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Weight and Body Composition Compartments do Not Predict Therapeutic Thiopurine Metabolite Levels in Inflammatory Bowel Disease

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OBJECTIVES: Thiopurine drugs are the most commonly used steroid-sparing therapies in moderate-to-severe inflammatory bowel disease (IBD). Their complex metabolism and their narrow therapeutic windows means that optimal dosing is difficult. However, weight-based dosing is the norm. Similar antimetabolites are dosed by body composition parameters. In IBD, treatment response and toxicity has been shown to correlate with thiopurine metabolite levels. We sought to determine whether weight or body composition parameters predicted therapeutic 6-thioguanine nucleotide (6TGN) or toxic 6-methylmercaptopurine (6MMP) levels.
METHODS: This single-center retrospective cohort study identified 66 IBD patients who had body composition analysis and thiopurine metabolite levels tested. Statistical analysis was performed using Spearman correlation, Kruskal–Wallis, Mann–Whitney, and unpaired *t* tests and receiver-operator operating characteristic curves. A *P* value of <0.05 was considered significant.
RESULTS: No correlation was identified between 6TGN and any body composition parameters, absolute drug dose or drug dose/kg of fat mass, fat-free mass (FFM), subcutaneous adipose tissue area, or visceral adipose tissue area. However, 6MMP correlated with azathioprine dose, thiopurine dose/kg of body weight, and with several body composition parameters.
CONCLUSIONS: No relationship was found between therapeutic metabolite levels and weight or body composition compartments. Higher thiopurine doses, especially in relation to FFM, are associated with higher levels of potentially hepatotoxic 6MMP and shunting toward this metabolite. Conventional weight-based dosing to attain therapeutic metabolite levels appears unreliable and may be replaced by metabolite level testing.

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INTRODUCTION

Azathioprine and its metabolite, 6-mercaptopurine (6MP), are the most commonly used steroid-sparing therapies in moderate-to-severe inflammatory bowel disease (IBD).[1,2] A total of 50–60% of patients respond to these treatments; the remainder will have refractory disease or adverse drug reactions.[3]

The complex metabolism of these drugs and their narrow therapeutic windows means that optimal dosing is difficult. Recent interest in the measurement of intracellular thiopurine metabolites has led to the development of treatment algorithms based on therapeutic 6-thioguanine nucleotide (6TGN) and toxic 6-methylmercaptopurine (6-MMP) levels,[3–6] with a meta-analysis demonstrating an association between the levels of 6TGN and likelihood of clinical response.[7] Combination therapy with allopurinol has been recommended in patients with an elevated 6MMP:6TGN ratio to optimize metabolite levels and treatment response.[8,9] A ratio of >11 has been described as abnormal,[4] with >20 clearly demonstrating skewed metabolism.[10] Sulfasalazine and aminosalicylate (5ASA) drugs, which are commonly prescribed in IBD, inhibit thiopurine S-methyltransferase (TPMT), an intestinal mucosa and liver enzyme, which is important in thiopurine metabolism.[11] This causes elevations of 6TGN in patients receiving concomitant thiopurines and may lower the 6MMP:6TGN ratio.[12]

However, weight-based dosing of thiopurines, without regard to 5ASA coprescription, remains the norm in clinical practice, with measurement of metabolites reserved for those who fail to respond to therapy.[1]

Body composition measurement with bioelectrical impedance analysis predicts pharmacokinetics of a similar antimetabolite fluorouracil more accurately than standard anthropometric parameters,[13] and low lean body mass has been shown to be a significant predictor of fluorouracil toxicity.[14] The most accessible[15] and accurate[16] means of determining whole-body and regional body composition compartments such as fat-free mass (FFM), fat mass (FM), lean tissue mass, and bone mineral density is dual-energy X-ray absorptiometry (DXA). DXA is often indicated for monitoring of bone mineral density in thiopurine-treated patients who may have disease-related malnutrition, cachexia, or significant corticosteroid exposure.[17] Despite recommendations that patients with IBD undergo regular DXA for monitoring bone mineral density,[18] screening prevalence is

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low: approximately one in five patients.[19] However, abdominal computed tomography (CT) scans are often obtained as part of routine care of Crohn's disease.[20] Analysis of a single cross-sectional image from CT or magnetic resonance imaging (MRI) provides an accurate estimate of total body FFM and FM, as well as visceral FM and subcutaneous FM.[21–24] We have validated this technique in patients with Crohn's disease,[25] with high degrees of correlation between CT or MRI and DXA.

