High salt intake causes leptin resistance and obesity in mice by stimulating endogenous fructose production and metabolism

Miguel A. Lanaspa[a,1,2], Masanari Kuwabara[a,1], Ana Andres-Hernando[a,1], Nanxing Li[a], Christina Cicerchi[a], Thomas Jensen[a], David J. Orlicky[b], Carlos A. Roncal-Jimenez[a], Takuji Ishimoto[a], Takahiko Nakagawa[a], Bernardo Rodriguez-Iturbe[c], Paul S. MacLean[d], and Richard J. Johnson[a]

[a]Division of Renal Diseases and Hypertension, University of Colorado Anschutz Medical Campus, Aurora, CO 80045; [b]Department of Pathology, University of Colorado Denver, Aurora, CO 80045; [c]Department of Medicine, Hospital Universitario, Maracaibo 4001, Zulia, Venezuela; and [d]Division of Endocrinology, Colorado Nutrition Obesity Research Center, University of Colorado Denver, Aurora, CO 80045

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Dietary guidelines for obesity typically focus on three food groups (carbohydrates, fat, and protein) and caloric restriction. Intake of noncaloric nutrients, such as salt, are rarely discussed. However, recently high salt intake has been reported to predict the development of obesity and insulin resistance. The mechanism for this effect is unknown. Here we show that high intake of salt activates the aldose reductase–fructokinase pathway in the liver and hypothalamus, leading to endogenous fructose production with the development of leptin resistance and hyperphagia that cause obesity, insulin resistance, and fatty liver. A high-salt diet was also found to predict the development of diabetes and nonalcoholic fatty liver disease in a healthy population. These studies provide insights into the pathogenesis of obesity and diabetes and raise the potential for reduction in salt intake as an additional interventional approach for reducing the risk for developing obesity and metabolic syndrome.

salt | fructose | obesity | metabolic syndrome | NAFLD

Salt (sodium chloride, NaCl) is an essential micronutrient commonly added to food to enhance taste, to preserve food, and to improve the appearance of processed foods. High intakes of salt are associated with increased risk for hypertension and for cardiovascular mortality, leading many professional societies to recommend limiting salt intake to 3.75–6 g/d. However, intake of salt is generally greater and can average >10 g/d in many populations (1).

Recently a high-salt diet has been reported to cause hyperphagia in mice and humans, but weight remains stable acutely (over 4 wk) under ad libitum dietary conditions due to a hypercatabolic state (2). When mice are switched to isocaloric diets, the mice on a high-salt diet lose weight over a 2-wk period (2).

In contrast to short-term studies, long-term intake of a high-salt diet is associated with increased frequencies of obesity, insulin resistance, nonalcoholic fatty liver disease (NAFLD), and metabolic syndrome (3–5). While these latter studies were cross-sectional in design, longitudinal studies have also reported that a high salt intake predicts development of obesity and diabetes even when total energy intake or intake of sugary beverages was controlled, and the development of diabetes was also independent of obesity or dietary factors (6). Experimentally, humans placed on a high-salt diet show reduced insulin sensitivity at 5 d (using the euglycemic insulin clamp) (7). Laboratory rats on a high salt intake also develop obesity with increased adipocyte size and leptin expression (8). Nevertheless, the mechanism(s) by which a high-salt diet may cause obesity and insulin resistance is not known.

Fructose is a component of table sugar (sucrose) and high fructose corn syrup and when administered to laboratory animals can induce the metabolic syndrome (9). While fructose exposure is usually from dietary intake, fructose is also produced endogenously from glucose via activation of the aldose reductase (AR)–sorbitol dehydrogenase (SDH) pathway. This is the only known mechanism whereby humans and most mammals generate fructose. Indeed, we reported that one of the key mechanisms by which high-glycemic diets induce NAFLD and hyperinsulinemia in mice was due to glucose-induced AR induction with the generation of endogenous fructose (10). High-salt diets, by virtue of raising serum osmolality, can also induce AR and theoretically lead to endogenous fructose generation. This led to the hypothesis that a high-salt diet may induce obesity by generating endogenous fructose (Fig. S1*A*).

Results

High Salt Intake Stimulates Endogenous Fructose Production. Our initial studies evaluated the biological effects of giving a mildly hypertonic solution of salt to mice. We first evaluated the effects of salt intake on portal vein osmolality. A 1% NaCl solution [600 μL, equivalent to 342 mOsm/kg or the content of a salty soup (11)] was administered to wild-type mice by gavage, resulting in a significant rise in portal vein osmolality (from 285 ± 8 to 332 ± 12 mOsm/kg H_2O) (Fig. 1*A*) that peaked at 15–30 min. In contrast, the gavage of regular water reduced portal vein osmolality.

Significance

High salt intake is common in Western diets and likely contributes to hypertension and cardiovascular disease. Recently high salt intake has also been found to both be associated and predict the development of obesity, insulin resistance, and metabolic syndrome. Here we show that high-salt diet activates the aldose reductase (polyol) pathway in the liver, resulting in endogenous fructose production that then induces leptin resistance and the development of metabolic syndrome and fatty liver. Blocking fructose metabolism blocks the effects of high-salt diet. High salt intake also predicts diabetes and nonalcoholic fatty liver disease in Japanese adults. Thus, high-salt diet, an essential micronutrient with no intrinsic caloric value, may have a contributory role in driving obesity and diabetes.

