Leptin enhances hypothalamic LDHA-dependent glucose sensing to lower glucose production in high-fat fed rats.

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\textbf{Abstract:} The responsiveness of glucose sensing \textit{per se} to regulate whole-body glucose homeostasis is dependent on the ability of a rise in glucose to lower hepatic glucose production and increase peripheral glucose uptake \textit{in vivo}. In both rodents and humans, glucose sensing is lost in diabetes and obesity but the site(s) of impairment remain elusive. We here first report that short-term high-fat feeding disrupts hypothalamic glucose sensing to lower glucose production in rats. Second, leptin administration into the hypothalamus of high-fat fed rats restored hypothalamic glucose sensing to lower glucose production during a pancreatic (basal insulin-euglycemic clamp) and increased whole-body glucose tolerance during an intravenous glucose tolerance test. Finally, both chemical inhibition of hypothalamic lactate dehydrogenase (LDH) (achieved via hypothalamic LDH inhibitor oxamate infusion) and molecular knockdown of LDHA (achieved via hypothalamic lentiviral-LDHA shRNA injection) negated the ability of hypothalamic leptin infusion to enhance glucose sensing to lower glucose production in high-fat fed rats. In summary, our findings illustrate that leptin enhances LDH-A-dependent glucose sensing in the hypothalamus to lower glucose production in high-fat fed rodents \textit{in vivo}.

The hallmark feature of Type 2 diabetes is traditionally viewed as insulin resistance resulting in elevated hepatic glucose production and impaired glucose uptake in peripheral tissues. However, a change in glucose responsiveness as defined as the ability of glucose sensing \textit{per se} in regulating glucose production and uptake could also potentially contribute to glucose dysregulation in diabetes.

For instance, studies conducted in both rodents and humans report that independent of changes in glucoregulatory hormones during the pancreatic clamps, a doubling of circulating glucose levels inhibits glucose production and stimulates peripheral glucose uptake in healthy conditions (1-3). However, the same increment in the plasma glucose levels fail to lower glucose production in diabetic rodents (1) and humans (2,3), thereby illustrating glucose unresponsiveness as a feature in diabetic conditions. Glucose unresponsiveness in obese individuals has been determined from a mathematical minimal model analysis following a frequently sampled intravenous (ivGTT) or oral glucose tolerance test. Indeed, a reduction in glucose responsiveness was associated with impaired glucose tolerance in obese individuals (4). As seen in humans, obese and diabetic rodents such as the leptin deficient \textit{ob/ob} mice also display a loss of glucose sensing (5). Thus, the ability of glucose sensing \textit{per se} to regulate its own metabolism is highly relevant in obesity and diabetes.

To date, the site(s) of glucose sensing impairment that lead to glucose unresponsiveness in...
obesity and diabetes remain elusive. In this regard, a hypothalamic glucose sensing mechanism has been documented to maintain glucose homeostasis in the context of hypoglycemic-induced counter regulation (6) as well as short-term hyperglycemia (7) in chow fed rodents. However, whether changes in hypothalamic glucose sensing mechanisms dysregulate glucose metabolism in obesity and diabetes remain unclear. Interestingly, direct restoration of leptin signaling in the hypothalamus of the leptin receptor deficient Koletsky (fa/−fa/) rats in parallel improves glucose tolerance (8), while central administration of FGF19 improves glucose effectiveness and glucose tolerance in ob/ob mice as estimated from the minimal model analysis (9). Based on these collaborative findings of glucose sensing and hormonal action in the hypothalamus, we hypothesize that hypothalamic leptin action enhances hypothalamic glucose sensing machinery to regulate glucose metabolism in obesity and diabetes in vivo.

