Reducing insulin via conditional partial gene ablation in adults reverses diet-induced weight gain

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ABSTRACT: Excess circulating insulin is associated with obesity in humans and in animal models. However, the physiologic causality of hyperinsulinemia in adult obesity has rightfully been questioned because of the absence of clear evidence that weight loss can be induced by acutely reversing diet-induced hyperinsulinemia. Herein, we describe the consequences of inducible, partial insulin gene deletion in a mouse model in which animals have already been made obese by consuming a high-fat diet. A modest reduction in insulin production/secretion was sufficient to cause significant weight loss within 5 wk, with a specific effect on visceral adipose tissue. This result was associated with a reduction in the protein abundance of the lipodystrophy gene polymerase I and transcript release factor (Ptrf/Cavin) in gonadal adipose tissue. RNAseq analysis showed that reduced insulin and weight loss also associated with a signature of reduced innate immunity. This study demonstrates that changes in circulating insulin that are too fine to adversely affect glucose homeostasis nonetheless exert control over adiposity.—Page, M. M., Skovso, S., Cen, H., Chiu, A. P., Dionne, D. A., Hutchinson, D. F., Lim, G. E., Szabat, M., Flibotte, S., Sinha, S., Nislow, C., Rodrigues, B., Johnson, J. D. Reducing insulin via conditional partial gene ablation in adults reverses diet-induced weight gain. FASEB J. 32, 1196–1206 (2018). www.fasebj.org

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Obesity and related diseases burden both society and the individual, increasing the prevalence and risk of comorbidities including diabetes, heart disease, and cancer (1). It is clear that obesity and hyperinsulinemia are closely related, although their causal relationship remains poorly defined, obscuring the molecular mechanisms underlying possible treatments for obesity. The common belief is that obesity precedes insulin resistance, which subsequently causes a compensatory increase in β-cell insulin secretion to prevent hyperglycemia (2). However, elevated circulating insulin levels have been reported prior to the onset of obesity (3, 4), and increasing evidence suggests that hyperinsulinemia is not simply an adaptive response to obesity (5). In support of this concept, adipose tissue–specific impairment of insulin signaling prevents obesity in mice (6–10). Furthermore, we recently reported that mice with lifelong prevention of diet-induced hyperinsulinemia by partial insulin gene deletion were protected against high-fat diet (HFD)–induced obesity (11, 12). These results provided the first evidence in mammals that hyperinsulinemia itself plays a causal role in obesity, yet this preventative approach could not determine whether insulin reduction could serve as a viable treatment in those individuals who are already obese. Suppressing hyperinsulinemia with drugs has been used as an obesity treatment in animal models and in some human studies (13–15). However, these results must be interpreted with caution, because these drugs directly affect other organs that are also implicated in weight regulation, including white adipose...
tissue (WAT) and the hypothalamus (13, 16). Herein, we report, that an adult-onset partial reduction of insulin (Ins)-2 gene dose in obese male mice caused significant weight loss within 5 wk, with specific effects on visceral adipose depots. The results demonstrate that adiposity and obesity can be controlled by modest changes in circulating insulin.

MATERIALS AND METHODS

Animals and in vivo physiology

Animal protocols were performed in accordance with the University of British Columbia Animal Care Committee. Ins1−/−:Ins2−/− mice have been described in Duvillié et al. (17). Pdx1CreERT mice (024968) and mice carrying the lineage-tracing marker membrane-targeted tdTomato/membrane-targeted enhanced green fluorescent protein; (mTmG; 007675) were purchased from The Jackson Laboratories (Bar Harbor, ME, USA). Before they were randomized into different groups, they were fed a 19% protein extruded rodent diet (Teklad; Envigo, Madison, WI, USA). At 6 wk of age, male mice were randomized and placed on 1 of 3 diets: 1) a 10% fat diet [total calories = 3.85 kcal/g; 10% calories from fat, 20% from protein, and 70% from carbohydrate; D12450B (Open Source/Research Diets, New Brunswick, NJ, USA)], 2) a 25% fat diet [total calories = 3.70 kcal/g; 25% calories from fat, 20% from protein, and 55% from carbohydrate; SL5J (Lab Diet, St. Louis, MO, USA)], 3) a 58% fat diet [total calories = 5.56 kcal/g; 58.0% calories from fat, 16.4% calories from protein, and 25.5% calories from carbohydrate; D12330 (Open Source Diets/Research Diets)]. Mice that were randomized were born from 5 breeding pairs, and we ensured that pups of subsequent litters were placed on each of the three different diets before repeating any diet twice. The body mass of the mice before randomization ranged from 21.4 to 33.2 g. Because our study primarily focused on the physiologic changes in mice fed an HFD, we can report that, immediately upon randomization, the average body weights were as follows: vehicle, 26.5 ± 0.5 g; control, 26.8 ± 0.5 g; and experimental, 26.1 ± 0.3 g. Twelve weeks after the initiation of this diet, control (Ins1−/−:Ins2−/−:mTmG) and experimental (Ins1−/−:Ins2−/−:Pdx1CreERT::mTmG) mice were injected intraperitoneally with tamoxifen (3 mg/40 g body weight) after the mice remained unfed for 4 h. Glucose tolerance and insulin sensitivity were assessed in mice injected with 20% glucose after 4 h without food. Insulin-stimulated serum insulin secretion was measured by perifusion and radioimmunoassay (19).

