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ORIGINAL ARTICLE

Obesity Biology and Integrated Physiology

High-fat and high-sodium diet induces metabolic dysfunction in the absence of obesity

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Abstract

Objective: Excess dietary fat and sodium (NaCl) are both associated with obesity and metabolic dysfunction. In mice, high NaCl has been shown to block high-fat (HF) diet–induced weight gain. Here, the impact of an HF/NaCl diet on metabolic function in the absence of obesity was investigated.

Methods: Wild-type mice were administered chow, NaCl (4%), HF, and HF/NaCl diets. Metabolic analysis was performed by measuring fasted blood glucose and insulin levels and by glucose tolerance test and insulin tolerance test.

Results: After 10 weeks on diets, male and female mice on the HF diet gained weight, and HF/NaCl mice had significantly reduced weight gain similar to chow-fed mice. In the absence of obesity, HF/NaCl mice had significantly elevated fasting blood glucose and impaired glucose control during glucose tolerance tests. Both NaCl and HF/NaCl mice had decreased pancreas and β-cell mass. Administration of NaCl in drinking water did not protect mice from HF-diet-induced weight gain and obesity. Further analysis revealed that longer administration of HF/NaCl diets for 20 weeks resulted in significant weight gain and insulin resistance.

Conclusions: The data demonstrate that despite early inhibitory effects on fat deposition and weight gain, an HF/NaCl diet does not prevent the metabolic consequences of HF diet consumption.

INTRODUCTION

Obesity is one of the most important risk factors for the development of metabolic dysfunction such as insulin resistance and type 2 diabetes mellitus. The Western diet, which includes highly processed foods containing a high percentage of saturated fats, sugars, and salt, contributes to obesity and metabolic disease not only through increased caloric content but also though systemic effects that induce inflammation and dysregulation of metabolic function. The mechanisms by which these nutrient components interact to alter metabolism and fat deposition are complex and remain incomplete.

Excess sodium intake is associated with high blood pressure and other cardiovascular consequences, and the vast majority of scientific literature has strongly supported the reduction of sodium to decrease the risk of cardiovascular disease and stroke (1-3). There is also a body of literature associating sodium with insulin resistance and metabolic disease. In human studies, high sodium intake has been shown to be associated with insulin resistance and metabolic syndrome (4-7). Similarly, in animal models, high sodium administration was reported to decrease glucose tolerance and insulin sensitivity (8). Consistent with these findings, a low-sodium diet can increase insulin-sensitizing adipokines while decreasing adipose tissue inflammation in rodents (9).

The mechanisms by which sodium affects insulin sensitivity and metabolic function are multifactorial and not fully understood. One potential mechanism is through activation of the renin–angiotensin system (RAS), which has been shown to be associated with insulin resistance and metabolic dysfunction in humans (10,11). A similar effect is thought to occur whereby short-term, highly restricted, low sodium increases insulin resistance through RAS activation (12). In contrast, mineralocorticoid receptor blockade was shown to enhance adiponectin and decrease adipose tissue inflammation (13). In other models, dietary sodium has been shown to have important pathophysiological effects through regulation of immune cell phenotypes. Sodium has been shown to drive Th17 differentiation, which exacerbates inflammatory diseases like multiple sclerosis (14), and it also enhances proinflammatory phenotypes in monocytes and macrophages (15-17). Overall, these cellular effects have translated into poor clinical outcomes in animal models of disease (14,18).

In addition to its effects on insulin sensitivity and metabolic function, sodium has also been shown to affect fat deposition and adiposity. In humans, sodium intake has been associated with obesity (19,20). However, the mechanistic understanding of this is confounded by conflicting reports in animal models. In one study, sodium intake was found to increase fat depot mass without affecting body weight (21), whereas others have indicated that sodium actually decreases body weight and fat depot mass (22,23). In the context of diet-induced obesity (DIO), sodium has been shown to dose-dependently prevent weight gain with the addition of 4% NaCl being sufficient in one study to completely block DIO (24,25).

Although high fat and high sodium were shown to dramatically prevent weight gain and adiposity, the impact that high fat and high sodium have on glucose regulation in the absence of obesity is unknown. In the present study, we examine the metabolic consequences of high-fat diet (HF)/high-sodium diet (NaCl) administration in mice. We tested the effect of HF/NaCl on glucose control and insulin sensitivity to determine whether chronic administration of HF/NaCl affects glucose homeostasis and metabolic function.

Study Importance

What is already known?

- ► Excess sodium intake is associated with increased risk of cardiovascular disease, obesity, and inflammation.
- ► Animal models have shown that high sodium can inhibit adiposity in mice.

What does this study add?

- ► High sodium causes impaired glucose control during a high-fat diet in the absence of obesity.
- ► Inhibitory effects of sodium on high-fat diet-induced weight gain are transient and are dependent on the route of sodium intake.

How might these results change the direction of research?

- ► These findings demonstrate an important interaction between a high-fat diet, sodium intake, and glucose regulation, and they indicate that a thorough understanding of this interaction is critical in our understanding of metabolic dysfunction.

METHODS

Animals

Wild-type male and female C57BL/6J mice were purchased from The Jackson Laboratory (Bar Harbor, Maine) and allowed to acclimate for 1 week prior to all studies. Mice were multihoused in static cages in a temperature-controlled specific pathogen free room (21°C to 23°C) with a light:dark cycle of 12:12 hours (lights on at 6 AM). Prior to experiments, mice were normalized by body weights and randomly assigned to treatment groups. Mice were maintained on standard laboratory chow or on sodium- and fat-adjusted diets and water ad libitum. Estimation of required sample size for all experiments was based on a priori power analysis calculations using expected standard deviations based on previous experiments and published literature. All animal procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals (8th edition) and were approved by the Institutional Animal Care and Use Committee of the University of Michigan.

Rodent diets

Standard laboratory chow diet was 5L0D (LabDiet, St. Louis, Missouri). A 4% NaCl diet was prepared in-house by adding an

additional 3% NaCl to powder chow (5001, LabDiet) and forming pellets. HF diet (D12492) and HF/NaCl diet (D06111102) were purchased from Research Diets, Inc. (New Brunswick, New Jersey). Calorie and sodium composition of all diets is provided in Supporting Information Table S1. For NaCl experiments administered in the water, mice were given 2% NaCl water ad libitum.

Body composition and indirect calorimetry

See Supporting Information Methods.

Food consumption

Food intake was repeatedly measured in both standard static cages and metabolic cages over 2- or 3-day intervals.

Glucose and insulin tolerance tests

For glucose tolerance tests (GTT), mice were fasted for 6 hours (5:00 AM to 11:00 AM) before receiving an intraperitoneal injection of glucose. Glucose dose (1.25 mg/g lean mass) was determined from lean body mass to avoid confounding effects from obesity. Blood glucose was measured at 0, 15, 30, 60, and 120 minutes after glucose injection using a Contour glucose meter (Bayer, Whippany, New Jersey). For insulin measurements, plasma was collected at 0, 30, and 60 minutes after glucose injection, and plasma insulin was assayed using an Ultra Sensitive Mouse Insulin ELISA Kit (Crystal Chem, Elk Grove Village, Illinois). For insulin tolerance tests (ITT), mice were injected with insulin (0.5 U/kg lean mass) (Humulin R, Eli Lilly and Company, Indianapolis, Indiana), and blood glucose was measured at 0, 15, 30, 60, and 120 minutes.

Gene expression analysis

See Supporting Information Methods.