Results
We first determined whether the hypothalamus is a site of glucose unresponsiveness in the short-term 3d-high-fat fed model as 3d of high-fat feeding in rats induces inflammation, ER stress and insulin resistance in the hypothalamus and the dorsal vagal complex (10-12) as well as hyperphagia, hyperinsulinemia and hyperleptinemia (10,12-14). We placed rats that received stereotaxic hypothalamic and vascular surgeries (Fig. 1A) on a high-fat vs. regular-chow diet and found that upon 3d of monitoring and feeding, the HFD rats became hyperphagic (Fig. 1B) and hyperinsulinemic (plasma insulin levels ng/ml; HFD, 0.7 +/- 0.2 vs regular-chow, 2.0 +/- 0.3, p<0.05) but with no differences detected in body weight (Fig. 1C), fat and lean mass (Fig. 1D), and free (mass of water in the bladder) and total water in the body (Fig. 1E).

We next administered glucose directly into the mediobasal hypothalamus of healthy chow-fed rats for 6 hrs and found that in consistent with previous studies (7), glucose infusion rate (Fig. 1F) was increased in parallel to a reduction of glucose production (Fig. 1G) but no changes detected in glucose uptake (Fig. 1H) during the pancreatic (basal insulin)-euglycemic clamps (Table 1). Importantly, repeating hypothalamic glucose administration into 3d high-fat fed rats completely failed to lower plasma glucose levels because of the inability of glucose production to be inhibited (Figs. 1F-H), independently of changes in plasma insulin and glucose levels (Table 1). Thus, we discovered, for the first time to our knowledge, that high-fat feeding disrupts glucose sensing within the hypothalamus that lower glucose production in vivo. It remains unknown whether the diet composition and/or the hyperphagic response of HFD is responsible for the impairment of hypothalamic glucose sensing, although it has been documented that the hyperphagic response, but not the diet composition, of HFD is responsible for the impairment of hypothalamic fatty acid sensing mechanisms (15).

Next, we assessed whether leptin can restore hypothalamic glucose sensing by infusing leptin directly into the mediobasal hypothalamus of glucose unresponsive-3d high-fat fed rats (Fig. 1A). During the pancreatic (basal insulin)-euglycemic clamps, we found that upon co-infusion with leptin, hypothalamic glucose infusion retained its ability to lower glucose production that led to an increase in glucose infusion rate to maintain euglycemia (Figs. 2A,B), while glucose uptake remained comparable (Fig. 2C). Although such pancreatic clamp studies illustrate that leptin is able to restore hypothalamic glucose sensing in lowering glucose production independently of changes in plasma insulin and glucose levels, one would wonder whether leptin can still impact glucose metabolism while the gluco-regulatory hormones are allowed to change at will within the circulation. Thus, we next evaluated whether direct leptin administration into the mediobasal hypothalamus of 3d high-fat fed rats alter whole-body glucose tolerance during an ivGTT (Fig. 2D). In spite of the fact that gluco-regulatory hormones are allowed to change at will, hypothalamic administration of leptin given at equidosage as the pancreatic clamp studies is sufficient to increase glucose tolerance during ivGTT (Fig. 2E). Collectively, these two sets of data show that hypothalamic leptin administration enhances gluco-regulatory control in 3d HFD-induced hypothalamic glucose-unresponsive rodents. Of note, hypothalamic leptin action remains intact in 3d HFD rats is consistent with the fact that central leptin delivery activates hypothalamic STAT3 in 3d HFD rats (16).

To begin evaluating the downstream molecular pathway of glucose sensing that is necessary for leptin to improve glucose sensing, we
tested whether lactate dehydrogenase (LDH) in the hypothalamus is necessary for leptin-glucose action (Fig. 3A). We hypothesize that LDH is the primary downstream target as chemical inhibition of LDH-dependent lactate metabolism in the hypothalamus negates glucose infusion to lower glucose production in healthy rodents (7), while shuttling of glucose-derived lactate from the astrocytes into neurons serve as an important step to provide neuronal fuel (Fig. 3A) (17-19). We reason that if leptin restores glucose sensing in the hypothalamus to control whole-body glucose metabolism, LDH-dependent lactate metabolism could be a crucial step. These LDH-targeted experiments would also be necessary to ensure that the currently described glucoregulatory effect of hypothalamic leptin infusion with glucose is specifically targeting glucose sensing mechanisms. This is because a previous study has documented that central leptin infusion into HFD rats in comparable pancreatic (basal insulin)-euglycemic clamp conditions as the current study would still lower glucose production in the absence of central glucose co-infusion (20), suggesting that hypothalamic leptin action lowers glucose production in hypothalamic glucose sensing-dependent and –independent pathways.