RNA isolation, real-time quantitative PCR, and transcriptome analysis

Total RNA was isolated from islets with an RNeasy Mini Kit (Qiagen, Mississauga, ON, Canada). Reverse transcription was used to generate cDNA (Superscript III; Thermo Fisher Scientific). TaqMan probes (Integrated DNA Technologies, Coralville, IA, USA) were used to measure Ins2 gene expression, with actin as a reference gene. Gonadal adipose tissue was dissected from a cohort of 23-wk-old (corresponding to 5 wk after tamoxifen injection) control littermate (n = 7) and experimental (n = 8) mice euthanized after remaining unfed for 4 h. RNA isolation was performed with Trizol reagent (Thermo Fisher Scientific, Waltham, MA, USA) and Qiagen RNeasy Mini Kit (Qiagen). In brief, tissue was homogenized in Trizol and centrifuged for 10 min at 4°C. Chloroform was added to the supernatant and
BioAnalyzer (Agilent Technologies, Santa Clara, CA, USA) from 400 ng of total RNA. Sample quality was assessed with a BioAnalyzer (Agilent Technologies, Santa Clara, CA, USA), and RNA was quantified with Qubit (Thermo Fisher Scientific). Libraries were multiplexed and sequenced over two rapid-run lanes on HiSeq2500, and raw data were converted to fastq format with bcl2fastq-v1.8.4 (Illumina). Kallisto software (https://pachterlab.github.io/kallisto/) was used for read alignment and expression quantification and the R package DESeq2 software (http://bioconductor.org/packages/release/bioc/html/DESeq.html) for differential expression analysis. In brief, kallisto (version 0.4.2.3) was used to build an index file for the mouse reference transcriptome GRCm38 [downloaded from Ensembl (www.ensembl.org)]. The sequence reads for each sample were then quantified with the quant function of kallisto. In-house Perl scripts were used to sum the read counts at the transcript level for each gene and to create a matrix comprising the read counts for all the genes for all the samples. Differential expression analysis was then performed on the data from that matrix by using DESeq2. Each sample was assessed with the quality control software RNA-SeQC (The Broad Institute, Cambridge, MA, USA; http://archive.broadinstitute.org/cancer/cga/rna-seqc). Quality control showed one control and two experimental outliers, and these samples were removed from differential expression analyses. Therefore, included in the analyses are expression from 6 control and 6 experimental mice. RNA sequencing transcriptome data were analyzed with GeneMania (http://genemania.org/), to construct a predicted protein–protein networking model which was manually arranged into groups based on predicted tissue origin.

Micro-computed tomography and dual-energy X-ray absorptiometry

Scans were performed with a TriFoil Micro-Computed Tomography (CT) scanner (Northridge Tri-Modality Imaging, Inc., Chatsworth, CA, USA) with the following settings: resolution, 50 μm; scan time, ~4 min. Each mouse was scanned 1 wk before and again 5 wk after tamoxifen injections. The mice were positioned on their backs in the scanner with a straight spine and a minimum 90° angle between the spine and the femur bone, which was confirmed by a scout scan. Animals were scanned while anesthetized (O2 at 1.0 L/min; 5% isoflurane). Analysis of the scanned images was performed with Amira 6.0.1 software (FEI, Hillsboro, OR, USA). The first transsectional scan showing the pelvis was set as the reference scan for each mouse. The inner layers of the abdominal wall were applied to distinguish visceral from subcutaneous fat depot volumes (μm³). Lean mass was determined in vivo by dual-energy X-ray absorptiometry (Lunar Piximus; no longer manufactured) densitometer in mice 5–7 wk after tamoxifen (from 15 control and 12 experimental mice).

