Investigating genetic determinants of plasma inositol status in adult humans

Eleanor Weston[1], Faith Pangilinan[2], Simon Eaton[1], Michael Orford[1], Kit-Yi Leung[1], Andrew J Copp[1], James L Mills[3], Anne M Molloy[4], Lawrence C Brody[2], Nicholas DE Greene[1]

[1] Developmental Biology and Cancer Department, Great Ormond Street Institute of Child Health, University College London, London, UK

[2] Genetics and Environment Interaction Section, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, USA

[3] Epidemiology Branch, Division of Population Health Research, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD, USA

[4] Department of Clinical Medicine, School of Medicine, Trinity College, Dublin, Ireland.

Sources of support: This study was funded by Sparks and Great Ormond Street Hospital Children's Charity (17ICH03), Action Medical Research (GN2656) and MRC (N003713). Funders had no involvement in study design, data collection and interpretation, writing of the report or submission of the report for publication.

Conflicts of Interest: The authors declare no conflicts of interest.

*Corresponding author: NDE Greene, Developmental Biology & Cancer Department, UCL Great Ormond Street Institute of Child Health, 30 Guilford Street, London, WC1N 1EH, UK.

n.greene@ucl.ac.uk Tel: +44 2079052217 orcid.org/0000-0002-4170-5248

Running title: Determinants of plasma inositol

Abbreviations: MI, *myo*-inositol; DCI, *D-chiro*-inositol; GC-MS, gas chromatography mass spectrometry; SNP, single nucleotide polymorphism; GWAS, genome-wide association study;

ISYNA1, inositol-3-phosphate synthase 1; IMPA1, inositol monophosphatase 1; LAPTM4B, lysosomal protein transmembrane 4 beta; MIOX, myo-inositol oxygenase; MTDH, metadherin; PIP_2, phosphatidylinositol (4,5)-biphosphate; SLC5A11, Solute Carrier Family 5 Member 11; SMIT2, sodium-dependent *myo*-inositol transporter 2; ZP2, Zona Pellucida 2.

Abstract

Background: *Myo*-inositol (MI) is incorporated into numerous biomolecules, including phosphoinositides and inositol phosphates. Disturbance of inositol availability or metabolism is associated with various disorders, including neurological conditions and cancers, while supplemental MI has therapeutic potential in conditions such as depression, polycystic ovary syndrome and congenital anomalies. Inositol status may be influenced by diet, synthesis, transport, utilisation and catabolism.

Objective: We aimed to investigate potential genetic regulation of circulating MI status and to evaluate correlation of MI concentration with other metabolites.

Methods: Gas chromatography mass spectrometry was used to determine plasma MI concentration of more than 2,000 healthy, young adults (aged 18-28 years) from the Trinity Student Study. Genotyping data was used to test association of plasma MI with SNPs in candidate genes, encoding inositol transporters and synthesising enzymes, and test for genome-wide association. We evaluated potential correlation of plasma MI with D-chiro inositol, glucose and other metabolites by Spearman's rank correlation.

Results: Mean plasma MI showed a small but significant difference between males and females (28.5 and 26.9 μM, respectively). Candidate gene analysis revealed several nominally significant associations with plasma MI, most notably for *SLC5A11*, encoding a sodium-coupled inositol transporter, also known as SMIT2 (sodium-dependent *myo*-inositol transporter 2). However, these did not survive correction for multiple testing. Subsequent testing for genome-wide association with plasma MI did not identify associations of genome-wide significance ($p< 5x10^{-8}$). However, 8 SNPs exceeded the threshold for suggestive significant association with plasma MI concentration ($p<1x10^{-5}$), 3 of which were located within or close to genes: *MTDH*, *LAPTM4B* and *ZP2*. We found significant positive

correlation of plasma MI concentration with concentration of *D-chiro*-inositol and several other biochemicals including glucose, methionine, betaine, sarcosine and tryptophan.

Conclusion: Our findings suggest potential for modulation of plasma MI in young adults by variation in *SLC5A11* which is worthy of further investigation.

Keywords: myo-inositol; chiro-inositol; genome-wide association study; mass spectrometry; glucose; inositol transporter

Introduction

Nutrient sufficiency is a key determinant of health during development and post-natal life. Among such molecules, numerous functions have been identified for inositol (cyclohexanehexol), a six-carbon sugar alcohol whose nine possible stereoisomers form a subgroup of cyclitols of which *myo*-inositol (MI) is the predominant naturally occurring form (1,2).

MI is incorporated into a wide range of molecules including phosphatidylinositol, inositol phosphates, inositol glycans and inositol sphingolipids (3,4). As a result, MI is involved in key physiological functions via several distinct signalling pathways (5-8). For example, classical phosphoinositide signalling involves cleavage of phosphatidylinositol (4,5)-biphosphate (PIP_2) to generate diacylglycerol and inositol triphosphate (IP_3), inducers of protein kinase C activity and calcium release, respectively. Further phosphorylation of PIP_2 generates PIP_3 that mediates additional signalling, including activation of AKT1 (serine threonine kinase 1). Phosphoinositides play additional roles in regulation of membrane trafficking and protein-membrane interactions. Inositol is also a component of glycosylphosphatidylinositol (GPI) anchors which mediate protein-membrane attachment or can be cleaved by GPI-specific phospholipases to generate inositol phosphoglycans which are reported to have second messenger properties, including as insulin mimetics (9).

Dysregulation of inositol availability, inositol phosphate metabolism or phosphoinositide signalling networks are associated with a range of disorders including neurological and psychiatric conditions, cancers and Lowe oculo-cerebro-renal syndrome (7,8,10). Enzyme components of inositol metabolism therefore represent drug targets in various conditions.

Supplemental MI may also have beneficial effects in conditions such as depression, anxiety and polycystic ovary syndrome, while supplementation during pregnancy may prevent or ameliorate gestational diabetes(11-13).

In addition, there is a functional requirement for MI during embryonic development. Inositol deficiency inhibits neural tube closure in mouse embryos, leading to cranial neural tube defects (NTDs), which are also common congenital anomalies of the central nervous system in humans. Conversely, MI supplementation in mice can prevent NTDs in genetic and diabetes-induced models of NTDs (4), as well in a mouse genetic model of NTDs induced by folate-deficiency (14). Clinical use of MI in pilot studies and a randomised clinical trial suggest that this protective effect may also be replicated in high-risk pregnancies in humans (15,16). The requirement for MI in neural tube closure is yet to be clearly defined and could potentially involve one or more of its intracellular functions. For example, possible involvement of inositol phosphate signalling is suggested by the finding that in mice cranial NTDs can be caused by mutation of *Itpk1* (inositol 1,3,4-triphopsphate 5/6 kinase), *Pip5k1c* (phosphatidylinositol-4-phosphate 5-kinase type gamma) or *Inpp5e* (inositol polyphosphate-5-phosphatase E), encoding an inositol phosphate kinase, inositol phosphoinositide kinase and an inositol phosphoinositide phosphatase, respectively (17-19). Moreover, a maternal polymorphism in *ITPK1* may be associated with human NTDs (20).