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Conflict of interest statement: M.A.L. and R.J.J. are members of Colorado Research Partners, LLC that is developing an inhibitor of fructose metabolism. R.J.J. is on the Scientific Board of Amway and has shares in XORT Therapeutics. R.J.J. has also written a layperson's book on sugar (*The Fat Switch*, Mercola.com, 2012).

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[1]M.A.L., M.K., and A.A.-H. contributed equally to this work.

[2]To whom correspondence should be addressed. Email: miguel.lanaspagarcia@ucdenver.edu.

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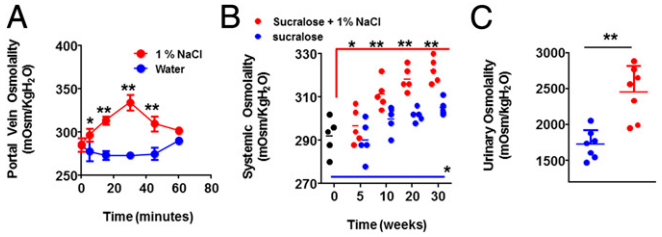

Fig. 1. Dietary salt raises portal, systemic, and urinary osmolality in mice. (*A*) Acute administration of salt results in a significant elevation of portal vein but not systemic osmolality in mice. $*P < 0.05$, $**P < 0.01$ vs. time 0 min, $n = 5$ mice per group, one-way ANOVA. (*B*) Chronic administration of 1% salt in water results in significant elevation of systemic osmolality in mice. $*P < 0.05$, $**P < 0.01$ vs. time 0 wk, $n = 5$ mice per group, one-way ANOVA. (*C*) At 30 wk, mice on salt demonstrated a significant elevation in 24-h urinary osmolality compared with control mice. $**P < 0.01$, $n = 7$ mice per group, two-tailed *t* test.

No significant changes were observed in systemic osmolality after acute gavage of the hypertonic solution or tap water (Fig. S1*B*). Thus, a 1% salt solution can induce transient hyperosmolality in the portal vein and liver without changes in serum osmolality.

Next, 1% hypertonic salt (NaCl) drinking solution or regular tap water was administered chronically (30 wk) to wild-type mice. To ensure equal volume intake between groups, the noncaloric sweetener sucralose (0.04% wt/vol) was added to the solutions to encourage fluid intake. Daily water intake was not different between groups (Fig. S1*C*). As expected, mice on salt demonstrated increased cumulative salt intake over time (Fig. S1 *D* and *E*).

Mice chronically receiving 1% hypertonic salt for 30 wk demonstrated significantly higher serum osmolality than control mice (316 ± 12 mOsm/kg H_2O vs. 302 ± 9 in water-sucralose–receiving animals) (Fig. 1*B*) and higher urinary osmolality (Fig. 1*C*). Mice receiving sucralose alone also showed mild increases in serum osmolality at the end of the 7-mo study period compared with baseline (302 ± 9 vs. 292 ± 5), which might relate to the impaired urinary concentration that occurs with aging (12).

TonEBP is a transcription factor that is induced by hypertonicity and activates AR (13). Low levels of TonEBP are constitutively expressed in mesenteric and epididymal adipose tissue, pancreas, kidney, and the hypothalamus of normal mice, consistent with the fact that multiple factors regulate TonEBP (Fig. S2) (14). Mice drinking the 1% hypertonic salt solution chronically showed increased TonEBP activation in the liver compared with control mice (Fig. 2 *A* and *B*), with increased nuclear localization as well as mRNA expression of established TonEBP target genes including AR, heat shock protein 70 (Hsp70), and the betaine-GABA transporter (BGT1) (Fig. 2*C* and Fig. S2 *A* and *B*).

AR is a rate-limiting enzyme of the polyol pathway (10). Consistently, salt-treated wild-type mice had significantly greater AR protein expression and activity and hepatic sorbitol accumulation in liver and peripheral tissues (Fig. S2) than sucralose-treated wild-type mice. Liver and serum fructose were also significantly greater in salt-treated wild-type mice compared with sucralose alone-treated animals (Fig. 2 *D*–*G* and Table S1). We also measured plasma cortisol but found no differences in obese salt-treated mice compared with controls (103 ± 16 vs. 116 ± 23 ng/mL).

The functional role of the AR pathway as a mechanism for inducing triglyceride accumulation was explored. In human HepG2 cells, forced expression of AR increased glucose-dependent fructose and triglyceride accumulation, while silencing AR prevented fat accumulation (Fig. S3). Thus, AR-induced fructose generation results in triglyceride accumulation in liver cells.

High-Salt Diet Induces Metabolic Syndrome in Wild-Type but Not Fructokinase-Deficient Mice. To test whether the endogenous fructose generated by a high-salt diet might have a role in obesity, we administered the high-salt diet to wild-type mice or mice that could not metabolize fructose via fructokinase (KHK), the primary enzyme that metabolizes fructose [fructokinase-deficient (FKA-A/C KO) mice]. Both groups were given drinking water containing 1% hypertonic salt solution or water for 30 wk; all groups received 0.04% (wt/vol) sucralose in the drinking water to ensure minimal differences in water intake between groups (Fig. S4*A*).

