cannot exclude the possibility that longer glutamine treatment or earlier intervention would have a more beneficial effect on adiposity and prevent obesity (11,29).

Low-grade inflammation during the early stages of obesity may be triggered by nutrient overload and metabolic dysfunction and thereby cause peripheral insulin resistance (5). We report that glutamine treatment improved the skeletal muscle expression profile of genes related to inflammatory responses as compared with HFD-fed mice, with decreased expression of the cytokine *Tnf* and the chemokine *Ccl2*. Accordingly, the expression of the monocyte marker *Cd68* and the M1 macrophage marker *Cd11c* were lower in glutamine-treated HFD-fed mice, suggesting a diminution in macrophage infiltration and/or activation in response to glutamine (36). In parallel, we show that glutamine treatment can directly regulate the expression and secretion of the cytokines IL-6 and CCL2 in human

myotubes. Interestingly, inhibition of glutaminase activity by BPTES profoundly increased *IL-6* expression in myotubes, suggesting that glutamine catabolism is important for its anti-inflammatory effect. Glutamine conversion by glutaminase generates glutamate, which can undergo metabolism in the TCA cycle (glutaminolysis) or be used as a precursor for the synthesis of glutathione, an antioxidant. Thus, the modulation of *NRF2* expression in response to glutamine suggests that lower oxidative stress levels may account for the decrease in cytokine expression in a high-glutamine condition. Conversely, *CCL2* expression, despite significant downregulation after a 48-h exposure of cultured cells to high glutamine, was not increased after inhibition of glutaminase activity. This suggests a differential temporal and mechanistic glutamine-dependent regulation of *IL-6* and *CCL2* expression in myocytes, consistent with an earlier report on isolated adipocytes, where IL-6, but not CCL2, was regulated by glutamine-induced *O*-GlcNAcylation (11). Collectively, our data indicate that increased glutamine availability may modulate cytokine secretion by myotubes and thereby reduce immune cell recruitment or proinflammatory activity. Of clinical relevance, dietary glutamine supplementation may potentially delay the onset of insulin resistance by modulating skeletal muscle metabolism toward an anti-inflammatory phenotype.

Glutamine levels can influence the inflammatory response of skeletal muscle and regulate the expression of multiple transcripts. In the current study, a limited number of transcripts were similarly altered in skeletal muscle following stratification by glutamine exposure in humans and mice. This could reflect both the cohort size and interspecies differences in muscle transcriptome (37,38). Therefore, the identified genes are likely glutamine-responsive genes that are conserved across species. Interestingly, we noted that *GRB10*, an adaptor protein that interacts with tyrosine kinase receptors, was downregulated in skeletal muscle of both humans and mice in conjunction with elevated glutamine levels. Furthermore, *Grb10* mRNA was correlated with HOMA-IR in mice, with a similar profile observed in humans. A genome-wide association study identified an association between the *GRB10* locus and type 2 diabetes in an Amish population (39). Of functional relevance, disruption of *GRB10* in peripheral tissues improves insulin sensitivity in skeletal muscle and adipose tissue (40–42). Moreover, downregulation of *GRB10* mRNA in human skeletal muscle cells increases insulin-induced PI3K/AKT signaling and glucose uptake (21), which is consistent with the increased AKT activity detected in the EDL muscle of glutamine-treated HFD-fed mice. *GRB10* can also be upregulated by mTORC1 activation (43). In our study, LPS treatment of primary human myotubes, which activates the mTORC pathway (44), did not increase *GRB10*, suggesting that a different mechanism is involved. In skeletal muscle cells, high-glutamine treatment downregulated *GRB10* expression, and BPTES inhibition of glutaminase 1 increased *GRB10* levels, indicating that *GRB10* expression is regulated through glutamine metabolism by glutaminase. Thus, transcription factors sensitive to metabolites, such as CTCF (regulated by α ketoglutarate, an intracellular metabolite of glutamine), are potential candidates for glutamine-dependent *GRB10* regulation (45,46). While we cannot exclude the possibility that changes in glutaminase activity increased *GRB10* levels in skeletal muscle from men with type 2 diabetes, glutamine supplementation of myotube cultures and in HFD-fed mice was associated with reductions in *GRB10* expression in skeletal muscle. Therefore, glutamine modulation of *GRB10* could represent a potential target to improve skeletal muscle insulin sensitivity in type 2 diabetes. Nevertheless, further validation by proteomic-based approaches may elucidate the link between glutamine and GRB10 regulation. Additional studies in both men and women may also clarify whether there is a sex-dependent effect of glutamine treatment on insulin sensitivity and inflammation.