MI status is determined by endogenous synthesis, intake in the diet, uptake and intracellular transport, utilisation within tissues and catabolism (1). *De novo* synthesis of MI from glucose is achieved in various tissues via sequential phosphorylation to glucose 6-phosphate, conversion to *myo*-inositol 1-phosphate and dephosphorylation to *myo*-inositol mediated by

action of hexokinase (HK), inositol-3-phosphate synthase 1 (ISYNA1) and inositol monophosphatase 1 (IMPA1), respectively (Figure 1A) (1,3,4). Free MI can also be recycled intracellularly from inositol phosphates by the action of inositol poly- and mono-phosphatases. It has been estimated that MI synthesis in the human kidney is around 2 g per day, which exceeds the typical dietary intake of around 1 g per day (1,21), and a minimal requirement for dietary MI has not been determined (13). Nevertheless, deficiency or supplementation is sufficient to modulate circulating MI levels in various animal models. Despite access to maternal inositol, MI synthesis within the embryo appears essential as *Impa1* null mice show partially penetrant lethality, which can be rescued by maternal MI supplementation, while surviving mice exhibit behavioural abnormalities (22). In humans, *IMPA1* mutation is associated with intellectual disability and abnormal EEG (23,24).

MI catabolism is mediated by *myo*-inositol oxygenase (MIOX) in the kidney, generating glucuronic acid (1,25). In addition to shared (and potentially competing) use of some transporters, a potential effect of glucose on MI status is further highlighted by the observation that hyperglycemia and/or diabetes are associated with up-regulation of *MIOX* (26).

MI and MI derivatives have been the primary focus of research on inositol functions and potential therapeutic application, and *D-chiro*-inositol (DCI) can have similar effects, while effects that are distinct from MI have also been observed. For example, like MI, DCI has been found to be effective in prevention of NTDs in mice (27) and combined treatment of DCI with MI may be beneficial in polycystic ovary syndrome although the ideal ratio and mechanism of action are still to be fully established (28,29).

Inositol transport is mediated by a proton-coupled inositol transporter (encoded by *SLC2A13*) and two higher affinity sodium ion-coupled transporters (sodium/myo-inositol cotransporter 1 and 2), encoded by *SLC5A3* and *SLC5A11* (30). Both SLC2A13 and SLC5A11 can also transport DCI, and SLC5A11 also transports some other hexoses including glucose. Exposure of the developing embryo or fetus to inositol is dependent on maternal circulating inositol and transport across the yolk sac and placenta (30). At pre-implantation stages, MI uptake is predominantly by sodium-coupled transport, and subsequently all three inositol transporters are expressed in the human yolk sac and placenta (30). Hence, all three transporters may contribute to support of MI-dependent processes required for embryonic development. In mice, loss of *Slc5a3* results in MI depletion in the fetus and early post-natal lethality (31,32).

Other than intake in the diet, the transport, synthesis and metabolism of MI represent multiple possible points at which inositol status could be modulated by functional variation in the molecular mediators of these processes. The combination of biochemical assays with genome-wide association studies offers potential to identify genetic determinants of nutrient status (33-35). In the current study, we adapted mass spectrometry-based methodology to enable high throughput quantification of inositol in plasma and examined potential genetic associations with plasma MI. Owing to the relationship of MI with glucose, and therefore other metabolites related to central carbon and one-carbon metabolism, we also evaluated the potential correlation of MI with glucose and other metabolites, as well as DCI.

Methods

Study Participants

Samples were collected as part of the Trinity Student Study (TSS), in which a cohort of healthy young adults (aged 18-28 years) were recruited at Trinity College Dublin over a one year period (2003-2004), as described previously (36-38). Briefly, 2,508 individuals were eligible to participate, gave written informed consent and completed a questionnaire including parameters of age, sex, height, weight, dietary habits (meat eating, vegetarian or vegan), micronutrient intake from supplements and fortified foods, usual alcohol intake, smoking status and contraceptive use among female participants. A non-fasting blood sample was collected for metabolite quantification and extraction of DNA. Blood samples were collected into serum tubes and EDTA-coated tubes for measurement of metabolites, processed and frozen at -80^{o}C within 3 hours of collection, and stored until analyzed. Ethical approval was obtained from the Dublin Federated Hospitals Research Ethics Committee, affiliated with Trinity College Dublin. The study was reviewed by the Office of Human Subjects Research at the NIH and by the Research and Innovation Office at UCL Great Ormond Street Institute of Child Health.

Quantification of *myo*- and *D-chiro*-Inositol

Among TSS samples, sufficient plasma was available for analysis of inositol in 2,064 individuals. MI and DCI were analysed by gas chromatography mass spectrometry (GC-MS), by adaptation of a published method, with generation of a hexa-acetyl derivative of inositol (39). In addition to inositol enantiomers, this method also allowed simultaneous quantification of glucose in plasma samples.

Sample preparation and derivatisation: Plasma samples (50 µl) were mixed with 50 µl internal standard (100 µM *myo*-inositol-d_6, 12.5 mM glucose-d_2; Sigma) and 1 ml methanol, and centrifuged. Supernatants were collected, dried by lyophilisation and stored at -70°C prior to analysis. Samples were derivatised by addition of 100 µl of 0.1 mg 4-(Dimethylamino)pyridine (DMAP; Fluka) and 100 µl acetic anhydride and incubation at 80°C for 30 minutes. A series of internal standards (0-200 µM MI and 0-25 mM glucose) were prepared and derivatised as above after addition of internal standard solution.

Gas chromatography mass spectrometry (GC-MS): Samples were analysed on a GC-MS system comprising Triplus sample autosampler, Trace GC Ultra Gas Chromatography, DSQII mass spectrometer, operated with XCalibur V 3.0.63 Software (all ThermoFisher Scientific). GC used an Rxi-5Sil MS fused silica (5% diphenyl / 95% dimethylpolysiloxane), 30 m x 0.25 mm I.D, 0.25 µm film thickness column (RESTEK). The inlet temperature, MS transfer line and ion source were set to temperatures of 280˚C, 250˚C and 225°C respectively. The split ratio was 1:12. The carrier gas (helium) was set to a flow rate of 0.8 ml/min. Detection was in positive chemical ionization mode with reagent gas (methane) at 2 ml/min. GC-MS was performed using a temperature gradient from 200-320 °C. Inositol enantiomers (*myo*, *D-chiro*, *allo*, *muco* and *scyllo*) were separated on the basis of retention time (Supplementary Fig. 1). The glucose in the sample was derivatised and showed different retention time to inositol (Supplementary Fig. 2).

Analysis: Selected Ion Monitoring (SIM) was used for collecting data on inositol enantiomers (M/Z 372.5 – 373.5), *myo*-inositol-d_6 (378.5 – 379.5), glucose (330-331.5) and glucose-d_2

(332.5-333.5). Peak areas corresponding to MI, DCI, MI-d_6, glucose and glucose-d_2 were determined by peak integration. Standard curves were constructed by plotting the ratio of inositol (MI or DCI)/MI-d_6 and glucose/glucose-d_2 (Supplementary Fig. 3).

Samples were analysed in batches of 100-200. As an internal quality control, two pools of plasma were prepared with inositol concentrations towards the upper and lower ends of the range. These samples were aliquoted and frozen for replicate analysis in each GC-MS run, for determination of between day (inter-assay) variance (CV was ≤ 7.8% for myo-inositol).

Genotyping

To ask whether there is a genetic influence on concentration of MI in plasma, we made use of the curated SNP genotyping dataset from the TSS cohort (33). Genotyping, quality control, and preparation of the data have been described in detail (33). Briefly, DNA samples from participants with high quality DNA (n = 2,490) were genotyped using Illumina 1M HumanOmni1Quad_v1-0_B chips at the Center for Inherited Disease Research (CIDR; Baltimore, MD). The call rate was ≥ 98% and minor allele frequency > 0.01. The final, clean dataset consists of genotype data for 757,577 single nucleotide polymorphisms (SNPs) for 2,232 participants. This has previously allowed study of genetic associations with multiple metabolites in plasma and serum in the TSS cohort (33).