Cumulative salt intake did not differ between wild-type and FKA-A/C KO mice. Similarly, systemic osmolality, urinary osmolality, and AR activity at 30 wk were not different between wild-type and FKA-A/C KO mice (Fig. S4 *B*–*E*). In contrast, intrahepatic accumulation of sorbitol and fructose as well as serum and urinary fructose levels were significantly higher in FKA-A/C KO mice than in wild-type animals (Fig. S4 *F*–*H*) as a consequence of decreased fructose metabolism.

As reported by others (15), a high-salt diet resulted in hyperphagia with increased total energy intake that occurred within the first weeks of the high-salt diet. Initially body weight was not different between wild-type mice on a high-salt diet and those on a control diet, consistent with a catabolic state as reported by Kitada et al. (2). However, significant differences in body weight gain between wild-type mice on a high-salt diet and the rest of the groups became apparent by week 13. After several months the wild-type mice on a high-salt diet showed marked weight gain compared with both low-salt-diet controls and FKA-A/C KO mice on salt or water + sucralose (Fig. 3*A*). At this time point, a significant correlation existed between salt intake and total caloric intake in individual wild-type mice, but not FKA-A/C KO mice, on salt (Fig. 4*C*).

Wild-type animals on salt developed features of metabolic syndrome including fatty liver, transaminitis, insulin resistance, elevated blood pressure, and obesity (Figs. 3 *B*–*G* and 4 *D*–*G* and Table S1). In contrast, the salt-fed mice that were unable to metabolize fructose (FKA-A/C KO mice) were protected from obesity, and analysis of their livers showed less triglyceride

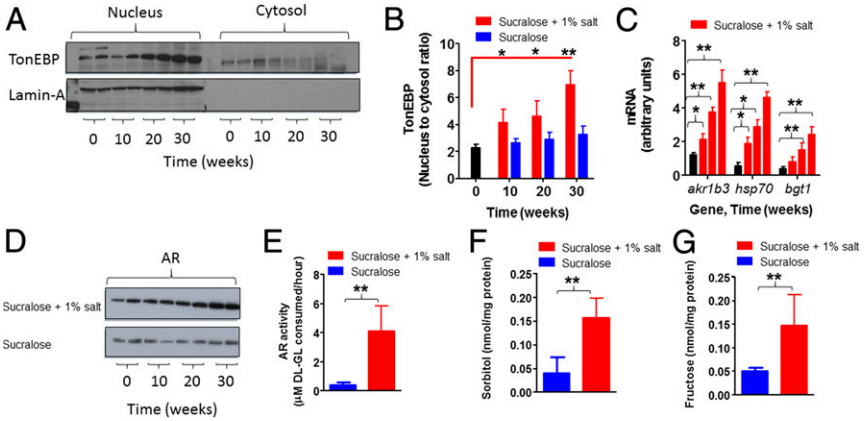

Fig. 2. The polyol pathway is activated in the liver of mice chronically exposed to salt. (*A* and *B*) TonEBP expression (*A*) and quantitation (*B*) in nuclear extracts of mice exposed to 1% NaCl in water at baseline (0) and at 10, 20, and 30 wk. $*P < 0.05$, $**P < 0.01$ vs. time 0 wk, $n = 5$ mice per group, one-way ANOVA. (*C*) mRNA expression of TonEBP target genes AR (*akr1b3*), *hsp70*, and *bgt1* in the liver. $*P < 0.05$, $**P < 0.01$ vs. time 0 wk, $n = 5$ mice per group, one-way ANOVA. (*D*–*G*) Hepatic AR expression (*D*) and AR activity (*E*) and sorbitol (*F*) and fructose (*G*) levels in the same groups. $*P < 0.05$, $**P < 0.01$, $n = 7$ mice per group, two-tailed *t* test.

