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FOOD & FUNCTION

Metabolomic analysis reveals differences in urinary excretion of kiwifruit-derived metabolites in a mouse model of inflammatory bowel disease

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The interleukin-10-deficient (IL-$10^{-/-}$) mouse, a model of inflammatory bowel disease (IBD), develops intestinal inflammation unless raised in germ-free conditions. The metabolic effects of consuming extracts from the fruits of yellow (*Actinidia chinensis*) or green-fleshed (*A. deliciosa*) kiwifruit that displayed in vitro anti-inflammatory activity were investigated in IL-$10^{-/-}$ mice by metabolomic analysis of urine samples. Kiwifruit-derived metabolites were detected at significantly higher levels in urine of IL-$10^{-/-}$ mice relative to those of wild-type mice, indicating that the metabolism of these metabolites was affected by IL-$10^{-/-}$-wild-type genotypic differences. Urinary metabolites previously associated with inflammation were not altered by the kiwifruit extracts. This study demonstrates the use of metabolomic analysis to study dietary effects and the influence of genotype on food metabolism, which may have implications on the development of functional foods for the treatment of IBD.

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Inflammatory bowel diseases (IBD), such as Crohn's disease and ulcerative colitis, are characterized by recurring intestinal inflammation which is attributed to a dysregulated immune response towards intestinal microbiota [1]. IBD patients require long-term anti-inflammatory medication to induce and sustain remission from intestinal inflammation. Enteral nutrition is also beneficial in inducing remission for some patients but patients cannot remain indefinitely on this type of diet [2]. Feeding studies on IBD animal models indicate that dietary modulation with foods containing immunomodulatory components may influence disease activity [3, 4].

The fruits of yellow (*Actinidia chinensis*) and green-fleshed (*A. deliciosa*) kiwifruit are rich in phytochemicals associated with health benefits including polyphenols [5, 6]. Aqueous and ethyl acetate extracts from the fruits of yellow and green-fleshed kiwifruit inhibit the production of cytokines by murine macrophage and intestinal cells stimulated with bacterial ligands [7, 8]. These findings suggest that bioactive compounds present in yellow and green-fleshed kiwifruits may be beneficial for IBD patients through the regulation of intestinal mucosal immunity towards intestinal microbiota, or the reduction of oxidative stress associated with chronic inflammation.

Metabolomic analysis, the parallel analysis of multiple small molecule metabolites using high-throughput analytical techniques, is potentially useful to identify new markers of dietary exposure and to assess the nutritional effects of bioactive food components [9]. Gas chromatography–mass spectrometry (GC-MS) metabolomic analysis was previously used to identify a urinary metabolite profile associated with intestinal inflammation in interleukin-10-deficient (IL-$10^{-/-}$) mice, a mouse model of IBD that

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Table 1. Kiwifruit-derived metabolites that occurred at higher concentrations in urine from IL-$10^{-/-}$mice

Metabolite	GCMS representative *m/z*	IL-$10^{-/-}$/wild-type fold difference (average of all three sampling time points)[a)]			
		Yellow kiwifruit experiment		Green kiwifruit experiment	
		Aqueous extract	Ethyl acetate extract	Aqueous extract	Ethyl acetate extract
Unknown RT1462	286	**10**	5	**544**	**34**
Unknown RT1565	286	**8**	5	**39**	1
Unknown RT1824	167	17	2	**24**	**13**
Unknown RT2330	81	N/D[b)]	N/D[b)]	6	1
Unknown RT2676	337	N/D[b)]	N/D[b)]	2	**2**

m/z, mass spectral ion used for calculating fold difference.
a) Bold: $p<0.05$ for genotype in two-factor ANOVA of genotype and time point.
b) Not Detected on the HP-5 GC column used for this experiment. Samples from the green kiwifruit experiment were analysed using a ZB-5MS GC column.

develops Crohn's-like colitis unless raised in germ-free conditions [10, 11]. This metabolite profile, consisting of 15 metabolites (xanthurenic acid, fucose, 5-aminovaleric acid, uracil and 11 unknowns) [10], could potentially be used to evaluate the anti-inflammatory effects of functional foods in IL-$10^{-/-}$ mice.