In conclusion, we show that lower plasma glutamine is associated with high BMI and HOMA-IR index in men. Moreover, we provide evidence of a direct role of glutamine in the prevention of inflammation and insulin resistance in skeletal muscle. Dietary glutamine supplementation could represent a potential therapeutic strategy to prevent or delay skeletal muscle insulin resistance by maintaining the homeostatic control of immunometabolism and through regulating GRB10 expression.

Funding. This work was supported by grants from the Swedish Research Council (2015-00165 and 2018-02389), the Novo Nordisk Foundation (Tripartite Immuno-metabolism Consortium grant NNF15CC0018486, MSAM Consortium grant NNF15SA0018346, and MeRIAD Consortium grant 0064142), the European Foundation for the Study of Diabetes Research Programme on New Targets for Type 2 Diabetes supported by an educational research grant from MSD, the Swedish Diabetes Foundation (DIA2018-357 and DIA2018-336), Diabetes Wellness, the Knut and Alice Wallenberg Foundation (2018-0094) (M.R., A.K, E.N., and J.R.Z.), Cimed (M.R.), the European Association for the Study of Diabetes/Novo Nordisk Foundation (NNF21SA0072747) (N.J.P.), the Strategic Research Programme in Diabetes at Karolinska Institutet (2009-1068), and the Stockholm County Council (SLL20170159). L.D. was supported by a Novo Nordisk postdoctoral fellowship run in partnership with Karolinska Institutet and by a Novo Nordisk Foundation postdoctoral fellowship for research abroad (NNF20OC0060969). J.R.Z., J.T., and E.D. were supported by the Novo Nordisk Foundation Center for Basic Metabolic Research (CBMR). CBMR is an independent Research Center at the University of Copenhagen that is partially funded by an unrestricted donation from the Novo Nordisk Foundation (NNF18CC0034900).

Duality of Interest. The costs of the human metabolomic study were supported by a grant from Daiichi Sankyo Co., Ltd. J.H. is an employee of Daiichi Sankyo Co., Ltd. No other potential conflicts of interest relevant to this article were reported.

Author Contributions. L.D. and A.K. conceived the idea, planned the experiments, collected and analyzed data, and wrote the manuscript. M.K., E.C., D.R.-R., L.P., and E.D. collected mouse data and analyzed tissue samples. E.C., A.M.A., H.K.R.K., M.B., J.T.T., J.H., and N.J.P. contributed to the scientific discussion. A.M.A. collected cell data. H.K.R.K. assisted with recruitment of participants and collection of human metabolite data. E.N. obtained

human skeletal muscle biopsies and blood samples. M.R., J.R.Z., and A.K. supervised the study, reviewed and edited the manuscript, and acquired funding. N.J.P. performed bioinformatic analysis. All authors edited and reviewed the manuscript. A.K. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