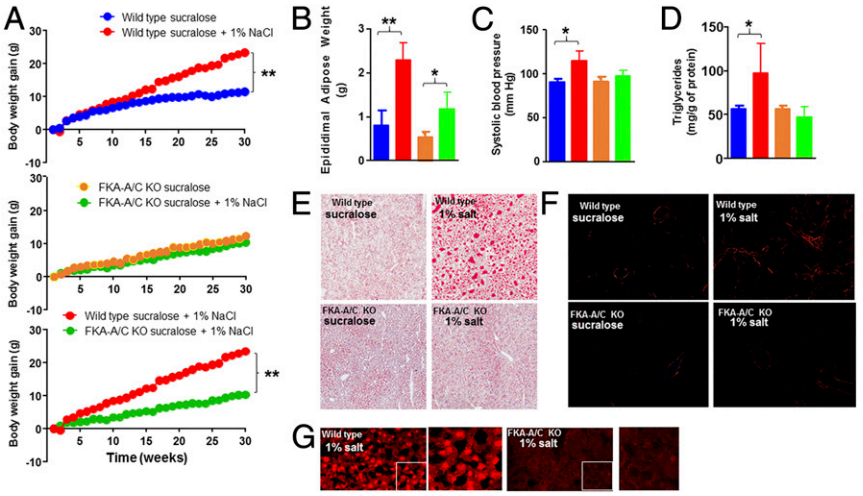

Fig. 3. FKA-A/C KO is associated with improved metabolic syndrome induced by salt. (*A*) Body weight gain in wild-type and FKA-A/C KO mice exposed to 0.04% sucralose (control) or sucralose + 1% NaCl ($n = 7$ mice per group, one-way ANOVA). (*B–D*) Epididymal fat weight (*B*), systolic BP (*C*), and liver triglycerides (*D*) in the same groups at week 30. $^{*}P < 0.05$, $^{**}P < 0.01$, $n = 7$ mice per group, one-way ANOVA. (*E–G*) Oil-Red O (*E*, 40×) and PicroSirius Red (*F*, 40×) staining under polarized light demonstrating collagen fibers and dihydroethidium (superoxide) staining (*G*, 60×) in livers of the same animal groups at 30 wk.

accumulation and lower levels of the purine by-products (inosine and uric acid) that are generated during fructose metabolism. Thus, FKA-A/C KO mice were protected from developing metabolic syndrome despite equivalent salt intake.

While FKA-A/C KO mice on a high-salt diet were protected from obesity and metabolic syndrome, there were slightly higher areas under the curve (AUC) for oral glucose (OGTT) and insulin (ITT) tolerance tests (Fig. 4*G*) compared with FKA-A/C KO mice on sucralose alone, suggesting a fructokinase-independent component in salt-induced insulin resistance. In addition, while there was significantly lower epididymal adipose accumulation in FKA-A/C KO mice on salt compared with wild-type animals (Fig. 3*B*), both groups showed similar relative increases in fat gain (2.82-fold in wild-type vs. 2.18-fold in FKA-A/C KO mice) suggestive of a fructokinase-independent pathway whereby salt promotes fat accumulation. Thus, FKA-A/C KO was largely but not completely protective against the metabolic effects of a high-salt diet.

Sucralose Alone Does Not Induce Features of Metabolic Syndrome. We also performed separate studies to investigate the effects of sucralose on metabolic syndrome, given that artificial sugars, and especially saccharin, have been reported to induce glucose intolerance in mice (16). However, in our hands, exposure of animals to sucralose in the drinking water for 30 wk did not induce features of metabolic syndrome, including body weight, food intake, epididymal fat weight, glucose and insulin levels, leptin levels, and other parameters, compared with mice drinking regular tap water (Table S1).

High Salt Intake Enhances Fructose-Induced Metabolic Syndrome. To better understand the interplay between fructose and salt, we also compared wild-type mice that were given fructose (15% wt/vol) in the drinking water in the presence or absence of 1% salt (NaCl). We found that the addition of salt to fructose in the drinking water significantly accelerated the development of metabolic syndrome (Fig. S5).

Fructose-Dependent Leptin Resistance as a Mechanism for Salt-Induced Obesity. We next evaluated why FKA-A/C KO mice on a high-salt diet were protected from weight gain. One potential mechanism could be the loss of fructose in the urine, as the lack of fructokinase results in enhanced fructosuria (17). Indeed, FKA-A/C KO mice on a high-salt diet had greater fructosuria than wild-type mice on salt, but the amount of fructose expelled in the urine accounted for less than 5% of the total caloric intake (Fig. 3*A* and Fig. S4*I*).

In contrast, FKA-A/C KO mice on a high-salt diet ingested 40–50% less energy than wild-type mice on a high-salt diet (Fig. 4). Thus, the primary mechanism accounting for differences in weight gain was the difference in energy intake. Based on previous studies linking salt intake with leptin expression and activation of TonEBP target genes such as Hsp70 (18), we analyzed leptin expression and leptin response to diet.

Plasma leptin levels were significantly higher in wild-type mice on salt compared with the rest of the groups when mice were killed (24.3 ± 5.8 vs. an average of 6.3 ± 2.3 ng/mL for the rest of the groups) (Fig. 5*A*). Adipose leptin mRNA levels were also

