Figure S1

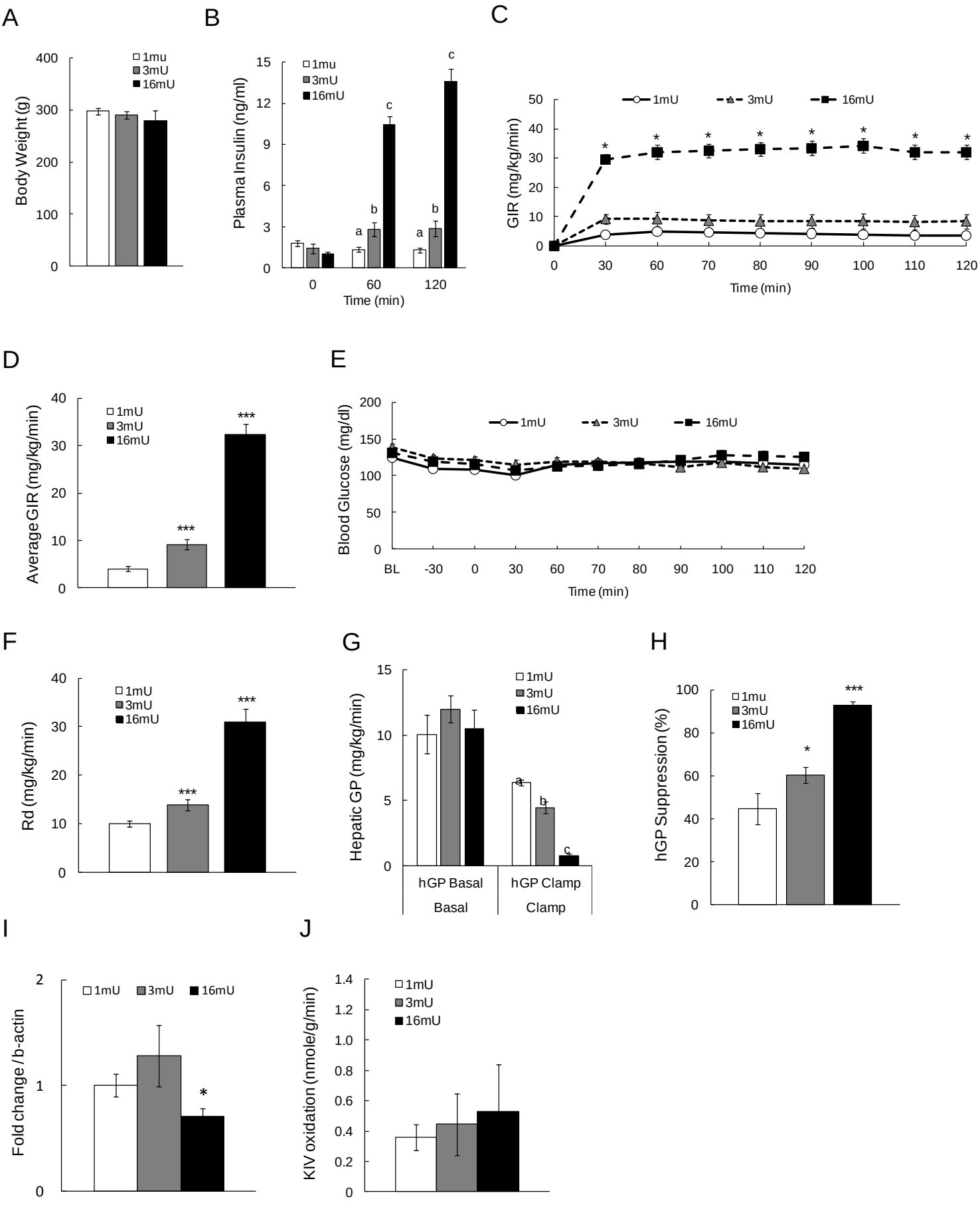
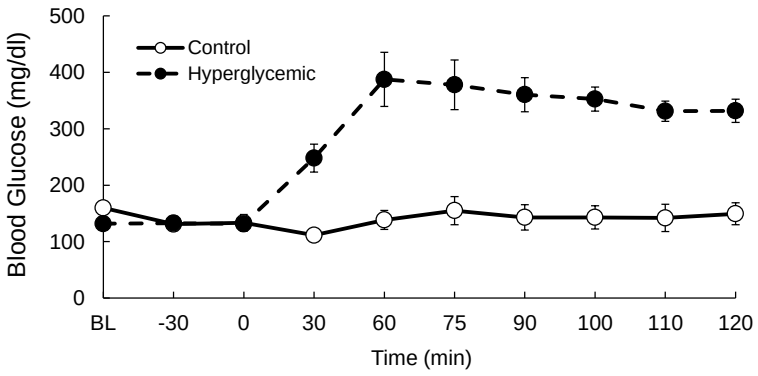
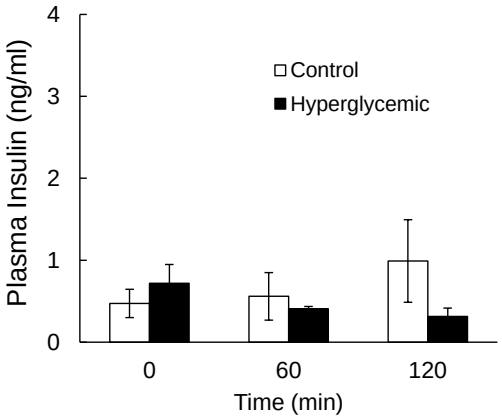