Biochemical analyses

Methionine (interassay CV = 3.4%), serine (interassay CV = 5.7%), glycine (interassay CV = 3.3%), and cystathionine (within-day CV = 4-6%) in serum were measured by GC-MS, serum

sarcosine and tryptophan (interassay CV ≤4.8%) were measured by GC-tandem MS, and serum betaine (within-day CV = 4-6%) and dimethylglycine (interassay CV <10%) were measured by liquid chromatography tandem MS. These measurements were performed by Bevital, Norway (www.bevital.no)(40). The remaining metabolites were measured as previously described. Briefly, serum folate, red cell folate, and serum vitamin B_{12} were measured by microbiological assay (41,42), with between-assay CVs of <11.0%. Serum holotranscobalamin was measured using an AxSYM analyzer (43); the between-assay CV was <11.1%. Serum methylmalonic acid and homocysteine were measured by automated isotope-dilution GC-MS as previously described (33), with inter-assay CVs of 8.1% and 2.2%, respectively. Formate (inter-assay variance of 7.4%) was measured in serum by GC-MS, as previously described (35).

Statistical analysis

Statistical analysis was performed using OriginPro 2019 and SigmaStat v3.5 software. Mean plasma concentrations were compared between sexes by t-test. Median plasma concentrations were compared between smoking/non-smoking and oral contraceptive/non-use groups by Mann Whitney Rank Sum test. The log transformed concentration of MI showed a normal distribution. As other metabolites did not all show a normal distribution, Spearman's rank correlation was used to test for correlation between MI and other metabolites.

Log-transformed MI measurements were used to identify genome-wide association signals. Linear regression with an additive genetic model incorporating age and sex as covariates was performed in PLINK v1.07 (http://zzz.bwh.harvard.edu/plink/ (44). Genome-wide

associations were considered significant at $p < 5x10^{-8}$ or suggestive at $p < 1x10^{-5}$. Ten candidate genes were selected based on potential involvement in inositol transport, synthesis and catabolism. Variants within each candidate gene and its 10 kb flanks were considered. Measures of linkage disequilibrium (D', r^2) were generated and tagSNPs (r^2<0.8) were identified based on genotyping data from the TSS using Haploview (https://www.broadinstitute.org/haploview/haploview)(45). This approach allows the evaluation of multiple SNPs in or near the same gene, while being able to discern the presence of independently acting SNPs. Significance was set at alpha=0.05 for all statistical tests. Evaluating significance in the context of multiple tests was performed by defining tagSNPs (r^2<0.8) as independent, and using Bonferroni correction to adjust the threshold for significance in each of the candidate gene groups.

Results

Inositol quantification

Sufficient samples were available for determination of the concentration of myo-inositol by GC-MS in plasma from 2,064 participants in the Trinity Student Study (TSS) (Figure 1B). Plasma myo-inositol concentration ranged from 10.3 to 83.4 µM (mean 27.6 ± 6.4 µM; median 26.6 µM) and log-transformed values showed a normal distribution (Figure 1C). This range of values was consistent with typically reported concentrations, and plasma MI values (26-43 µM) which we previously determined using LC-MS/MS methodology in a control group of healthy adults (30,46).

Among the study cohort of 1258 females and 806 males, we observed a significant difference in mean plasma MI between sexes, with a shift towards higher values in males

(mean ± standard error, 28.5 ± 0.2 vs 26.9 ± 0.2 μM; p <0.001)(Fig. 1D). Correlating with this observation, we found positive correlations of plasma MI with height and weight, but not with BMI, in the overall cohort (but not within sexes)(Supplementary Table 1). There was also a correlation of plasma MI with age in the overall cohort (Table 1). On the basis of these findings, sex and age were included as covariates in genetic association studies. No association with plasma MI concentration was found for alcohol intake, smoking, or use of oral contraceptives (in females) (Table 1-2).

Investigating genetic association with plasma *myo*-inositol status

SNP genotype data were available for 2,064 individuals for whom MI was determined. We first investigated candidate genes selected on the basis of encoding proteins involved in inositol transport, as well as glucose transporters owing to the potential antagonistic interplay between inositol and glucose transport (Table 3). Genotyped SNPs within 10 kb of these genes were extracted for candidate gene analysis, encompassing 176 SNPs of which 106 were considered independent tagSNPs (r^2<0.8).

Among these candidate genes, the most striking finding was for *SLC5A11*, for which nominal association with plasma MI was found for four SNPs (RS234605, p = 0.0247; RS1718990, p = 0.0032; RS28540434, p = 0.0008; RS4788439, p = 0.0015); the latter three SNPs are not independent (r^2 > 0.8). However, none of these SNPs showed significant association with MI, following adjustment of the threshold for significance to account for multiple tests (106 independent SNPs; Bonferroni-adjusted p-value is p<0.0005 by study), although RS28540434 approached significance. Nominally significant association with MI (p<0.05) was also found

for five SNPs in *SLC2A13*, and one SNP in each of *SLC5A1* and *SLC5A2* but none survived correction for multiple testing.

We also assessed additional candidate genes related to inositol synthesis (*ISYNA1*, *IMPA1*) and catabolism (*MIOX*). Among 37 SNPs in these genes, 24 SNPs represented independent tagSNPs (r^2<0.8). A nominally significant association between MI and *ISYNA1* (p<0.05 for each of 6 SNPs; represented by two tagSNPs) was identified but was not significant following correction for multiple testing. We also selected two genes encoding enzymes that are responsible for production of highly phosphorylated inositol (Table 3). These include *ITPK1*, encoding an inositol triphosphate kinase, which has a possible association with NTDs, and *IMPK*. Among 65 SNPs in these two genes, 44 SNPs represented independent tagSNPs (r^2<0.8). Nominally significant association with MI (p<0.05) was found for two SNPs in strong linkage disequilibrium (r^2 = 0.93) in *ITPK1* but did not survive correction for multiple testing. Hence, we did not find strong evidence to suggest that common variation in these genes influences circulating MI levels.

We next tested for genome wide association with MI concentration. A quantile-quantile (Q-Q) plot of p-values for SNP associations with log-transformed MI, adjusted for age and sex did not show deviation between expected and observed values, until the very lowest observed p-values, suggesting that there are no major confounders in the data (Figure 2B). Statistical significance in the GWAS is shown in a Manhattan plot (Figure 2A). No SNPs showed p-values above the threshold for genome-wide statistical significance ($p < 5x10^{-8}$). However, 8 SNPs exceeded the threshold that defined suggestive significant association with MI concentration in plasma (at $p<1x10^{-5}$)(Table 4). The genes associated with three of these

SNPs encode metadherin (*MTDH*, chromosome 8), lysosomal protein transmembrane 4 beta (*LAPTM4B*, chromosome 8) and zona pellucida 2 (*ZP2*, chromosome 16). Additional SNPs that showed suggestive significance were in intergenic regions (more than 10 kb from the closest annotated genes).

