Supplemental Material

Supplemental Methods:

Mice

Age-matched male apoA-I deficient (apoA-I ko), human apoA-I transgenic (apoA-I tg), and control (wt) C57/Bl6J mice (The Jackson Laboratories, Bar Harbor, ME) were housed in specific pathogen–free facilities with a 12-hour light/12-hour dark cycle and were fed1 basal rodent chow 5058 PicoLab Mouse Diet 20 (LabDiet, Richmond, IN). Mice that underwent the diet-induced obesity study were fed a low fat diet containing 4.8% fat by weight (D12328; Research Diets, New Brunswick, NJ) or a high-fat diet containing 35.8% fat by weight (D12330; Research Diets) for 12 weeks. All experimental procedures conformed to institutional guidelines for animal experiments and were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Cincinnati. Body fat mass was measured in conscious mice using 1H magnetic resonance spectroscopy (EchoMRI-100; Echo- medical Systems). Body weight and body composition measurements were performed monthly.

For the exercise study, mice were accustomed to treadmill running for 3 days as described previously [1] and then an exercise exhaustion test was performed in all mice. Next, mice were randomized for body weight and divided into two groups. The ‘runner’ group was subjected to treadmill running (Simplex II metabolic rodent treadmill, Columbus Instruments, Columbus, OH) during the first 4 h of the dark phase of the circadian cycle, five times a week. During the 12 months of training time (30 – 45 min), speed (5 – 7 m/min), and treadmill inclination (10 - 20°) of exercise was adjusted according to the fitness level resulting in a covered distance of 200 - 315 m/day. The ‘sedentary’ group was not forced to run except during exercise exhaustion tests. Exhaustion tests were performed during the first four hours in the dark phase of the circadian cycle at weeks 0

and 8 of the study as described previously [1]. Briefly, animals ran on the treadmill tilted 20° uphill starting at a warm-up speed of 5 m/min for 4 min after which speed was increased to 14m/min for 2 min. Every subsequent 2 min, the speed was increased by 2 m/min until mice were exhausted. Exhaustion was defined as the inability of the animal to return to running within 10 s after direct contact with an electric-stimulus grid and/or terminal glucose levels below 100 mg/dl. Running time was measured and running distance calculated. Distance is the product of time and speed of the treadmill. Outside of the training schedule, all mice had unlimited access to food and water.

For the determination of downstream insulin-signaling target AKT we fasted mice for 2 hours prior to intraperitoneal injection of insulin (2 IU/kg body weight) or saline (equal volumes). 5 minutes after injection, mice were sacrificed by cervical dislocation, liver and quadiceps muscles were immediately harvested and snap frozen in liquid nitrogen for later determination of AKT phosphorylation.

Euglycemic-hyperinsulinemic clamp studies

Mice underwent vascular surgery under anesthesia with Isoflurane (1,5% ; Air : 0,4l/min) at least 6 days before the experiment. The right jugular vein was catheterized for infusion with a silastic catheter. The free end of the catheter was tunneled under the skin to the back of the neck. Catheters are flushed daily with ~50µl of 0,9% NaCl containing 5mg/ml ampicillin and 20U.I/ml heparin.After surgery animals were individually housed and their body weight was monitored daily. Mice that lost more than 10% of presurgery weight by postsurgery day 6 were excluded from the study. At the day of the clamp study, conscious mice were place in their home cage for the duration of the clamp experiment and food-deprivated for 5 hours. After a bolus infusion of (5 µCi) of D-[3-3H] glucose (Perkin Elmer, Courtaboeuf, France) tracer solution and 80 mU/kg insulin, the tracer was infused