In this study, the potential of kiwifruit as a functional food for IBD was investigated by evaluating the dietary effects of extracts of yellow or green-fleshed kiwifruit using the IL-$10^{-/-}$ mouse model and metabolomic approaches. Two separate feeding experiments were conducted on male IL-$10^{-/-}$ (B6.129P2-Il10t^{m1Cgn}) and wild-type C57BL/6 mice (The Jackson Laboratory, USA). In the first (yellow kiwifruit) experiment, 4.6-wk-old mice were fed with powdered AIN76A diets containing 5% w/w aqueous or 0.1% w/w ethyl acetate extracts from yellow-fleshed kiwifruit (*A. chinensis*, 'Hort16A'). In the second (green kiwifruit) experiment, 5.9-wk-old mice were fed with AIN76A diets containing 5% w/w aqueous or 0.1% w/w ethyl acetate extracts from green-fleshed kiwifruit (*A. deliciosa*, 'Hayward'). Each dietary treatment group consisted of 6–15 mice. Full details of experiment conditions, diets and ethical approval are included in Supporting Information. At the start of each experiment, all mice were dosed orally with a mixture of intestinal microflora to ensure the same microbial exposure [12]. Spot urine samples were collected when mice were 5.5, 7, 8.5-wk old for the yellow kiwifruit experiment; and 7, 9 and 11.5-wk old for the green kiwifruit experiment. Urine samples were subjected to GC-MS metabolomic analysis as described previously [10]. Both feeding experiments lasted 6 wk, before mice were euthanased and their intestinal tissues subjected to histological examination [12].

The global urinary metabolite profiles (represented by GC-MS mass spectral ion data) of IL-$10^{-/-}$ mice differed from wild-type mice irrespective of dietary treatments and sampling time (Fig. 1), indicating that the kiwifruit extracts did not restore the metabolite profile of IL-$10^{-/-}$ mice to that of wild-type. The individual levels of the 15 metabolites associated with intestinal inflammation in IL-$10^{-/-}$ mice [10] were not significantly different between IL$10^{-/-}$ mice fed with kiwifruit extracts and those fed with control diet (Supporting Information). Histology examination of the colon tissue, the main site of inflammation, revealed that the histology injury scores were not significantly different between IL-$10^{-/-}$ mice fed with kiwifruit extracts and those fed with control diet (Supporting Information). Overall, these findings indicate that the kiwifruit extracts did not reduce intestinal inflammation in IL-$10^{-/-}$ mice.

The global urinary metabolite profiles of mice differed for the kiwifruit extracts relative to control diets (Fig. 1), suggesting the bioavailability of some of the kiwifruit metabolites. Nine metabolites (represented by groups of GCMS mass spectral ions with the same chromatographic retention time, Supporting Information) were present at higher levels in the urine of mice fed with kiwifruit extracts compared with those fed with control diet, indicating that these metabolites are likely to be derived from the kiwifruit extracts. Among these kiwifruit-derived metabolites, only hippuric acid was identified with confirmation by an authentic chemical standard. Hippuric acid is produced by intestinal microbial metabolism of phenolic compounds [13], such as chlorogenic acid [14] which is present in kiwifruits [5]. The remaining metabolites may be derived from polyphenols, as high-resolution mass spectrometry showed that their fragment ions may have aromatic structures (data not shown). However, none could be identified from mass spectral libraries or as previously reported metabolites from polyphenol metabolism. The levels of these kiwifruit-derived metabolites were generally higher in urine from mice fed with aqueous extracts compared with those fed with ethyl acetate extracts, reflecting presumably differences in phytochemicals extracted by water and ethyl acetate.

Interestingly, the levels of five of the nine kiwifruit-derived urinary metabolites were significantly higher in urine from IL-$10^{-/-}$ relative to that of wild-type mice ($p<0.05$, Table 1, Fig. 2), indicating genotypic differences

