Overnutrition Stimulates Intestinal Epithelium Proliferation Through β-Catenin Signaling in Obese Mice

Jiaming Mao,1 Xiaomin Hu,1 Yao Xiao,1 Chao Yang,1 Yi Ding,1 Ning Hou,1 Jue Wang,1 Heping Cheng,1,2 and Xiuqin Zhang1

Obesity is a major risk factor for type 2 diabetes and cardiovascular diseases. And overnutrition is a leading cause of obesity. After most nutrients are ingested, they are absorbed in the small intestine. Signals from β-catenin are essential to maintain development of the small intestine and homeostasis. In this study, we used a hyperphagic db/db obese mouse model and a high-fat diet (HFD)-induced obesity mouse model to investigate the effects of overnutrition on intestinal function and β-catenin signaling. The β-catenin protein was upregulated along with inactivation of glycogen synthase kinase (GSK)-3β in the intestines of both db/db and HFD mice. Proliferation of intestinal epithelial stem cells, villi length, nutrient absorption, and body weight also increased in both models. These changes were reversed by caloric restriction in db/db mice and by β-catenin inhibitor JW55 (a small molecule that increases β-catenin degradation) in HFD mice. Parallel, in vitro experiments showed that β-catenin accumulation and cell proliferation stimulated by glucose were blocked by the β-catenin inhibitor FH535. And the GSK-3 inhibitor CHIR98014 in an intestinal epithelial cell line increased β-catenin accumulation and cyclin D1 expression. These results suggested that, besides contribution to intestinal development and homeostasis, GSK-3β/β-catenin signaling plays a central role in intestinal morphological and functional changes in response to overnutrition. Manipulating the GSK-3β/β-catenin signaling pathway in intestinal epithelium might become a therapeutic intervention for obesity induced by overnutrition. *Diabetes* 62:3736–3746, 2013

---

Obesity, affecting ~30% of the world population, is a major risk factor for metabolic syndrome, inflammation, type 2 diabetes (T2D), and cardiovascular diseases (1). Epidemiological evidence suggests that body weight is regulated by complex physiological mechanisms (2,3). However, environmental factors, especially when the energy intake from food exceeds normal physiological needs, are considered to be culprits for becoming overweight and then obese. Conversely, caloric restriction (CR) significantly reduces obesity and incidences of T2D and cardiovascular disease in rodents, primates, and humans (4–6).

Appetite and food intake are a complex physiologic process. Regulation of appetite involves numerous hormones and signals, and defects of these appetite-related molecules and related signaling pathways cause severe obesity (7–9). These findings strongly suggest a prominent role for excess food intake and an oversupply of nutrients in obesity and related diseases. Studies show that high-fat diet (HFD) could induce intestinal epithelial proliferation, absorption, and adiposity (10–12). However, the underlying mechanisms remain poorly understood.

The internal surface of the mammalian intestine is covered by a single layer of epithelial cells that protrude into the intestinal lumen to form finger-like villi that absorb nutrients from food. This single layer of cells is renewed every 3–5 days. Besides these villi, other specialized structures have evolved in the intestinal epithelium, termed crypts, which contain multipotent stem cells and are responsible for intestinal epithelial cell renewal. This cell-renewal process is strictly controlled through a series of coordinated signaling pathways (13,14).

In mammals, the canonical Wnt signaling pathway is essential for maintaining intestinal crypt cell proliferation during development and for intestinal epithelium homeostasis during adulthood (14–16). As a core effector of the Wnt signaling pathway, β-catenin is regulated mainly at the protein level by a proteolytic degradation complex that consists of adenomatous polyposis coli, casein kinase I, glycogen synthase kinase (GSK)-3β, and axin. When the complex is assembled, the GSK-3β will effectively phosphorylate β-catenin, leading to β-catenin protease hydrolysis (17). However, GSK-3β is inactivated by phosphorylation at Ser37/38, leading to cytoplasmic β-catenin accumulation and nuclear translocation, resulting in an increase of β-catenin target gene, such as cyclin D1 expression and cell proliferation (18).