Statistical analysis

Data are expressed as means ± SEM unless otherwise indicated. Results were considered statistically significant at P < 0.05, by 2-tailed, unequal Student’s t test. Statistical analyses were performed with Microsoft Excel (Redmond, WA, USA).

RESULTS AND DISCUSSION

Inducible, partial Ins2 gene deletion as an experimental model to reduce insulin production

To directly assess the sustained effects of hyperinsulinemia on obesity and weight loss, we generated a mouse model in which the insulin gene dose could be reduced by using an inducible Cre-loxP system (Fig. 1A). At 6 wk of age, mice were fed 1 of 3 diets; low fat (10%), moderate fat (25%), or high fat (58%). After 12 wk of consuming these diets, littermate control (Ins1+/+;Ins2−/−;Pdx1CreERT:mTmG) and experimental (Ins1+/−;Ins2−/−;Pdx1CreERT:mTmG) male mice were injected intraperitoneally with tamoxifen, resulting in a near complete recombination on this genetic background (Fig. 1B) (20).

Inducible partial loss of Ins2 does not affect the weight of mice fed a moderate- or low-fat diet

Manipulation of Ins2 gene dose in experimental mice fed a moderate-fat diet did not significantly affect islet insulin levels (Fig. 1C), nor did this manipulation lead to a statistically significant alteration in circulating insulin in experimental mice throughout the study (Fig. 1D). Consistent with these findings, glucose tolerance, insulin sensitivity, and glucose-stimulated insulin secretion were not impaired in experimental mice when compared to control littermates in the context of this diet (Fig. 1E–G). Reduction of Ins2 gene dose in male mice did not affect body mass in mice fed a moderate-fat diet (Fig. 1H). Similarly, body mass was unchanged after a reduction in the Ins2 gene dose in experimental mice fed a low-fat diet (Fig. 1I). These results are in line with previous evidence from other genetic manipulations that do not affect body mass in mice fed low- or moderate-fat diets (21).

Inducible Ins2 gene reduction modestly reduces islet insulin secretion in mice fed an HFD

Ins2 gene reduction in male mice fed an HFD resulted in a significant (30%) reduction of Ins2 expression (Fig. 2A) and a similar magnitude, but nonsignificant, reduction in insulin protein content from isolated islets (Fig. 2B) compared to littermate controls. The modest reduction in insulin gene expression in experimental mice led to a significant reduction in the second phase of high glucose-stimulated insulin secretion (Fig. 2C). We detected significantly lower levels of fasting insulin in vivo at 25 wk after tamoxifen injection, although we noted high variability in the fasting insulin measurements at all ages (Fig. 2D). These modest reductions in insulin secretion were not sufficient to alter glucose tolerance, insulin sensitivity, or glucose-stimulated insulin secretion (Fig. 2E–G). We were also unable to detect any differences in the levels of several other circulating hormones, including leptin, resistin,
Figure 1. Adult reduction of Ins2 gene dose in mice consuming low- and moderate-fat diets does not reverse weight gain. A) Schematic of our hypothesis that reduced insulin would reverse weight gain and obesity in adult male mice fed a low-fat (10%, yellow), moderate-fat (25%, light blue), or high-fat (58%, dark blue) diet. B) Tamoxifen induction of Pdx1CreERT led to near complete, as evidenced by membrane GFP expression (bottom). C) Insulin content in mice (n = 7, 6; control n is listed first throughout) fed a moderate-fat diet were measured from isolated islets 40 wk after tamoxifen injections. For insulin content measurements, n represents individual mice. D) Circulating insulin at 5, 25, and 40 wk after tamoxifen injection in control littermates (n = 10–15) and experimental mice (n = 7–12) fed a moderate-fat diet. E, F) Glucose tolerance (E) and insulin sensitivity (F) in mice (n = 15, 12). Insets: area under the curve (AUC) (E) and area over the curve (AOC) (F). G) Glucose-stimulated (continued on next page)
ghrelin, GIP, GLP-1, IL-6, and PYY (Fig. 2H). Thus, in these experimental conditions, partial ablation of the Ins2 gene in adult mice results in small but significant reductions in basal and glucose-stimulated second-phase insulin release that remain within the normal range for glucose homeostasis. The maintenance of normal glucose homeostasis within our model allowed us to test for the causality of hyperinsulinemia in obesity, without confounding changes in glucose tolerance or insulin sensitivity.