β-cell mass

Cohorts of male mice were used for these experiments. The entire pancreas was excised, weighed, and fixed in 4% formaldehyde overnight. Tissue was then processed and embedded in paraffin, and 5 serial sections were cut throughout the pancreas approximately 100 μm apart. Sections were immunostained for insulin and then scanned and analyzed. The total pancreas area and the insulin positive regions were measured using ImageJ software (version 1.52; National Institutes of Health, (NIH) Bethesda, Maryland) to determine the β-cell percentage, and the β-cell percentage was then multiplied by the pancreas weight to determine the β-cell mass.

Statistical analysis

See Supporting Information Methods.

RESULTS

Incorporation of NaCl into HF diet suppresses diet-induced obesity in both male and female mice

Wild-type mice were placed on chow, NaCl, HF, and HF/NaCl diets for 10 weeks. Consistent with what has been previously reported in male mice, incorporation of 4% NaCl into a 60% HF diet effectively prevented weight gain during 10 weeks on HF (Figure 1A,B). We extended this finding to females and found that sodium also significantly prevented HF-induced weight gain in female mice (Figure 1B). There was a significant reduction in total fat mass as well as fat deposition in select adipose tissue depots in both male (Figure 1C) and female mice (Figure 1D). No statistically significant differences were detected in lean body mass or fluid percent as a result of NaCl administration (Figure 1E,F). Mice on HF and HF/NaCl had similar food and calorie intake, and mice on NaCl and HF/NaCl had expected increases in water consumption (Supporting Information Figure S1).

Similar to what has been reported previously, HF significantly reduced the respiratory exchange ratio and resulted in a change in energy substrate use from glucose to fatty acids. These differences occurred rapidly by 1 week (Supporting Information Figure S2A) and remained constant at 8 weeks (Supporting Information Figure S2B). No differences were detected between HF- and HF/NaCl-treated mice in males (Supporting Information Figure S2A-B) and females (Supporting Information Figure S2C). No significant changes were detected in energy expenditure when normalized to lean body mass. When normalized to total body weight, HF-treated mice had significantly lower energy expenditure compared with HF/NaCl mice (Figure S2).

Incorporation of NaCl into HF diet causes glucose intolerance in the absence of obesity

Although HF/NaCl mice are protected from HF-induced weight gain, they have similar food consumption compared with HF-treated mice and they are therefore exposed to similar caloric composition. To determine whether HF/NaCl has an effect on glucose homeostasis in the absence of obesity, we measured glucose levels during treatment. Both HF and HF/NaCl significantly increased fasted blood glucose levels in female mice after 2 weeks on diets (Figure 2A). After 8 weeks on diets, both HF and HF/NaCl significantly increased fasted blood glucose in male and female mice (Figure 2B). Only HF resulted in significantly elevated fed blood glucose levels after 8 weeks on diets (Figure 2C).

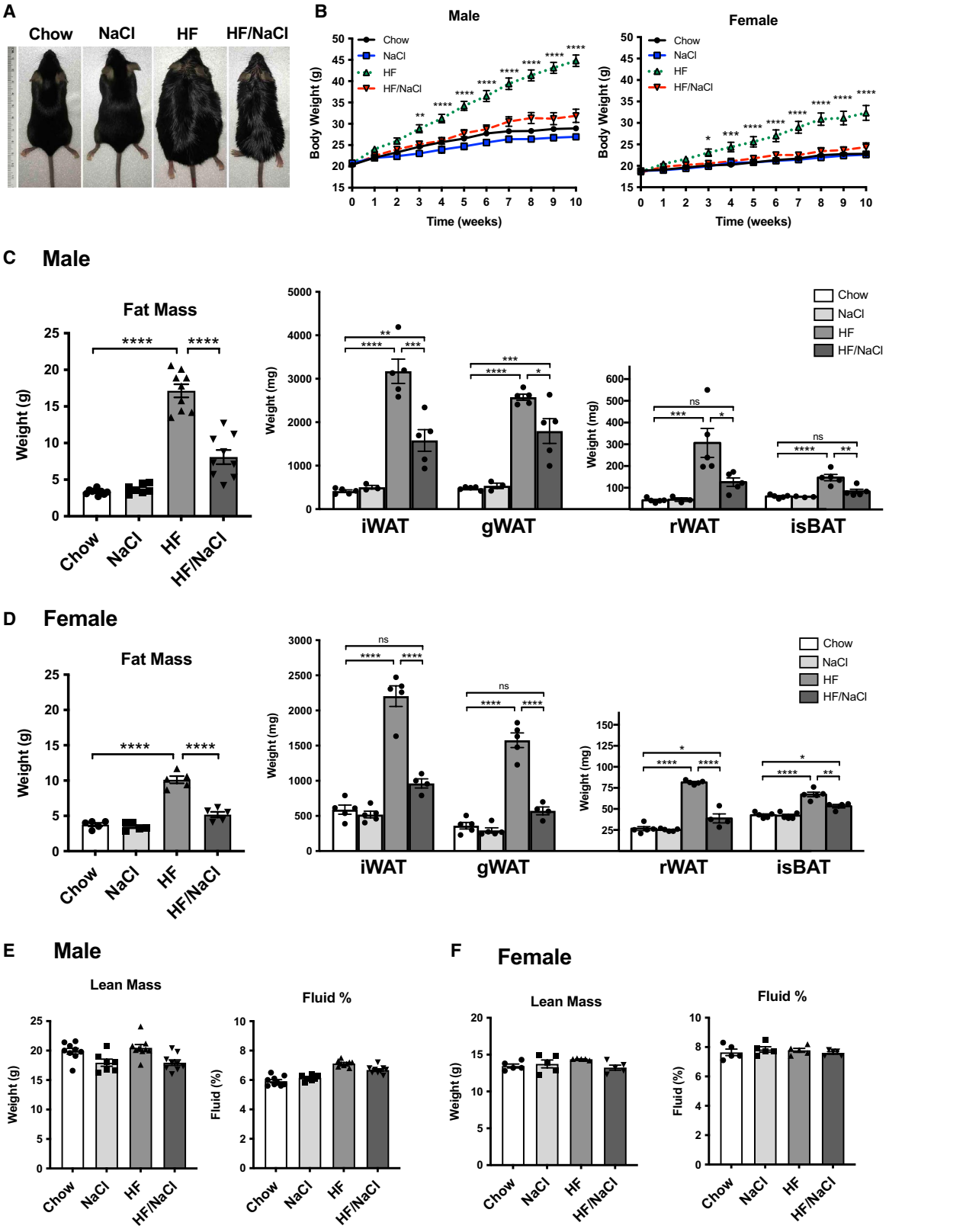

FIGURE 1 HF/NaCl diet prevents weight gain in male and female mice. (A) Photograph of mice after 10 weeks on diet. (B) Body weight in male and female mice. Total fat mass from body composition analysis and fat depot mass in (C) male and (D) female mice after 10 weeks on diet. Lean body mass and fluid % in (E) male and (F) female mice at week 10. Data represented as mean ± SEM. $N = 4$ to 9 per group. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$. gWAT, gonadal white adipose tissue; HF, high fat; isBAT, interscapular brown adipose tissue; iWAT, inguinal white adipose tissue; NaCl, high sodium; rWAT, renal white adipose tissue [Color figure can be viewed at wileyonlinelibrary.com]