To better understand whether and how small intestine homeostasis is involved in its morphological and functional changes induced by excess food intake and HFD, we used a hyperphagic db/db obese mouse model and a model of obesity induced by HFD to investigate the changes in absorptive surface area and related signaling in the small intestine during the occurrence of obesity. We found that intestinal epithelial cell proliferation induced by excess food intake was correlated with activation of the GSK-3β/β-catenin signaling pathway, suggesting that nutrient-induced activation of GSK-3β/β-catenin signaling in the intestinal epithelium may contribute to increased nutrient absorption and obesity development.

---

RESEARCH DESIGN AND METHODS

Male and female db/+ mice of a hyperphagic db/db obese mouse model, obtained from The Jackson Laboratory (Bar Harbor, ME), were mated to generate db/db mice. Mice were fed with a standard chow diet containing 4% fat and 50% carbohydrate. Age-matched male ad libitum db/+ and db/db mice were used for further studies (n = 8 per group). For embryonic intestine sample collection, the pregnant female db/+ mice were killed at the time that embryos became 18.5 days old.

In the CR group, 4-week-old male db/db mice (n = 5) were housed in individual cages and fed twice daily with a restricted amount (60% of ad libitum) of the standard chow diet; these mice were killed after 4 weeks of CR, and intestinal samples were harvested for further studies.
For the HFD-induced obesity model, at the age of 4 weeks, male C57BL/6 mice were fed an HFD (cat. no. D12492; Research Diets, New Brunswick, NJ), containing 55 kcal/kg fat, 10 kcal/kg protein, and 10 kcal/kg carbohydrate. The mice were housed with free access to water, and body weight was measured weekly or twice a week. The current study was approved by the Institutional Animal Care and Use Committee, and all experiments were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee for the care and use of laboratory animals.

Ringer buffer (in mM: NaCl 115, NaHCO3 25, MgCl2 1.2, CaCl2 1.2, K2HPO4 2.4, and KH2PO4 0.4, pH 7.3) was supplemented with phenylmethylsulfonyl fluoride. Small intestines were dissected, and the jejunal mucosa was minced in liquid nitrogen for isolation of total RNA and protein. The entire intestine was removed, weighed, and frozen with liquid nitrogen immediately after dissection.

Results

Intestinal Absorption and CR.

The mouse colon epithelial cell line (CT26) was obtained from the American Type Culture Collection (Manassas, VA). Cells were cultured at 37°C under 5% CO2 and 5% O2 in RPMI 1640 medium containing 10% heat-inactivated FBS, 100 units/mL penicillin, and 100 μg/mL streptomycin (Gibco). For determination of the effect of high-glucose exposure on cell proliferation, after 24 h culturing, cells were serum starved for 0 or 20 mM/L-D-glucose. For proliferation assays, MTT assay was performed as previously described (24, 25). Absorbance was measured at 490 nm and converted into cell number. Three independent experiments were performed.

For the determination of the signaling pathways involved in CT26 cell proliferation in response to high glucose or free fatty acid (FFA) (palmitic acid; Sigma), cells were seeded in six-well plates in RPMI 1640 medium to reach 70–80% confluence. After 24 h of serum starvation, the cells were incubated with serum-free RPMI 1640 medium containing 20 mM/L-D-glucose or serum-free RPMI 1640 medium containing 2% BSA and 0.5 mM/L FFA for 15, 30, 60, 120, or 240 min. The cells then were lysed in radioimmunoprecipitation assay buffer to extract protein.

Statistical analysis. All data were expressed as mean ± SEM. Student t test was used to compare the differences between groups. A P value <0.05 was considered statistically significant.

Results

Intestinal growth and glucose absorption increased in db/db mice and was reversed by CR. Nutrient absorption in the intestine after food intake is the gateway for the body’s energy supply. Energy overload in excess of expenditure is the basis of obesity, while CR significantly reduces the incidence of obesity and its related diseases (4, 5, 6). To investigate changes in intestinal absorption related to excess food intake and developing obesity, we first looked at hyperphagic db/db mice. We found that, from the age of 4 weeks, food intake in db/db mice was increased significantly compared with age-matched lean db/+ mice (Fig. 1A) in concordance with body weight (Fig. 1B).

Restricting food intake caused a distinct reduction in body weight in db/db mice compared with their ad libitum db/db littermates (Fig. 1B). Intestinal length and weight also dramatically increased in db/db mice. CR did not alter db/db mouse intestinal length in db/db mice but robustly decreased intestine weight (Fig. 1C and D).