Inducible insulin reduction causes weight loss in mice fed an HFD

To test our hypothesis that obesity could be reversed by acutely suppressing insulin, we examined the body weight of HFD-fed control littermates and experimental mice 5 wk after tamoxifen injections. In a pilot study, we found that tamoxifen injection resulted in a slight reduction in body mass compared to that in vehicle-treated mice (Supplemental Fig. S1). To be conservative, we felt it was most appropriate to compare groups of mice that were both injected with tamoxifen. Using this conservative approach, we found that acute reduction in Ins2 gene dose resulted in an ~5% reduction in body mass (Fig. 3A). Control mice had body weights ranging from 42 to 44 g, whereas experimental mice weighed between 39 and 41 g 5 wk after the tamoxifen injection. The weight loss we observed in the experimental mice was primarily related to the reduced mass of gonadal and perirenal fat pads, which were significantly lighter in experimental mice (30.9 ± 0.6 g vs. 29.7 ± 0.7 g). There were no differences in daily food intake before or after recombination (Fig. 3D), suggesting the absence of a robust action on the brain. Analysis of gonadal and perirenal fat pad cross sections did not reveal robust differences in adipocyte morphology between control littermate and experimental mice (30.9 ± 0.6 g vs. 29.7 ± 0.7 g). There were no differences in daily food intake before or after recombination (Fig. 3D), suggesting the absence of a robust action on the brain. Analysis of gonadal and perirenal fat pad cross sections did not reveal robust differences in adipocyte morphology between control littermate and experimental mice (30.9 ± 0.6 g vs. 29.7 ± 0.7 g). There were no differences in daily food intake before or after recombination (Fig. 3D), suggesting the absence of a robust action on the brain. Analysis of gonadal and perirenal fat pad cross sections did not reveal robust differences in adipocyte morphology between control littermate and experimental mice (30.9 ± 0.6 g vs. 29.7 ± 0.7 g). There were no differences in daily food intake before or after recombination (Fig. 3D), suggesting the absence of a robust action on the brain.

Markers of lipid mobilization are altered after induced insulin reduction

Insulin regulates lipid metabolism at multiple steps, including by inhibiting lipolysis (23). Basal levels of NEFA, free glycerol, or glycerides (Fig. 3F–H) did not differ between control littermates and experimental mice, indicating that the reduction of circulating insulin in the experimental mice did not grossly impair lipid metabolism and that insulin sensitivity remained similar between these two groups of mice. Insulin is well known to regulate WAT lipid accumulation (24). This, along with the reduction of fat mass in the experimental mice, led us to explore proteins involved in adipocyte energy storage and lipid mobilization. First, we examined the protein abundance of Ptf/Cavin, given that both mice and humans with loss-of-function mutations in this gene display a lipodystrophic phenotype (25, 26). Indeed, Ptf protein levels were significantly lower in experimental mice after reduction of Ins2 gene dose (Fig. 4A). Ptf is known for its role in the formation and organization of caveolae, together with caveolin proteins, which have also been implicated in lipodystrophy (27–29). In addition to cavin, phosphorylated caveolin-1 has been implicated recently in insulin uptake and insulin receptor internalization (30, 31). However, we did not observe significant differences in the protein abundances of cavin, caveolin 1 (Cav1), Y14 phosphorylated Cav1, insulin receptor, phosphorylated-Akt, or phosphorylated-Erk between control littermates and experimental mice. This result suggests that Ptf either acts downstream of reduced insulin independent from Cav1 or in a manner that does not necessitate changes in Cav1 protein levels or phosphorylation at the Y14 site. It is also consistent with our observations that insulin sensitivity was not influenced by our mild and acute reductions in insulin production. Nevertheless, caveolin proteins have been implicated in the activation of lipolysis (32) and Hsl (33). Phospho-Hsl did not differ statistically between control littermates and experimental mice, consistent with the similarities in plasma levels of free fatty acids and glycerol (Fig. 3F, G). We observed a slight reduction (P = 0.06) in Lpl in our experimental mice compared with their control littermates, suggesting another mechanism associated with altered lipid uptake into adipose tissue in the context of reduced insulin production and secretion. Given the role of Lpl as an important marker for adipogenesis (34), the reduced levels in our study may reflect impaired adipogenesis related to reduced insulin production and secretion (35).