References

1. Hu FB, Manson JE, Stampfer MJ, et al. Diet, lifestyle, and the risk of type 2 diabetes mellitus in women. N Engl J Med 2001;345:790–797
2. Willett WC, Dietz WH, Colditz GA. Guidelines for healthy weight. N Engl J Med 1999;341:427–434
3. DeFronzo RA, Tripathy D. Skeletal muscle insulin resistance is the primary defect in type 2 diabetes. Diabetes Care 2009;32(Suppl. 2):S157–S163
4. Shoelson SE, Lee J, Goldfine AB. Inflammation and insulin resistance. J Clin Invest 2006;116:1793–1801
5. Wu H, Ballantyne CM. Skeletal muscle inflammation and insulin resistance in obesity. J Clin Invest 2017;127:43–54
6. Ferrante AWJr. Macrophages, fat, and the emergence of immunometabolism. J Clin Invest 2013;123:4992–4993
7. Mathis D, Shoelson SE. Immunometabolism: an emerging frontier. Nat Rev Immunol 2011;11:81
8. McArdle MA, Finucane OM, Connaughton RM, McMorrow AM, Roche HM. Mechanisms of obesity-induced inflammation and insulin resistance: insights into the emerging role of nutritional strategies. Front Endocrinol (Lausanne) 2013;4:52
9. Li P, Oh DY, Bandyopadhyay G, et al. LTB4 promotes insulin resistance in obese mice by acting on macrophages, hepatocytes and myocytes. Nat Med 2015;21:239–247
10. Wang H, Knaub LA, Jensen DR, et al. Skeletal muscle-specific deletion of lipoprotein lipase enhances insulin signaling in skeletal muscle but causes insulin resistance in liver and other tissues. Diabetes 2009;58:116–124
11. Petrus P, Lecoutre S, Dollet L, et al. Glutamine links obesity to inflammation in human white adipose tissue. Cell Metab 2020;31:375–390.e11
12. Gonzalez-Franquesa A, Burkart AM, Isganaitis E, Patti ME. What have metabolomics approaches taught us about type 2 diabetes? Curr Diab Rep 2016;16:74
13. Yoneshiro T, Wang Q, Tajima K, et al. BCAA catabolism in brown fat controls energy homeostasis through SLC25A44. Nature 2019;572:614–619
14. Berger SL, Sassone-Corsi P. Metabolic signaling to chromatin. Cold Spring Harb Perspect Biol 2016;8:a019463
15. Sjögren RJO, Rizo-Roca D, Chibalin AV, et al. Branched-chain amino acid metabolism is regulated by ERRα in primary human myotubes and is further impaired by glucose loading in type 2 diabetes. Diabetologia 2021;64: 2077–2091
16. Okita K, Iwahashi H, Kozawa J, et al. Usefulness of the insulin tolerance test in patients with type 2 diabetes receiving insulin therapy. J Diabetes Investig 2014;5:305–312
17. Al-Khalili L, Chibalin AV, Kannisto K, et al. Insulin action in cultured human skeletal muscle cells during differentiation: assessment of cell surface GLUT4 and GLUT1 content. Cell Mol Life Sci 2003;60:991–998
18. Savikj M, Kostovski E, Lundell LS, Iversen PO, Massart J, Widegren U. Altered oxidative stress and antioxidant defence in skeletal muscle during the first year following spinal cord injury. Physiol Rep 2019;7:e14218
19. Menni C, Fauman E, Erte I, et al. Biomarkers for type 2 diabetes and impaired fasting glucose using a nontargeted metabolomics approach. Diabetes 2013;62:4270–4276
20. Chen S, Wasserman DH, MacKintosh C, Sakamoto K. Mice with AS160/TBC1D4-Thr649Ala knockin mutation are glucose intolerant with reduced insulin sensitivity and altered GLUT4 trafficking. Cell Metab 2011;13:68–79
21. Edick AM, Auclair O, Burgos SA. Role of Grb10 in mTORC1-dependent regulation of insulin signaling and action in human skeletal muscle cells. Am J Physiol Endocrinol Metab 2020;318:E173–E183
22. Brasse-Lagnel C, Lavoinne A, Husson A. Control of mammalian gene expression by amino acids, especially glutamine. FEBS J 2009;276:1826–1844
23. Cheng S, Rhee EP, Larson MG, et al. Metabolite profiling identifies pathways associated with metabolic risk in humans. Circulation 2012; 125:2222–2231
24. Cauchi S, Nead KT, Choquet H, et al. The genetic susceptibility to type 2 diabetes may be modulated by obesity status: implications for association studies. BMC Med Genet 2008;9:45
25. Wajngot A, Roovete A, Vranić M, Luft R, Efendić S. Insulin resistance and decreased insulin response to glucose in lean type 2 diabetics. Proc Natl Acad Sci USA 1982;79:4432–4436
26. Nurjhan N, Bucci A, Perriello G, et al. Glutamine: a major gluconeogenic precursor and vehicle for interorgan carbon transport in man. J Clin Invest 1995;95:272–277
27. Cruzat V, Macedo Rogero M, Noel Keane K, Curi R, Newsholme P. Glutamine: metabolism and immune function, supplementation and clinical translation. Nutrients 2018;10:1564
28. Leite JS, Raizel R, Hypólito TM, Rosa TD, Cruzat VF, Tirapegui J. L-glutamine and L-alanine supplementation increase glutamine-glutathione axis and muscle HSP-27 in rats trained using a progressive high-intensity resistance exercise. Appl Physiol Nutr Metab 2016;41(8):842–849
29. Abboud KY, Reis SK, Martelli ME, et al. Oral glutamine supplementation reduces obesity, pro-inflammatory markers, and improves insulin sensitivity in DIO Wistar rand reduces waist circumference in overweight and obese humans. Nutrients 2019;11:536
30. Opara EC, Petro A, Tevrizian A, Feinglos MN, Surwit RS. L-glutamine supplementation of a high fat diet reduces body weight and attenuates hyperglycemia and hyperinsulinemia in C57BL/6J mice. J Nutr 1996;126: 273–279
31. Laviano A, Molfino A, Lacaria MT, et al. Glutamine supplementation favors weight loss in nondieting obese female patients. A pilot study. Eur J Clin Nutr 2014;68:1264–1266
32. Mansour A, Mohajeri-Tehrani MR, Qorbani M, Heshmat R, Larijani B, Hosseini S. Effect of glutamine supplementation on cardiovascular risk factors in patients with type 2 diabetes. Nutrition 2015;31:119–126
33. de Souza AZ, Zambom AZ, Abboud KY, et al. Oral supplementation with L-glutamine alters gut microbiota of obese and overweight adults: a pilot study. Nutrition 2015;31:884–889
34. Melis GC, Boelens PG, van der Sijp JR, et al. The feeding route (enteral or parenteral) affects the plasma response of the dipetide Ala-Gln and the amino acids glutamine, citrulline and arginine, with the administration of Ala-Gln in preoperative patients. Br J Nutr 2005;94:19–26
35. Song XM, Kawano Y, Krook A, et al. Muscle fiber type-specific defects in insulin signal transduction to glucose transport in diabetic GK rats. Diabetes 1999;48:664–670
36. Liu PS, Wang H, Li X, et al. α-ketoglutarate orchestrates macrophage activation through metabolic and epigenetic reprogramming. Nat Immunol 2017;18:985–994
37. Abdelmoez AM, Sardón Puig L, Smith JAB, et al. Comparative profiling of skeletal muscle models reveals heterogeneity of transcriptome and metabolism. Am J Physiol Cell Physiol 2020;318:C615–C626
38. Kelahmetoglu Y, Jannig PR, Cervenka I, et al. Comparative analysis of skeletal muscle transcriptional signatures associated with aerobic exercise capacity or response to training in humans and rats. Front Endocrinol (Lausanne) 2020;11:591476
39. Rampersaud E, Damcott CM, Fu M, et al. Identification of novel candidate genes for type 2 diabetes from a genome-wide association scan in the Old Order Amish: evidence for replication from diabetes-related quantitative traits and from independent populations. Diabetes 2007;56:3053–3062