To test whether the absorption capacity per unit length of intestine also increased in db/db mice, we carried out glucose uptake assays in jejunal rings of 8-week-old db/db, db/+, and db/CR mice using a H3-labeled glucose tracer (21). The data showed that the intestinal glucose absorption of the jejunal rings increased in db/db mice compared with the same length of db/+ mouse intestine (Fig. 1E). Consistently, the GLUT2 protein, which mediates glucose transport from the intestinal lumen to the blood stream (21, 27), was increased dramatically in db/db intestines (Fig. 1F and G). CR significantly reduced glucose uptake and GLUT2 expression in db/db mice (Fig. 1E, F, and G). These results suggested that excess food intake, along with the increased intestinal absorption capacity, may contribute to obesity progression and that this can be reversed by CR.

Intestinal absorption area increased in db/db mice, and CR reduced absorption area in db/db mice. The intestinal mucosa is the main site of nutrient absorption. Villi in the mucosa increase the surface area over which absorption takes place. We studied morphological changes to determine whether the overall absorption surface area of the small intestine in db/db mice was increased and the influence of CR on the absorption surface area. H&E staining of the jejunum showed that the villi lengthened by approximately one-third (36.65%, P < 0.001) in db/db mice, and CR significantly reduced the length of villi (Fig. 2A and B). However, villi in db/db mice were more irregular than those in db/CR mice. This suggested that the increased absorption area may have contributed to the increased absorption capacity in db/db mice, and CR reduced absorption by reducing growth of villi.

In adult mice, epithelial cells in the villi are renewed every 3–5 days through cell proliferation, differentiation, and
migration, and apoptosis from multipotent stem cells located in the intestinal crypts. During the cell-renewal process, these stem cells proliferate, differentiate, and convert into several types of functional mature epithelial cells that migrate along the villi (15). To evaluate the epithelial cell proliferation and migration in the small intestinal villi, we injected BrdU (intraperitoneally) into 8-week-old mice to label S-phase cells. One hour after BrdU was injected, the BrdU-positive rate in the jejunal crypts was significantly higher in db/db mice. The increase in BrdU-positive cells was reduced by CR in db/db mice (Fig. 2C and D). In addition, we performed immunohistochemistry 24 h after BrdU was injected to trace the proliferation and migration of BrdU-labeled cells. After 24 h, the number of BrdU-positive cells increased significantly in db/db mice (Fig. 2E and F) and the distance of BrdU-positive cells from the crypt/villi axis was significantly longer in the db/db mice. Nearly 100% of BrdU-positive cells in the control mice were distributed within 25 cells from the base of the crypt, while more than half of the BrdU-positive cells were located between cell positions 25 and 40 in db/db mice (Fig. 2G). These data suggest that increased proliferation and migration of stem cells from the crypts were likely responsible for the observed villi elongation and increased absorption in db/db mice.

Activation of GSK-3β/β-catenin axis in the mucosa of small intestine of db/db mice. Cellular proliferation regulated by β-catenin plays a pivotal role in crypt stem cells and epithelial progenitor cells during gut development and in colon cancer (14,15,28). To assess whether β-catenin signaling also is involved in villi elongation in db/db mice, we examined β-catenin expression and signaling in the jejunum of 8-week-old db/db and db/+ mice. β-catenin mRNA expression within the intestine did...
not differ between these mice when assayed by quantitative RT-PCR (Fig. 3A). However, the protein level was significantly increased in \( \text{db/db} \) mice compared with \( \text{db/+} \) mice (Fig. 3B and C). This suggested that \( \beta \)-catenin proteins accumulation is increased in the \( \text{db/db} \) mouse intestine and that this accumulation was reduced with CR (Fig. 3B and C). Analysis of GSK-3\( \beta \) phosphorylation showed that the ratio of Ser\(^9\) phosphorylation (p-GSK-3\( \beta \))...
to total GSK-3β levels was upregulated in intestines of db/db. While the total GSK-3β (t-GSK-3β) protein expression did not change, the ratio of p-GSK-3β to t-GSK-3β was reduced by CR (Fig. 3B and D). As expected, when using immunohistochemistry to labeling the β-catenin target gene cyclin D1, positive cells also increased in the intestine of db/db mice and decreased after CR (Fig. 3E and F). These results mean that excess food intake–stimulated GSK-3β/β-catenin signaling activation might be involved in proliferation of intestinal epithelium.