A
Body Weight (g)
1mu
3mU
16mU
B
Plasma Insulin (ng/ml)
Time (min)
C
GIR (mg/kg/min)
Time (min)
D
Average GIR (mg/kg/min)
E
Blood Glucose (mg/dl)
Time (min)
F
Rd (mg/kg/min)
G
Hepatic GP (mg/kg/min)
hGP Basal
hGP Clamp
Basal
Clamp
H
hGP Suppression (%)
I
Fold change / b-actin
J
KIV oxidation (nmole/g/min)

Figure S2

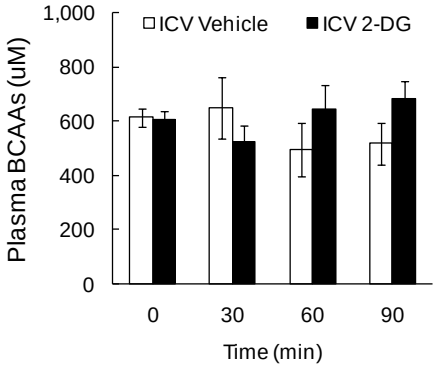
A



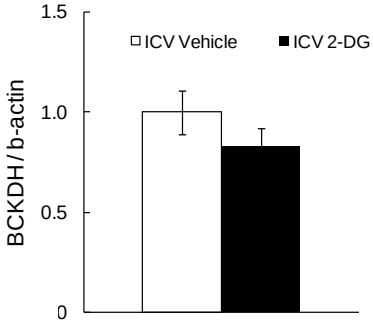
B



C



D



E

	WT	ΔPER	WT	ΔWB
Glucose (mg/dl)	156±6	488±24	149±14	470±42
Insulin (ng/ml)	0.7±0.1	62±18.3	1.1±0.1	69±3.4