Correlation of myo-inositol concentration with D-chiro inositol and other metabolites in plasma Among individuals for whom MI was determined, D-chiro-inositol (DCI) concentration in plasma was above the limit of detection for 1,066 of the samples. The mean plasma concentration of DCI among these samples was 8.5 ± 9.2 µM (with a range from 0.04-89 µM, median 5.6 µM) compared with 29.5 ± 6.8 µM (range 15.4- 70.3 µM, median 28.4 µM) for MI in this group. GWAS analysis did not reveal potential genetic modifiers of DCI with genome wide or nominal significance. However, DCI concentration showed a significant positive correlation with MI ($P<6 \times 10^{-90}$, Spearman rank correlation)(Figure 3A).

Owing to the biochemical link between MI and glucose as well as sharing of some transporters, we next asked whether MI status correlates with non-fasting glucose (Figure 3B). We also examined possible correlation with other metabolites. We particularly focussed on metabolites linked to one-carbon metabolism owing to the potential biochemical relationship between MI and serine, which share a biosynthetic precursor in glucose 6-phosphate (Table 5 and examples in Figure 3C-D). Interestingly, we observed correlation of MI with glucose, methionine, cystathionine, betaine, dimethylglycine, sarcosine, tryptophan, serum and red cell folate, glycine, formate and holotranscobalamin but not serum vitamin B_{12}, serine, methylmalonic acid or homocysteine.

Discussion

Myo-inositol, via phosphoinositides and inositol phosphates, plays a key role in numerous cellular functions and is implicated in a range of diseases. We found a small but significant difference in plasma MI in females and males. The biological significance of this difference is as yet unknown. High throughput analysis of plasma MI by GC-MS provided an opportunity to investigate potential genetic modulators of MI status. Key candidate genes included those encoding the sodium-coupled (SMIT1 and SMIT2) and proton coupled (HMIT) active MI co-transporters, encoded by *SLC5A3*, *SLC5A11* and *SLC2A13*, respectively (47). Among these candidate genes, four SNPs covered by two tagSNPs and linked to *SLC5A11* were nominally associated with plasma MI (e.g. p = 0.0008 for rs28540434). *SLC5A11* is expressed in the gastrointestinal tract and kidney and has been found to be responsible for apical MI transport in the rat intestine (48,49). Hence, variation in expression or function of this gene represents a plausible mechanism by which plasma MI could be modulated.

Another *SLC5A11* variant, rs11074656 has been examined for potential effects on blood MI among children with spina bifida and their mothers (50). Although MI did not differ by analysis of all genotypes, a sub-analysis suggested an association of the TT genotype (encoding V182A) with lower MI compared with the CC genotype among the mothers (50). No genetic association of this polymorphism with spina bifida has been reported to date but evaluation of *SLC5A11* in larger scale studies, that also include cranial NTDs, may be warranted.

Unbiased investigation of potential genetic modifiers of plasma MI by GWAS did not reveal loci of genome-wide significance. However, we found nominally significant associations of plasma MI with SNPs located in or near three genes. *MTDH*, which encodes metadherin, also known as *AEG1* (Astrocyte Elevated Gene 1) or *LYRIC* (lysine-rich CEACAM-1 co-isolated protein), is a transmembrane protein that has been implicated in multiple biological processes (51,52). In particular, MTDH is over-expressed in many cancers, including liver, brain and breast cancers, and is associated with metastasis and tumour progression with a direct role being indicated by experimental models in mouse (51,53). In addition to cancer, over-expression of MTDH has been noted in CNS disease including Huntington's disease and migraine (52,54). MTDH may act via multiple downstream pathways with functional effects having been identified in Wnt/β-catenin, NF-κB, MAPK/ERK, retinoid and PI3-kinase/Akt signalling (53). Although functionally linked to PI3-kinase signalling it is unclear whether MTDH could affect MI status, but the pleiotropic effects of MTDH suggest multiple possible mechanisms by which such an effect could occur. It is not yet clear whether and how plasma MI is associated with cancer risk but supplemental MI and (inositol hexaphosphate (phytic acid; IP_6) have been suggested to be protective against several cancer types, including colorectal colon, mammary and liver (2).

LAPTM4B is a transmembrane protein involved in endosomal sorting and regulation of proteins such as EGFR, whose degradation it suppresses (55). LAPTM4B over-expression has been identified as a prognostic marker in various cancers, with potential effects via modulation of cell proliferation and/or autophagy (56-58). A potential interaction between LAPTM4B and phosphoinositide metabolism is shown by the finding that it can bind and is

regulated by the endosomal PIP kinase, PIPKIγi5, and its product $PI(4,5)P_2$ (55), providing a means which by EGFR trafficking is modulated.

The other gene with nominally significant association with plasma MI, *ZP2*, encodes one of the glycoprotein components of the zona pellucida, the extracellular matrix that surrounds the mammalian oocyte. Human ZP2 appears to have a particular function in sperm-oocyte binding (59,60), and mutation may cause infertility. Although MI signalling, via the IP_3 receptor, is involved in oocyte maturation, and inositol status is thought to modulate fertility in females and males (12,61), it is not clear how a *ZP2*-associated variant could affect circulating MI.

Overall, our study did not indicate a strong genetic modifier of plasma MI. We speculate that tissue MI may be subject to greater influence of genetic variation as concentration in tissues may be significantly higher than blood owing to active transport (47). For example, some tissues are reported to have a higher intracellular MI concentration than plasma, including brain, pituitary and kidney, suggesting tissue-specific regulation (21,25). The relative contribution of endogenous synthesis (active in kidney for example) and MI from dietary sources also varies between tissues. Although dietary intake may account for less than half of MI tissue content, this source is likely to vary widely between individuals. Dietary MI comes from free MI, which is abundant in citrus fruits, IP_6 which is enriched in nuts and seeds, and inositol-containing phospholipids present in animal and plant tissue. We did not have dietary data on the TSS cohort that would allow us to consider this variability.

In addition to potential genetic modulators, we also examined potential correlation of plasma MI with other metabolites and nutrients. Correlation, rather than regression analysis, was performed to encompass the possibility that plasma MI influenced or was influenced by the other variable or that both were influenced by a third variable. Each of the correlations that we observed were positive. While r-values were generally low, this was not unexpected, given the inherent diversity in a human cohort and use of non-fasting samples. Nevertheless, the sample size was large enough to identify significant correlations.

The closest correlation was observed between MI and DCI, in terms of p-value and r. This suggests potential for shared regulation of circulating levels and/or overlap of dietary sources. A possible direct link between MI and DCI concentration within tissues could result from interconversion of MI and DCI which has been reported to occur via epimerase action (62,63), although the extent to which this activity occurs *in vivo* is not well understood. Analysis of plasma DCI in MI-supplemented individuals and vice versa will provide further insight into the relationship of circulating MI and DCI.

The relationship between glucose and MI status has been unclear. MI can be synthesised from D-glucose via glucose 6-phosphate (Figure 1A), suggesting the potential for positive co-regulation of MI and glucose. On the other hand, hyperglycemia is associated with depletion of tissue MI in cultured rodent embryos (64) and diabetes models (65). This could potentially result from competition for the same transporter, SLC5A11 being an example of both an inositol and glucose transporter. We therefore explored the relationship between plasma MI and glucose concentration and found a significant positive correlation of MI and

glucose concentration in plasma. However, the Spearman's r value of <0.2 suggests that the plasma myo-inositol concentration is not highly dependent on glucose concentration.