**Leptin signaling defects did not affect proliferation of intestinal epithelium in the embryonic stage.** Because db/db mice are leptin receptor mutant mice, we investigated whether increased intestinal epithelial proliferation was derived from abnormal gut development caused by the loss of leptin signaling. E18.5 db/db and db/+ littermates showed no difference in body weight or intestinal length (Fig. 4A and B). H-E staining also did not show gross differences in intestinal histology and villi length (Fig. 4C and D). In addition, there was no difference in cryptal BrdU labeling between the two groups (Fig. 4E and F). β-Catenin protein level, GSK-3β phosphorylation (Fig. 4G, H, and I), and cyclin D1–positive cells (data not shown) were similar in embryonic db/db and db/+ mouse intestines. These findings indicated that the genetic defect in the leptin receptor did not affect β-catenin signaling and cell proliferation in the embryonic intestinal epithelium. Combined with the earlier results, these results support a model in which increased intestinal epithelial cell proliferation was due to the excess food intake stimulation in adult db/db mice and suggested that compensatory intestinal epithelial cell proliferation/migration was important in increasing intestinal absorption and might contribute to obesity.

**HFD increased intestinal epithelial cell proliferation through activation of GSK-3β/β-catenin signaling pathway.** As described above, we observed that proliferation of epithelial cells in the intestine of db/db mice was increased significantly and could be reversed by CR. Because the db/db mice were leptin receptor mutant and hyperphagia, the intestinal changes in adult mice also could have been caused either by genetic background or physical stimulation due to the increased volume of food consumed.
To confirm this, we subjected 4-week-old, wild-type C57BL/6 mice to HFD. After 8 weeks of HFD, the mice had significantly greater body weight than mice fed a standard chow diet (Fig. 5A and B). Concurrently, increased villi length and epithelial cell proliferation were found in the small intestines of HFD mice (Fig. 5C–F). Moreover, GSK-3β phosphorylation, β-catenin protein, and cyclin D1 level all were increased (Fig. 5G–J). These results confirmed that it was the energy density in the food behind the pronounced signaling of GSK-3β/β-catenin and stimulated intestinal epithelial proliferation and absorption rather than genetic background and volume of food intake.

To investigate further whether β-catenin was responsible for proliferation and morphological changes of intestinal cell from over nutrition, we treated mice fed the HFD with JW55. JW55 is a small-molecule inhibitor of the β-catenin signaling pathway. It stabilizes AXIN2, a member of the β-catenin destruction complex, and increases degradation of β-catenin (19). After 3 weeks’ treatment with JW55, β-catenin and cyclin D1 were significantly lower compared with the HFD DMSO control treatment group, but no differences were found with the CD group (Fig. 6A–D). Also, villi length and BrdU-positive cells were lower in the JW55-treated group (Fig. 6E–H). The absolute increased body weight in mice treated with JW55 was lower, but not significantly, compared with mice treated with DMSO (8.25 ± 0.37 g and 9.23 ± 0.46 g for JW55 and DMSO treatments, respectively). In our prior studies, significant differences in body weight between chow diet– and HFD-fed C57BL/6 mice usually appeared after 4 weeks. The present result might be due, in part, to the shorter duration of HFD feeding and treatment. These data indicated that an
activated β-catenin signaling pathway was essential for proliferation intestinal epithelium in response to HFD feeding. High glucose and FFA induce GSK-3β phosphorylation and β-catenin accumulation in epithelial cells. Carbohydrate is the main component of regular mouse chow. Most ingested carbohydrate is digested and broken down into glucose in the small intestine and transported into the bloodstream. Previous studies have reported that exposure to high glucose levels induces proliferation in some cell types (29,30). In addition, high-sucrose diets promote intestinal epithelial cell proliferation and tumorigenesis in APC<sup>min</sup> mice (31). Therefore, we performed an in vitro study to determine whether glucose directly stimulates intestine epithelial cell proliferation. When CT26 epithelial cells were exposed to high glucose (20 mmol/L), phosphorylation of GSK-3β increased, and the expression of β-catenin and its target gene, cyclin D1, was upregulated after 1 h of high-glucose stimulation (Fig. 7A and B). After mice were fed an HFD, the primary nutrient absorbed in the intestine was FFA. To confirm whether FFA directly activates GSK-3β/β-catenin signaling, we added FFA to CT26 cell culture medium. GSK-3β phosphorylation and β-catenin accumulation were increased after 1 h of FFA stimulation. The expression of β-catenin, GSK-3β Ser<sup>9</sup> phosphorylation, and cyclin D1 expression. Western blot data from at least three independent experiments and expressed as mean ± SEM; mice n = 6–8 per group. *P < 0.05, **P < 0.01. Scale bar, 50 μm.
Cyclin D1 all were upregulated significantly (Fig. 8E–H). These data support the supposition that high nutrient levels directly activate GSK-3β/β-catenin signaling and stimulate intestinal epithelial cell proliferation.