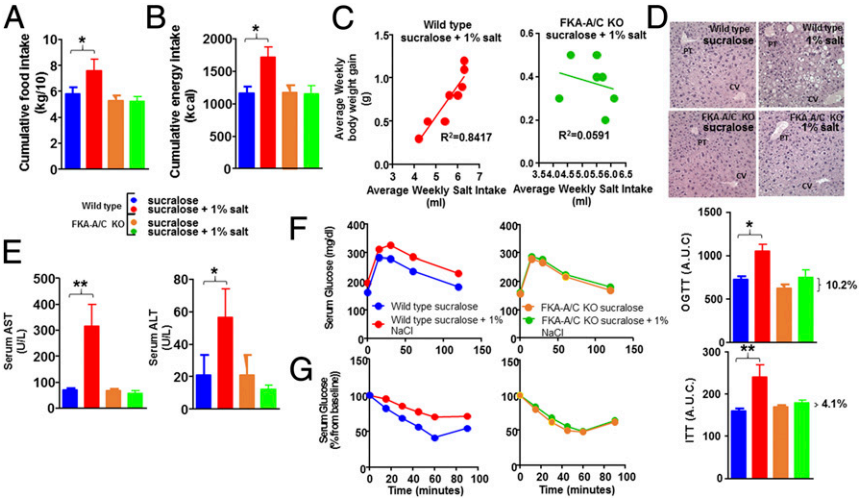

Fig. 4. Increased salt intake is associated with metabolic syndrome in wild-type but not FKA-A/C KO mice. (*A* and *B*) Total cumulative food (*A*) and caloric (*B*) intake in wild-type and FKA-A/C KO mice exposed to 0.04% sucralose (control) or sucralose + 1% NaCl in water for 30 wk. $^{*}P < 0.05$, $n = 7$ mice per group, one-way ANOVA. (*C*) Linear correlation between salt intake and body weight gain in wild-type (*Left*) and FKA-A/C KO (*Right*) mice ($n = 7$ mice per group). (*D* and *E*) Liver H&E staining (*D*) and serum transaminases (*E*) in wild-type and FKA-A/C KO mice as in *C*. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. wild-type sucralose, $n = 7$ mice per group, one-way ANOVA. (*F*) Serum glucose and AUC in wild-type and FKA-A/C KO mice as in *C* during an OGTT. $^{*}P < 0.05$, $n = 4$ mice per group, one-way ANOVA. (*G*) Serum glucose and AUC in wild-type and FKA-A/C KO mice as in *C* during an ITT. $^{**}P < 0.01$, $n = 4$ mice per group, one-way ANOVA.

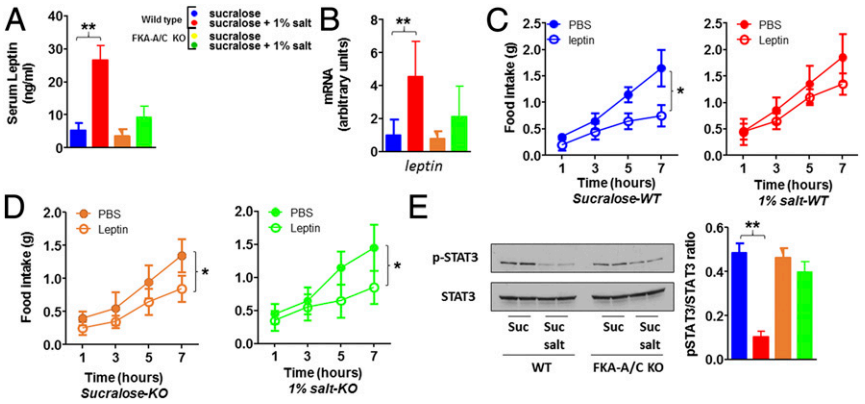

Fig. 5. Increased salt intake is associated with hyperleptinemia and reduced hypothalamic leptin sensitivity in wild-type but not FKA-A/C KO mice. (*A*) Fasting serum leptin in wild-type and FKA-A/C KO mice exposed to 0.04% sucralose (control) or sucrose + 1% NaCl in the drinking water for 30 wk. $^{**}P < 0.01$, $n = 7$ mice per group, one-way ANOVA. (*B*) Adipose *leptin* mRNA levels in the same groups as in *A*. $^{**}P < 0.01$, $n = 7$ mice per group, one-way ANOVA. (*C* and *D*) Cumulative food intake in wild-type (*C*) and FKA-A/C KO (*D*) mice exposed to 0.04% sucralose (control) or sucrose + 1% NaCl in the drinking water for 30 wk and injected with either PBS or leptin. $^{*}P < 0.05$, $n = 4$ mice per group, two-way *t* test. (*E*) Western blot and quantitation for total and phosphorylated STAT3 in the hypothalamus of wild-type and FKA-A/C KO mice exposed to sucralose or sucralose + 1% NaCl 1 h after i.v. leptin injection.

higher in wild-type mice on salt compared with the other groups, indicating increased transcriptional activity (Fig. 5*B*).

To determine if the increased leptin was mediated by endogenous fructose, differentiated murine 3T3-L1 adipocytes were placed under hypertonic conditions (350 mOsm/kg H_2O), resulting in significantly increased accumulation of sorbitol and fructose and leptin mRNA levels (Fig. S6 *A–D*). The increased transcriptional leptin activity by hypertonicity was dependent on both Na^+ and Cl^-, as substitution by choline or acetate did not significantly reduced leptin transactivation in differentiated 3T3-L1 cells (Fig. S6*B*). The increased transcriptional leptin activity was also dependent on endogenous fructose metabolism, as leptin up-regulation was less in FKA-A/C KO 3T3-L1 cells exposed to high salt (Fig. S6*E*).

To determine if the wild-type mice on salt had impaired leptin sensitivity, leptin was injected (150 μg/kg i.p.) at 12 wk after the high-salt diet was initiated (i.e., before the onset of metabolic syndrome) and also at 25 wk (after obesity developed). Total food intake was monitored for 7 h. At both 12 and 25 wk, leptin injection caused significant reduction in 7-h food consumption in control mice and FKA-A/C KO mice on salt compared with vehicle-injected mice. In contrast, wild-type animals on salt did not significantly reduce food intake compared with vehicle-injected mice (Fig. 5 and Fig. S7). Furthermore, when body weight was normalized in a subset of animals to the same body weight at week 12 (32.1 ± 0.5 in wild-type vs. 32.2 ± 0.7 g in FKA-A/C KO mice, $P = 0.85$), the wild-type mice, but not FKA-A/C KO animals, on a high-salt diet showed reduced leptin sensitivity (1.1% reduction in food intake in wild-type mice vs. 41.2% reduction in food intake in FKA-A/C KO mice post leptin injection, $P < 0.01$).