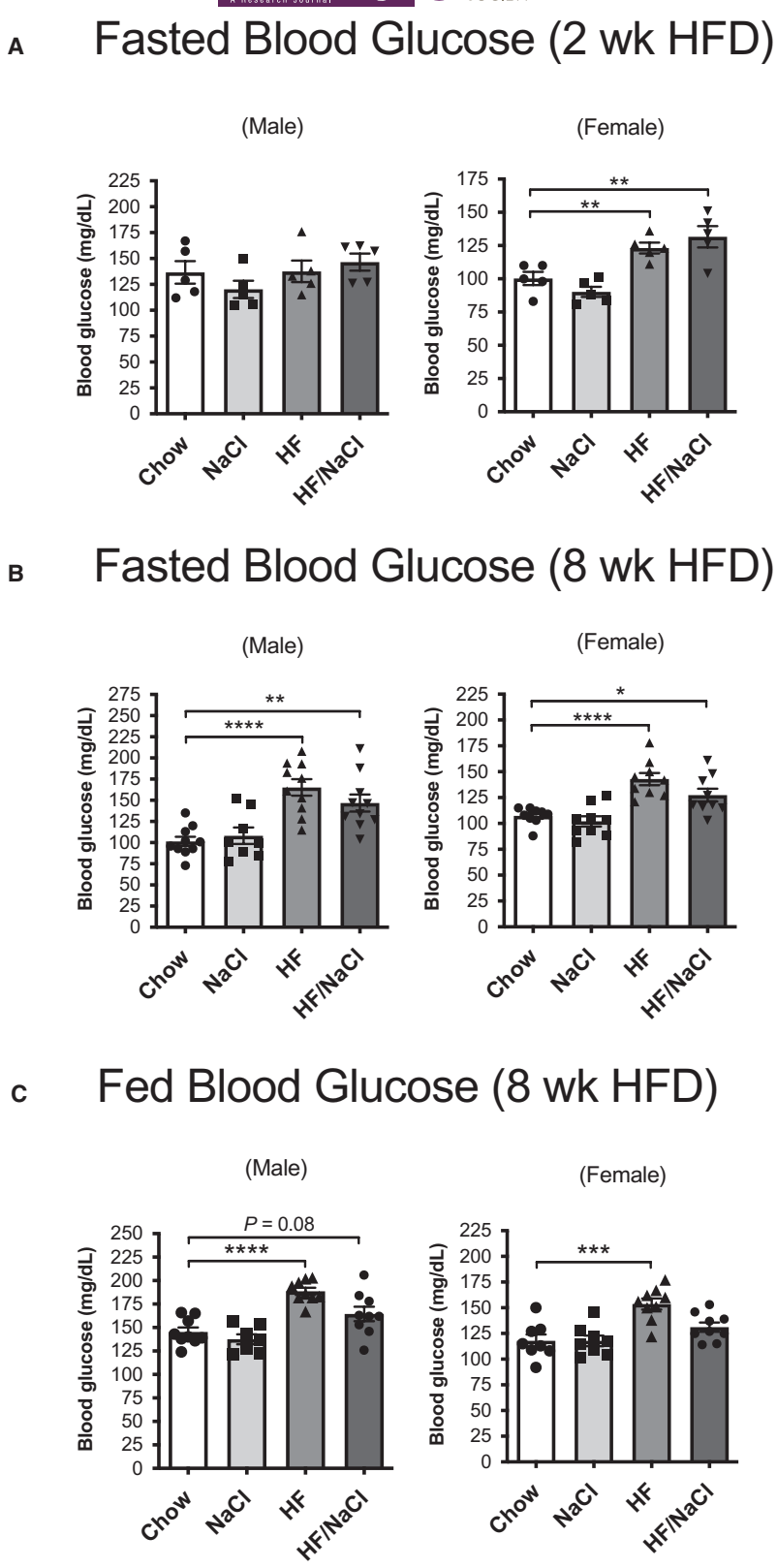

FIGURE 2 Mice on HF/NaCl diet have elevated blood glucose. Fasted blood glucose levels at (A) 2 weeks and (B) 8 weeks on diet. (C) Fed blood glucose at 8 weeks. Data represented as mean ± SEM. $N = 5$ to 9 per group. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$. HF, high fat; HFD, high-fat diet; NaCl, high sodium

We next tested whether sodium also affected glucose homeostasis during GTT. After 2 weeks on sodium- and fat-adjusted diets in which mice had not gained significant weight, both HF and HF/NaCl treatment significantly elevated blood glucose after glucose injection in male mice (Figure 3A). After 8 weeks on diets in which HF mice had significant adiposity and HF/NaCl mice were normal weight, a similar effect in GTT was detected wherein both HF and HF/NaCl mice had significantly elevated blood glucose after GTT when compared with chow- and NaCl-treated mice (Figure 3B). In female mice, only HF/NaCl resulted in significantly elevated blood glucose levels during GTT after both 2 weeks and 8 weeks on diets (Figure 3C,D).

To determine whether HF/NaCl resulted in impaired insulin response in the absence of obesity, we measured plasma insulin levels. After 2 weeks on diets, none of the treatments resulted in increased fasted insulin levels. By 8 weeks on diets, only HF mice had significantly elevated fed and fasted insulin levels (Figure 4A). We performed ITT on the mice at 8 weeks on diets and found that again only HF-treated male mice had impaired insulin responses with increased blood glucose after insulin injection (Figure 4B). Although females had significantly high blood glucose during ITT, the percent reduction in blood glucose was similar (Figure 4C). This likely reflects the reduced obesity response seen in female mice during shorter lengths of HF treatment.

Upon dissection, histological assessment of gonadal white adipose tissue revealed increased adipocyte hypertrophy in HF-treated mice and comparatively normal adipocyte size in HF/NaCl-treated mice (Figure 5A,B). Similarly, hepatosteatosis was present in HF mice, whereas HF/NaCl mice had normal liver histology. To assess whether changes in adipose depot function contribute to impaired glucose homeostasis in HF/NaCl mice, we analyzed adipokine and inflammatory cytokine gene expression in gonadal white adipose tissue (gWAT) depots after 10 weeks on diets. The expression of inflammatory genes tumor necrosis factor α (TNFα) and interleukin 1β (IL-1β) was modestly increased in HF-treated mice but not in mice on HF/NaCl (Figure 5B). Adiponectin expression was significantly decreased only with HF treatment, although peroxisome proliferator-activated receptor γ (PPARγ) was significantly decreased in mice on both HF and HF/NaCl (Figure 5B).

Mice on HF/NaCl diet have decreased pancreas and β-cell mass

To determine whether HF/NaCl has an effect on metabolic capacity and glucose control through effects on pancreas function, we first measured pancreas mass. Treatment with HF/NaCl for 10 weeks resulted in a small but statistically significant reduction in pancreas mass in both male and female mice (Figure 6A,B). Female mice on NaCl also had a reduction in pancreas mass. We next assessed β-cell mass in male mice after 10 weeks on diets. HF mice had compensatory increases in β-cell mass compared with chow control mice (Figure 6C). Mice on NaCl and HF/NaCl had a statistically significant decrease in β-cell mass compared with both chow control and HF-treated mice.

NaCL administered in water does not prevent HF-diet-induced obesity and insulin resistance

We next assessed whether sodium would also prevent DIO when administered in the drinking water. We selected a 2% NaCl water dose based on pilot experiments in which we tested multiple concentrations of NaCl water (1%, 1.5%, and 2%) and measured water consumption to estimate NaCl intake. The 2% NaCl water was determined to provide an equivalent NaCl intake; therefore, wild-type mice were placed on chow or HF, and sodium was administered as 2% NaCl water. Food consumption, kilocalorie intake, water consumption, and total NaCl intake were equivalent with HF/NaCl treatment whether NaCl was administered in diet or in water (Figure 7A). In the absence of HF, administration of NaCl in water did result in a significant increase in water consumption and NaCl intake compared with NaCl administration in the diet.