Figure S3

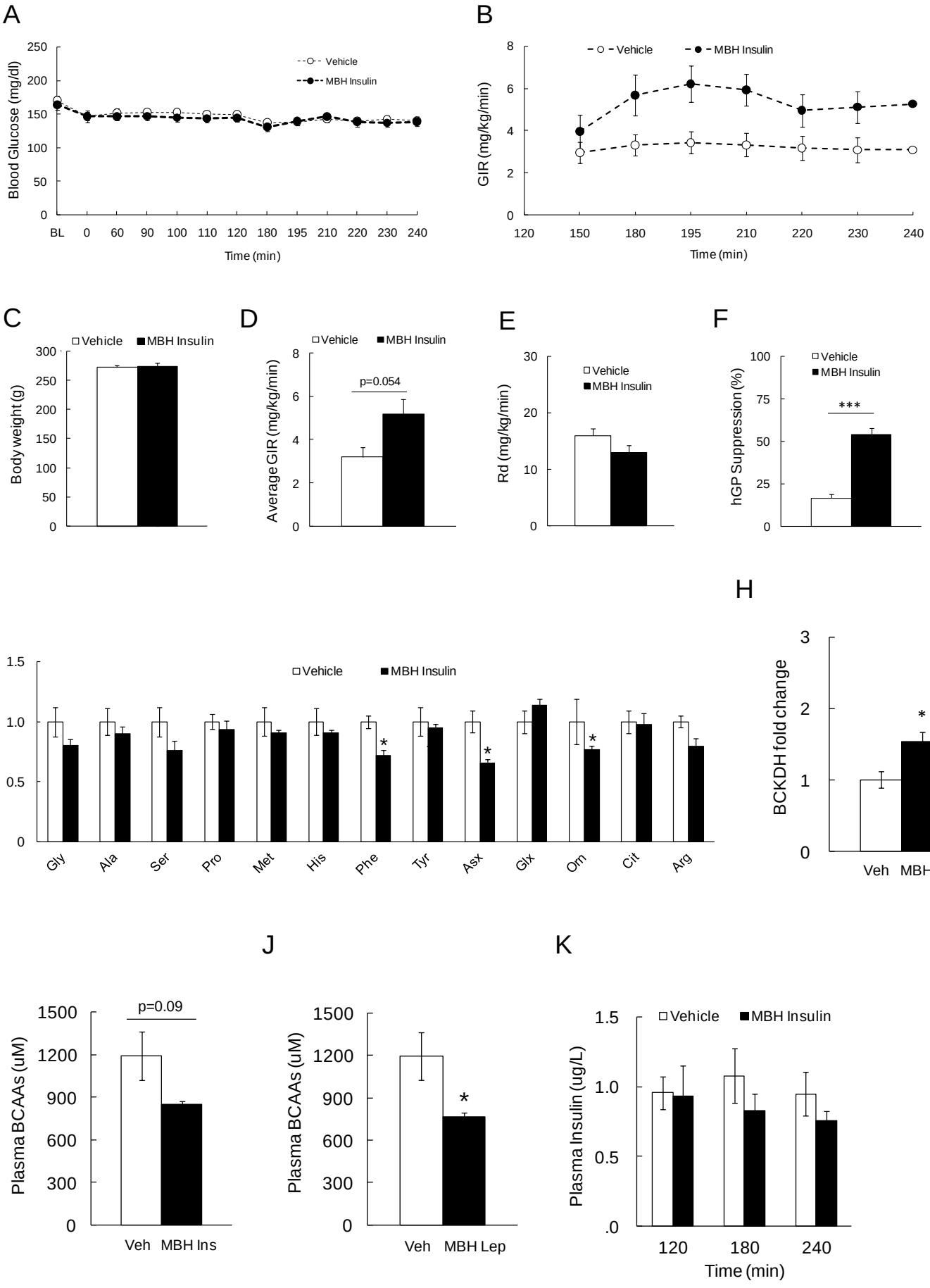
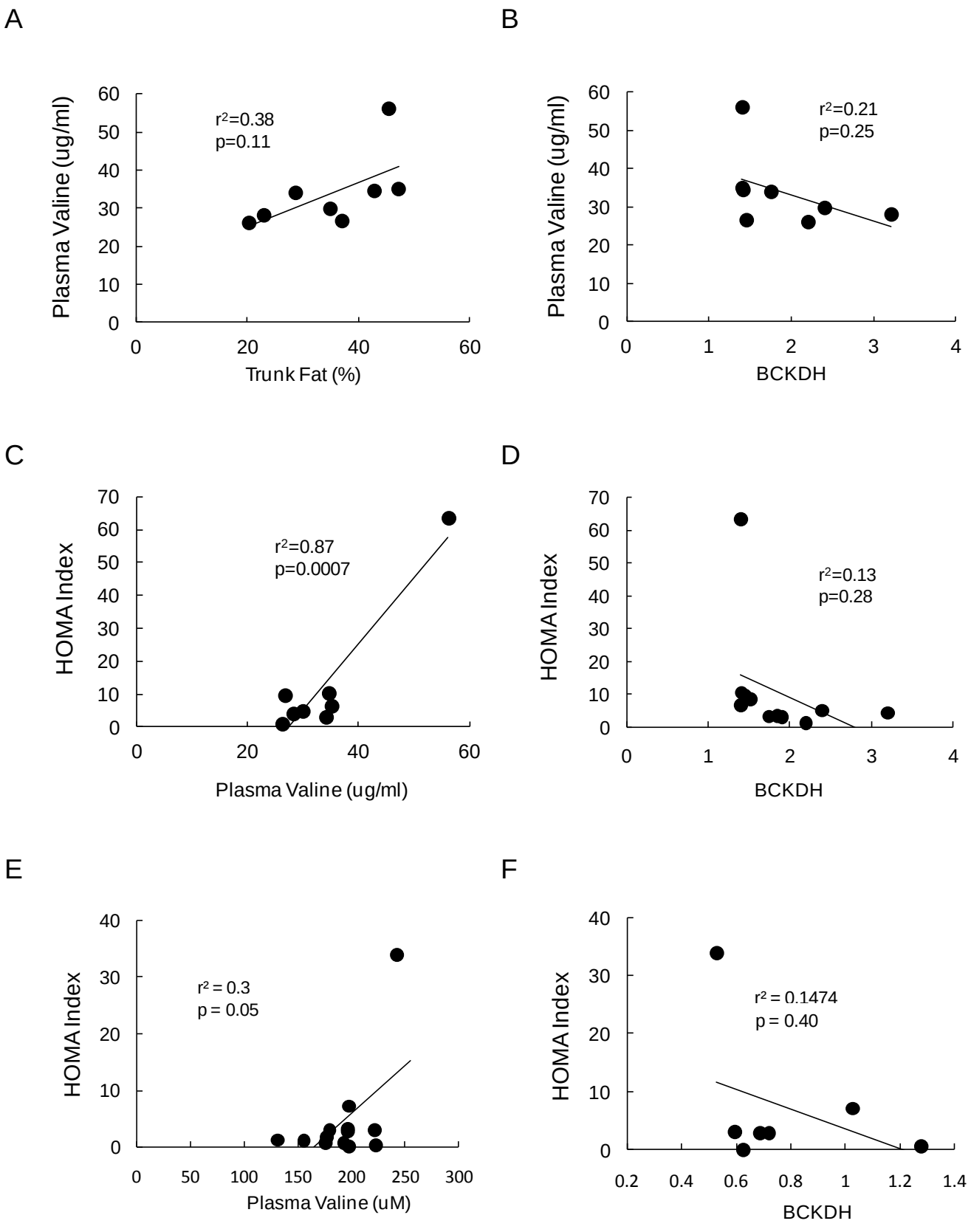