STAT3 phosphorylation, a marker of JAK-STAT–dependent leptin signaling, was evaluated in the hypothalamus 30 min after injection in the set of mice at 25 wk and was significantly down-regulated in wild-type mice on salt compared with the other groups (Fig. 5*E*). These data document reduced hypothalamic leptin sensitivity in wild-type mice on a high-salt diet.

High Salt Intake Exacerbates Western Diet-Induced Metabolic Syndrome. To evaluate the effects of salt intake on metabolic syndrome induced by a Western diet, wild-type and FKA-A/C KO mice received a Western diet (high fat and high sucrose) containing either modest [0.25%, which corresponds to an average daily salt intake of 6.8 g in an individual with mean food intake of 2,500 g (19)] or low salt (0.1%, which corresponds to the American Heart Association recommended daily salt intake of 3 g in an individual with average food intake) with ad libitum access to water. Similar our observations in mice exposed to 1% salt in the drinking water, wild-type mice on 0.25% salt developed hyperleptinemia and increased food and total caloric intake as well as body weight gain compared with wild-type mice fed a low-salt diet (Fig. 6 *A–D*). Furthermore, hepatic AR expression and sorbitol and fructose levels as well as serum levels of the liver-injury markers [alanine aminotransferase (ALT) and aspartate aminotransferase (AST)] were significantly higher in animals on the 0.25% salt diet compared with mice on a low-salt diet (Fig. 6 *E–I* and Fig. S8). In contrast, FKA-A/C KO mice were completely protected from the effects of the higher-salt diet to stimulate energy intake and weight gain.

High Salt Intake Predicts Diabetes and NAFLD. To determine if a high salt intake predicts diabetes in humans, we evaluated medical records and questionnaires from 13,000 healthy adults undergoing an annual medical examination at St. Luke's International Hospital Center for Preventive Medicine in Tokyo, Japan in 2004 for whom follow-up data were available in 2009 (Table S2) (details of this cohort are described in ref. 20). Risk factors for developing diabetes identified by single-regression analysis included older age, male gender, higher body mass index (BMI), smoking habit, hypertension, dyslipidemia, hyperuricemia, chronic kidney disease, high total caloric intake, and high salt intake (Table S3). Multivariable adjustment identified the following risk factors for developing diabetes: older age [odds ratio (OR): 1.045 per 1 y increased, 95% CI: 1.033–1.057], higher BMI (OR: 1.182 per 1 kg/m^2 increased, 95% CI: 1.140–1.227), smoking habit (OR: 1.338, 95% CI: 1.031–1.738), dyslipidemia (OR: 1.451, 95% CI: 1.142–1.843), and high salt intake of >11 g/d (OR: 1.330 per 1 mOsm/kg increased, 95% CI: 1.023–1.730). There were no significance differences in gender, hypertension, hyperuricemia, chronic kidney disease, and total caloric intake after multiple adjustments (Table S3, Adjusted columns).

Total calorie and salt intake were correlated ($r = 0.52$, $P < 0.001$) (Fig. 7*A* and Fig. S9). We then compared the prevalence of diabetes in relation to total calorie and salt intake. Dichotomous variables were used to assess salt intake [based on 11 g/d as the mean intake in the Japanese population (21)] and total calorie intake (using a cutoff of 2,150 calories/d, the mean intake in this study). The group with high calorie and high salt intake had a significantly higher cumulative incidence of diabetes compared with subjects in the high-calorie and low-salt-intake group ($P = 0.005$, logistic regression adjusted with Tukey's post hoc test) (Fig. 7*B*).

Furthermore, we analyzed the cumulative incidence of NAFLD over 5 y and NAFLD score between sodium intake ≤11 and >11 g/d. The results showed that the cumulative incidence of NAFLD (greater than −0.675) over 5 y is higher in the high-salt-intake group than in the low-salt-intake group [22.3% (167/7,486) vs. 15.5% (75/4,839), $P = 0.008$] (Fig. 7*C*). The high-salt-intake group was associated with a significantly higher NAFLD score than the low-salt-intake group (−2.0793 vs. −2.2124, $P < 0.001$) (Fig. 7*C*). Moreover, the change in NAFLD score over 5 y was also significantly higher in the high-salt-intake group than in the low-salt-intake group (0.359 ± 0.578 vs. 0.324 ± 0.565, $P < 0.001$).