**DISCUSSION**

The increasing incidence of obesity and its association with disease have become a great challenge to global health. Obesity increases the risk of cardiovascular disease, premature death, insulin resistance, T2D, and many types of cancer (32,33). However, few drugs for antiobesity are approved by the U.S. Food and Drug Administration, such as phentermine and orlistat (34). Unfortunately, because of insufficient weight loss and significant gastrointestinal side effects, these antiobesity drugs are less than ideal. So, there is an urgent need to find new therapeutic targets for obesity.

The etiology of obesity is very complex, and pathological mechanisms have been studied widely in adipose tissue, liver, and muscle (35,36). Several genes have been identified that regulate adipose mass and are associated with the development of obesity. Recent findings regarding the role of intestine-secreted hormones in metabolic diseases and the effects of gastric bypass bariatric surgery make the intestine a primary site in the pathophysiology of obesity and T2D (37–39). However, aside from the impact of gut-derived endocrine hormones on T2D, there has been...
little study of the contribution of the intestine to excess food intake–related obesity.

Some studies describing gene regulation in the intestine during the development of obesity induced by an HFD found that many dietary fat–induced molecular changes are associated with lipid metabolism, the cell cycle, inflammation, and the immune response (40–42). But details of mechanisms that affect the functionality of intestine during the progression of obesity have not been studied. In a regular diet, carbohydrates are the most prevalent nutrient. Monosaccharides in the intestinal lumen interact with the intestinal epithelium after carbohydrate digestion. Whether this interaction affects intestinal function is unknown. β-Catenin–dependent intestinal stem cell proliferation is a prerequisite for the maintenance of epithelial homeostasis and absorption (13).

Free β-catenin proteins accumulating in cells are an important feature of activating β-catenin downstream signaling (16). Chen et al. found that retinal tissue sections from patients with diabetes displayed increased β-catenin expression and nuclear translocation (43). Anagnostou and Shepherd also verified that high glucose induces upregulation of β-catenin in two macrophage cell lines (44). In this study, nutrient overload in animals and high glucose or FFA in cultured intestinal epithelial cells induced β-catenin accumulation and intestinal epithelial cell proliferation. In confirmation of this, high glucose did not stimulate intestinal cell proliferation or cyclin D1 expression after treatment with the inhibitor of β-catenin transcription, FH535. Giving a β-catenin inhibitor, JW55, to mice fed an HFD also prevented intestinal epithelial cell proliferation. Together, these results indicate that nutrients may prevent β-catenin degradation and promote downstream transcriptional activation that increases intestinal epithelial cell proliferation. Functionally, GLUT2, a main transporter of dietary sugar in the intestine (27), increased in hyperphagic obese db/db mice. On the contrary, food restriction in db/db mice reduced intestinal cell proliferation and absorption, the indication being that intestinal absorptive capacity was increasing along with the increased food intake and intestinal epithelial proliferation.

GSK-3β–based protein degradation is a key event in regulating intracellular β-catenin protein accumulation in canonical Wnt signaling (14,16). The ninth serine in the NH2-terminus is very important for GSK-3β activity. Once this serine is phosphorylated, GSK-3β fails to bind and phosphorylate β-catenin for degradation. We found that excess food intake promoted phosphorylation of the ninth serine of GSK-3β in intestine. Adding CHIR98014 (a GSK-3β inhibitor) alone, or with high glucose, to epithelial cells could have increased GSK-3β phosphorylation and then induced β-catenin accumulation and cyclin D1 expression. This suggests that the increased β-catenin protein in intestinal epithelial cells may depend on phosphorylation of the ninth serine of GSK-3β.