A
Blood Glucose (mg/dl)
Vehicle
MBH Insulin
BL 0 60 90 100 110 120 180 195 210 220 230 240
Time (min)
B
GIR (mg/kg/min)
Vehicle
MBH Insulin
Time (min)
C
Body weight (g)
D
Average GIR (mg/kg/min)
p=0.054
E
Rd (mg/kg/min)
F
hGP Suppression (%)
***
G
Fold change
Gly Ala Ser Pro Met His Phe Tyr Asx Glx Orn Cit Arg
H
BCKDH fold change
Veh MBH Ins
I
Plasma BCAAs (uM)
p=0.09
Veh MBH Ins
J
Plasma BCAAs (uM)
Veh MBH Lep
K
Plasma Insulin (ug/L)
Time (min)

Figure S4


A
Plasma Valine (ug/ml)
r²=0.38
p=0.11
Trunk Fat (%)
B
Plasma Valine (ug/ml)
r²=0.21
p=0.25
BCKDH
C
HOMA Index
r²=0.87
p=0.0007
Plasma Valine (ug/ml)
D
HOMA Index
r²=0.13
p=0.28
BCKDH
E
HOMA Index
r² = 0.3
p = 0.05
Plasma Valine (uM)
F
HOMA Index
r² = 0.1474
p = 0.40
BCKDH

Supplemental Legends

Figure S1, related to Figure 1. Hyperinsulinemic euglycemic clamp data in male SD rats. A) Body weight, B) Plasma insulin levels before and during clamps, C) Glucose infusion rate (GIR) required to maintain euglycemia, D) Mean GIR, E) Blood glucose levels from the baseline until the end of clamps, F) Rate of disappearance (Rd), G) Hepatic glucose production (hGP) before and during clamps, and H) Percent suppression of hGP (n ≥ 5 per group). I) BCKDH protein expression in pergonadal WAT at the end of the clamps (n ≥ 5 per group). J) BCKDH activity in the white adipose tissue as measured by α-keto isovalerate (KIV) oxidation assay using 1-^{14}C isotope. (n ≥ 5 per group). * $p<0.05$ compared to control group. *** $p<0.01$ compared to the control group. Values are mean±SEM.