Among the other metabolites for which we observed significant correlations in plasma or serum concentrations, the majority of those with higher r values are related to the methionine cycle, including methionine itself. These include betaine, which is a methyl donor in generation of methionine from homocysteine, and its products dimethylglycine and sarcosine (methylglycine) as well as cystathionine, which is generated from homocysteine in the transulfuration pathway. Future studies should examine potential links between inositol and one-carbon metabolism.

Acknowledgements: The Trinity Student GWAS and metabolite analysis was supported by the Health Research Board, Dublin, Ireland and the Intramural Research Programs of the National Human Genome Research Institute and the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health, USA. Research at UCL was supported by the National Institute for Health Research Biomedical Research Centre at Great Ormond Street Hospital for Children NHS Foundation Trust and University College London.

Contributions: Experimental design: NG, LB, AM, FP, JM, AC; Experimental work; EW, SE, MO, KYL; Data analysis: EW, FP, LB, NG; Data interpretation: FP, LB, NG, AC, JM, AM; Drafting manuscript: EW, NG, FP: All authors contributed to edits and content of final manuscript. The authors have no conflicts of interest to declare.

References

1. Holub BJ. Metabolism and function of myo-inositol and inositol phospholipids. Annu Rev Nutr 1986;6:563-97.

2. Weinberg SE, Sun LY, Yang AL, Liao J, Yang GY. Overview of Inositol and Inositol Phosphates on Chemoprevention of Colitis-Induced Carcinogenesis. Molecules 2020;26.

3. Livermore TM, Azevedo C, Kolozsvari B, Wilson MS, Saiardi A. Phosphate, inositol and polyphosphates. Biochem Soc Trans 2016;44:253-9.

4. Greene ND, Leung KY, Copp AJ. Inositol, neural tube closure and the prevention of neural tube defects. Birth Defects Res 2017;109:68-80.

5. Sun Y, Thapa N, Hedman AC, Anderson RA. Phosphatidylinositol 4,5-bisphosphate: targeted production and signaling. Bioessays 2013;35:513-22.

6. Bilanges B, Posor Y, Vanhaesebroeck B. PI3K isoforms in cell signalling and vesicle trafficking. Nat Rev Mol Cell Biol 2019;20:515-34.

7. Ramos AR, Ghosh S, Erneux C. The impact of phosphoinositide 5-phosphatases on phosphoinositides in cell function and human disease. J Lipid Res 2019;60:276-86.

8. Mandal K. Review of PIP2 in Cellular Signaling, Functions and Diseases. Int J Mol Sci 2020;21.

9. Suzuki S, Suzuki C, Hinokio Y, et al. Insulin-mimicking bioactivities of acylated inositol glycans in several mouse models of diabetes with or without obesity. PLoS One 2014;9:e100466.

10. Wise HM, Hermida MA, Leslie NR. Prostate cancer, PI3K, PTEN and prognosis. Clin Sci (Lond) 2017;131:197-210.

11. Croze ML, Soulage CO. Potential role and therapeutic interests of myo-inositol in metabolic diseases. Biochimie 2013;95:1811-27.

12. Dinicola S, Unfer V, Facchinetti F, et al. Inositols: From Established Knowledge to Novel Approaches. Int J Mol Sci 2021;22.

13. DiNicolantonio JJ, J HOK. Myo-inositol for insulin resistance, metabolic syndrome, polycystic ovary syndrome and gestational diabetes. Open Heart 2022;9.

14. Burren KA, Scott JM, Copp AJ, Greene ND. The genetic background of the curly tail strain confers susceptibility to folate-deficiency-induced exencephaly. Birth Defects Res A Clin Mol Teratol 2010;88:76-83.

15. Cavalli P, Tonni G, Grosso E, Poggiani C. Effects of inositol supplementation in a cohort of mothers at risk of producing an NTD pregnancy. Birth Defects Res A Clin Mol Teratol 2011;91:962-5.

16. Greene ND, Leung KY, Gay V, et al. Inositol for the prevention of neural tube defects: a pilot randomised controlled trial. Br J Nutr 2016:1-10.

17. Wilson MP, Hugge C, Bielinska M, Nicholas P, Majerus PW, Wilson DB. Neural tube defects in mice with reduced levels of inositol 1,3,4-trisphosphate 5/6-kinase. Proc Natl Acad Sci U S A 2009;106:9831-5.

18. Wang Y, Lian L, Golden JA, Morrisey EE, Abrams CS. PIP5KI gamma is required for cardiovascular and neuronal development. Proc Natl Acad Sci U S A 2007;104:11748-53.

19. Jacoby M, Cox JJ, Gayral S, et al. INPP5E mutations cause primary cilium signaling defects, ciliary instability and ciliopathies in human and mouse. Nat Genet 2009;41:1027-31.

20. Guan Z, Wang J, Guo J, et al. The maternal ITPK1 gene polymorphism is associated with neural tube defects in a high-risk Chinese population. PLoS One 2014;9:e86145.

21. Clements RS, Jr., Diethelm AG. The metabolism of myo-inositol by the human kidney. J Lab Clin Med 1979;93:210-9.

22. Cryns K, Shamir A, Van AN, et al. IMPA1 is essential for embryonic development and lithium-like pilocarpine sensitivity. Neuropsychopharmacology 2008;33:674-84.

23. Figueiredo T, Melo US, Pessoa AL, et al. A homozygous loss-of-function mutation in inositol monophosphatase 1 (IMPA1) causes severe intellectual disability. Mol Psychiatry 2016;21:1125-9.

24. Walker CP, Pessoa ALS, Figueiredo T, et al. Loss-of-function mutation in inositol monophosphatase 1 (IMPA1) results in abnormal synchrony in resting-state EEG. Orphanet J Rare Dis 2019;14:3.

25. Troyer DA, Schwertz DW, Kreisberg JI, Venkatachalam MA. Inositol phospholipid metabolism in the kidney. Annu Rev Physiol 1986;48:51-71.

26. Nayak B, Kondeti VK, Xie P, et al. Transcriptional and post-translational modulation of myo-inositol oxygenase by high glucose and related pathobiological stresses. J Biol Chem 2011;286:27594-611.

27. Cogram P, Tesh S, Tesh J, et al. D-c*hiro*-inositol is more effective than m*yo*-inositol in preventing folate-resistant mouse neural tube defects. Human Reproduction 2002;17:2451-8.

28. Dinicola S, Chiu TT, Unfer V, Carlomagno G, Bizzarri M. The rationale of the myo-inositol and D-chiro-inositol combined treatment for polycystic ovary syndrome. J Clin Pharmacol 2014;54:1079-92.

29. Monastra G, Vucenik I, Harrath AH, et al. PCOS and Inositols: Controversial Results and Necessary Clarifications. Basic Differences Between D-Chiro and Myo-Inositol. Front Endocrinol (Lausanne) 2021;12:660381.

30. D'Souza SW, Copp AJ, Greene NDE, Glazier JD. Maternal Inositol Status and Neural Tube Defects: A Role for the Human Yolk Sac in Embryonic Inositol Delivery? Adv Nutr 2020.

31. Berry GT, Wu S, Buccafusca R, et al. Loss of murine Na+/myo-inositol cotransporter leads to brain myo-inositol depletion and central apnea. J Biol Chem 2003;278:18297-302.

32. Buccafusca R, Venditti CP, Kenyon LC, et al. Characterization of the null murine sodium/myo-inositol cotransporter 1 (Smit1 or Slc5a3) phenotype: myo-inositol rescue is independent of expression of its cognate mitochondrial ribosomal protein subunit 6 (Mrps6) gene and of phosphatidylinositol levels in neonatal brain. Mol Genet Metab 2008;95:81-95.