Importantly, there are no published data regarding thiopurine metabolite levels and body composition parameters aside from body weight. We sought to determine whether body composition analysis may provide a more accurate means of dosing to achieve therapeutic metabolite levels.

METHODS

All IBD patients who had undergone thiopurine metabolite level testing at a single tertiary care hospital were identified from pathology databases; this was cross-referenced with radiology records. Local practice is to initiate recommended doses of thiopurines (1.0–1.5 mg/kg body weight for 6MP, 2.0–2.5 mg/kg body weight for azathioprine).[26]

Body composition studies were performed using either whole-body DXA or single-slice analysis of abdominal CT scans. DXA scans were performed on a GE Lunar Prodigy DXA scanner (GE Healthcare, Little Chalfont, UK) with reported body composition data, including weight, height, body mass index, body surface area, appendicular skeletal muscle index, appendicular muscle mass, total body FFM, total body FM, percentage body fat, trunk lean tissue mass, trunk FM, android FM, and gynoid FM. From CT and MRI studies obtained as part of routine clinical care, cross-sectional images from the level of the third lumbar vertebra (L3) and L4–L5 intervertebral disc were analyzed by a single experienced operator using SliceOMatic v.4.3 (Tomovision, Montreal, Quebec, Canada) to measure the area of skeletal muscle, subcutaneous visceral adipose tissue, visceral adipose tissue, and intermuscular adipose tissue. Using published techniques,[22–24,27] estimates of total body FM, FFM, appendicular skeletal muscle index, and waist circumference were reported. CT and DXA data was pooled for analyses. Four subjects in this group had contemporaneous CT and DXA scans, which demonstrated a high degree of correlation, consistent with previously published studies[23,25] (for FFM, Spearman $r=0.97$, $P=0.004$). Chart review was used to obtain weight, height, and thiopurine dose at the time of metabolite measurement. Erythrocyte concentrations of 6TGN, 6MMP, and the ratio between these two measures were reported. Thiopurine S-methyltransferase activity phenotype or genotype was not available for many patients. Clinical response was not recorded in this cohort.

Statistics. Prism 6 (GraphPad Software, La Jolla, CA) was used to perform Spearman correlation analysis between variables, with Kruskal–Wallis, Mann–Whitney and unpaired *t* tests as appropriate to determine differences between subjects grouped by category of metabolites. Receiver-operator characteristic curves were used to identify cutoff drug doses. A P value of <0.05 was considered significant.

Table 1 Demographics of thiopurine-treated patients

Indication	*n*
Crohn's disease	52
Ulcerative colitis	14
Gender	
Male	44
Female	22
Drug	
Azathioprine	49
6MP	17
Drug dose (median (IQR))	
Azathioprine	150 (125–200)
6MP	75 (50–75)
5ASA or sulfasalazine prescribed	26 (42%)
Weight (mean ± s.d.)	75.2 ± 17.6
BMI (mean ± s.d.)	25.4 ± 5.0
Age (mean ± s.d.), years	35.6 ± 14.1
Body composition technique	
CT	48
DXA	18

BMI, body mass index; CT, computed tomography; DXA, dual-energy X-ray absorptiometry; IQR, interquartile range; 5ASA, aminosalicylate; 6MP, 6-mercaptopurine.

Ethics. This project was approved by the Monash Health Human Research Ethics Committee (project 15056Q).