To address whether leptin enhances hypothalamic LDH-dependent glucose sensing mechanisms, chemical and molecular approaches targeted to the mediobasal hypothalamus were used. We first infused oxamate, a competitive inhibitor of both LDHA (expressed in astrocytes (21-24)) and LDHB (expressed in neurons (22-24)), into the mediobasal hypothalamus (Fig. 1A) at a dose that would negate hypothalamic glucose infusion to lower glucose production in rodents (7). We here found that infusion of oxamate together with leptin prevented the ability of leptin to enhance hypothalamic glucose sensing in increasing glucose infusion rate (Fig. 3B) and lowering glucose production (Fig. 3C), without any detectable differences in glucose uptake (Fig. 3D), plasma insulin and glucose levels (Table 2). Importantly, co-infusion of oxamate with only leptin into the hypothalamus did not negate leptin’s ability to alter glucose kinetics (Fig. 3B-D). Two important implications are derived from this control experiment; (i) Consistent with previous literature, hypothalamic leptin administration into 3 d HFD rats during the pancreatic basal insulin clamp lowers glucose production (20) in the absence of glucose co-infusion into the MBH indicating that leptin action in the brain lowers glucose production in a hypothalamic glucose sensing-independent fashion. (ii) The presence of oxamate did not negate such leptin-glucose production-lowering effect in the absence of glucose-co-infusion, indicating that oxamate administration is selectively blocking the glucose-sensing dependent pathway and indicates that leptin in the brain can also in parallel lower glucose production in a hypothalamic glucose-sensing dependent fashion. Thus, LDH-dependent lactate metabolism in the hypothalamus is a necessary step for leptin to enhance glucose sensing.

Next, we selectively evaluated for a necessary role of LDHA-dependent lactate metabolism by injecting a lentivirus expressing the shRNA of LDHA into the mediobasal hypothalamus of rats. We first confirmed that LDHA expression was reduced by ~40% in mediobasal hypothalamic tissues harvested from HFD rats injected with LDHA shRNA compared with the mismatch (MM) control, while LDHB expression was unaltered (Fig. 4A). Second, we found that hypothalamic glucose vs. saline infusion into MM-injected chow-fed rats was equally effective as non-injected rats (Figs. 1F-H) to increase glucose infusion rate (Fig. 4B) and lower glucose production (Fig. 4C), independently of changes in plasma insulin and glucose levels (Table 3). However, hypothalamic glucose infusion failed to alter glucose metabolism in LDHA shRNA injected rats (Figs. 4B-C), illustrating for the first time that hypothalamic LDHA-dependent pathway is necessary for glucose sensing to lower glucose production in chow-fed rats. Although hypothalamic glucose infusion failed to alter glucose metabolism in HFD-fed MM or LDHA shRNA injected rats (Figs. 4B-C) as well, hypothalamic glucose-infused and LDHA shRNA injected HFD rats also failed to respond to hypothalamic leptin administration to alter glucose metabolism (Figs. 4B-C). In direct contrast, hypothalamic leptin infusion was effective in restoring glucose sensing in HFD-MM injected rats (Figs. 4B-C). Glucose uptake, plasma insulin and glucose levels were comparable among groups (Fig. 4D & Table 3). Thus, molecular knockdown of LDHA in the hypothalamus negates leptin to enhance glucose sensing mechanism.
Discussion