Figure S2, related to Figure 1 and Figure 3. Hyperglycemic clamps, ICV 2-DG infusion study, and whole-body vs. peripheral insulin receptor KO study. A) Blood glucose levels and B) Plasma insulin levels before and during clamps in SD male rats (n ≥ 4 per group). C) Plasma BCAA levels before and after ICV 2-DG infusion (n ≥ 4 per group). D) Hepatic BCKDH protein expression 90 min after ICV 2-DG infusion in male SD rats (n ≥ 4 per group). E) Systemic glucose and insulin levels in IRΔWB and IRΔPER mice (n ≥ 7 per group). Values are mean±SEM.

Figure S3, related to Figure 2. Data from euglycemic clamps with MBH insulin infusion. A) Blood glucose levels before and during clamps, B) GIR during clamps, C) Body weight, D) Mean GIR, E) Rd, and F) Percent suppression of hGP (n ≥ 7 per group). G) Relative quantification of plasma amino acid levels after MBH insulin infusion (n = 4 per group). H) BKCDH protein expression in perigonadal WAT after MBH insulin infusion (n ≥ 5 per group). I) Plasma BCAA levels after MBH insulin infusion in a separate cohort (n ≥ 4 per group). J) Plasma BCAAs after MBH leptin infusion (n = 4 per group). K) Plasma insulin levels during euglycemic clamp with MBH insulin infusion (n ≥ 7 per group). * $p<0.05$ compared to control group. *** $p<0.01$ compared to the control group. Values are mean±SEM.

Figure S4, related to Figure 4. Correlations between parameters of metabolic control and BCAA catabolism in male Macaque and obese and diabetic human studies. Correlations between A) % trunk fat vs. plasma valine. B) Hepatic BCKDH protein levels vs. plasma valine. C) Plasma valine vs. HOMA index and D) Hepatic BCKDH protein vs. HOMA index in male Macaque monkeys after 1.5 years of either regular chow or HF feeding. E) Correlations

between plasma valine vs. HOMA index and F) Hepatic BCKDH protein levels vs. HOMA index in non-obese, obese, or obese and diabetic male subjects. n ≥ 7 per group.

Supplemental Experimental Procedures

Pancreatic Clamps

SD hyperinsulinemic euglycemic clamps:The animals were clamped at either basal (1mU/kg/min), mild (3mU/kg/min), or markedly high (8 or 16mU/kg/min) insulinemia along with somatostatin (3ug/kg/min) to suppress endogenous insulin and control counter-regulatory hormones. Basal and moderate hyperinsulinemic clamps consisted of 4h of basal state (t=-120 to 120 min) followed by 2h of insulin (t=120 to 240 min) clamp along with somatostatin and 25% glucose infusion, while marked hyperinsulinemic clamps lasted for 150 min with t=-30 min as baseline and insulin clamp starting at t=0 min until t=120 min. 5% glucose was used to maintain euglycemia.

SD hyperglycemic clamps: Glucose levels were clamped at 330-360 mg/dl by infusing a variable rate of 45% glucose intravenously while maintaining basal insulin levels (1mU/kg/min) with somatostatin infusion as above. At the end of clamps, animals were decapitated and their organs including liver and kidney were harvested and freeze-clamped for western blot analysis.

SD MBH insulin infusion: Euglycemic clamps with [3-^{3}H]-glucose tracer were performed in nonrestrained, conscious rats as previously described[24]. During the 6 hr experimental protocol (Fig. 2A), we either infused a vehicle (aCSF; Harvard Apparatus, Holliston, MA) or human insulin (2 μU; Humulin R diluted in aCSF; Lilly, Indianapolis, IN) at a rate of 0.18 μl/h per side for 6h into the MBH. Somatostatin was infused (3 μg/kg/min) to prevent insulin and glucagon secretions from pancreas. At each sampling time, collected erythrocytes were re-suspended in saline and given back to the test animal during the study.