Interestingly, administration of sodium in drinking water (NaCl-W) did not prevent HF-induced weight gain (Figure 7B). Both HF and HF/NaCl-W mice had significantly increased fat deposition in major fat depots, although no differences were detected between the 2 treatments (Figure 7C). Both HF and HF/NaCl-W treatment resulted in significantly increased fasted and fed blood glucose (Figure 7D) and significantly elevated fed and fasted insulin levels (Figure 7E). We next challenged mice with GTT and ITT to test glucose tolerance and insulin sensitivity. Both HF and HF/NaCl-W resulted in significantly increased glucose levels during both GTT (Figure 7F) and ITT (Figure 7G), although no differences were present as a result of increased sodium.

Incorporation of NaCl into HF diet only protects from obesity and insulin resistance during short-term feeding

Since incorporation of sodium in HF protected mice from weight gain and development of insulin resistance, we tested whether administration of HF/NaCl to obese mice would result in weight loss and normalization of insulin sensitivity. Mice were treated with chow, NaCl, HF, and HF/NaCl, and after 10 weeks, obese mice on HF were switched to HF/NaCl. The switch to HF/NaCl resulted in an initial decrease in weight, but mice eventually resumed gaining weight (Figure 8A). Mice on HF/NaCl also gained a significant amount of weight from week 10 until week 20 and they were obese by the end of the 20-week treatment. There were no differences in food consumption when obese mice were on HF or HF/NaCl (Figure 8B). As expected, HF- and HF/NaCl-treated mice had increased fasted blood glucose levels, and only obese HF-treated mice had increased fasted insulin after 10 weeks on diets (Figure 8C). At 10 weeks after the diet switch, both HF to HF/NaCl and HF/NaCl treatments resulted in increased fasted insulin levels (Figure 8D). When subjected to GTT, both mice on HF to HF/NaCl and HF/NaCl for 20 weeks had increased blood glucose (Figure 8E). After 20 weeks, mice on HF/NaCl also had significantly reduced insulin sensitivity during ITT (Figure 8F).

DISCUSSION

Excess sodium has been implicated in the development of obesity and metabolic dysfunction, and more recently, studies have shown that sodium can dose-dependently suppress HF-diet-induced weight

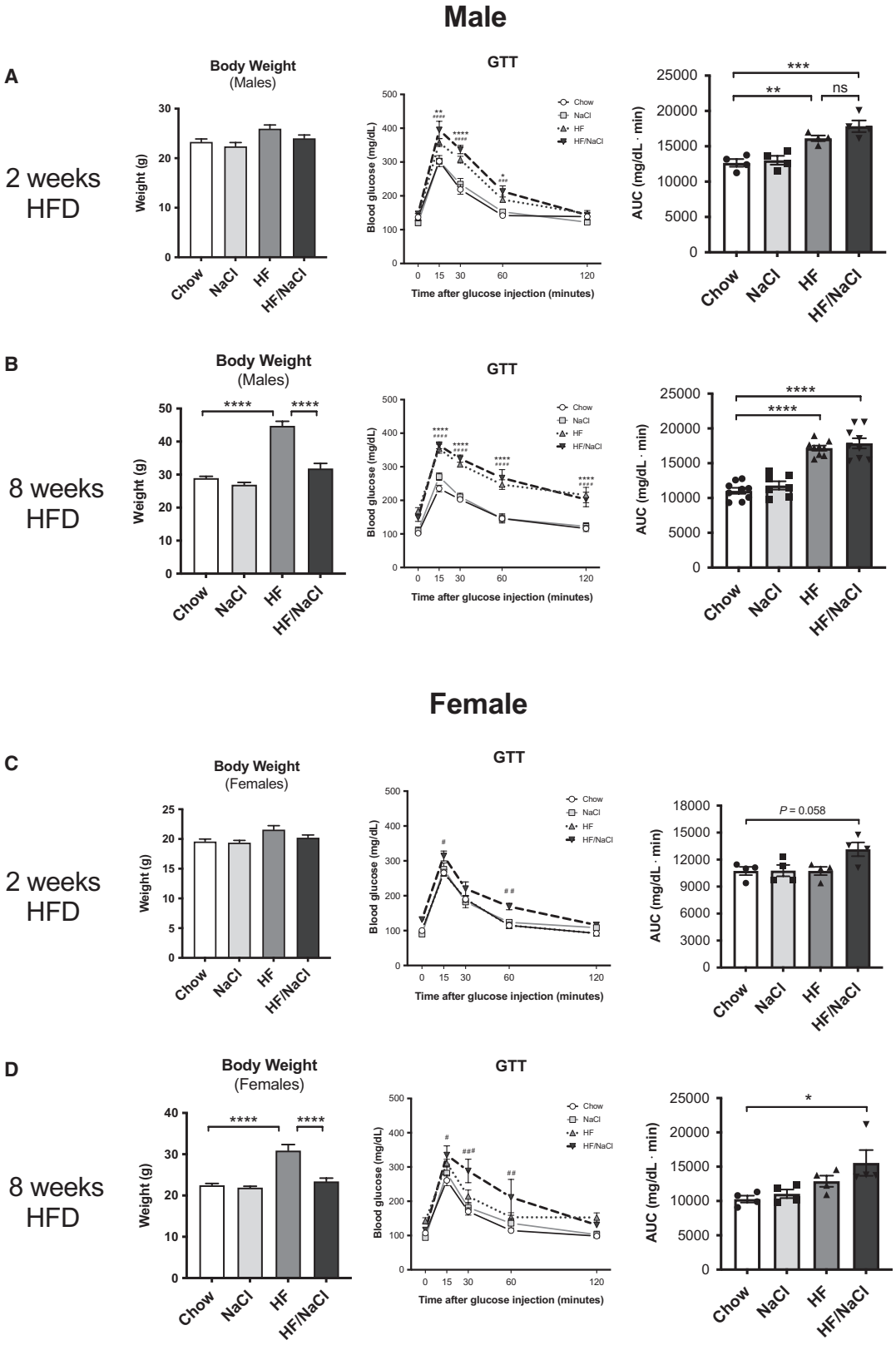

FIGURE 3 HF/NaCl diet induces glucose intolerance in the absence of obesity. Body weight and GTT at 2 weeks on diet in (A) male and (C) female mice. Body weight and GTT at 8 weeks on diet in (B) male and (D) female mice. Data represented as mean ± SEM. $N = 4$ to 9 per group. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. * Chow vs. HF. # Chow vs. HF/NaCl. AUC, area under the curve; GTT, glucose tolerance test; HF, high fat; HFD, high-fat diet; NaCL, high sodium