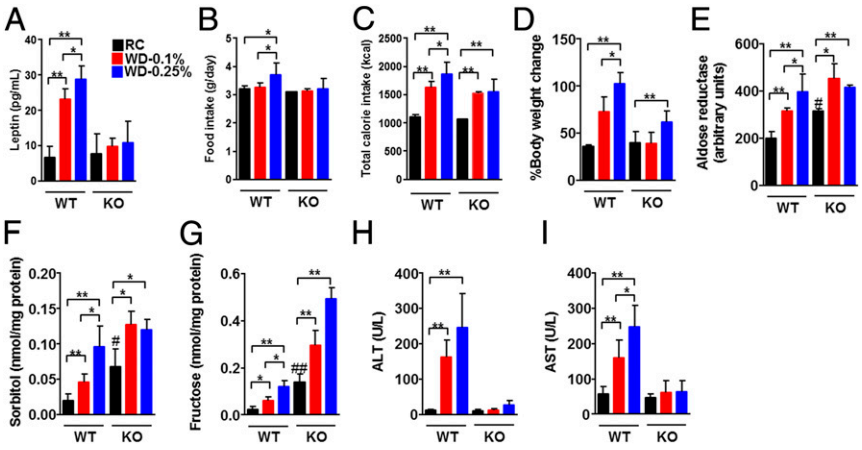

Fig. 6. Increased salt intake in the chow is associated with hyperleptinemia, hyperphagia, and increased body weight gain in Western diet-fed wild-type but not FKA-A/C KO mice. (*A*) Fasting serum leptin in wild-type and FKA-A/C KO mice exposed to the control diet (RC) or a Western diet (WD) containing either 0.1% or 0.25% salt. $*P < 0.05$, $**P < 0.01$, $n > 5$ mice per group, one-way ANOVA. (*B–I*) Daily food intake (*B*), total caloric intake (*C*), increased body weight gain (*D*), AR hepatic expression (*E*), hepatic sorbitol (*F*), hepatic fructose (*G*), serum ALT (*H*), and serum AST (*I*) in the same animal groups as in *A*. $*P < 0.05$, $**P < 0.01$, $\#P < 0.05$, $\#\#P < 0.01$ vs. wild-type mice on regular chow, $n = 6$ mice per group, one-way ANOVA.

Discussion

Salt is an essential micronutrient with no caloric value, but recently it has been associated with the development of obesity and diabetes. First, salt intake has increased in parallel with increasing rates of obesity (22). Second, high salt intake also is associated with increased frequency of obesity, insulin resistance, metabolic syndrome, and NAFLD (23), and subjects with obesity have higher serum osmolality than lean subjects (24). Third, high salt intake independently predicts the development of obesity and diabetes in longitudinal studies (6). Serum copeptin (a biomarker of vasopressin) is induced by increased serum osmolality, and elevated copeptin levels both predict and are elevated in subjects with metabolic syndrome (25, 26). Experimentally, a high-salt diet rapidly induces reduced insulin sensitivity in humans (7). Furthermore, a randomized clinical study has reported that a low-salt diet causes weight loss in hypertensive subjects (−2.1 kg in the low-salt group vs. +0.3 kg in control group, $P < 0.001$) (27). Likewise, increasing water intake has also been reported to reduce total energy intake and cause weight loss (28).

Here we investigate potential mechanisms by which salt may cause obesity and metabolic syndrome. Similar to recent studies, we found that a high-salt diet caused hyperphagia in mice. Acutely, weight gain did not increase, consistent with a hypercatabolic state, but after a few months progressive weight gain and metabolic syndrome developed. Investigations showed that high salt intake led to an increase in osmolality in the liver, resulting in the induction of TonEBP, activation of AR, and generation of endogenous fructose. These studies were confirmed in cell culture, which also showed that the AR response drove intracellular triglyceride accumulation. Next, we showed that mice lacking the ability to metabolize fructose (FKA-A/C KO mice) were protected from salt-induced metabolic syndrome despite similar salt intake. These studies identified endogenous fructose production and metabolism as the primary mechanism by which salt induces obesity.

The primary experiments utilized salt in the drinking water at an osmolality just slightly higher than serum, as this allowed a simple way to directly test the hypothesis that mild hyperosmolality might induce obesity. However, we also performed studies in which salt intake was adjusted in the chow to mimic the human condition. Again, the observation that increasing the salt content resulted in greater weight gain in the wild-type mouse on a Western diet but not in a mouse that cannot metabolize fructose provided evidence that salt may drive obesity and NAFLD. The clinical study also found that high salt intake increased the risk for diabetes independent of energy intake.

Finally, we addressed the mechanism for salt-induced hyperphagia, and we found that high salt intake was associated with elevated leptin levels and with leptin resistance as noted by leptin injection studies. Importantly, these studies showed that leptin resistance preceded weight gain.

A limitation of our study was that we used a genetically modified (fructokinase knockout) mouse to show a functional role of fructokinase in high-salt-mediated obesity. However, these mice show normal activity and development and have a normal life span (29). They are also protected against fructose-induced obesity (9) but not against obesity driven by a high-fat diet (30), which supports the finding that high-salt diets may induce obesity via endogenous fructose production. A second limitation is that we encouraged high salt intake by including sucralose in the drinking water, and there is a study that suggests artificial sweeteners may cause insulin resistance and possibly weight gain via effects on the microbiome (16). However, we extensively studied sucralose and found that it did not induce any features of metabolic syndrome (Table S1). Finally, there are studies in mice and rats that suggest that high-salt diets may block high-fat-diet–induced obesity either