RESULTS

Sixty-six IBD patients were identified as having had either a CT, MRI, or whole-body DXA scan and thiopurine metabolite level testing while being treated with azathioprine or 6MP (Table 1). The mean azathioprine dose was 2.18 mg/kg body weight (s.d. 0.71); the mean 6MP dose was 0.92 mg/kg body weight (s.d. 0.33).

Thiopurine metabolite levels. No correlation was identified between 6TGN levels and any parameter of body composition, absolute drug dose, or drug dose/kg of FM, fat free mass, subcutaneous adipose tissue area, or visceral adipose tissue area.

There were no significant differences in mean 6TGN (309.2 (pmol/8×10^8 red blood cells (RBC) ± 237.7 vs. 394.6 ± 233.1, $P=0.164$), 6MMP (2,465.8 (pmol/8×10^8 RBC) ± 3,210.6 vs. 3,542.7 ± 5,884.4, $P=0.403$), and 6MMP:6TGN ratio (11.3 ± 15.9 vs. 9.6 ± 16.8, $P=0.696$) between those who were not prescribed 5ASA or sulfasalazine and the 42% of patients who were; similarly, the thiopurine dose/kg of body weight was not different between these groups.

6MMP levels showed a weak but statistically significant correlation with dose of azathioprine (Spearman $r=0.409$, $P=0.004$), azathioprine dose/kg of body weight ($r=0.489$, $P<0.001$), and dose of 6MP/kg body weight ($r=0.520$, $P=0.032$).