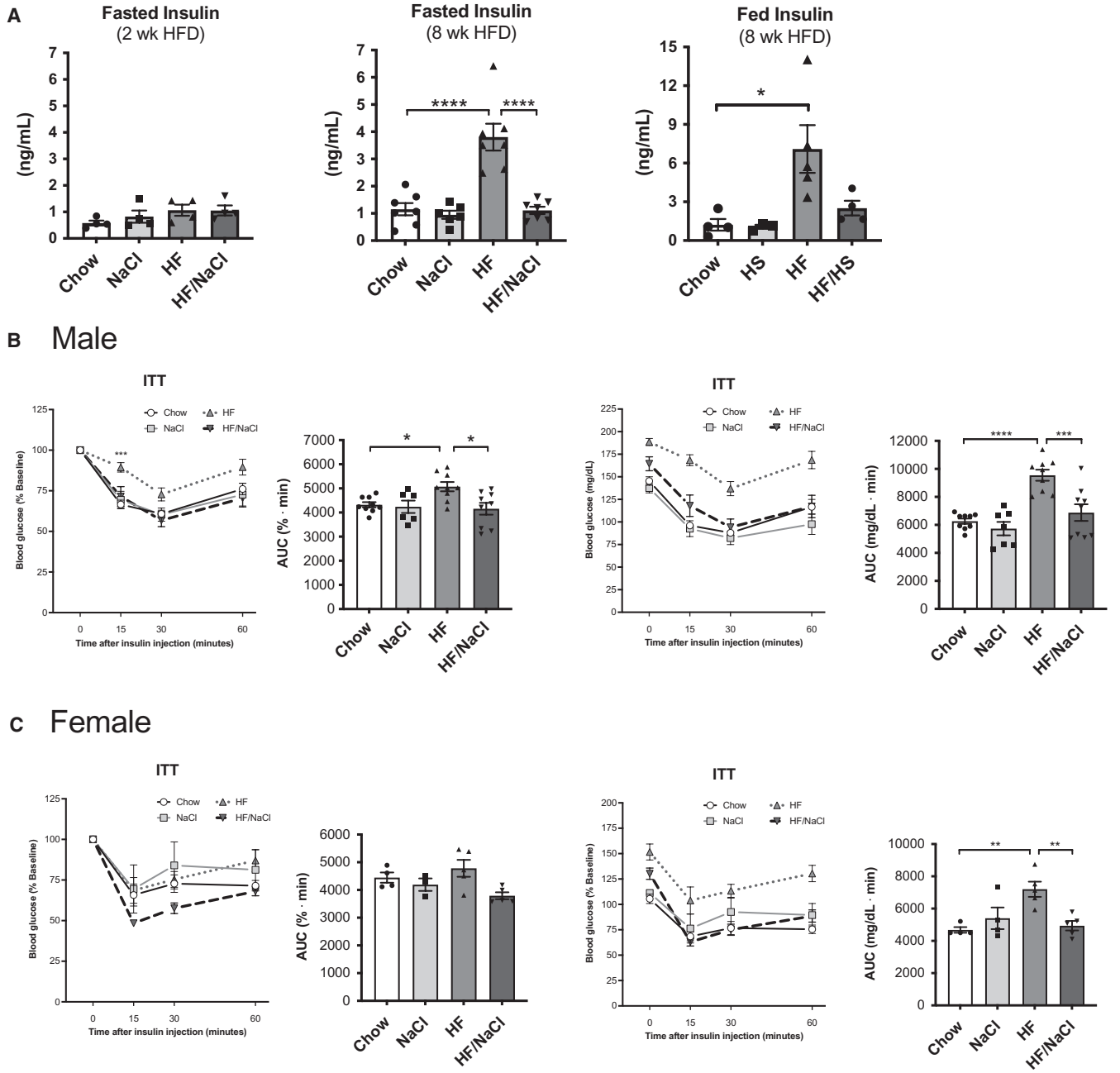

FIGURE 4 HF but not HF/NaCl diet induces insulin resistance at 8 weeks on diet. (A) Fasted and fed insulin levels at 2 and 8 weeks on diet. ITT at 8 weeks on diet in (B) male and (C) female mice. Data represented as mean ± SEM. $N = 4$ to 9 per group. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$. AUC, area under the curve; HF, high fat; HFD, high-fat diet; ITT, insulin tolerance test; NaCL, high sodium

gain. In the present study, we examined the metabolic consequence of HF/NaCl diet in the absence of obesity. Similar to what was reported previously (25), we found that HF/NaCl diet effectively prevented weight gain in male and female mice. However, when we extended the diet administration and body weight analysis to 20 weeks, HF/NaCl mice had significant adiposity. This indicates that the suppressive effects on weight gain are only transient.

HF mice had decreased energy expenditure when normalized to body weight. This is consistent with what has been reported in the literature, and it is thought that this significantly contributes to observed obesity changes. However, white adipose tissue often has lower metabolic activity than other tissues, and therefore, normalization to body weight or lean body mass may not accurately reflect the correct expenditure rate. Although it is possible that the significant increase in energy expenditure in mice on HF/NaCl diet at the later 8 weeks' analysis could be responsible for the transient inhibition of weight gain, more detailed and rigorous analysis will be required to elucidate this mechanism (26).

Further analysis of early administration (10 weeks) revealed that despite the lack of weight gain and obesity, mice on HF/NaCl diet