33. Molloy AM, Pangilinan F, Mills JL, et al. A Common Polymorphism in HIBCH Influences Methylmalonic Acid Concentrations in Blood Independently of Cobalamin. Am J Hum Genet 2016;98:869-82.

34. Brosnan JT, Mills JL, Ueland PM, et al. Lifestyle, metabolite, and genetic determinants of formate concentrations in a cross-sectional study in young, healthy adults. Am J Clin Nutr 2018;107:345-54.

35. O'Reilly J, Pangilinan F, Hokamp K, et al. The impact of common genetic variants in the mitochondrial glycine cleavage system on relevant metabolites. Mol Genet Metab Rep 2018;16:20-2.

36. Mills JL, Carter TC, Scott JM, et al. Do high blood folate concentrations exacerbate metabolic abnormalities in people with low vitamin B-12 status? Am J Clin Nutr 2011;94:495-500.

37. Desch KC, Ozel AB, Siemieniak D, et al. Linkage analysis identifies a locus for plasma von Willebrand factor undetected by genome-wide association. Proc Natl Acad Sci U S A 2013;110:588-93.

38. Carter TC, Pangilinan F, Molloy AM, et al. Common Variants at Putative Regulatory Sites of the Tissue Nonspecific Alkaline Phosphatase Gene Influence Circulating Pyridoxal 5'-Phosphate Concentration in Healthy Adults. J Nutr 2015;145:1386-93.

39. Shetty HU, Holloway HW. Assay of myo-inositol in cerebrospinal fluid and plasma by chemical ionization mass spectrometry of the hexaacetate derivative. Biol Mass Spectrom 1994;23:440-4.

40. Ueland PM, Midttun O, Windelberg A, Svardal A, Skalevik R, Hustad S. Quantitative profiling of folate and one-carbon metabolism in large-scale epidemiological studies by mass spectrometry. Clin Chem Lab Med 2007;45:1737-45.

41. Molloy AM, Scott JM. Microbiological assay for serum, plasma, and red cell folate using cryopreserved, microtiter plate method. Methods Enzymol 1997;281:43-53.

42. Kelleher BP, Broin SD. Microbiological assay for vitamin B12 performed in 96-well microtitre plates. J Clin Pathol 1991;44:592-5.

43. Brady J, Wilson L, McGregor L, Valente E, Orning L. Active B12: a rapid, automated assay for holotranscobalamin on the Abbott AxSYM analyzer. Clin Chem 2008;54:567-73.

44. Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007;81:559-75.

45. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005;21:263-5.

46. Leung KY, Mills K, Burren KA, Copp AJ, Greene ND. Quantitative analysis of myo-inositol in urine, blood and nutritional supplements by high-performance liquid chromatography tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2011;879:2759-63.

47. Coady MJ, Wallendorff B, Gagnon DG, Lapointe JY. Identification of a novel Na+/myo-inositol cotransporter. J Biol Chem 2002;277:35219-24.

48. Aouameur R, Da Cal S, Bissonnette P, Coady MJ, Lapointe JY. SMIT2 mediates all myo-inositol uptake in apical membranes of rat small intestine. Am J Physiol Gastrointest Liver Physiol 2007;293:G1300-7.

49. Lahjouji K, Aouameur R, Bissonnette P, Coady MJ, Bichet DG, Lapointe JY. Expression and functionality of the Na+/myo-inositol cotransporter SMIT2 in rabbit kidney. Biochim Biophys Acta 2007;1768:1154-9.

50. Groenen PM, Klootwijk R, Schijvenaars MM, et al. Spina bifida and genetic factors related to myo-inositol, glucose, and zinc. Mol Genet Metab 2004;82:154-61.

51. Dhiman G, Srivastava N, Goyal M, et al. Metadherin: A Therapeutic Target in Multiple Cancers. Front Oncol 2019;9:349.

52. Noch EK, Khalili K. The role of AEG-1/MTDH/LYRIC in the pathogenesis of central nervous system disease. Adv Cancer Res 2013;120:159-92.

53. Banerjee I, Fisher PB, Sarkar D. Astrocyte elevated gene-1 (AEG-1): A key driver of hepatocellular carcinoma (HCC). Adv Cancer Res 2021;152:329-81.

54. Emdad L, Das SK, Hu B, et al. AEG-1/MTDH/LYRIC: A Promiscuous Protein Partner Critical in Cancer, Obesity, and CNS Diseases. Adv Cancer Res 2016;131:97-132.

55. Tan X, Sun Y, Thapa N, Liao Y, Hedman AC, Anderson RA. LAPTM4B is a PtdIns(4,5)P2 effector that regulates EGFR signaling, lysosomal sorting, and degradation. EMBO J 2015;34:475-90.

56. Gu S, Tan J, Li Q, et al. Downregulation of LAPTM4B Contributes to the Impairment of the Autophagic Flux via Unopposed Activation of mTORC1 Signaling During Myocardial Ischemia/Reperfusion Injury. Circ Res 2020;127:e148-e65.

57. Hashemi M, Bahari G, Tabasi F, et al. LAPTM4B gene polymorphism augments the risk of cancer: Evidence from an updated meta-analysis. J Cell Mol Med 2018;22:6396-400.

58. Meng Y, Wang L, Chen D, et al. LAPTM4B: an oncogene in various solid tumors and its functions. Oncogene 2016;35:6359-65.

59. Avella MA, Baibakov B, Dean J. A single domain of the ZP2 zona pellucida protein mediates gamete recognition in mice and humans. J Cell Biol 2014;205:801-9.

60. Gupta SK. Human Zona Pellucida Glycoproteins: Binding Characteristics With Human Spermatozoa and Induction of Acrosome Reaction. Front Cell Dev Biol 2021;9:619868.

61. De Luca MN, Colone M, Gambioli R, Stringaro A, Unfer V. Oxidative Stress and Male Fertility: Role of Antioxidants and Inositols. Antioxidants (Basel) 2021;10.

62. Pak Y, Huang LC, Lilley KJ, Larner J. In vivo conversion of [3H]myoinositol to [3H]chiroinositol in rat tissues. J Biol Chem 1992;267:16904-10.

63. Pak Y, Hong Y, Kim S, Piccariello T, Farese RV, Larner J. In vivo chiro-inositol metabolism in the rat: a defect in chiro- inositol synthesis from myo-inositol and an increased incorporation of chiro-[3H]inositol into phospholipid in the Goto- Kakizaki (G.K) rat. Mol Cells 1998;8:301-9.

64. Hod M, Star S, Passonneau JV, Unterman TG, Freinkel N. Effect of hyperglycemia on sorbitol and myo-inositol content of cultured rat conceptus: failure of aldose reductase inhibitors to modify myo-inositol depletion and dysmorphogenesis. Biochem Biophys Res Commun 1986;140:974-80.

65. Sussman I, Matschinsky FM. Diabetes affects sorbitol and myo-inositol levels of neuroectodermal tissue during embryogenesis in rat. Diabetes 1988;37:974-81.

Table 1. Correlation of plasma myo-inositol with BMI, age, and alcohol intake in young adults (Trinity Student Study)[1]

Parameter	Mean (± SD)	*Myo*-inositol (Spearman's *p*)	*P*
BMI (kg/m^2)	23.0 ± 3.1	0.012	0.600
Age (years)	22.4 ± 1.7	0.072	0.001 [1]
Alcohol intake (g/day)	24.4 ± 21.1	0.058	0.0082

[1] Correlation was determined using raw MI data in the cohort of 2064 individuals (1258 females and 806 males).