40. Shiura H, Miyoshi N, Konishi A, et al. Meg1/Grb10 overexpression causes postnatal growth retardation and insulin resistance via negative modulation of the IGF1R and IR cascades. Biochem Biophys Res Commun 2005;329: 909–916
41. Smith FM, Holt LJ, Garfield AS, et al. Mice with a disruption of the imprinted Grb10 gene exhibit altered body composition, glucose homeostasis, and insulin signaling during postnatal life. Mol Cell Biol 2007;27:5871–5886
42. Wang L, Balas B, Christ-Roberts CY, et al. Peripheral disruption of the Grb10 gene enhances insulin signaling and sensitivity in vivo. Mol Cell Biol 2007;27:6497–6505
43. Yu Y, Yoon SO, Poulogiannis G, et al. Phosphoproteomic analysis identifies Grb10 as an mTORC1 substrate that negatively regulates insulin signaling. Science 2011;332:1322–1326
44. Ribeiro MC, Peruchetti DB, Silva LS, et al. LPS induces mTORC1 and mTORC2 activation during monocyte adhesion. Front Mol Biosci 2018;5:67
45. Chisolm DA, Savic D, Moore AJ, et al. CCCTC-binding factor translates interleukin 2- and alpha-ketoglutarate-sensitive metabolic changes in T cells into context-dependent gene programs. Immunity 2017;47:251–267.e7
46. Plasschaert RN, Bartolomei MS. Tissue-specific regulation and function of Grb10 during growth and neuronal commitment. Proc Natl Acad Sci USA 2015;112:6841–6847