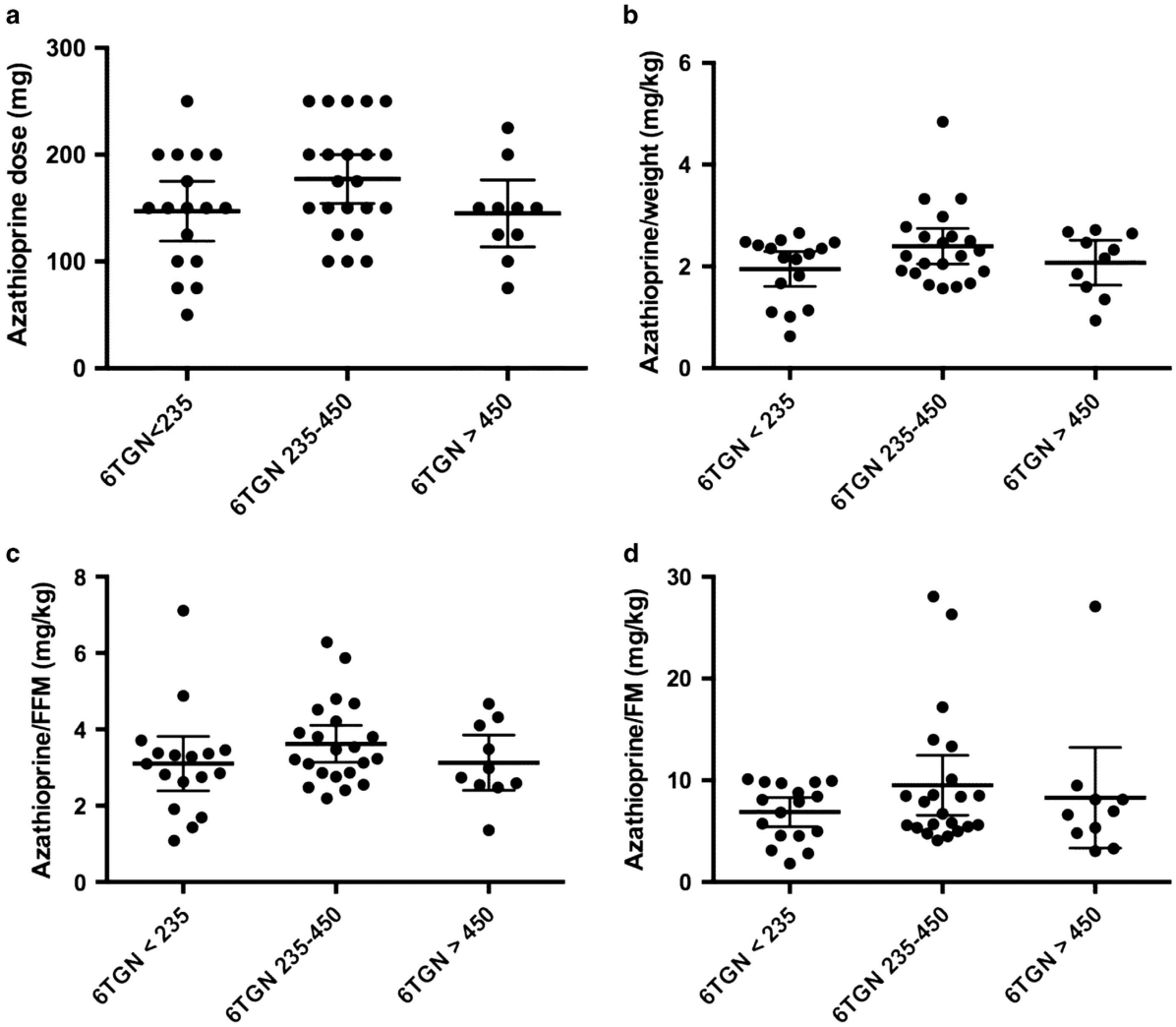

Figure 1 Categories of 6-thioguanine nucleotide (6TGN) levels and dose of azathioprine (the most commonly prescribed thiopurine) (lines: mean ± 95% confidence interval of the mean): (**a**) total azathioprine dose; (**b**) azathioprine dose/kg body weight; (**c**) azathioprine dose/fat-free mass (FFM); (**d**) azathioprine dose/fat mass (FM).

Similarly, there was a weak correlation between 6MMP and several body composition parameters: azathioprine dose/kg of FFM ($r=0.481$, $P<0.001$), azathioprine dose/body surface area ($r=0.507$, $P<0.001$), azathioprine dose/body mass index (BMI) ($r=0.425$, $P=0.002$), and 6MP dose/kg of FFM ($r=0.573$, $P=0.016$), 6MP/BSA ($r=0.513$, $P=0.032$), and 6MP/BMI ($r=0.491$, $P=0.013$).

Using previously defined,[4,28,29] clinically relevant categories of thiopurine metabolites, subjects were classified as having:

(1) Therapeutic 6TGN (6TGN 235–450 pmol/8×10^8 RBC);
(2) Subtherapeutic 6TGN (<235 pmol/8×10^8 RBC);
(3) Supratherapeutic 6TGN (>450 pmol/8×10^8 RBC);

Between these categories, there was no difference in the following parameters: body weight; azathioprine dose; 6MP dose; and dose of either thiopurine per kg of body weight or FM or FFM (Figure 1 and Table 2).

A further two categories were defined utilizing 6MMP levels:
(4) Skewed metabolism,[10] ratio 6MMP:6TGN >20;
(5) Potentially hepatotoxic[5] 6MMP ($>5{,}700$ pmol/8×10^8 RBC)
These categories were not mutually exclusive.

Patients with a 6MMP:6TGN ratio >20 had a higher mean azathioprine dose/FFM (4.12 mg/kg ± 0.97 vs. 3.20 mg/kg ± 1.17, $P=0.011$), with a higher median azathioprine dose (200 mg vs. 150 mg; $P=0.03$); mean azathioprine dose/FFM was higher in the group of patients with 6MMP $>5{,}700$ pmol/8×10^8 RBC (4.15 mg/kg ± 0.97 vs. 3.21 mg/kg ± 1.18, $P=0.017$) (Figure 2,Table 2).