[2] Indicates significant correlation (*P*<0.0023 set as level of statistical significance accounting for Bonferroni correction). BMI, body mass index; SD, standard deviation.

Table 2. Plasma *myo*-inositol concentration does not differ with smoking or use of contraceptives in young adults (Trinity Student Study)

Group	Participants, n (%)	Median plasma MI (μM) [1]
Total cohort	2059	26.6 ± 0.18
Smoking	648 (31.5%)	26.5 ± 0.30
Non-smoking	1411 (68.5%)	26.6 ± 0.22
P		0.448[2]
Total women	1258	25.9 ± 0.22
Oral contraceptive use	339 (26.9%)	25.7 ± 0.43
No oral contraceptive	919 (73.1%)	26.1 ± 0.26
P		0.434[3]

[1] Totals represent participants for whom information was provided and median values of MI concentration are shown (± standard error of the median).

[2, 3] No significant difference between smoking and non-smoking groups or between contraceptive use and non-use groups (Mann-Whitney test). MI, *myo*-inositol.

Table 3. Candidate genes tested for association with plasma MI concentration in young adults (Trinity Student Study) [1]

Gene symbol	Protein	Function	SNPs, n	tagSNPs, n
Candidate genes tested for association with plasma MI				
SLC2A13	Solute Carrier Family 2 Member 13	Inositol transport	113	65
SLC5A11	Solute Carrier Family 5 Member 11	Inositol transport	31	24
SLC5A1	Solute Carrier Family 5 Member 13	Glucose transport	18	10
SLC5A2	Solute Carrier Family 5 Member 13	Glucose transport	5	3
SLC5A3	Solute Carrier Family 5 Member 13	Glucose/inositol transport	9	4
Candidate genes relating to inositol synthesis and catabolism				
ISYNA1	inositol-3-phosphate synthase 1	MI synthesis	7	3
IMPA1	inositol monophosphatase 1	MI synthesis & IP recycling	17	8
MIOX	Myo-inositol oxygenase	MI catabolism	13	13
Candidate genes relating to production of highly phosphorylated inositol				
ITPK1	Inositol-Tetrakisphosphate 1-Kinase	Inositol phosphate kinase	48	36
IMPK	Inositol polyphosphate multikinase	Inositol phosphate kinase	17	8

[1] Data for individual SNPs in candidate genes is provided in Supplementary Table 2. IP, inositol phosphate; MI, *myo*-inositol; SNP, single nucleotide polymorphism.

Table 4. SNPs showing suggestive significant association with plasma *myo*-inositol in GWAS in young adults (Trinity Student Study)

Chromosome	SNP	BP	Allele [1]	Beta [2]	*P*	Gene [3]
5	RS13436726	3308137	G	0.0748	1.10×10^{-6}	
5	RS10037610	3321365	A	0.0704	2.59×10^{-6}	
7	RS342301	106164564	T	0.0140	9.08×10^{-6}	
8	RS2448193	98716623	A	0.0311	6.96×10^{-6}	*MTDH*
8	RS2002450	98856971	T	0.0290	1.28×10^{-6}	*LAPTM4B*
12	RS28390364	131460020	A	0.0592	1.73×10^{-6}	
16	RS7189430	21117507	C	0.0130	8.44×10^{-6}	*ZP2*[3]
22	RS135374	45961150	T	0.0204	2.46×10^{-7}	

[1] Allele is minor allele.

[2] Beta is the regression coefficient.

[3] Among the 8 SNPs which show suggestive significance ($P<1 \times 10^{-5}$), RS2448193 lies at -8.95 kb from *MTDH*, RS2002450 lies at -0.013 kb from *LAPTM4B* and RS7189430 lies within the *ZP2* gene.

BP, base-pair (physical position); GWAS, genome-wide association study; SNP, single nucleotide polymorphism.

Table 5. Correlation of circulating MI with DCI and other metabolites in young adults (Trinity Student Study) [1]

Metabolite [2]	**Concentration (mean ± SD)**	**Spearman's *p***	***P***
Chiro-inositol, µM	8.5 ± 9.2	0.422	5.8×10^{-90} *
Glucose, mM	4.7 ± 1.0	0.161	1.8×10^{-13} *
Serum Folate, nM	22.4 ± 1.7	0.128	4.7×10^{-9} *
Red Cell Folate, nM	1070 ± 427	0.117	1.1×10^{-7} *
Serum Vitamin B_{12}, pM	332 ± 147.0	0.030	0.169
Serine, µM	147 ± 24.0	0.048	0.029
Glycine, µM	293 ± 64.1	0.080	2.9×10^{-4} *
Formate, µM	30.0 ± 13.6	0.203	1.1×10^{-20} *
Holotranscobalamin, pM	59.3 ± 31.2	0.090	4.7×10^{-5} *
Methylmalonic Acid, µM	0.2 ± 0.1	0.064	0.004
Homocysteine, µM	8.6 ± 3.0	0.054	0.014
Methionine, µM	29.3 ± 8.3	0.206	4.2×10^{-21} *
Cystathionine, µM	0.14 ± 0.08	0.203	2.3×10^{-20} *
Betaine, µM	37.5 ± 14.2	0.230	4.3×10^{-26} *
Dimethylglycine, µM	4.2 ± 1.2	0.108	8.0×10^{-7} *
Sarcosine, µM	1.5 ± 6.3	0.261	3.3×10^{-33} *
Tryptophan, µM	70.6 ± 13.1	0.244	3.7×10^{-29} *

[1] Potential correlation of MI with other metabolites was examined by Spearman's rank correlation using raw data, with statistical significance (*) set at $P<0.0023$ following

Bonferroni correction for multiple testing. The number of individuals was 1,066 for MI vs DCI and 2,064 for MI vs other metabolites.

[2] Analytes were analysed in plasma unless otherwise noted e.g. serum folate and vitamin B_{12} and red blood cell folate.

DCI; D-*chiro*-inositol; MI, *myo*-inositol.