The current set of findings collectively indicates that leptin enhances hypothalamic LDHA-dependent glucose sensing to lower glucose production in HFD rats in vivo, and highlights several important implications. First, the defects of glucose unresponsiveness in regulating hepatic glucose production and glucose tolerance as previously reported in rodents (1,5) and humans (2-4) with diabetes and obesity could lie within the hypothalamus, although future studies are warranted in assessing hypothalamic glucose sensing mechanisms in chronic high-fat fed, obese, and/or diabetic rodent models. Second, the loss of glucose sensing in leptin-deficient ob/ob mice (5) could be restored by direct hypothalamic leptin administration as restoration of hypothalamic leptin action is effective in improving glucose tolerance in leptin receptor-deficient Koletsky (fa/faq) rats (8) as well. Importantly, such postulated leptin-dependent rescue of glucose sensing could be due to an enhancement of hypothalamic glucose sensing. However, this postulation is cautioned by the fact that central leptin delivery may not restore hypothalamic glucose sensing in rats fed a HFD for more than 5 weeks as central leptin action fails to lower food intake in 5 week-HFD fed rats (25) and that central leptin resistance may additionally exists at the level of the leptin blood-brain barrier transport (26). Third, given that LDHA is selectively expressed in the astrocytes (21-24) and that leptin enhances LDHA-dependent glucose sensing in the hypothalamus as currently described, leptin signaling and glucose-lactate metabolism could intersect within the astrocytes to improve whole-body metabolic control. This postulation is collaborative with findings indicating that leptin signaling in the astrocytes regulates feeding (27) and that insulin signaling in the astrocytes enhances central glucose sensing (28). In summary, we here report, for the first time to our knowledge, that leptin enhances LDHA-dependent hypothalamic glucose sensing to regulate glucose production in high-fat fed in vivo conditions.

Experimental procedures:
Animal preparation and surgeries
Adult male Sprague-Dawley rats (Charles River Laboratories, Saint-Constant, QC, Canada) initially weighing 280-300g were studied. Rats were housed in individual cages and subjected to a standard light-dark cycle (0700 light, 1900 dark), and had ad libitum access to drinking water and regular chow or a HFD. The regular chow (Teklad Diet #7002, Harlan Laboratories, Madison, WI, USA) contained 18% fat, 33% protein and 49% carbohydrate content (3.1 kcal/g total metabolizable energy), whereas the 10% lard oil-enriched HFD (TestDiet #571R, Purina Mills, IN, USA) contained 34% fat, 22% protein and 44% carbohydrate (3.9 kcal/g total metabolizable energy). Ketamine (60 mg/kg) and xylazine (8 mg/kg) were used to anesthetize rats during surgeries. A 26-gauge stainless steel bilateral guide catheter (C235G, Plastics One Inc. Virginia, USA) was stereotaxically placed into the mediobasal hypothalamus (MBH) using the coordinates 3.1 mm posterior to bregma, 0.4 mm lateral of midline and 9.6 mm below skull surface as described (29). After recovery for 7-8 days, vascular catheters were inserted into the internal jugular vein and carotid artery for infusion and blood sampling (29). Post-surgical body weight and food intake were checked daily and only those rats that attained a minimum of 90% of their pre-vascular surgery body weight underwent subsequent in vivo studies. All procedures were approved by the Institutional Animal Care and Use Committee of the University Health Network.

Lentivirus injection
Immediately following brain surgery, a group of rats received 3 μL of lentivirus expressing shRNA to LDH-A (1.0 x 10^6 infectious units, sc-270631-V, Santa Cruz Biotechnology, Inc., Dallas, TX), or a mismatch sequence as a control (1.0 x 10^6 infectious units, Sc-108080, Santa Cruz Biotechnology) through each side of the MBH catheters that target the arcuate nucleus (29,30). Eight days after MBH cannulation and virus injection, vascular catheterization was performed as described above in rats that would undergo clamp experiments.