Anthropometry and body composition categories. Categorization of subjects by weight or muscle mass did not predict metabolite profiles. There was no difference in the levels of 6TGN and 6MMP between patients with a healthy range BMI (18.5–24.9 kg/m^2)[30] and those with BMI $\geqslant$ 25 kg/m^2; ($P=0.484$ for 6TGN and $P=0.484$ for 6MMP) nor for patients with sarcopenia—defined as an appendicular

Table 2 Body composition parameters, drug dosing and clinical categories of thiopurine metabolites

	6TGN < 235	235 ≤ 6TGN ≤ 450	6TGN > 450	*P* value
		Mean ± s.d.		
Weight (kg)	75.7 ± 18.1	75.8 ± 17.7	72.4 ± 17.4	0.96
BMI (kg/m^2)	25.72 ± 5.26	25.37 ± 5.12	24.75 ± 4.47	0.96
BSA (m^2)	1.87 ± 0.22	1.88 ± 0.24	1.83 ± 0.25	0.89
Aza (mg; median (IQR))	150 (100–200)	175 (144–213)	150 (119–163)	0.16
Aza/weight (mg/kg)	1.95 ± 0.64	2.40 ± 0.77	2.07 ± 0.62	0.43
Aza/FFM (mg/kg)	3.10 ± 1.39	3.62 ± 1.08	3.13 ± 1.01	0.30
Aza/FM (mg/kg)	6.88 ± 2.80	9.51 ± 6.64	8.29 ± 6.94	0.64
6MP dose (mg; median (IQR))	50 (38–75)	75 (50–106)	50 (25–75)	0.38
6MP/weight (mg/kg)	0.74 ± 0.18	1.03 ± 0.31	0.79 ± 0.68	0.22
6MP/FFM (mg/kg)	1.26 ± 0.34	1.63 ± 0.58	1.40 ± 1.17	0.63
6MP/FM (mg/kg)	2.44 ± 0.89	3.64 ± 1.54	2.19 ± 2.13	0.38
	(6MMP:6TGN) < 20		(6MMP:6TGN) > 20	*P* value
		Mean ± s.d.		
Weight (kg)	75.9 ± 18.0		70.3 ± 14.0	0.59
BMI (kg/m^2)	25.55 ± 5.14		24.28 ± 3.99	0.59
BSA (m^2)	1.88 ± 0.24		1.81 ± 0.22	0.51
Aza (mg; median (IQR))	150 (118–200)		200 (175–200)	0.03
Aza/weight (mg/kg)	2.09 ± 0.72		2.68 ± 0.45	0.02
Aza/FFM (mg/kg)	3.12 ± 1.17		4.20 ± 0.97	0.01
Aza/FM (mg/kg)	8.01 ± 5.41		10.38 ± 7.29	0.33
6MP dose (mg; median (IQR))	75 (50–75)		62.5 (50–75)	0.97
6MP/weight (mg/kg)	0.89 ± 0.34		1.12 ± 0.06	0.29
6MP/FFM (mg/kg)	1.47 ± 0.60		1.69 ± 0.42	0.62
6MP/FM (mg/kg)	2.98 ± 1.32		4.18 ± 2.98	0.62
	6MMP < 5,700		6MMP > 5,700	*P* value
		Mean ± s.d.		
Weight (kg)	76.9 ± 17.6		65.4 ± 14.3	0.09
BMI (kg/m^2)	25.72 ± 5.06		23.41 ± 4.36	0.20
BSA (m^2)	1.90 ± 0.23		1.73 ± 0.22	0.06
Aza (mg; median (IQR))	150 (125–200)		175 (150–200)	0.36
Aza/weight (mg/kg)	2.10 ± 0.72		2.64 ± 0.50	0.05
Aza/FFM (mg/kg)	3.21 ± 1.18		4.15 ± 0.97	0.02
Aza/FM (mg/kg)	8.07 ± 5.42		10.04 ± 7.35	0.46
6MP dose (mg; median (IQR))	69.6 ± 35.6		66.7 ± 14.4	0.92
6MP/weight (mg/kg)	0.87 ± 0.35		1.13 ± 0.05	0.15
6MP/FFM (mg/kg)	1.44 ± 0.61		1.76 ± 0.32	0.36
6MP/FM (mg/kg)	2.95 ± 1.36		3.90 ± 2.16	0.68

Aza, Azathioprine; BMI, body mass index; BSA, body surface area; FFM, fat-free mass; FM, fat mass; IQR, interquartile range; 6MP, 6-mercaptopurine; 6MMP, 6-methylmercaptopurine; 6TGN, 6-thioguanine nucleotide.

skeletal muscle index (calculated from CT or MRI or measured by DXA) >2 s.d. below a young adult mean (i.e., 7.26 kg/m^2 for men and 5.45 kg/m^2 for women[31]) ($P = 0.429$ for 6TGN and $P = 0.607$ for 6MMP).