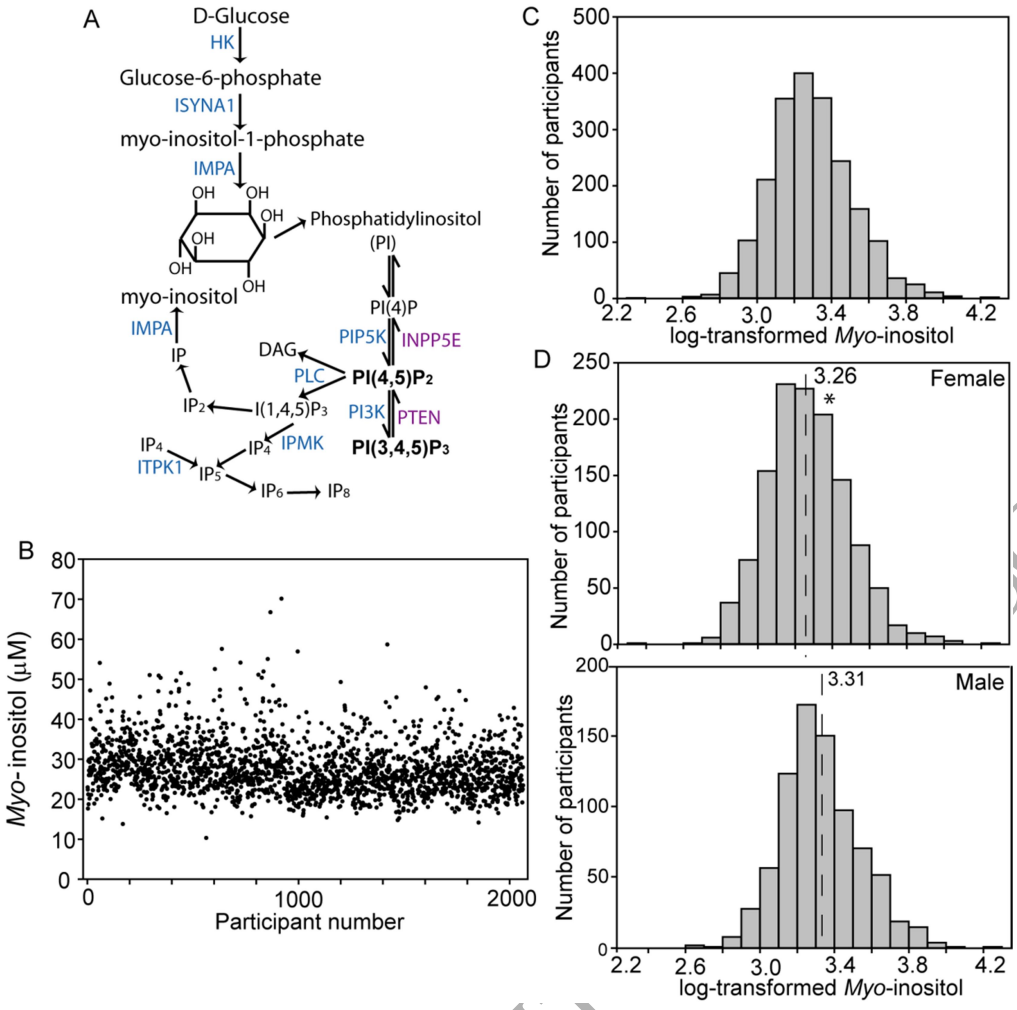

Figure 1. Plasma *myo*-inositol concentration in young adults (Trinity Student Study). (A) Summary of MI synthesis pathway, phosphoinositide (PI) signalling and recycling of inositol phosphates (IPs). (B) Concentration of MI in plasma by participant number. (C) Distribution of log-transformed values for MI concentration (all 2,064 individuals). (D) Distribution of log-transformed MI values in females and males with median values indicated by dotted line. Mean plasma MI (± standard error) was 26.9 ± 0.18 μM in females (n = 1258) and 28.5 ± 0.23 μM in males (n = 806). * different from males (t-test; $P<0.0010$). MI, *myo*-inositol; PI, phosphoinositide.

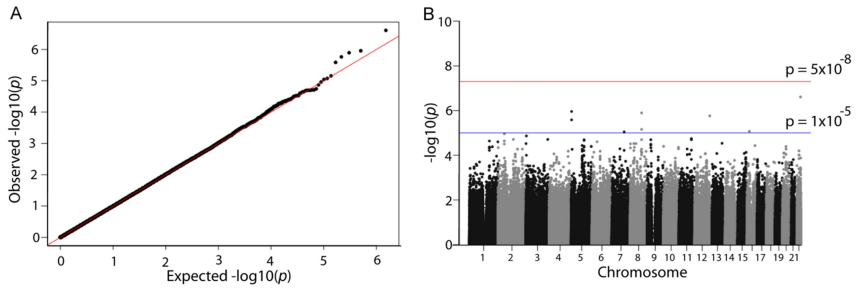

Figure 2. Genome-wide association study of plasma *myo*-inositol in young adults (Trinity Student Study). (A) Quantile-quantile (Q-Q) plot shows distribution of observed vs expected *P*-values. (B) Manhattan plot showing -$\log_{10}$(p) for each SNPs ordered by chromosome, indicated by alternating black and grey colour. Lines indicate cut-off for genome-wide statistical significance ($P = 5x10^{-8}$; line at $-\log_{10}(P)=7.3$) and suggestive significance ($P = 1x10^{-5}$; line at $-\log_{10}(P)=5.0$). Data was obtained from 2,064 participants.

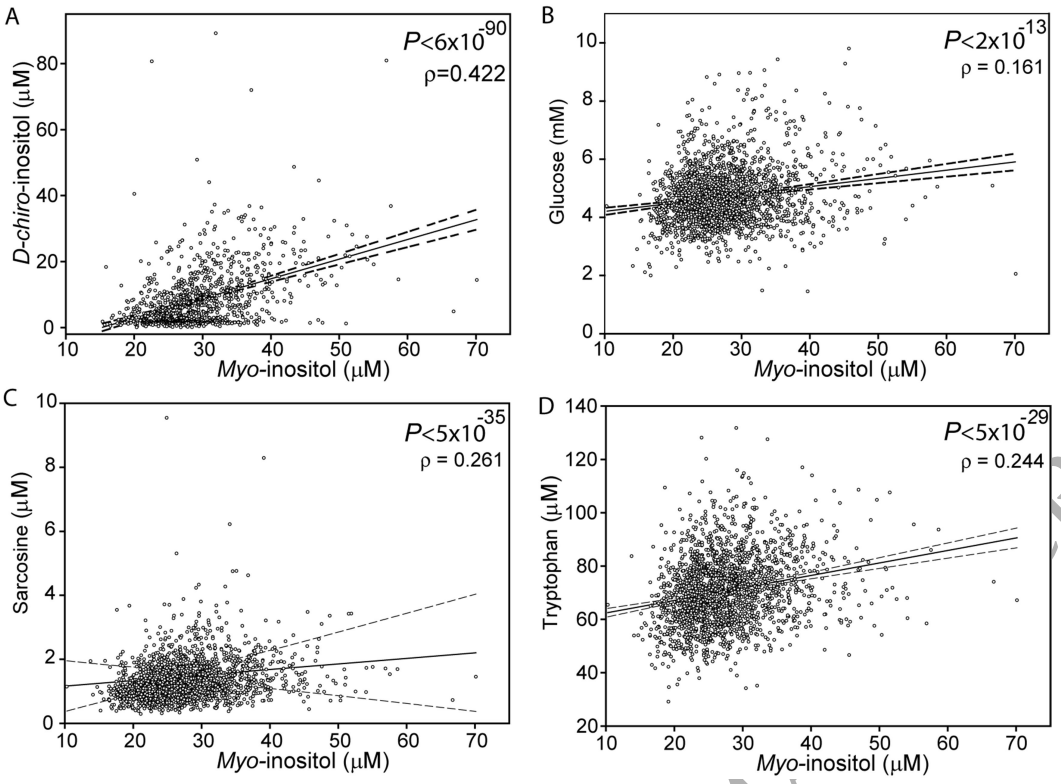

Figure 3. Correlation of plasma *myo*-inositol with *D-chiro* inositol, glucose, sarcosine and tryptophan in young adults (Trinity Student Study). Plasma MI concentration shows significant correlation with (A) DCI, (B) glucose, (C) sarcosine, and (D) tryptophan. Solid line represents regression line and dashed line indicates 95% confidence intervals. The number of participants was 1,066 for MI vs DCI and 2,064 for MI vs other metabolites. *P* values for Spearman's rank correlation and Spearman's *p* are shown in each panel. MI, *myo*-inositol; DCI, *D-chiro* inositol.