Pancreatic (basal insulin)-euglycemic clamps
Four days following vascular catheterization, rats were subjected to 4-6 h fast before the clamp experiments. On the day of the clamp, conscious, unrestrained rats received the following MBH infusions at 0.33μl/h (CMA 400 syringe pump, CMA Microdialysis, Inc., North Chelmsford, MA): i) 0.9% Saline, ii) Glucose (Sigma-Aldrich, St. Louis, MO, 2mM for 6h; previously documented to lower glucose production (7)), iii) Glucose (2 mM) + leptin (R&D systems, Minneapolis, MN, 46
ng/ul) (iv) lactate dehydrogenase blocker, oxamate (Millipore Sigma, Oakville, ON, Canada, 50 mM; first given as bolus (0.33ul)), (v) Glucose (2 mM) + oxamate (50 mM) or (vi) Glucose (2 mM) + leptin (46 ng/ul) + oxamate (50 mM). Leptin was infused at 33ng/h for 5h and this dose was chosen based on a previous study that reports leptin administered at 33 ng/h into the MBH for 6h exhibit metabolic control (31). In addition, we have chosen to infuse leptin 1h after glucose was started as we attempted to have the HFD rats first engage in glucose sensing mechanisms before examining whether leptin alters glucose sensing. Oxamate concentration was based on a study that report MBH oxamate at 50 mM sufficiently negated glucose sensing in healthy rats (7). Infusions of oxamate were started at t= -180 min followed by glucose + oxamate at t=-150 min. Leptin + glucose + oxamate was then started at t= -90 min and maintained until the end of the clamps, t= 210 min. Clamp methodology was performed as follows. At t=0 min, a primed, continuous infusion (PHD2000 syringe pump, Harvard Apparatus, Saint Laurent, QC, Canada) of [3-$^3$H]-glucose (PerkinElmer; 40 uCi bolus+ 0.4 uCi min$^{-1}$ infusion) was started and maintained until the end of the clamps (t=210 min) to measure glucose kinetics using tracer-dilution methodology. The glucose turnover was calculated using the steady-state formulae, where the rate of appearance of glucose is calculated using [3-$^3$H]-glucose. The total rate of appearance of endogenous glucose production is equivalent to the rate of glucose utilization during the basal period (t=60–90 min). The pancreatic (basal insulin)–euglycemic clamp was initiated at t=90 min with a primed continuous infusion of insulin (1.2mU kg$^{-1}$ min$^{-1}$), somatostatin (3 ug kg$^{-1}$ min$^{-1}$) and a variable infusion of 25% glucose to maintain glycemia at a similar level to the basal period and was maintained until t=210 min as described (29). Plasma samples were obtained every 10 min for the determination of [3-$^3$H]-glucose concentrations. At the end of the experiment, rats were anesthetized and injected with 3µl bromophenol blue through each side of the MBH catheter to verify the correct placement of the catheter. The MBH wedges were then collected, frozen in liquid nitrogen and stored at -80 °C for subsequent analysis.

**Intravenous glucose tolerance test**
Following 1 day after vascular catheterization, separate groups of MBH rats were placed on HFD for 3 consecutive days and subsequently subjected to an overnight (16 to 18 h) fast to undergo the intravenous glucose tolerance test (ivGTT) as described (32,33). MBH infusions (0.33 µL/h, CMA 400 syringe pump, CMA Microdialysis, Inc., North Chelmsford, MA) of 0.9% saline or leptin (R&D systems, Minneapolis, USA, 33ng/h/5h) were commenced at t=-240 min and maintained until the end of the experiment at t = 60 min to ensure that rats received the same duration of MBH leptin treatment as clamp experiments. After t = 0 min blood samples were obtained, an intravenous bolus of glucose (20% glucose, 0.25 g/kg) was injected and flushed with saline. The jugular vein catheter was used to administer the injections and the carotid artery catheter was used to sample blood to measure plasma glucose for 60 min following the bolus glucose injection.