DISCUSSION

Optimal dosing of thiopurines in IBD is difficult owing to wide variation in metabolism, relatively long time to efficacy, and the high incidence of adverse effects. There is comparatively little research regarding the pharmacokinetics of thiopurine drugs. Absorption from the gut is variable (5–37%).[32] Area under the curve of plasma mercaptopurine concentration over time has been shown to correlate with clinical response and toxicity in a similar clinical setting,[33] but no correlation exists between oral 6MP dose (in mg/m^2 of body surface area) and area under the curve,[34] implying weight and height may not be significant factors in determining therapeutic dosing. In individual patients, a large degree of variability (up to eightfold) has been observed from day-to-day in area under the curve,[35] which may reflect the variable effect of food intake and metabolic enzyme activity. TPMT activity does not predict preferential metabolism to 6MMP; however, the largest study correlating metabolite profile and TPMT activity found a skewed metabolism at all activity levels.[10] On an individual patient basis, 6TGN levels remain stable throughout the dosing interval.[35] There is considerable interpatient variability in 6TGN levels with thiopurine dosing based on weight[36] or body surface area,[37] with only a weak correlation ($r = 0.28$) demonstrated between thiopurine dose (mg/kg) and 6TGN levels in a group of patients who had ongoing symptoms despite stable thiopurine therapy.[3] A randomized control trial examining clinical outcomes in patients dosed by weight compared with those dosed by metabolite level monitoring found no difference in thiopurine metabolite levels between

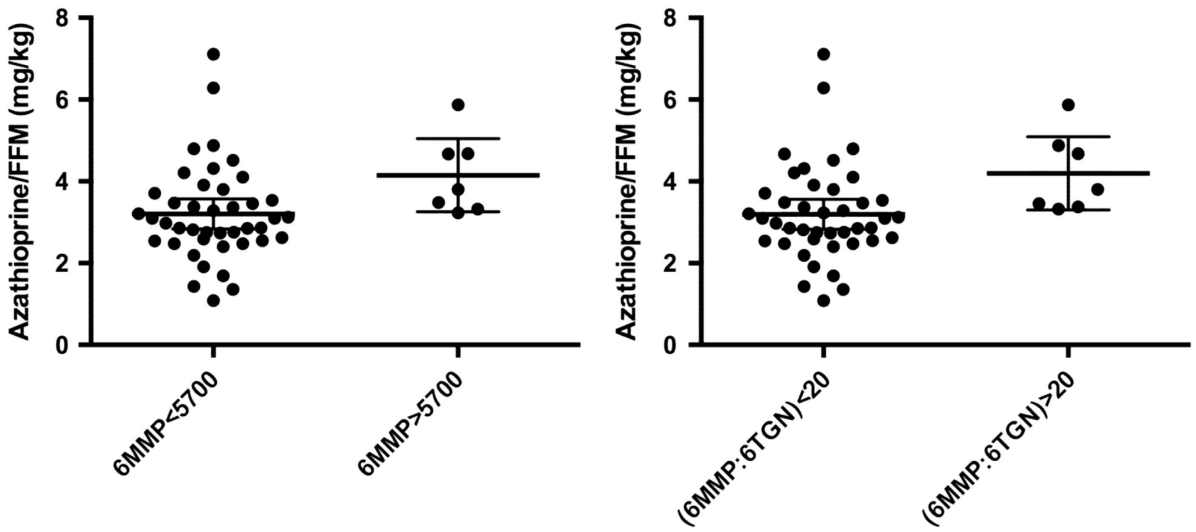

Figure 2 Azathioprine dose per kg of fat-free mass (FFM) by categories of 6-methylmercaptopurine (6MMP) and 6MMP:6TGN (6-thioguanine nucleotide) (lines: mean ± 95% confidence interval of the mean).