Macaque monkey study

Male rhesus macaque monkeys were fed a diet high in sucrose and fat (HF), manufactured by TestDiet (Diet 5L0P; 36.1% of energy from animal fat, 45.7% from

carbohydrates and 18.2% from protein; cholesterol 661p.p.m.; TestDiet, Richmond, IN, USA) and food was available *ad libitum* from 1000h to 1630h. The animals did not have access to food overnight. Animals on the HF diet were supplemented with 500ml of Kool-Aid (Kraft Foods Group, Glenview, IL, USA) drink mix+20% fructose three times a week. The control animals were fed regular chow (TestDiet 5052, 14.6% of energy from fat, 58.5% from carbohydrates and 26.8% from protein). Water was provided *ad libitum* at all times. Lights were on from 0700h to 1900h. Body composition was determined by dual-energy X-ray absorptiometry (DEXA) scan. Briefly, animals were sedated with Telazol (3mg/kg) and the sedation was maintained with ketamine (5–10mg/kg). The animals were laid flat on the DEXA (Hologic Discovery A, Bedford, MA, USA) and a whole body scan performed. Data were imported into an excel file and analyzed with Graphpad Prism software (GraphPad Software, Inc., La Jolla, CA, USA). Glucose homeostasis was determined via IVGTTs, which measures blood glucose clearance after an intravenous bolus infusion of a sterile 50% dextrose solution (600mg/kg). Animals were not fed on the morning of IVGTTs. Blood glucose was measured immediately after the bolus with a glucometer (Onetouch Ultra Blood Glucose Monitor, LifeScan, Milpitas, CA, USA). Plasma was assayed for insulin by the ONPRC/OHSU Endocrine Services Laboratory using an Immulite 2000 (Siemens USA, Deerfield, IL, USA). Protocols involved in this study were approved by the Institutional Animal Care and Use Committee at Mount Sinai School of Medicine and at the Oregon National Primate Research Center at Oregon Health & Science University in accordance with guidelines established by the National Institutes of Health.

Human subjects

11 male and 11 female subjects between 46 and 58 of age were recruited to participate in the study. Exclusion criteria were type I diabetes and acute inflammatory disease. Healthy, non-obese subjects had BMI of 25.9 ± 0.6, while the obese or obese and diabetic groups had 52.1 ± 2.0 and 54.8 ± 1.9, respectively. The HOMA index, an indicator of insulin

sensitivity, was 1.4 ± 0.3 for non-obese, and 5.1 ± 0.5 for obese and 12.4 ± 2.6 for obese+diabetic group. This study was approved by the Medical Ethical Board of the Maastricht University Medical Centre in line with the revised version of the Declaration of Helsinki (October 2008, Seoul), and informed consent in writing was obtained from each subject. Liver samples from the study cohort were obtained for protein expression analysis.

LC-MS/MS quantitative proteomics

Quadruplicate samples for the control and MBH insulin-stimulated conditions were utilized for proteomic analyses. Liver tissues were initially subjected to subcellular fractionation and cytosolic fractions were used for proteomics. Equal amounts of proteins (~500µg) from each of cytosolic fractions were used and proteins were denatured by 8 M Urea and reduced by 10 mM dithiothreitol (DTT) at 37 °C for 1h. Protein cysteine residues were further alkylated by 40 mM iodoacetamide at 37 °C for 1h. Samples were then diluted 10-fold with 50 mM NH_4HCO_3 and digested by sequencing-grade trypsin (Promega, Madison, WI) for 3h at 37°C at a 1:50 trypsin to protein ratio (w:w). The digested sample was then cleaned up with a SPE C-18 column (Supelco, Bellefonte, PA) using a protocol previous described(Qian et al., 2008), and dried using a speed-vac concentrator. The peptide samples were re-dissolved in 100 mM triethylammonium bicarbonate (TEAB) buffer, pH 8.0, and the peptide concentration was determined using a BCA protein assay from Pierce (Rockford, IL).

To enable multiplexed quantification, 8-plex iTRAQ labeling was applied to four control samples and four MBH insulin-stimulated samples. To prepare the iTRAQ reagents, 70µL ethanol was added to each iTRAQ reagent vial. For each sample labeling, 100µg peptides in ~30µl dissolution buffer (100 mM TEAB) were mixed with 70 µl iTRAQ labeling reagent from one of the eight labeling channels. The labeling was carried out at room temperature for 1 hr according to the manufacturer's instructions. The eight samples in the 8-plex experiment

were subsequently mixed and concentrated using a SpeedVac. The 8-plexed labeled samples were then cleaned up using C18 SPE columns.