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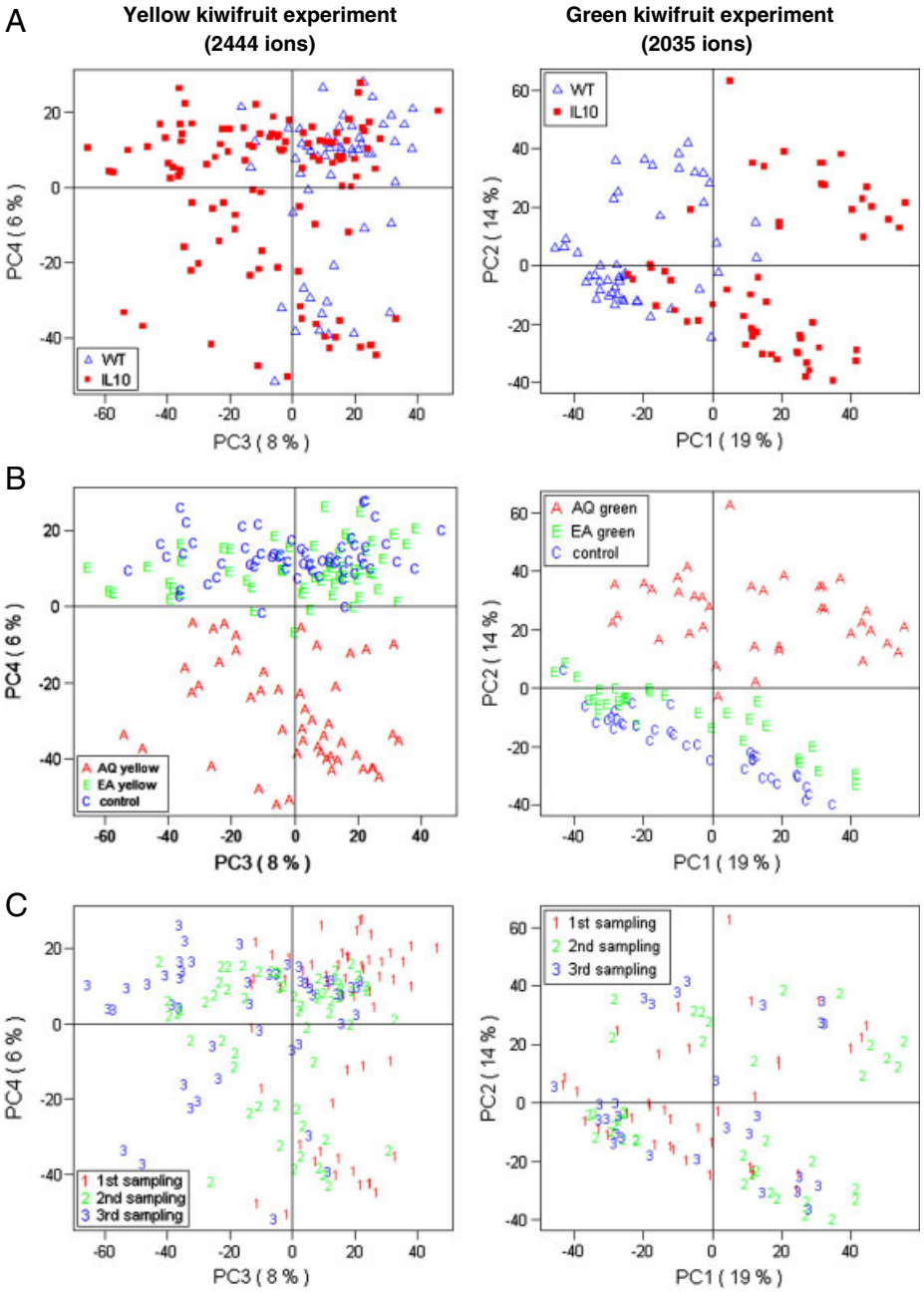

Figure 1. Principal components analysis score plots of the intensities of total GCMS mass spectral ions detected by the XCMS data analysis software, representing the global urinary metabolite profile. Each data point represents a urine sample, labeled according to mouse strain (A), dietary treatments (B) or urine sampling time points (C). WT, wild-type; IL-10, IL10$^{-/-}$ mice; AQ, aqueous extract; EA, ethyl acetate extract; yellow, yellow-fleshed kiwifruit; green, green-fleshed kiwifruit.

between the two mouse strains in the excretion of these metabolites. Weighing of leftover food in the cages over five days before each urine sampling showed that food intake between IL-10$^{-/-}$ and wild-type mice was overall not significantly different. On two occasions before each urine sampling, the average food intake (adjusted to body weight over five days) was in fact higher for wild-type mice relative to IL-10$^{-/-}$ mice. Therefore, the higher urinary excretion of the kiwifruit-derived metabolites by IL-10$^{-/-}$ mice was not caused by differences in food intake.

IL-10$^{-/-}$ mice are congenic strains of C57BL/6 background but their genome contains some DNA of 129 strain origin [15], thus C57BL/6 and 129 strain differences in xenobiotic metabolism may be responsible for IL-10$^{-/-}$ and wild-type differences in the excretion of these kiwifruit-derived metabolites. For example, the UDP glucuronosyltransferase gene family is located in a region of 126 strain-derived DNA and may contribute to genotype differences in glucuronide excretion [16]. Impaired gut function arising from IL-10-deficiency may also be contributing to the differences in urinary excretion of the kiwifruit-metabolites. The colonic intestinal permeability of IL-10$^{-/-}$ mice is higher than wild-type even before the onset of intestinal inflammation [17]. Therefore, a higher diffusion of kiwifruit