**Body composition**
On the morning before the clamp experiments, the body composition (fat mass, lean mass, free water and total body water) of the rats was assessed with an EchoMRI$^{\text{TM}}$ (Echo Medical Systems, Houston, TX, USA).

**Western blot analyses**
MBH samples from rats that received lentiviral injections of LDH-A shRNA or a MM sequence were lysed on ice with a handheld homogenizer in a lysis buffer containing 50 mM Tris-HCl (pH 7.5), 1 mM EGTA, 1 mM EDTA, 1% (w/v) Nonidet P40, 1 mM sodium orthovanadate, 50 mM sodium fluoride, 5 mM sodium pyrophosphate, 0.27 M sucrose, 1 µM dithiothreitol (DTT) and protease inhibitor cocktail (Roche). The protein concentration of homogenized tissues was determined using the Pierce 660 nm protein assay (Thermo Scientific). Ten to 30 µg of lysates obtained as described above were subjected to electrophoresis on a 10% polyacrylamide gel and transferred to nitrocellulose membranes. The membranes were incubated with blocking solution (5% BSA in Tris-buffered saline containing 0.2% Tween-20 (TBS-T)) for 1h at room temperature followed by primary antibody incubation with antibodies diluted 1/1,000 in 5% BSA in TBS-T for anti-LDHA (ab135366 rabbit, Abcam, Cambridge, MA, USA; same antibody as previously reported (34) overnight at 4°C, or anti-LDHIB (ab85318 mouse, Abcam, Cambridge, MA, USA) and anti-β-tubulin (MAB1637 mouse, Millipore Sigma, Oakville, ON, Canada) for 2 h at 4°C. The blots
were then washed 4 times with TBS-T and incubated with secondary HRP-conjugated antibodies in 5% skimmed milk for 1h. After repeating the washing steps, the signal was detected with the enhanced chemiluminescence reagent (ECL). Immunoblots were detected using a MicroChemi 4.2 chemiluminescent imaging system and quantified with GelQuant image analysis software (DNR Bio-Imaging Systems, Jerusalem, Israel).

Statistics
Unpaired Student’s t-tests were performed in the statistical analysis of two groups. Where comparisons were made across more than two groups, ANOVA was performed, and if significant, followed by Tukey’s post hoc tests. Significance was accepted as \( P<0.05 \).

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Author Contributions: M.A.A conducted and designed experiments, performed data analyses and wrote the manuscript. M.R., and P.V.B assisted with the experiments. T.K.T.L. supervised the project, designed experiments, and edited the manuscript.

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Table Legends:

Table 1. Plasma insulin and glucose concentrations during the basal and clamp periods relating to Figure 1. Values are presented as mean ± SEM (Basal: 60-90; Clamp: 180-210).

Table 2. Plasma insulin and glucose concentrations during the basal and clamp periods relating to Figure 3. Values are presented as mean ± SEM (Basal: 60-90; Clamp: 180-210).

Table 3. Plasma insulin and glucose concentrations during the basal and clamp periods relating to Figure 4. Values are presented as mean ± SEM (Basal: 60-90; Clamp: 180-210).
|               | RC Saline | RC Glucose | HFD Saline | HFD Glucose |
|---------------|-----------|------------|------------|-------------|
| **Basal**     |           |            |            |             |
| Insulin (ng/ml) | 0.7 ± 0.1 | 0.5 ± 0.1 | 1.5 ± 0.2 | 1.6 ± 0.2   |
| Glucose (mM)  | 7.1 ± 0.2 | 6.2 ± 0.2 | 7.2 ± 0.3 | 7.6 ± 0.4   |
| **Clamp**     |           |            |            |             |
| Insulin (ng/ml) | 0.8 ± 0.1 | 0.9 ± 0.1 | 1.0 ± 0.3 | 0.8 ± 0.3   |
| Glucose (mM)  | 7.3 ± 0.2 | 7.0 ± 0.3 | 7.3 ± 0.3 | 7.2 ± 0.3   |

