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**Objective** To investigate whether a whole grain diet alters the gut microbiome and insulin sensitivity, as well as biomarkers of metabolic health and gut functionality. Design 60 Danish adults at risk of developing metabolic syndrome were included in a randomised cross-over trial with two 8-week dietary intervention periods comprising whole grain diet and refined grain diet, separated by a washout period of ≥6 weeks. The response to the interventions on the gut microbiome composition and insulin sensitivity as well on measures of glucose and lipid metabolism, gut functionality, inflammatory markers, anthropometry and urine metabolomics were assessed. Results 50 participants completed both periods with a whole grain intake of 179±50 g/day and 13±10 g/day in the whole grain and refined grain period, respectively. Compliance was confirmed by a difference in plasma alkylresorcinols (p<0.0001). Compared with refined grain, whole grain did not significantly alter glucose homeostasis and did not induce major changes in the faecal microbiome. Also, breath hydrogen levels, plasma short-chain fatty acids, intestinal integrity and intestinal transit time were not affected. The whole grain diet did, however, compared with the refined grain diet, decrease body weight (p<0.0001), serum inflammatory markers, interleukin (IL)-6 (p=0.009) and C-reactive protein (p=0.003). The reduction in body weight was consistent with a reduction in energy intake, and IL-6 reduction was associated with the amount of whole grain consumed, in particular with intake of rye. **Conclusion** Compared with refined grain diet, whole grain diet did not alter insulin sensitivity and gut microbiome but reduced body weight and systemic low-grade inflammation.

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