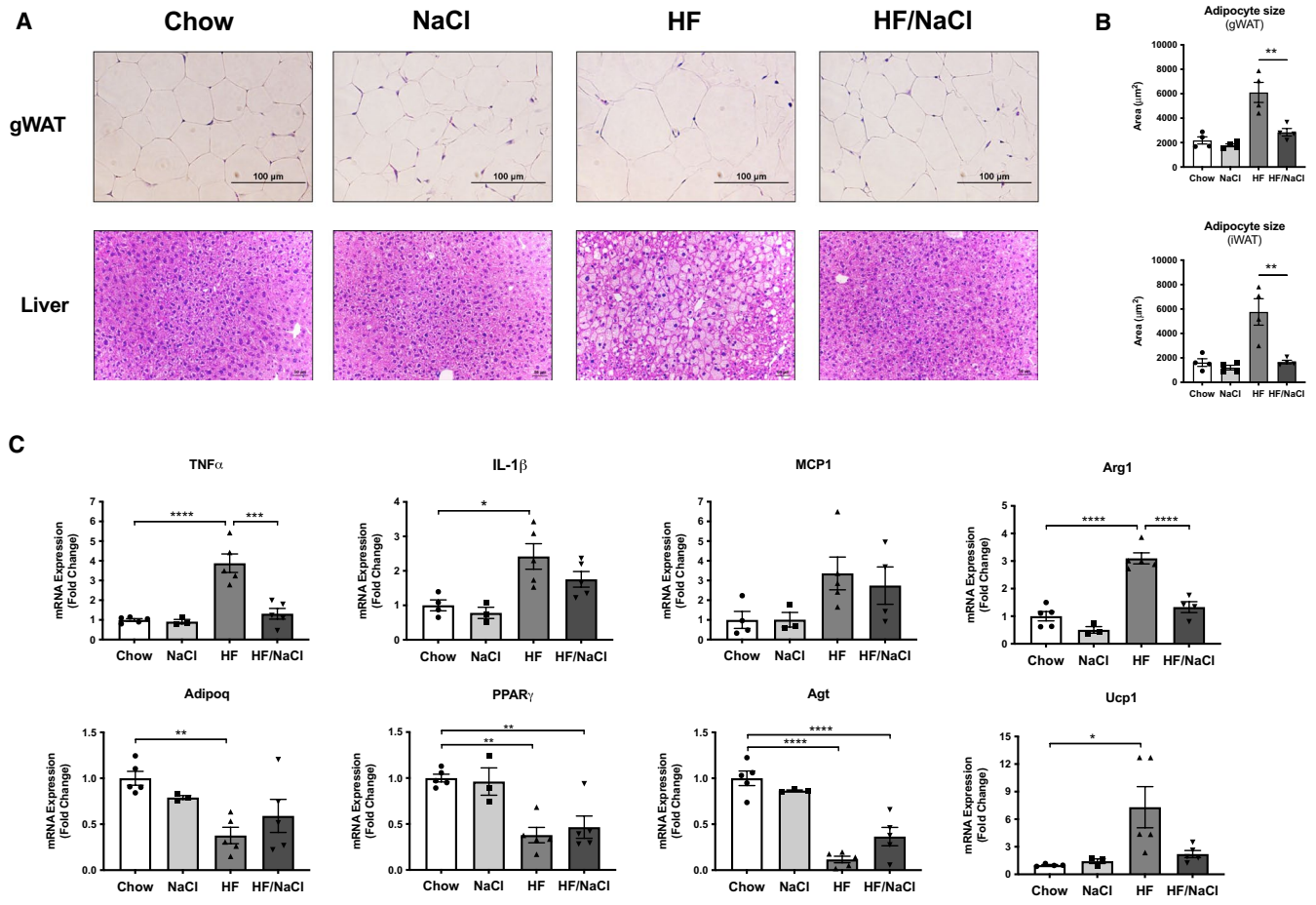

FIGURE 5 Adipose tissue inflammation during HF diet. (A) Histology of gWAT and liver from male mice after 10 weeks on diet. (B) Adipocyte size (cross-sectional area μm^2). (C) Gene expression analysis measured by quantitative real-time PCR of inflammatory cytokines and adipokines from gWAT at 10 weeks on diet. Data represented as mean ± SEM. $N = 3$ to 5. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$. Adipoq, adiponectin; Agt, angiotensinogen; Arg1, arginase 1; gWAT, gonadal white adipose tissue; HF, high fat; IL-1β, interleukin 1β; iWAT, inguinal white adipose tissue; MCP1, monocyte chemoattractant protein 1; NaCl, high sodium; PPARγ, peroxisome proliferator-activated receptor γ; TNFα, tumor necrosis factor α; Ucp1, uncoupling protein 1 [Color figure can be viewed at wileyonlinelibrary.com]

had significantly elevated fasted blood glucose and GTT after 2 weeks' administration. This early effect on glucose levels by HF diet is consistent with literature in which it has been shown that nearly maximal glucose intolerance and insulin resistance can be achieved within 3 days of HF diet administration (27). We did not detect any differences in blood glucose during ITT in HF/NaCl mice compared with chow, suggesting that HF/NaCl mice still maintain normal insulin sensitivity. These results indicate that the lack of obesity in HF/NaCl mice does not completely protect them from the metabolic consequences of HF diet.

In female mice, HF/NaCl diet also resulted in a modest increase in glucose intolerance compared with HF diet controls. It is well known that female mice have a delayed and blunted response to HF diet, and female mice do not exhibit the same degree of insulin resistance compared with male mice (28). Increased glucose intolerance in female mice treated with HF/NaCl diet may indicate a sexually dimorphic effect with respect to salt sensitivity and glucose regulation; however, an overwhelming majority of studies have found that female mice actually have decreased salt sensitivity with respect to blood pressure elevation (29,30). This is in contrast with humans in which there is evidence that women are more salt sensitive than men (31-33). In addition, it has been found that women have an increased taste for salt and are more likely to excessively consume salt than men. (34,35). Similarly, female rodents have been reported to consume more salt even though they are less salt sensitive to hypertension (36,37). There is insufficient evidence in animal models to draw strong conclusions on whether a sexual dimorphism exists with regard to salt sensitivity, glucose regulation, and insulin resistance.

Sodium administration in water has been reported to cause impaired glucose control in mice in the absence of HF diet (8). However, we did not detect any signs of glucose intolerance or insulin resistance in our NaCl-diet-treated group. A previous study that showed sodium can induce insulin resistance used BALB/c mice, which are known to have significantly different responses in a variety of animal models possibly because of differences in immune cell phenotypes.

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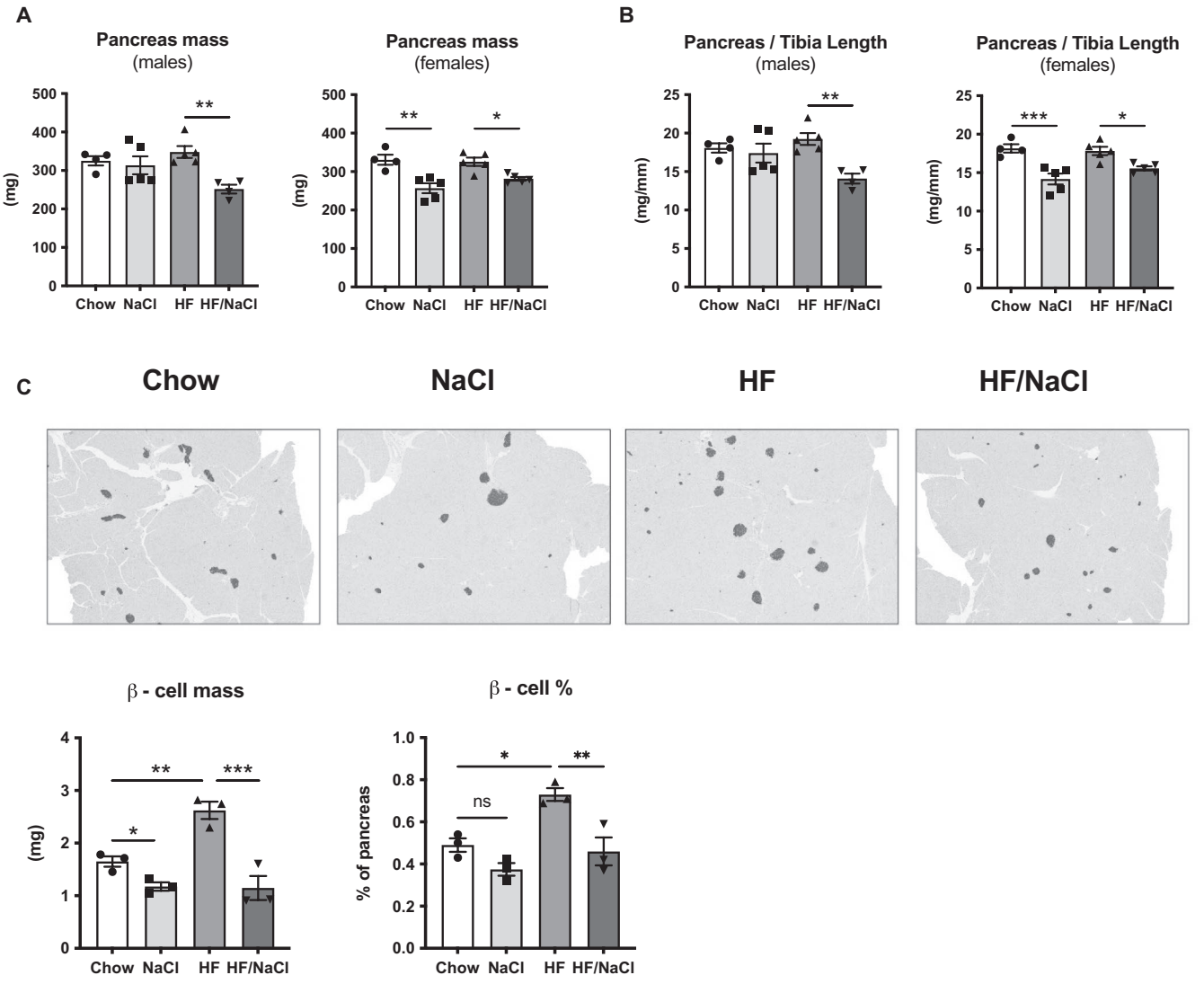

FIGURE 6 Decreased pancreas and β-cell mass in mice on HF/NaCl diet. (A) Pancreas mass in male and female mice at 10 weeks on diet. (B) Pancreas mass normalized to tibia length at 10 weeks on diet. (C) Photomicrographs of pancreas with immunohistochemical staining for insulin and quantification of β-cell mass from male mice. Data represented as mean ± SEM. $N = 3$ to 5. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. HF, high fat; NaCL, high sodium

However, when we assessed the effect of sodium in BALB/c mice, we also did not detect any signs glucose intolerance or insulin resistance (data not shown). This could be due to differences in the microbiota, which can arise in different animal vivaria. Animal microbiota has been shown to affect glucose control and insulin resistance (38), and it is also known that housing conditions can significantly affect the microbiota and can alter the phenotypes in many disease models.