Recently, in addition to Wnts, a variety of signaling molecules involved in the regulation of intracellular transcriptional activity of β-catenin/TCF in different contexts have been found, including insulin, IGF-1, platelet-derived growth factor, glucagon-like peptide (GLP)-2, and forkhead...
Studies have shown that, in breast cancer cells, IGF-1 stabilizes intracellular β-catenin and promotes the transcription activity of β-catenin/TCF. IGF-1 and insulin are high in the plasma of obese animals and are closely related to nutrient status. GLP-2 is one of the best studied peptide secreted from enteroendocrine L cells of the intestine that are closely related to intestinal function (48,49). Studies show that GLP-2 could stimulate cell proliferation of intestinal epithelium through IGF-1 signaling and β-catenin alterations (46,47,50). And GLP-2 is also involved in intestinal epithelial proliferation after HFD stimulation (49). After 3 weeks of HFD feeding, blood glucose and insulin were increased significantly, though GLP-2 had no clear increase in the HFD group. Phosphorylation of Akt increased significantly in adipose tissue but not in the intestine (data not shown). Nutrients seemed to have a direct and important effect on the proliferation of intestinal epithelial cells, at least in the early period of nutrient overload. However, we cannot exclude that GLP-2, in inflammation, hyperglycemia, or hyperinsulinemia might also have contributed to intestinal epithelial cell proliferation in long-term nutrient overload or obesity status.

Taken together, our findings confirm the importance of nutrition on initiating proliferation of intestinal epithelium and provide a possible link between intestinal metabolism and obesity development. Targeting epithelial GSK-3b/β-catenin signaling in the small intestine may provide a novel strategy to prevent obesity related to diet. Further investigation into the mechanisms responsible for interactions between diet and the intestine will advance our knowledge of the pathogenesis of obesity.

ACKNOWLEDGMENTS

This study was supported by the National Natural Science Foundation of China (30870996, 81270883, and 31221002).

No potential conflicts of interest relevant to this article were reported.

J.M. contributed to the experiments and data analysis and wrote the manuscript. X.H. contributed to the experiments and contributed to all revised experiments. Y.X., C.Y., Y.D., and N.H. contributed to the experiments. J.W. and H.C. contributed to the discussion and reviewed and edited the manuscript. X.Z. contributed to the study design, data collection, and interpretation and wrote the manuscript. X.Z. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

The authors thank Dr. Iain C. Bruce (Institute of Molecular Medicine, Peking University), Dr. David M. Ornitz (Department of Developmental Biology, Washington University School of Medicine), and Jon K. Moon, PhD, for help with editing English. The authors also thank Drs. Ruiping Xiao, Xiaojun Zhu, Chunmei Cao, Ruisheng Song, and Yan Zhang (Institute of Molecular Medicine, Peking University) for scientific discussion and critical comments, and Hui Wang, Jun Zhang, and Yuli Liu (Institute of Molecular Medicine, Peking University) for technical support.