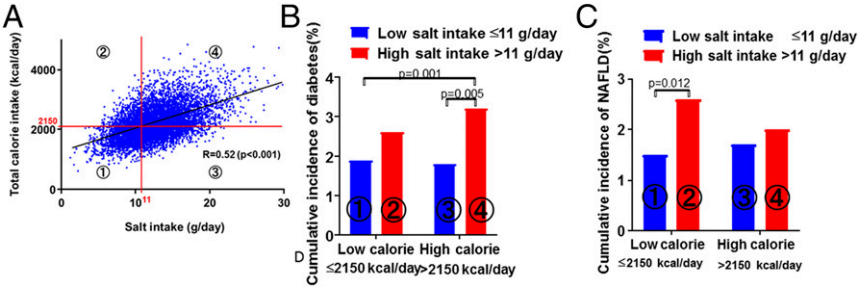

Fig. 7. High salt intake positively correlates with increased BMI and development of type 2 diabetes. (*A*) Significant positive correlation between total calories and salt intake by Pearson's correlation coefficient ($R = 0.52$, $P < 0.001$). The vertical red line shows the cutoff level of 11 g/d salt intake; the horizontal red line shows the cutoff level of 2,150 kcal/d. (*B*) Prevalence of developing diabetes mellitus associated with combined total calories and salt intake. The high-calories/high-salt-intake group demonstrated significantly higher prevalence for developing diabetes compared with the low-calories/low-salt-intake group ($P = 0.001$) and the high-calories/low salt-intake-group ($P = 0.005$) by using a logistic regression model adjusted with Tukey's post hoc test. (*C*) Prevalence for developing NAFLD associated with combined total calories and salt intake. The high-salt-intake group demonstrated a significantly higher prevalence for developing NAFLD compared with the low-salt-intake group in the setting of low caloric intake ($P = 0.012$), and the high-caloric-intake group showed a tendency for developing NAFLD. Groups were compared by using a logistic regression model adjusted with Tukey's post hoc test.

by impairing fat absorption or by an unknown mechanisms (31). These differences may relate in part to the fact that the typical high-fat diet and high-salt diet used in mice are relatively extreme compared with the human diet and with the diets we used. In addition, we found that the effect of the high-salt diet in increasing weight was delayed and did not manifest until mice had been on the diet for 4 mo or longer. Finally, our clinical study is a retrospective single-center study, and it may not be generalizable to other populations. Moreover, this study population is a generally healthy population, because all the subjects came to our center independently for their medical check-up rather than being referred for an existing medical condition. Further multicenter studies across the world involving groups with different salt intake should be studied to confirm our findings.

In summary, our studies provide a mechanism to explain why high-salt diets are associated with obesity and provide a framework for future investigation of the role of osmolality in weight gain. Importantly, we hope that providing a scientific basis for how salt and water may regulate food intake will lead to clinical trials that can test these simple interventions in humans. Finally, these studies emphasize how noncaloric substances can influence the risk for obesity and diabetes, thereby emphasizing that factors other than altering the protein, fat, and carbohydrate content of food or the counting of calories should be considered in promoting weight loss and preventing metabolic consequences.

Materials and Methods

More detailed materials and methodology are included in *Supporting Information*.

Animal Studies. All experiments were conducted with adherence to the NIH *Guide for the Care and Use of Laboratory Animals* (32). The animal protocol was approved by the Institutional Animal Care and Use Committee of the University of Colorado.

Fructokinase-knockout (KHK-A/C KO, C57BL/6 background) mice and wild-type litter mates (male, 8 wk old) were provided water containing 15% fructose, 0.04% sucralose, 0.04% sucralose and 1% NaCl, or tap water for 30 wk. Body weight was measured every week, and energy intake was measured two times per week.

Insulin sensitivity was determined by OGTT and ITT as previously described (10).

Low-salt (0.1% TD.150613) and medium-salt (0.25% TD.88137) Western diets were purchased from Envigo.

Human Studies. We adhered to the principles of the Declaration of Helsinki. All data were collected and compiled in a protected computer database. Individual data were anonymous without identifiable personal information. Informed consent was obtained from all subjects by a comprehensive agreement method provided by St. Luke's International Hospital. St. Luke's International Hospital Ethics Committee approved the protocol for this study. This was a large-scale, single-center, retrospective cohort study (20) based on medical records and questionnaires collected in healthy adults undergoing an annual medical examination at St. Luke's International Hospital Center for Preventive Medicine, Tokyo in 2004 with follow-up in 2009.

The study consisted of 13,070 subjects who were between 30 and 85 y old and for whom records were available in 2004 and at follow-up in 2009.

Dichotomous variables were used to assess salt intake [based on 11 g/d as the mean intake in the Japanese population (21)] and total calorie intake (using a cutoff of 2,150 calories/d based on that being the mean intake in this study). Diabetes was defined as a current history of diabetes mellitus and/or hemoglobin A1c (HbA1c) of ≥6.5%. A NAFLD score was calculated using a previously accepted formula (33). Hypertension was defined as the current use of medication for hypertension and/or systolic blood pressure (BP) of ≥140 mmHg and/or diastolic BP of ≥90 mmHg.

Statistical Analysis. All data are presented as the mean ± SEM. Independent replicates for each data point (n) are identified in figure legends. Data without indications were analyzed by two-tailed t test, one- or two-way ANOVA, and Tukey's post hoc test. $P < 0.05$ was regarded as statistically significant and as ensuring similar data variance between the groups being analyzed.

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