It is also possible that sodium-induced changes in gut microbiota could be important in the observed weight gain inhibition and glucose intolerance phenotype. Several studies have shown that NaCl diet can elicit significant changes in the intestinal microbiota and cause dysbiosis through alteration of Lactobacillus or other bacterial species. These sodium-induced changes in microbiota have been found to exacerbate numerous animal models of disease including colitis, experimental autoimmune encephalomyelitis (EAE), hypertension, and autism (39-41). Wilck and colleagues found that decreased Lactobacillus resulted in enhanced Th17 cell activation, which exacerbated EAE and hypertension. Importantly, Th17 activation also contributes to obesity-associated inflammation and type 2 diabetes (42). NaCL diet has also been found to induce changes in fecal short chain fatty acids (SCFAs) (43,44). SCFAs can alter host metabolism, and total levels of SCFAs correlate with insulin sensitivity; therefore, sodium-induced changes could have important implications in both glucose control and obesity.

Although NaCl diet alone did not result in impaired glucose control, both NaCl and HF/NaCl mice had decreased pancreas mass and decreased β-cell mass. This may indicate that impaired glucose control in mice on HF/NaCl diet is in part due to reduced insulin secretion as a result of decreased β-cell mass. This would also be

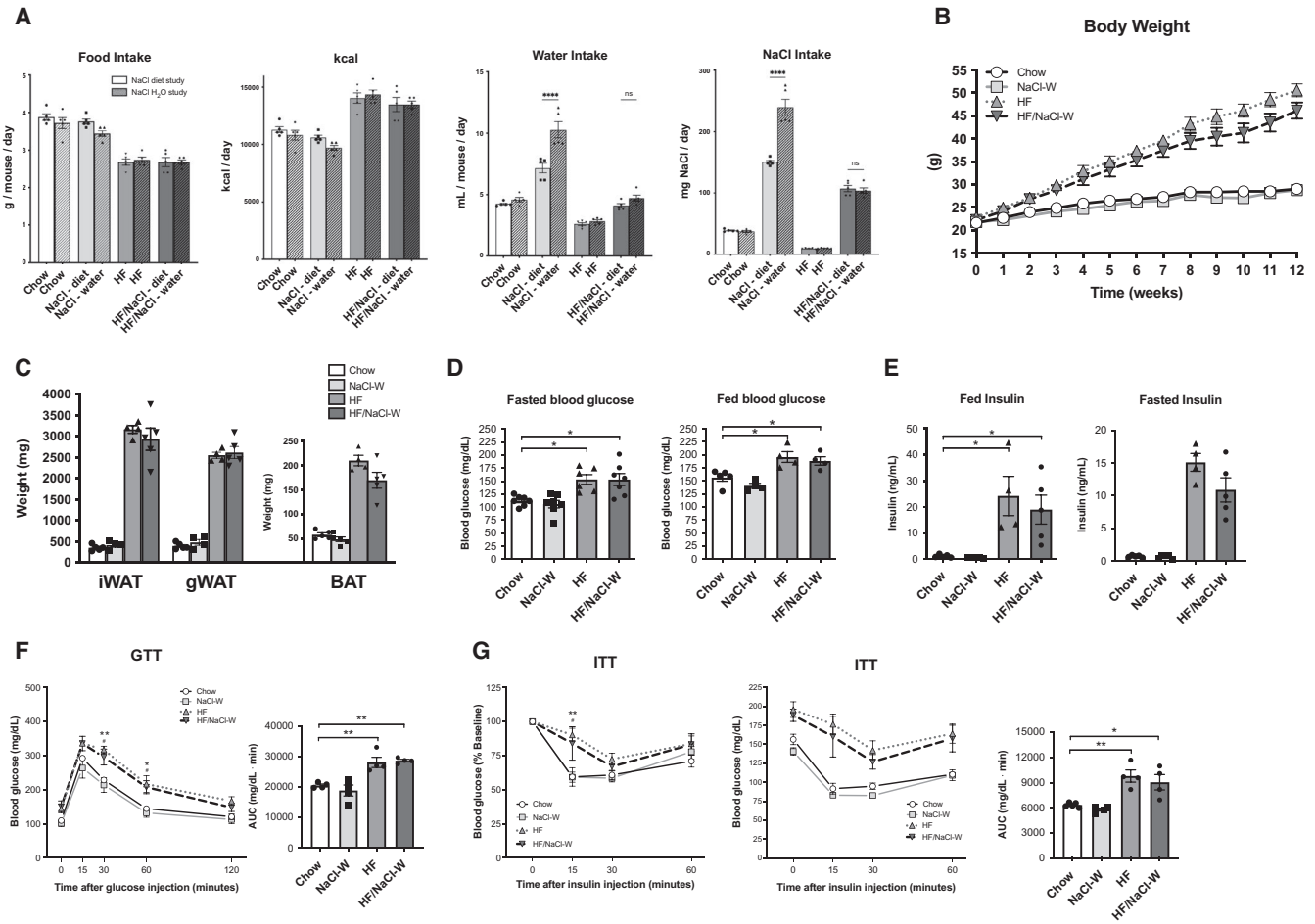

FIGURE 7 High salt administered in water does not prevent weight gain in male mice. (A) Food consumption, kilocalorie intake, water consumption, and NaCl intake. (B) Weight gain of male mice. (C) Fat depot mass at 12 weeks. (D) Fasted and fed blood glucose at 12 weeks on diet. (E) Fed and fasted insulin at 12 weeks on diet. (F) GTT at 12 weeks on diet and (G) ITT at 12 weeks on diet. Data represented as mean ± SEM. $N = 4$ to 7 per group. $^{*}p < 0.05$, $^{**}p < 0.01$. *Chow vs. HF. #Chow vs. HF/NaCl. AUC, area under the curve; BAT, brown adipose tissue; GTT, glucose tolerance test; gWAT, gonadal white adipose tissue; HF, high fat; ITT, insulin tolerance test; iWAT, inguinal white adipose tissue; NaCL, high sodium

consistent with our results showing increased glucose intolerance with apparently normal insulin sensitivity during ITT. However, the literature on the effects of high and low sodium intake on β-cell mass and insulin secretion is limited. In humans, sodium restriction has actually been shown to be associated with decreased insulin secretion (45).

The presence of glucose intolerance may also suggest that significant fat absorption is occurring and that other metabolic factors may be contributing to inhibition of weight gain. Weidemann et al. found that HF/NaCl mice had significantly decreased fat absorption using fecal steatocrit measurements, and they concluded that suppression of absorption was through RAS activation. Our early metabolic analysis at 1 week on diets using Comprehensive Lab Animal Monitoring System did indicate that HF/NaCl mice had similar energy substrate use as mice on HF diet whereby HF diet resulted in increased fatty acid oxidation and reduced glucose oxidation. Although it is possible that macronutrient composition and not quantity could account for our observed glucose intolerance, we also did not detect differences in absorption using bomb calorimetry (data not shown).