REFERENCES

1. Grundy SM. Metabolic syndrome pandemic. Arterioscler Thromb Vasc Biol 2008;28:629–636
2. Barsh GS, Schwartz MW. Genetic approaches to studying energy balance: perception and integration. Nat Rev Genet 2002;3:589–600
3. Spiegelman BM, Flier JS. Obesity and the regulation of energy balance. Cell 2001;104:531–543
4. Redman LM, Ravussin E. Caloric restriction in humans: impact on physiological, psychological, and behavioral outcomes. Antioxid Redox Signal 2011;14:275–287
5. Capel F, Klimcáková E, Viguier N, et al. Macrophages and adipocytes in human obesity: adipose tissue gene expression and insulin sensitivity during caloric restriction and weight stabilization. Diabetes 2009;58:1558–1567
6. Anderson RM, Shammuganayagam D, Weindruch R. Caloric restriction and aging: studies in mice and monkeys. Toxicol Pathol 2009;37:47–51
7. Murphy KG, Bloom SR. Gut hormones and the regulation of energy homeostasis. Nature 2006;444:854–859
8. Sun J, Jin T. Both Wnt and mTOR signaling pathways are involved in human obesity: adipose tissue gene expression and insulin sensitivity. PLoS ONE 2011;6:e26340
9. trayhurn P, Bing C. Appetite and energy balance signals from adipocytes. Philos Trans R Soc Lond B Biol Sci 2006;361:1237–1249
10. Keelan M, Cheeseman CI, Chlandinin MT, Thomson AB. Intestinal morphology and transport after ileal resection in rat is modified by dietary fatty acids. Clin Invest Med 1996;19:63–70
11. Petit V, Arnould L, Martin P, et al. Chronic high-fat diet affects intestinal fat absorption and postprandial triglyceride levels in the mouse. J Lipid Res 2007;48:276–287
12. Scoaris CR, Rizo GV, Roldi LP, et al. Effects of cafeteria diet on the jejunal morphology and transport after ileal resection in rat is modified by dietary fatty acids. Philos Trans R Soc Lond B Biol Sci 2006;361:1237–1249
13. Clevers H. Wnt/beta-catenin signaling in development and disease. Cell 2001;104:531
14. Gouyon F, Caillaud L, Carriere V, et al. Simple-sugar meals target GLUT2 at the apical membranes to improve sugar absorption: a study in GLUT2-null mice. J Physiol 2003;552:823–832
15. van der Flier LG, Clevers H. Stem cells, self-renewal, and differentiation in the intestinal epithelium. Annu Rev Physiol 2009;71:241–260
16. Beda S, Kishida S, Yamamoto H, Murai H, Koyama S, Kikuchi A. Axin, a negative regulator of the Wnt signaling pathway, forms a complex with GSK-3beta and beta-catenin and promotes GSK-3beta-dependent phosphorylation of beta-catenin. EMBO J 1998;17:1371–1384
17. Ikeda S, Kishida S, Yamamoto H, Murai H, Koyama S, Kikuchi A. Axin, a negative regulator of the Wnt signaling pathway, forms a complex with GSK-3beta and beta-catenin and promotes GSK-3beta-dependent phosphorylation of beta-catenin. EMBO J 1998;17:1371–1384
18. Karrasch T, Spaeath T, Allard B, Jobin C. PSEK-dependent GSK3beta(ser9)-phosphorylation is implicated in the intestinal epithelial cell wound-healing response. PLoS ONE 2011;6:e20340
19. Waaler J, Machon O, Tumova L, et al. A novel tankyrase inhibitor decreases canonical Wnt signaling in colon carcinoma cells and reduces tumor growth in conditional APC mutant mice. Cancer Res 2012;72:2822–2832
20. Geske MJ, Zhang X, Patel KK, Ornitz DM, Stappenbeck TS. Fgf9 signaling regulates small intestinal elongation and mesenchymal development. Development 2008;135:2699–2708
21. Gouyon F, Caillaud L, Carriere V, et al. Simple-sugar meals target GLUT2 at the apical membranes to improve sugar absorption: a study in GLUT2-null mice. J Physiol 2003;552:823–832
22. Tanaka S, Terada K, Nohno T. Canonical Wnt signaling is involved in inflammation and metabolic complications in obese mice. Am J Physiol Endocrinol Metab 2009;296:E216–E2171
23. Kellett GL, Brot-Laroche E. Apical GLUT2: a major pathway of intestinal sugar absorption. Diabetes 2005;54:3056–3062
24. de Wetere M, Sancho E, Verweij C, et al. The beta-catenin/TCF-4 complex imposes a crypt progenitor phenotype on colorectal cancer cells. Cell 2002;111:241–250
25. Yamamoto M, Acevedo-Duncan M, Chalante CF, Patel NA, Watson JE, Cooper DR. Acute glucose-induced downregulation of PFK-beta2/accelerates cultured VSMC proliferation. Am J Physiol Cell Physiol 2006;291:C587–C595