Table 1
### Table 2

| Condition  | HFD Glucose | HFD Glucose + Leptin | HFD Glucose + Oxamate | HFD Glucose + Leptin |
|------------|-------------|----------------------|-----------------------|----------------------|
| Basal      | 1.5 ± 0.2   | 1.0 ± 0.2            | 1.5 ± 0.2             | 1.7 ± 0.3            |
|            | 7.7 ± 0.3   | 7.0 ± 0.1            | 7.4 ± 0.0             | 7.9 ± 0.4            |
| Clamp      | 0.9 ± 0.1   | 1.0 ± 0.3            | 1.0 ± 0.2             | 0.9 ± 0.1            |
|            | 7.1 ± 0.4   | 7.3 ± 0.3            | 7.2 ± 0.3             | 7.0 ± 0.2            |
|               | RC + MM shRNA + Saline | RC + MM shRNA + Glucose | RC + LDH-A shRNA + Glucose | HFD + MM shRNA + Saline | HFD + MM shRNA + Glucose | HFD + LDH-A shRNA + Glucose | HFD + MM shRNA + Glucose + Leptin | HFD + MM shRNA + Glucose + Leptin |
|---------------|------------------------|-------------------------|---------------------------|-------------------------|-------------------------|-----------------------------|---------------------------------|---------------------------------|
| **Basal**     |                        |                         |                           |                         |                         |                             |                                 |                                 |
| Insulin (ng/ml) | 0.8 ± 0.2              | 0.7 ± 0.1               | 1.0 ± 0.4                 | 1.5 ± 0.3               | 1.4 ± 0.3               | 0.8 ± 0.0                   | 1.9 ± 0.4                        |                                 |
| Glucose (mM)   | 7.3 ± 0.2              | 6.4 ± 0.2               | 7.2 ± 0.3                 | 7.3 ± 0.4               | 7.0 ± 0.2               | 7.0 ± 0.2                   | 7.8 ± 0.6                        |                                 |
| **Clamp**     |                        |                         |                           |                         |                         |                             |                                 |                                 |
| Insulin (ng/ml) | 0.8 ± 0.1              | 0.8 ± 0.1               | 0.9 ± 0.3                 | 0.8 ± 0.2               | 0.8 ± 0.1               | 1.0 ± 0.4                   | 1.1 ± 0.2                        |                                 |
| Glucose (mM)   | 7.0 ± 0.2              | 6.7 ± 0.3               | 6.9 ± 0.6                 | 6.9 ± 0.1               | 6.5 ± 0.5               | 7.2 ± 0.3                   | 7.4 ± 0.5                        |                                 |

Table 3
Figure Legends:

**Fig 1. High fat feeding impairs hypothalamic glucose sensing.** (A) Experimental protocol. (B) Cumulative food intake, *p<0.05 compared to regular chow fed (RC), (C) Body weight, (D), Fat and lean mass, (E) Free water and total water mass of HFD (n=7) vs RC (n=5)-fed rats. (F) Glucose infusion rate, (G) Glucose production in basal (white square) and clamp (black square) conditions, and (H) Glucose uptake during the clamps. n=7 for RC-MBH saline, n = 6 for RC-MBH glucose, n=7 for HFD-MBH saline and n=7 for HFD-MBH glucose. Values are presented as mean ± SEM, *p<0.05 compared to RC-saline, HFD-saline and HFD-glucose.