continuously (0.05 µCi/min, at a constant rate of 1µl/min) for the duration of the experiment and insulin infusion was kept constant at 0,2UI/kg/h (3,33mU/kg/min). Blood glucose levels were determined from tail blood samples (1-2 µl) at t =0 and then every 20 min using the glucose analyzer Glucofix® (A. Menarini, Rungis, France). Steady state was ascertained when glucose measurements were constant for at least 20 min at a fixed glucose infusion rate and this was achieved within 50 to 80min. At steady state, 3 blood sample (10µl) were collected for determination of basal parameters, followed by a bolus injection of 2-deoxy-D-[1-14C] glucose (2DG) (3 µCi, Perkin Elmer, Courtaboeuf, France). Blood samples (10 µl) were collected from the tail at 0, 10, 20, 30, 40, 60 min until the end of the experiment where mice were sacrified by elongation and tissues were collected. Basal and steady state plasma [3-3H] glucose radioactivity were measured as described [2]. Tissue Glucose turnover rate (mg/kg/min) were calculated as described [2]. In vivo glucose uptake (mg/mg of tissue/min) for muscle (Tibialis Anterior, Soleus, Gastrocnemius, Extensor Digitorum Longus), subcutaneous adipose tissue, and liver were calculated based on the accumulation of 2DG6P in the respective tissue and the disappearance rate of 2DG from plasma as described [2]. For [3-3H] glucose determination, plasma was deproteinized with Ba(OH)2 and ZnSO4. For each sample, an aliquot of the supernatant was dried to remove 3H20. Immunoreactive insulin was determined by Elisa kit from Crystal Chem (Crystal Chem, Chicago, IL). Rate of glucose appearance (Ra) and rate of glucose dissappearance (Rd) is determined at steady state. Endogenous glucose production (endoGP, given as g/kg/min) is determined by subtracting the glucose infusion rate (GIR; rate of cold glucose required to sustain glycemia) from total Ra.

Biochemical Assays

Animals were fasted for 6 hours prior to blood collection from the tail vein. Plasma cholesterol levels were determined using the Infinity Cholesterol Kit (Thermo Scientific Inc, Rockford, IL). Plasma FFA levels were determined using the NEFA HR (2) kit (Wako Chemicals USA, Inc., Richmond, VA). Plasma insulin levels were measured with the Ultra Sensitive Rat Insulin ELISA kit (Crystal Chem, Chicago, IL) using rat insulin as the standard. Plasma lactate levels were determined with the lactometer Lactate Pro (ARKRAY Inc., Kyoto, Japan). Intraperitoneal glucose tolerance test (ipGTT) was performed by injection of glucose (2 g/kg, 50% wt/vol. d-glucose [Sigma, St Louis, MO] in 0.9% wt/vol. NaCl) after a 5-h fast as described previously [3]. Tail blood glucose levels [mg/dl] were measured with glucometer TheraSense Freestyle (Abbott Diabetes Care, Inc., Alameda, CA) before (0 min) and at 15, 30, 45, 60 and 120 min after injection. Glycated hemoglobin was measured in whole blood using the mouse hemoglobin A1c (HbA1c) kit from Crystal Chem (Crystal Chem, Chicago, IL). Fibroblast growth factor (FGF21) levels were measured with the rat/mouse FGF21 ELISA kit (EMD Millipore Corporation, Billerica, MA) after an overnight fast of 15 hours. Samples were analyzed individually except for lipoprotein separation in which pooled samples were subjected to fast-performance liquid chromatography (FPLC) as described previously [3].

Glycogen content in snap-frozen quadriceps muscles of ad libitum fed mice was measured with the glycogen assay kit from Abcam (Abcam plc, Cambridge, UK) according to the manufacturer's instructions. Liver glycogen content was determined with aminoglucosidase according to the method of Passoneau J. et al.[4]. Briefly, tissues were harvested and snap-frozen in liquid nitrogen and stored at -80C. 30 mg of polverized liver were homogenized in 1ml of ice-cold 0.3 M percloric acid. After centrifugation for 15 min at 3,000g, 50 ul of the homogenate was incubated with 500 ul of 50 mM sodium acetate containing 50ug/ml aminoglucosidase with shaking at room temperature for 2 hours. After centrifugation for 15 min at 3000g, 20 ul of samples were used for the determination of

glucose with the LabAssay Glucose kit from Wako (Wako Chemicals USA, Inc., Richmond, VA).

Liver lipid content of ad libitum fed mice was assessed by extraction as described previously [5], In summary, 50 mg of snap-frozen liver was homogenated with 1 ml cloroform. Lipids were extracted by overnight shaking at room temperature. For phase separation, 700 ul of ddH2O were added, samples were then centrifuged at 2000 rpm for 20 min at 4C and organic layer was collected. For second extraction 700 ul cloroform/methanol (2:1 vol/vol) was added to the remaining homogenate and lipid extraction, phase separation and organic layer collection were perfomed as described above. 15 ul of both extractions were then transfered to glass tubes, evaporated and measured with Infinity Trigliceride Kit (Thermo Scientific Inc, Rockford, IL).

Respiration studies in isolated skeletal muscle mitochondria and cultured muscle cells.