insulin secretion (n = 15, 12). H) Percentage change in body mass of male mice fed a moderate-fat diet; control littermates (n = 20; Ins1+/−:Ins2+/−:mTmG; gray dashed line) and experimental mice (n = 20; Ins1+/−:Ins2+/−:PdxIKCreERT:mTmG; blue dashed line). I) Percentage change in body mass of male mice fed a low-fat diet; control littermates (n = 8; Ins1+/−:Ins2+/−:mTmG; gray dashed line) and experimental mice (n = 2; Ins1+/−:Ins2+/−:PdxIKCreERT:mTmG; yellow line). Unless otherwise indicated, measurements were conducted from samples collected from mice between 5 and 7 wk after tamoxifen injection. Data are means ± SEM.
Inducible insulin reduction is associated with an altered immune profile in adipose tissue

We performed RNA sequencing to further examine the effects of inducible insulin reduction on the transcriptome of gonadal fat tissue 5 d after tamoxifen injection. After correction for multiple testing, we did not find any individual genes that were significantly differentially expressed when the Ins2 gene dose was reduced. However, ordering the genes by P-value before correction for multiple testing revealed that some gene families were overrepresented at the top of that list (Supplemental File S1). We therefore selected the top 24 genes, all with uncorrected \( P > 0.01 \), for protein–protein network modeling, to visualize the relationships between the expressed mRNAs; these networks were manually arranged to reflect the most likely cell source, based on the GTEx (http://www.gtexportal.org) resource. Six...
Figure 3. Adult reduction of Ins2 gene dose reverses obesity in mice fed an HFD, primarily because of reduced mass of gonadal and perirenal fat pads. A) Percentage change in mass of control littermate (n = 39) and experimental (n = 29) mice 5 wk after tamoxifen injection. B) Inguinal, gonadal, perirenal, and mesenteric WAT depots and BAT from mice were weighed after 16 h without food and again at 15 min after an insulin stimulation (n = 16, 11). C) Representative transsectional images obtained by micro-CT (left) and quantification (right) of percentage change in subcutaneous and visceral fat depots after tamoxifen injection (continued on next page)
immune-associated genes, including chemokine ligand (Ccl25), interferon-induced protein with tetratricopeptide repeats (Ifit)-2, Ifit3, Ifit3b, Ig-like domain-containing receptor (Ildr)-2, and inter-α-trypsin inhibitor heavy chain (Itih)-3 were down-regulated in the experimental mice compared to control littermates within the subnetwork of a predicted leukocyte source (Fig. 4B). Enriched pathways in the protein–protein network highlighted changes in immune function as well as lipid transport (Table 1). Ccl25 is primarily involved in leukocyte migration and its elevated expression is associated with chronic inflammatory conditions (36). Inflammatory conditions linked to the onset of diabetes in nonobese Goto-kakizaki rats are also associated with elevated expression of Ifit genes (37) and Itih3 and Itih4 genes have been linked to obesity (38) and diabetes (39) in mouse genetic studies. These genes bind to locally synthesized hyaluronan and, as a complex, have been reported to be involved in inflammatory diseases (40). Levels of hyaluronan, an extracellular matrix component, have been reported to increase in adipose tissue (41) as well as insulin-resistant skeletal muscle in mice with diet-induced obesity (42). Our control littermates are both

Figure 4. Network analysis of gonadal fat pad from adult mice with reduced Ins2 gene dose. A) Representative immunoblots (left) and quantification (right) of key proteins involved in lipid metabolism. B) Analysis of a protein–protein interaction network assembled from RNAseq data. Node color reflects whether mRNA is increased (green) or decreased (red); gray represents linking genes. C) IL-1β, TNF-α, IL-2, IL-6, and IL-12p70 levels in control littermate (n = 8–9) and experimental (n = 7–12) mice. D) Daily food intake was measured for 2 wk before, and 4 wk after, tamoxifen injection (n = 14, 19; control n is listed first throughout). Arrow: the week of tamoxifen injection. E) Representative sections of gonadal (n = 7, 10) and perirenal (n = 8, 11) adipose tissue (top; hematoxylin and eosin staining) and analysis of adipocyte size distribution (bottom; perilipin staining). F–H) NEFA (F), free glycerol (G), and glyceride (H) levels (n = 6, 8) were measured after food was withheld for 16 h and again 15 min after insulin stimulation. Unless otherwise indicated, measurements were conducted in samples collected from mice between 5 and 7 wk after tamoxifen injection. Data are means ± SEM. *P < 0.05.
TABLE 1. Enriched pathways in the protein—protein network in gonadal fat from mice with reduced insulin