Administration of an equivalent dose of sodium in drinking water did not suppress HF-diet-induced body weight gain. HF/NaCl-W mice had elevated fasted insulin and glucose levels and also had increased GTT and ITT at similar levels as the HF diet group. If suppression of weight gain is through systemic RAS activation, it would be expected that sodium administered in water should have a similar effect. A previous report found that a concentration of NaCl diet (4.3%) similar to what we used, inhibited weight gain in rats on a normal, low-fat diet (23). Interestingly, administration of sodium in water (1%) that provided a similar sodium intake did not result in inhibition of body weight gain. Although we did not detect any differences in body weight gain when sodium was administered during low-fat chow diet, our result in HF diet administration is very similar. In this previous study, it was found that when sodium was administered in the diet versus water, rats had decreased water intake and reduced energy retention, and it was suggested that this effect is due to decreased energy efficiency as a result of changes in electrolyte concentration. However, in our study, the sodium intake and water intake

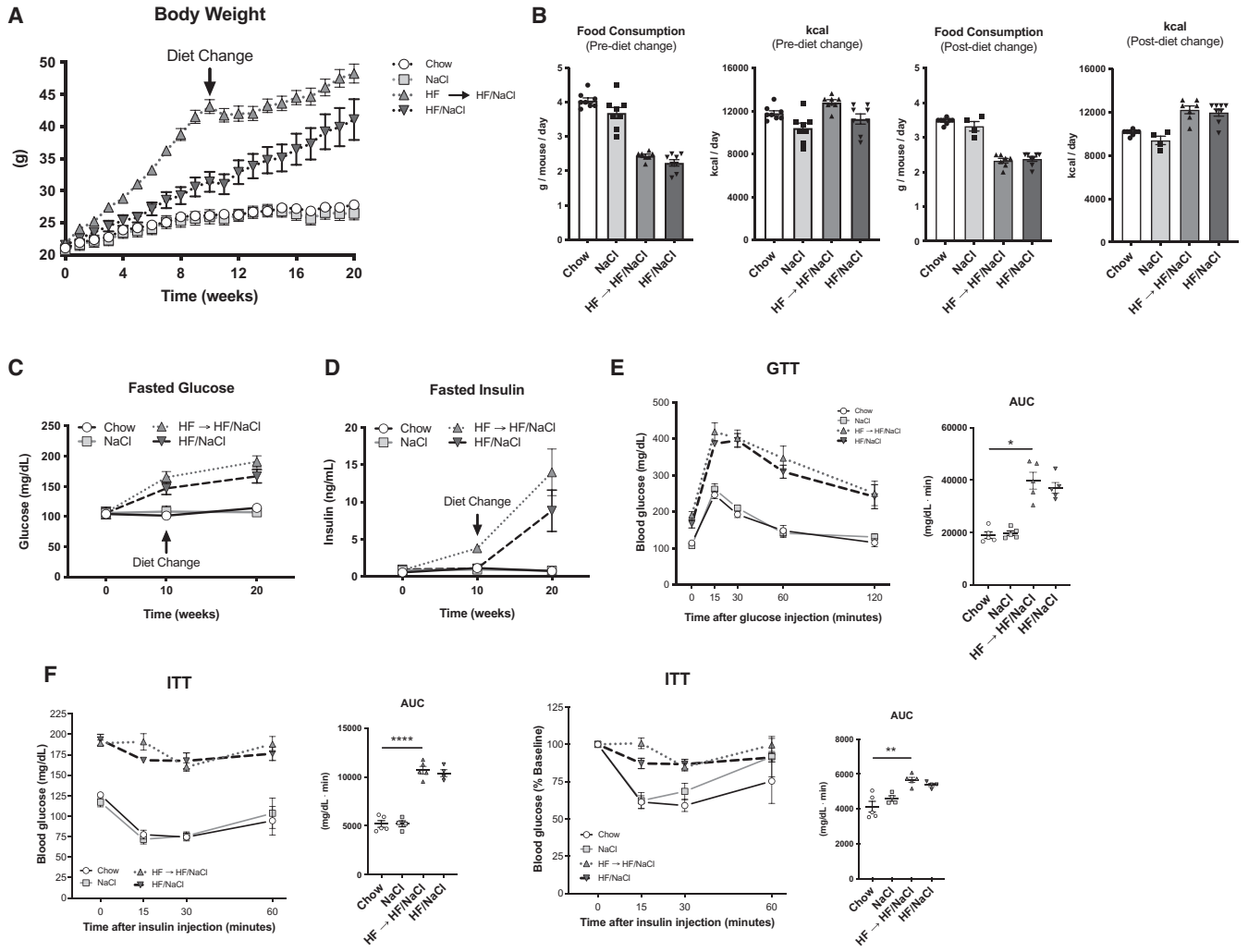

FIGURE 8 Dietary switch from HF to HF/NaCl diet does not reverse weight gain in male mice. (A) Body weight of male mice. (B) Food consumption and kilocalorie intake before and after diet switch. Time course of (C) fasted blood and (D) fasted insulin. (E) GTT and (F) ITT at 20 weeks after diet switch. Data represented as mean ± SEM. $N = 4$ to 9 per group. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{****}p < 0.0001$. AUC, area under the curve; GTT, glucose tolerance test; HF, high fat; ITT, insulin tolerance test; NaCL, high sodium

were not different whether sodium was administered in diet or water. This may suggest a different mechanism, although a more thorough analysis of calorie absorption and energy efficiency is necessary.

Although HF/NaCl mice have delayed weight gain, they did eventually begin to gain weight, which resulted in obesity and insulin resistance after 20 weeks of administration. Given that sodium has direct proinflammatory effects on immune cells like macrophages and lymphocytes, it could be hypothesized that the addition of sodium may further enhance metabolic or cardiovascular complications after mice have reached an equivalent degree of obesity. This could potentially lead to more severe metabolic and cardiovascular consequences than HF diet alone.

The suppressive effects of sodium on weight gain in rodents contrasts what has been observed in humans. In our study and most studies in the literature, high sodium has inhibitory effects on weight gain in rodents (22-25). In contrast, almost all human data has shown that sodium intake is associated with weight gain or obesity (19,20). These comparisons are difficult to interpret because the levels of sodium intake in humans may not be sufficient to achieve a similar effect. Humans consume an average of 3,600 mg of sodium (9,144 mg NaCl) per day, which is approximately 130 mg/kg. In comparison, mice on a high-NaCl diet consume approximately 100 mg of NaCl per day, which is roughly 4,000 mg/kg. These concentrations of sodium would most certainly not be palatable in most humans.

In summary, several major observations have emerged from our analysis of an HF/NaCl diet: (1) nonobese mice on HF/NaCl diet have impaired glucose control, (2) NaCl diet decreases pancreas and β-cell mass, (3) prolonged administration of HF/NaCl diet (>12 weeks) leads to obesity, (4) administration of HF/NaCl diet to obese animals does not reverse weight gain, and (5) administration of sodium in drinking water does not prevent HF-diet-induced weight gain.O

CONFLICT OF INTEREST

The authors declared no conflict of interest.

AUTHOR CONTRIBUTIONS

RAF and RMM conceived and designed the studies; RAF, CL, TMV, and JS performed the experiments and acquired the data; RAF, CNL, and RMM analyzed and interpreted the data; RAF constructed all graphs and figures and wrote the manuscript. All authors read and approved the final manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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