**Fig 2. Leptin enhances hypothalamic glucose sensing.** (A) Glucose infusion rate, (B) Glucose production in basal (white square) and clamp (black square) conditions, and (C) Glucose uptake during the clamps (n= 7 per group). Values are presented as mean ± SEM, *p<0.05 compared to HFD-MBH glucose. (D) Experimental protocol for the ivGTT experiments on HFD rats that received MBH saline (n=6) or MBH leptin (n=9). (E) Percent change in plasma glucose levels during ivGTT and integrated area under the curve (AUC). Values are presented as mean ± SEM, †p<0.05 compared to HFD-MBH saline.

**Fig 3. Chemical inhibition of hypothalamic LDH negates leptin’s impact on glucose sensing.** (A) Schematic of hypothesis. (B) Glucose infusion rate, (C) Glucose production in basal (white square) and clamp (black square) conditions, and (D) Glucose uptake during the clamps (n=7 per group). Values are presented as mean ± SEM, *p<0.05 compared to all other groups except leptin + oxamate; †p<0.05 compared to all other groups except leptin + glucose.

**Fig 4. Molecular knockdown of hypothalamic LDH negates leptin’s impact on glucose sensing.** (A) Representative Western blot of LDH-A and LDH-B in the MBH of rats that received MBH injections of either MM (n=4) or LDH-A shRNA lentivirus (n=5). Quantification of LDH-A or LDH-B levels normalized to β-tubulin expressed as fold change over MM, shown below. Values are presented as mean ± SEM, *p<0.05 compared to MM. (B) Glucose infusion rate, (C) Glucose production in basal (white square) and clamp (black square) conditions, and (D) Glucose uptake during the clamps (n=7 per group). Values are presented as mean ± SEM, *p<0.05 compared to RC-MBH saline, RC-MBH glucose + LDH-A shRNA, †p<0.05 compared to HFD-MBH saline + MM, HFD-MBH glucose + MM, HFD-MBH glucose + LDHA shRNA and HFD-MBH glucose + leptin + LDH-A shRNA.
**Figure 1**

**A**

- MBH surgery
- Vascular surgery
- Clamp
- MBH infusion: oxamate
- MBH infusion: glucose + saline/glucose + leptin/glucose + leptin + oxamate

**B**

- Food intake (kcal)
- Body weight (g)

**C**

- Fat mass (g)
- Lean mass (g)

**D**

- Total water (g)

**E**

- Free water (g)

**F**

- Glucose infusion rate (mg/kg/min)

**G**

- Glucose production (mg/kg/min)

**H**

- Glucose uptake (mg/kg/min)

**Note:** The figure includes various experimental conditions and outcomes related to metabolic studies, including MBH surgery, vascular surgery, clamp infusion conditions, and metabolic parameters such as food intake, body weight, fat mass, lean mass, total water, free water, glucose infusion rate, glucose production, and glucose uptake.
Figure 2

A. Glucose infusion rate (mg/kg/min)
B. Glucose production (mg/kg/min)
C. Glucose uptake (mg/kg/min)

D. Timeline:
- Day 0
- MBH surgery
- Vascular surgery
- HFD
- i.v. glucose (0.25 g/kg)

E. Plasma glucose (% change in baseline)

Legend:
- HFD saline
- HFD leptin
- Saline
- Leptin

AUC
Figure 3
Figure 4

A. Western blot showing the expression of LDH-A or LDH-B/β-tubulin in MM and LDH-A shRNA treated conditions. The graph below shows the fold change in expression compared to MM.

B. Bar graph showing the glucose infusion rate with different treatments: RC, HFD, Saline, Glucose, Leptin, MM shRNA, and LDH-A shRNA. The bars indicate the glucose infusion rate in mg/kg/min.

C. Bar graph showing the glucose production with different treatments: Basal and Clamp conditions. The bars indicate the glucose production in mg/kg/min.

D. Bar graph showing the glucose uptake with different treatments. The bars indicate the glucose uptake in mg/kg/min.
Leptin enhances hypothalamic LDHA-dependent glucose sensing to lower glucose production in high-fat fed rats.
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