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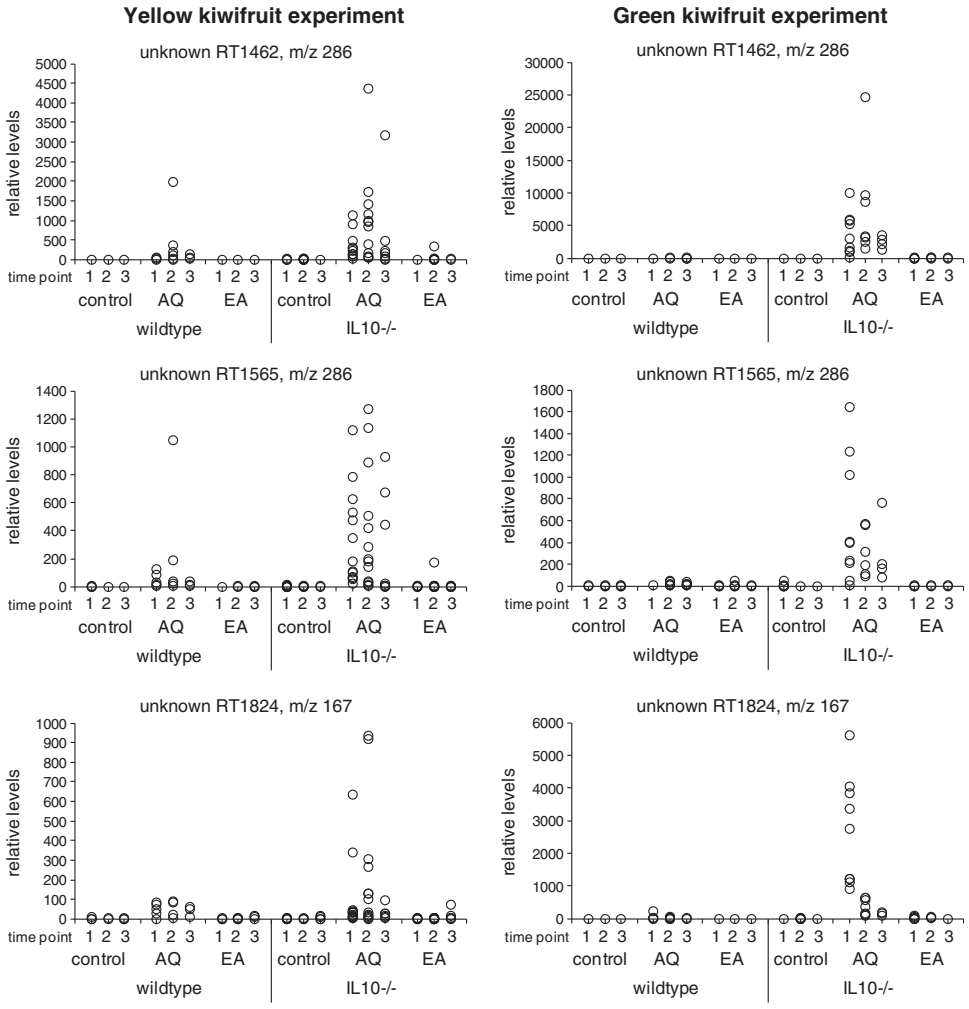

Figure 2. Relative levels of some kiwifruit-derived metabolites demonstrating IL-$10^{-/-}$-wild-type genotypic differences in urinary levels. (Levels are relative to a quality control urine sample, normalized but not log transformed. AQ, aqueous extract; EA, ethyl acetate extract.)

metabolites may be occurring across the intestinal epithelial barrier of IL-$10^{-/-}$ mice. Not all the kiwifruit-derived metabolites however show genotypic differences, which may in turn result from the selective expression of transmembrane transporters that selectively transport kiwifruit metabolites. For example, the expression of a monocarboxylic acid transporter which is selective for the phenolic metabolites ferulic acid and *p*-coumaric acid [18], is decreased in inflamed colonic mucosa of rats with chemically induced colitis and in IBD patients [19].

The metabolism of kiwifruit phytochemicals may also be affected by changes in the composition of the intestinal microbiota induced by host-mediated inflammation [20], as the intestinal microbiota is involved in the metabolism of phytochemicals [21]. Differences in intestinal microbial populations between IL-$10^{-/-}$ and wild-type mice have been observed [20, 22] and may result in the increased biosynthesis of some kiwifruit-derived metabolites in IL-$10^{-/-}$ mice.

IBDs are associated with increased intestinal permeability [23] and differences in the composition and metabolic activity of intestinal microbiota [24]. Various studies have demonstrated the influence of intestinal microbiota on drug metabolism and response [25]. The possible effects of these factors on the metabolism of exogenous metabolites, as demonstrated by the IL-$10^{-/-}$ mice, may have implications on the bioavailability and efficacy of functional foods for the treatment of IBD or other diseases with impaired gut function or altered intestinal microbiota. While the identities of the kiwifruit-derived metabolites and the mechanism for genotypic differences in the levels of these metabolites remain unanswered, this study highlights the possibilities of metabolomic analysis in nutritional research which include early non-invasive determination of treatment efficacy and the discovery of unexpected metabolic effects that may have implications for disease treatment.

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