Dried labeled peptide samples were dissolved in 10 mM Ammonium Formate, pH 10, and subjected to high pH reversed phase LC fractionation on an Agilent 1100 HPLC System (Agilent, Palo Alto, CA) as preciously described(Wang et al., 2011). Briefly, the LC separation was performed at a flow rate of 0.5 mL/min on a XBridge C18 column (250 × 4.6mm, Waters, Milford, MA). The solvent consisted of 10 mM ammonium formate (pH 10) as the mobile phase A and 10 mM ammonium formate and 90% acetonitrile (pH 10) as the mobile-phase B. 96 fractions through 96 min of the gradient were collected and the fractions were combined into 24 final concatenated fashions prior to LC-MS/MS analysis(Wang et al., 2011).

All the fractions from the iTRAQ labeled samples were analyzed by LC-MS/MS on a custom-built automated HPLC LC system coupled on-line to a LTQ-Orbitrap Velos mass spectrometer (Thermo Scientific, San Jose, CA). The LC system was operated the same as previously described(Qian et al., 2008). Full MS spectra were recorded at a resolution of 100K (for ions at *m/z* 400) over the range of *m/z* 400–2000 with an automated gain control (AGC) value of 1×10^6. MS/MS was performed in the data-dependent mode with an AGC target value of 3×10^4. The six most abundant parent ions were selected for MS/MS using high-energy collisional dissociation (HCD) with a normalized collision energy setting of 40%. A dynamic exclusion time of 45s was used.

LC-MS/MS raw data were converted into .dta files using Extract_MSn (version 3.0) in Bioworks Cluster 3.2 (Thermo Fisher Scientific, Cambridge, MA), and the SEQUEST algorithm(Yates et al., 1995) (version 27, revision 12) was used to search all MS/MS spectra against the Rat protein sequence database (Uniprot, released on June 28, 2011). The key search parameters used were: 50 ppm tolerance for precursor ion masses, 0.05 Da for fragment ion masses, a maximum of 2 missed tryptic cleavages, dynamic oxidation of methionine (+15.9949 Da), static cysteine carbamidomethylation (+57.0215), and static

iTRAQ modification of lysine and N-termini (+304.2022 Da). The decoy-database searching methodology(Qian et al., 2005) was used to control the false discovery rate at the unique peptide level to <1%. MS Generating-Function (MSGF) scores were generated for each identified spectrum by computing rigorous p-values (spectral probabilities)(Kim et al., 2008). For quantification, an in-house software MASIC was utilized to extract iTRAQ reporter ion intensities (114-121 in *m/z*) from each MS/MS fragmentation spectrum. Only spectra where the expected iTRAQ reporter ions detected were used for quantification. For each protein, all reporter ion intensities in each channel from different peptides originated from the same protein were summed to represent the relative abundance of the given protein. The raw intensity data were then transformed in Log_2 format and normalized by centering the median Log_2Ratio to zero. A student t-test was applied to identify proteins with significant abundance differences between control and the stimulated condition. Proteins with significant abundance changes were identified by requiring the p-value of <0.05.

BCKDH Activity Assay

Hepatic and white adipose tissue BCKDH activity was determined using a modified version of the method described by Tso(Tso et al., 2013). Briefly, frozen samples were pulverized in liquid nitrogen, then 250mg of tissue was homogenized in 1mL for liver or 250ul for white adipose tissue of ice cold homogenization buffer (30mM KPi pH7.5, 3mM EDTA, 5mM DTT, 1mM α-ketoisovalerate, 3% FBS, 5% Triton X-100, 1uM Leupeptin) using a QIAGEN TissueLyser II set at a frequency of 15/s for 1 minute. Homogenized samples were centrifuged for 10 minutes at 10 000 x g and the supernatant was collected. 50ul of supernatant was added to 300ul of assay buffer (50mM HEPES pH 7.5, 30mM KPi pH7.5, 0.4mM CoA , 3mM NAD+, 5% FBS, 2mM Thiamine Pyrophosphate, 2mM MgCl2, 7.8μM α-keto [1-^{14}C] isovalerate) in a polystyrene test tube containing a raised 1M NaOH CO_2 trapping system. The tubes were capped and placed in a shaking water bath set at 37°C for 30 min. Tubes were then placed on ice and the reaction mixture was acidified by injection of

100ul of 70% perchloric acid followed by shaking on an orbital shaker at room temperature for 1h. The $^{14}CO_2$ contained in the 1M NaOH trap was counted in a liquid scintillation counter.

Supplemental References

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