groups despite those in the weight-based group receiving more drug; there was no difference in clinical outcomes.[38]

Of interest in our study, the dose of azathioprine per kilogram of body weight—the accepted method of dosing azathioprine in IBD[39]—did not predict whether subjects were likely to have metabolites in the therapeutic range. This finding mirrors similar data from a number of retrospective studies[40] and a recent randomized controlled trial.[38] We found that no other body composition parameter predicted therapeutic metabolites.

This study is novel in seeking to determine a relationship between body composition compartments and thiopurine metabolite levels. Limitations of this retrospective cohort study are the lack of thiopurine S-methyltransferase genotyping or phenotyping, clinical efficacy end points, medication compliance, and data regarding the reason for metabolite testing. Although routine thiopurine dosing according to metabolite levels is becoming more common,[41] some of the subjects may have been tested owing to treatment failure or intolerance—with a possible inherent selection bias toward non-therapeutic or toxic metabolite levels.

A recent retrospective cohort study suggested an inverse relationship between BMI and 6TGN levels,[40] with the authors surmising that adipose tissue distribution of thiopurines may be an important factor in metabolism. Visceral and subcutaneous adipose tissue have distinct metabolic profiles,[42] with cross-sectional measurement predictive of total body volumes.[21,43] However, our analysis did not find a relationship between metabolites and total adipose tissue mass, visceral adipose tissue area, or subcutaneous adipose tissue area or dose of thiopurine divided by these areas.

Prediction of drug toxicity may help to avoid adverse effects causing delayed or discontinued therapy. We found that the likelihood ratio of a 6MMP > 5,700 pmol/8×10^8 RBC was 2.00 at a cutoff azathioprine dose > 3.04 mg/kg FFM (100% sensitivity, 50% specificity, $P = 0.019$). In a similar clinical setting, weight-based dosing was again found not to improve rates of therapeutic 6TGN levels but was associated with shunting toward 6MMP;[41] this finding supports those of our study, although external validation is required owing to potential overlap of subjects between data sets. Identification of patients at higher risk of toxicity by pretreatment anthropometric or body composition measures would be useful: although no such predictors were found in this study, a trend toward lower body weight and BMI in patients with undesirable metabolite profiles further implies that larger relative drug doses may cause shunting toward 6MMP.

No relationship was found between therapeutic metabolite levels and weight or body composition compartments. Higher doses of thiopurines, especially in relation to FFM, are associated with higher levels of potentially hepatotoxic 6MMP and shunting toward this metabolite. Conventional weight-based dosing to attain therapeutic metabolite levels appears unreliable and may be replaced by metabolite level testing.

CONFLICT OF INTEREST

Guarantor of the article: Darcy Q. Holt, MBBS, FRACP.
Specific author contributions: Darcy Q. Holt, Boyd J.G. Strauss and Gregory T. Moore conceived the study. Darcy Q. Holt collected and analyzed the data and wrote the paper, with assistance and revision from Boyd J.G. Strauss and Gregory T. Moore. All authors approved the final version of the article, including the authorship list.
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Potential competing interests: None.

Study Highlights

WHAT IS CURRENT KNOWLEDGE

- ✓ Thiopurines are accepted treatment for inflammatory bowel diseases and are conventionally dosed according to body weight.
- ✓ Erythrocyte concentrations of thiopurine metabolites are associated with treatment response or toxicity.
- ✓ Previous small studies have shown a lack of association between thiopurine metabolite levels and dose by body weight, but body composition parameters have not been examined.

WHAT IS NEW HERE

- ✓ This study demonstrates that therapeutic metabolite levels do not correlate with thiopurine dose by body weight or body composition parameters.
- ✓ Potentially hepatotoxic metabolites correlate with dose by weight and fat-free mass.

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