After sacrifice, gastrocnemius muscles were excised and mitochondria were isolated immediately as described previously [6]. For isolated muscle mitochondria all respiration measurements were made in triplicate and followed this protocol: resting respiration (state II, absence of adenylates) was assessed by the addition of 5 mM glutamate and 2 mM malate as the complex I supply. State III respiration was assessed by the addition of 1 mM ADP and subsequent state IV (uncoupling respiration) was determined by adding 2 uM of oligomycin. The integrity of the outer mitochondrial membrane was established by the addition of 10 uM cytochrome c. Measurements of oxygen consumption rate (OCR) by the Seahorse 24XF analyzer (Seahorse Biosciences Inc., North Billerica, MA) were completed within a 8- to 12-min period.

To investigate whether HDL directly enhances glycolysis via the extracellular acidification rate (ECAR) and/or mitochondrial oxygen consumption rate (OCR), murine skeletal muscle C2C12 cells were incubated for 4 hours with increasing amounts of human HDL and 4,5 mg/ml glucose utilizing the Seahorse XF24 analyzer as described previously [7]. To determine whether the observed effect on glycolysis is specifically mediated by HDL and/or its major protein constituent apoA-I, we incubated C2C12 cells with glucose (4,5 mg/ml) and with or without HDL, LDL, phospholipid vesicles (all at the same phospholipid concentration 100 ug/ml), and apoA-I (which was used at the same protein concentration as for HDL, 50 ug/ml) for 4 hours prior to determining glycolysis (ECAR). HDL, LDL, phospholipid vesicles and human apoA-I were prepared as described previously [8]***.***

Immuno blot analysis:

Whole gastrocnemius and quadriceps homogenates in lysis buffer from Pierce (Thermo Scientific Inc, Rockford, IL) containing protease and phosphates inhibitors were prepared with tissue lyzer from Qiagen (Qiagen Inc, Valencia, CA) and protein concentration was determined by BCA assay from Pierce. 80 ug of protein content from each sample were loaded on precast SDS-Tris gels from Pierce and separated by SDS-PAGE, transferred to polyvinylidene fluoride paper (EMD Millipore Corporation, Billerica, MA), and blotted with antibodies against the subunit a and b of the ATP synthase (Mitosciences, Eugene, OR), or with antibodies against all AKT isoforms (pan AKT) and against AKT phosphorylated around the Thr308 phosphorylation site (Cell Signaling Technology, Inc, Danvers, MA). Immunoreactive proteins were detected by incubating the blots with flourescently labeled species-specific secondary antibodies (Molecular Probes; Life Technologies, Grand Island, NY) and visualized by the Odyssee Infrared Imaging System (Li-Cor Biosciences, Eugene, NE) as described previously [5]***.***

Statistical analysis:

Unpaired student-t test with two-tailed P values was used for analysis of 1-group variables and one-way ANOVA was used for comparison between 3 or more groups followed by Bonferroni or Dunnet's post hoc tests. Genotype differences in glycogen levels between wt mice, apoA-I tg and apoA-I ko mice within one activity level of the two possible conditions (sed or exe) were analysed by one-way ANOVA followed by Bonferroni's multiple comparisons tests (Fig 1I and J). Multiplicity adjusted P values were calculated with 3 comparisons for figure 1I and J per family and a familiy-wise significance and confidence level of 0.05. The effect of exercise on glycogen levels (Fig 1I and J) within one genotype of mice (e.g. sed wt mice vs exe wt mice) was determined by unpaired student t test with two-tailed P values. The effect of HDL and apoA-I on ECAR and OCR (Figure 3 A, B and C) was analysed using one-way ANOVA followed by Dunnett's Multiple Comparison Test comparing cells incubated with glucose only (0+) vs. cells incubated with glucose and HDL, LDL, PL, and ApoA-I. Multiplicity adjusted P values were calculated with 6 comparisons for figure 3A and B and 4 comparisons for figure 3C per family and a familiy-wise significance and confidence level of 0.05. All data presented are raw values and are expressed as mean ± SEM and the 0.05 level of probability was accepted to indicate statistical significance. Statistical analyses were performed with GraphPad Prism version 6.0.