26. Li YM, Schilling T, Bensich P, et al. Effects of high glucose on mesenchymal stem cell proliferation and differentiation. Biochem Biophys Res Commun 2007;363:209–215
27. Wang B, Bobe G, LaPres JJ, Bourquin LD. High sucrose diets promote intestinal epithelial cell proliferation and tumorigenesis in APC(Min) mice by increasing insulin and IGF-I levels. Nutr Cancer 2009;61:81–93
28. E1171
29. Van de Wetering M, Acevedo-Duncan M, Chalante CF, Patel NA, Watson JE, Cooper DR. Acute glucose-induced downregulation of PFK-beta2/accelerates cultured VSMC proliferation. Am J Physiol Cell Physiol 2006;291:C587–C595
30. Li YM, Schilling T, Bensich P, et al. Effects of high glucose on mesenchymal stem cell proliferation and differentiation. Biochem Biophys Res Commun 2007;363:209–215
31. Wang B, Bobe G, LaPres JJ, Bourquin LD. High sucrose diets promote intestinal epithelial cell proliferation and tumorigenesis in APC(Min) mice by increasing insulin and IGF-I levels. Nutr Cancer 2009;61:81–93
32. Khandekar MJ, Cohen P, Spiegelman BM. Molecular mechanisms of cancer development in obesity. Nat Rev Cancer 2011;11:866–885
33. Kahn SE, Hull RL, Utschneider KM. Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature 2006;444:840–846
34. Powell AG, Apovian CM, Aronne LJ. New drug targets for the treatment of obesity. Clin Pharmacol Ther 2011;90:40–51
35. Samuel VT, Petersen KP, Shulman GL. Lipid-induced insulin resistance: unraveling the mechanism. Lancet 2010;375:2267–2277
36. Henriksen EJ. Dysregulation of glycogen synthase kinase-3 in skeletal muscle and the etiology of insulin resistance and type 2 diabetes.Curr Diabetes Rev 2010;6:285–293
37. Takashi S, Shihata W, Tomita H, et al. In vivo analysis of mouse gastric gene regulation in enhanced GFP-BAC transgenic mice. Am J Physiol Gastrointest Liver Physiol 2011;300:G334–G344
38. Hansen EN, Tamboli RA, Isbell JM, et al. Role of the foregut in the early improvement in glucose tolerance and insulin sensitivity following Roux-en-Y gastric bypass surgery. Am J Physiol Gastrointest Liver Physiol 2011;300:G795–G802
39. Gautier JF, Choukem SP, Girard J. Physiology of incretins (GIP and GLP-1) and abnormalities in type 2 diabetes. Diabetes Metab 2008;34(Suppl. 2):S65–S72
40. de Wit NJ, Boekschoten MV, Bachmair EM, et al. Dose-dependent effects of dietary fat on development of obesity in relation to intestinal functional gene expression in C57BL/6J mice. PLoS ONE 2011;6:e19145
41. Al-Dwairi A, Pabona JM, Simmen RC, Simmen FA. Cytosolic malic enzyme 1 (me1) mediates high fat diet-induced adiposity, endocrine profile, and gastrentestinal tract proliferation-associated biomarkers in male mice. PLoS One 2012;7:e46716
42. de Wit NJ, Bosch-Vermueulen H, de Groot PJ, et al. The role of the small intestine in the development of dietary fat-induced obesity and insulin resistance in C57BL/6J mice. DMC Med Genomics 2009;8:14
43. Chen Y, Hu Y, Zhou T, et al. Activation of the Wnt pathway plays a pathogenic role in diabetic retinopathy in humans and animal models. Am J Pathol 2000;157:2676–2685
44. Anagnostou SH, Shepherd PR. Glucose induces an autocrine activation of the Wnt/beta-catenin pathway in macrophage cell lines. Biochem J 2008;416:211–218
45. Rosner G, Rozen P, Bercovich D, et al. A protocol for genetic evaluation of patients with multiple colorectal adenomas and without evidence of APC gene mutation. Isr Med Assoc J 2010;12:540–553
46. Rowland KJ, Trivedi S, Lee D, Wan K, Kulkarni RN, Holzenberger M, Brubaker PL. Loss of glucagon-like peptide-2-induced proliferation following intestinal epithelial insulin-like growth factor-1 receptor deletion. Gastroenterology 2011;141:2166–2175
47. Dubé PE, Rowland KJ, Brubaker PL. Glucagon-like peptide-2 activates beta-catenin signaling in the mouse intestinal crypt: role of insulin-like growth factor-I. Endocrinology 2008;149:291–301
48. Sinclair EM, Drucker DJ. Proglucagon-derived peptides: mechanisms of action and therapeutic potential. Physiology (Bethesda) 2005;20:357–365
49. Baldassano S, Amato A, Cappello F, Rappa F, Mulè F. Glucagon-like peptide-2 and mouse intestinal adaptation to a high-fat diet. J Endocrinol 2011;217:11–20
50. Dubé PE, Forse CL, Bahrami J, Brubaker PL. The essential role of insulin-like growth factor-I in the intestinal tropic effects of glucagon-like peptide-2 in mice. Gastroenterology 2006;131:589–605