| Gene feature                                      | FDR     |
|---------------------------------------------------|---------|
| Blood microparticle                               | 4.24E−09|
| Lipid transporter activity                        | 1.73E−02|
| Lipid transport                                   | 2.62E−02|
| Steroid binding                                  | 2.62E−02|
| Triglyceride-rich lipoprotein particle            | 4.78E−02|
| Very-low-density lipoproteins particle            | 4.78E−02|
| Neutral lipid metabolic process                   | 6.20E−02|
| Response to virus                                 | 6.20E−02|
| Extracellular matrix                              | 6.20E−02|
| Cyclin-dependent protein kinase                   | 6.20E−02|
| Acetyl-CoA metabolic process                      | 6.20E−02|
| Lipid localization                                | 6.20E−02|
| Plasma lipoprotein particle                      | 6.51E−02|
| Phospholipid binding                              | 6.97E−02|
| Protein–lipid complex                             | 7.08E−02|
| Intestinal absorption                             | 7.74E−02|
| Adenylyltransferase activity                      | 7.74E−02|
| Lipoprotein particle receptor binding             | 8.96E−02|

Obese and hyperinsulinemic compared with our experimental mice, which is consistent with altered expression of proinflammatory genes. The down-regulation we observed of immune-related genes in mice with reduced insulin, however, did not correspond to statistically significant changes in individual cytokines measured within gonadal tissue homogenates (Fig. 4C). Although we did not observe the stereotypical up-regulation in uncoupled protein 1 expression (Supplemental File S1) in our experimental mice, we noted differential expression of Ildr2, a gene associated with white adipose browning. Specifically, the expression level of Ildr2 has been noted to be significantly lower in BAT than in subcutaneous and visceral WAT depots (43). Furthermore, we noted an increase in neuregulin (Nrg2) gene expression within the predicted adipocyte source. Nrg2 belongs to the neuregulin family of proteins (Nrg1–4) which shares N-terminal epidermal growth factor (EGF)-like domains, which activates membrane-associated tyrosine kinases related to the EGF receptors (44). Nrg4 has been characterized as a WAT browning adipokine (43, 45), and its over-expression has been linked to reduced chronic inflammation in preventative and treatment studies (46). Nrg2, like Nrg4, has been implicated in neurite outgrowth and may increase adipose tissue innervation, which is necessary for the initiation of WAT lipolysis (47). The RNA-sequencing data and network analysis suggest the potential for interesting differences in adipose tissue function and composition, although, clearly, additional targeted loss-of-function studies are needed to establish if any of these gene products mediate the effects of reduced insulin on body weight.

Our data provide the first molecular genetic evidence that obesity can be reversed by acutely and modestly reducing insulin production alone. The results of this treatment study, combined with our previous prevention studies (11, 12), clearly demonstrate the causal control of obesity by hyperinsulinemia in a genetically tractable mammalian system. The magnitude of insulin gene dose reduction achieved in our current study (~30%) does not cause changes in glucose homeostasis and is attainable in human populations as a preventative measure or weight-loss strategy. For example, intermittent and continuous energy restriction over a 6-mo period resulted in similar reductions in circulating insulin in obese patients, coupled with a 5–10% loss in body weight (48). Although such dietary restriction interventions are often associated with high dropout rates (49), isocaloric restriction of daily fructose intake from 28 to 10% is an alternate approach that may mitigate high dropout rates and has successfully been shown to achieve similar reductions in circulating insulin and weight loss in obese adolescent patients (50). The magnitude of weight loss in our study, when translated to humans, has been associated with improvements to risk factors linked to cardiovascular disease, as well as reductions in the risk of developing type 2 diabetes by more than 50% (51).

CONCLUSIONS

Our results have profound implications for nutritional guidelines and therapeutic efforts to combat obesity and its comorbidities. Our findings support the concept that an individual’s hyperinsulinemia status should be the target for lifestyle modifications, macronutrient dietary composition, and drug development for the purposes of weight loss. Studies where individualized dietary responses are assessed (52) and mechanistically defined will be useful for the design of personalized nutrigenomic strategies. Approaches that physiologically modulate insulin levels may also have therapeutic utility beyond weight loss, including in the improvement of insulin sensitivity, and perhaps even longevity (53).

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AUTHOR CONTRIBUTIONS

M. M. Page designed and performed experiments, analyzed results, and wrote the manuscript; S. Skovsø...
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