Supplemental References:

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Supplemental FIgures:

Figure 1S:

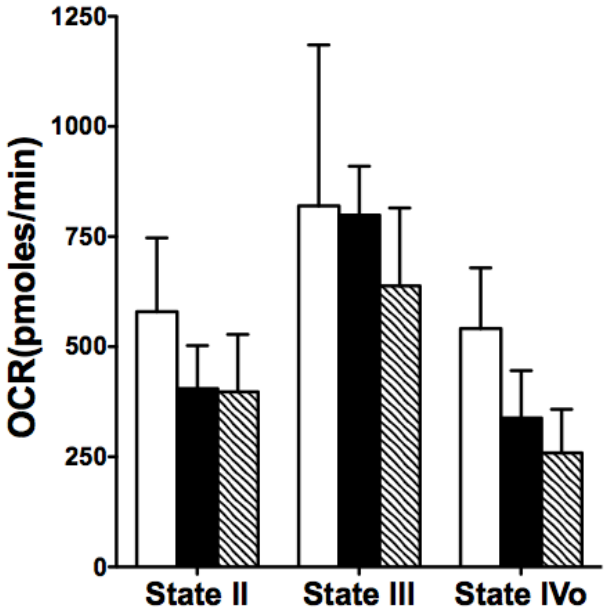

Figure 1S: Hepatic mitochodrial function is not altered by circulating HDL levels.

Oxygen consumption rate (OCR) in mitochondria isolated from liver in basal state (State II), after addition of ADP (State III), and after addition of of the ATP synthase inhibitor oligomycin (State IVo) of chow fed wt (open bars), apoA-I tg (filled bars) and apoA-I ko mice (hatched bars) (n = 4). Data are expressed as means ± SEM.

Figure 2:

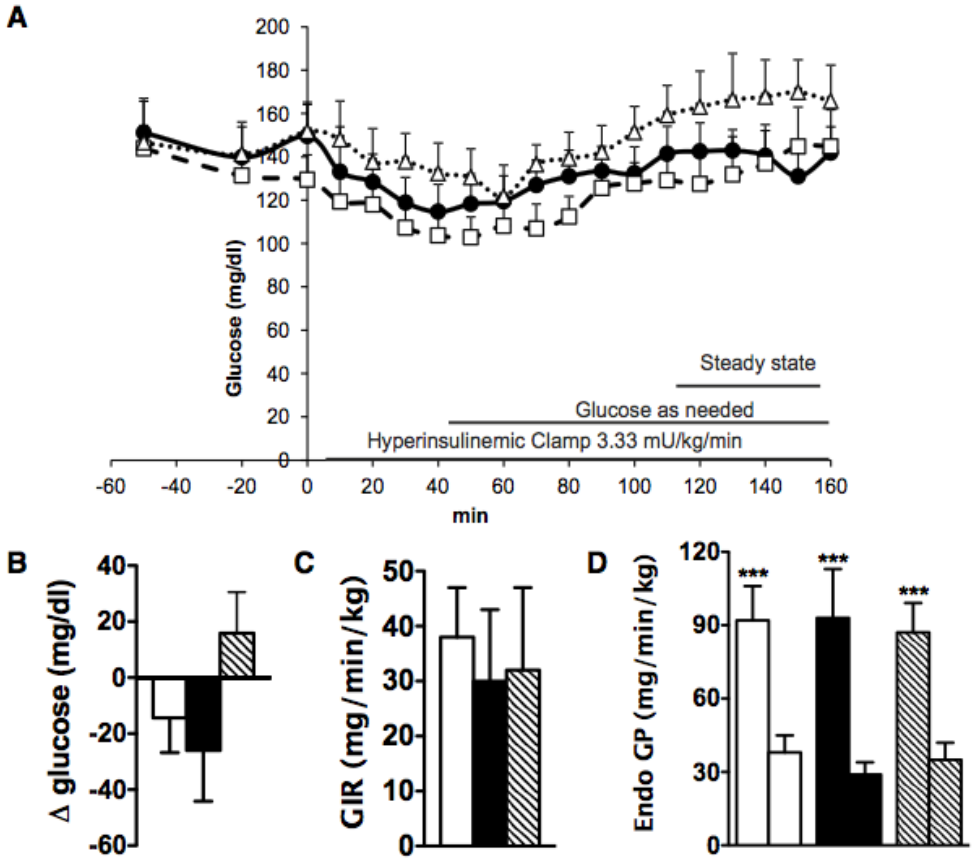

Figure 2S: Circulating HDL levels do not modulate insulin sensitivity: Absolute glucose levels (A) and change in glucose levels during basal clamp period (B), glucose infusion rates (GIR) (C) and hepatic glucose production (Endo GP) (D) during hyperinsulinemic-euglycemic clamp studies of chow fed and age-matched male wt (open bars, filled circles), apoA-I tg (filled bars; open squares) and apoA-I ko mice (hatched bars; open triangles) (n= 6-8). Data are expressed as means ± SEM ***P < 0.0005.